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TITLE: Use of the Abdominal Aortic Tourniquet for Hemorrhage Control

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CONTRACTING ORGANIZATION: Georgia Health Sciences University
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14. ABSTRACT Overall the project has made good progress despite multiple administrative hurdles. Delays occurred due to a temporary shortage of Veterinarians at Georgia Regents University. The research was to be completed at Fort Gordon which required going through a third party not for profit foundation. These delays have necessitated a no cost extension on the project. We have accomplished the administrative requirements and are now ready to begin the animal research protocols in the next 60 days. The wounding device (Blade Lever Apparatus) has been developed. The protocol has IAUC and ACURO approval. We have established a CRADA and have drafted a subcontract with Geneva Foundation to allow us to complete the research at Eisenhower Army Medical Center.					
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1. Project Status

a. Outcome Summary and Issues

ACCOMPLISHMENTS:

Overall the project has made good progress despite multiple administrative hurdles. Delays occurred due to a temporary shortage of Veterinarians at Georgia Regents University. The research was to be completed at Fort Gordon which required going through a third party not for profit foundation. These delays have necessitated a no cost extension on the project. We have accomplished the administrative requirements and are now ready to begin the animal research protocols in the next 60 days. The wounding device (Blade Lever Apparatus) has been developed. The protocol has IAUC and ACURO approval. We have established a CRADA and have drafted a subcontract with Geneva Foundation to allow us to complete the research at Eisenhower Army Medical Center.

The following milestones have been completed as listed in the proposal:

1. Milestone 4. IAUC approved protocol. Protocol approved by ACURO.

PRODUCTS: None

ISSUES: Coordination with Fort Gordon has taken a longer time than originally anticipated. The proper process has now been identified and the investigators at GRU are working closely with the Fort Gordon investigators. Protocol now ready and anticipate starting animal research with in the next 60 days.

b. Progress Summary

Please include a brief summary concerning the status of your project. Address technical progress aligned with schedule, but exclude discussion of costs in this section. Add additional row for each quarter to create a table showing all quarters to date.

Quarter 1:	-Kick off meeting with TATRC -The wounding model has been developed and tested using dead pigs -Investigators have had periodic meetings -Protocol 90% complete and will be submitted to IACUC early second quarter
Quarter 2:	-Protocol completed and submitted to IACUC with one revision -Coordination between GRU and Fort Gordon investigators

Quarter 3: Protocol approved by IACUC and ACURO
Quarter 4: No Cost Extension submitted. CRADA in place. Subcontract with Geneva Foundation drafted.

c. Progress Detail

Describe each task or logical segment on which effort was expended during this quarterly reporting period only, including pertinent data and graphs in sufficient detail to explain any significant results achieved. Methodology used should be succinct.

Approved Protocol Below:

PROTOCOL TITLE: Evaluation of traumatic porcine wounding model on hemodynamic and intracranial physiology.

PRINCIPAL INVESTIGATOR(S): Richard B. Schwartz, MD (Georgia Regent's University) and James McPherson (Clinical Investigations DDEAMC)

CO INVESTIGATOR (S): Matt Lyon MD (Georgia Regent's University), Brad Reynolds MD (Georgia Regent's University), Steve Shiver MD (Georgia Regents University), Steve Holsten MD (Georgia Regents University), Troy Akers DO (C Company DDEAMC), James Black MD (Georgia Regents University), Stefan Kazacos MD (C Company DDEAMC), Charlie Moore MD (C Company DDEAMC)

I. NONTECHNICAL SYNOPSIS:

This study is designed to evaluate the effectiveness of a new device to control and stop severe bleeding from a realistic combat wound (similar to those encountered on the battlefield). Additionally this study functions as a pilot study to evaluate the use of an ultrasound technique to diagnose increased intracranial pressure which is a hallmark of head injury. Patient medical indicators will be evaluated to determine if the device is effective compared to standard treatments employed by battlefield medical personnel.

II. BACKGROUND:

II.1. Background:

Section 1. Abdominal Tourniquet

Uncontrolled hemorrhage remains the leading cause of preventable death on the battlefield (1, 2, 3). While body armor protects vital organs in the torso, injuries to the unprotected inguinal and other junctional regions can lead to rapid exsanguination (4). Penetrating injuries involving the proximal femoral and iliac vasculature are particularly difficult to control as they often are not amenable to tourniquet or hemostatic agent application (5, 6). Currently there is no consistently effective method to provide hemorrhage control in these non-compressible areas. Hemorrhage control can be difficult in ideal circumstances

and nearly impossible in the austere battlefield environment. Prior studies have shown that flow in the common femoral artery (CFA) can be stopped through the application of 80 to 140 pounds of external pressure over the distal abdominal aorta (AA) (7). As there are limited options in the setting of inguinal injury (8), external abdominal pressure has multiple potential advantages. Blood flow to the injury site is limited and peripheral vascular resistance is increased, thus maximizing perfusion to vital organs such as the heart and brain. While applying external pressure to the abdomen is an effective technique to limit or eliminate blood flow to the inguinal region, it is highly provider dependent and potentially dangerous. Too little pressure results in inadequate hemorrhage control, whereas excessive or misplaced pressure may result in injury to bowel or other structures. Furthermore, the provider must maintain adequate pressure during transport until the time of definitive treatment. In many scenarios, providing this type of external abdominal pressure would be extremely difficult if not impossible due to operational constraints. The abdominal aortic tourniquet (AAT) is a pneumatic belt that allows for the constant delivery of pressure over a specific area for a prolonged period of time. The device is considered a Class 2 device by the FDA and has received FDA 510-k pre-market approval. The device is commercially available and has been fielded by US Army units in Afghanistan.. The device has shown efficacy and safety in a porcine model for aortic occlusion for up to 60 minutes. The data from this study was presented to the committee on tactical combat casualty care in November 2009. Feedback from this presentation highlighted the need for further study to evaluate the device in a hypotensive model. While it is intuitive that abdominal aortic occlusion will decrease or eliminate flow in the iliac and femoral arteries, it is unknown what the hemodynamic effect will be on the hypotensive patient, which the committee felt was important for further evaluation of the device. The device is placed on the lower abdomen at the level of the umbilicus. This location causes compression of the distal aorta below the renal arteries. The compression of the abdomen the device does cause a degree of increased respiratory effort. This study will quantify the degree of respiratory difficulty before and after device application.



Abdominal Aortic Tourniquet (AAT)

Section 2. Evaluation of Ultrasound (U/S) technique to determine intracranial pressure from the Optic Nerve Sheath Size

Head injury can be difficult to diagnose, especially in difficult battlefield settings.

Imaging techniques can reveal head injuries that patients are not clinical for, though a more rapid technique would be more preferable. It has been shown that the optic nerve sheath (ONS) dilates with increasing intracranial pressure (ICP). We did a cadaveric experiment that showed that this dilation occurs immediately with increasing pressure but the amount of change of the ONS is not proportional to the change in ICP. This cadaver experiment, however does not take into account auto regulation and other physiologic processes. Other studies have evaluated 2D ultrasound to evaluate the relationship and strong correlation was identified in severe cases in TBI, but proved ineffective in identifying mild cases. 3D evaluation of the ONS might provide more geometric changes that could allow for better detection of mild cases of TBI. The purpose of the animal experiment is to see if the relationship (non-uniform expansion of the ONS with elevating ICP) holds true in a live model using the 3D ultrasound technique.

II.2. Literature Search for Duplication:

II.2.1. Literature Source(s) Searched: MEDLINE, PubMed, NIHReporter, DTIC, Agricola

II.2.2. Date of Search: 20 MAR 2013

II.2.3. Period of Search: 1946 to MAR 2013

II.2.4. Key Words of Search: intracranial pressure, intracranial hypertension, optic nerve, myelin sheath, ONS, animal welfare, animal testing alternative, computer simulation, in vitro, virtual, cadaver, mannequin, abdominal aortic tourniquet, hemorrhage, bleeding, swine, sus, scrofa, femoral artery, tourniquets, hypotensive, iliac artery

II.2.5. Results of Search: A lot of work is being done to investigate tourniquet application in a pre-hospital setting. However none of the studies were specifically testing the efficacy of a novel new device. Since this is a new device there is an expectation of duplication. For ICP and ONS articles there are several clinical reports in humans but no experimental studies have been conducted to attempt to devise a mathematical relationship between the two using 3D U/S for making geometric measurements.

III. OBJECTIVE/HYPOTHESIS:

Hypothesis #1: The AAT device shall maintain a mean arterial higher over the course of the 60 minutes than other devices, treatments applied after wounding.

Hypothesis #2: There is a linear relationship between ONS diameter and ICP.

This study will utilize a porcine experimental model to evaluate the AAT and ONS U/S technology with the following objectives:

1. Demonstrate the effectiveness of hemorrhage control of a complex hemi-pelvectomy injury utilizing the AAT.
2. Measure the effect on blood pressure following application of the AAT and aortic occlusion in a hypotensive hypovolemic porcine model.
3. Measure the effect on peak airway pressure before and after the application of the AAT.
4. Confirm the utility of U/S for relating ONS size to ICP.
5. Further clarify how unilateral solitary hematomas impact ICP and ONS measurement.

IV. MILITARY RELEVANCE:

Hemorrhage in non-compressible sites such as the junction of the torso to the lower extremities continues to be a major cause of death on the modern battlefield. Currently, there is no consistently effective method to control this hemorrhage until arrival to definitive operative care. This device is designed to control hemorrhage of these otherwise fatal injuries at the point of injury to allow time for transport to definitive care. If effective a device of this kind has the potential to save the lives of many US military personnel.

The ONS ultrasound technique would give physicians in the field the ability to rapidly diagnose TBI and refer for neurological consult and ensure the patient received definitive care more quickly.

V. MATERIALS AND METHODS:

VI. Experimental Design and General Procedures:

Up to 20 25-50 kg female pigs will be obtained and utilized. Venous and Arterial catheters will be placed for IV access, fluid therapy and hemodynamic monitoring. The animals will be intubated and undergo general anesthesia and first set of procedures will be performed.

Section 1. Measurement of the ONS and relation to ICP

To measure the ONS, we use a linear array transducer placed on the closed eyelid. The ONS is visible posterior to the globe as a linear shadow. An intraventricular catheter will be placed and instill normal saline in 5 to 10 ml increments. This catheter will be connected to a transducer element to monitor ICP. We would instill fluid, measure the ICP and ONS width until there was a stabilization in the pressure (may take a few minutes). Then repeat.

On another subset of the pigs have an epidural mass created (to simulate an epidural hematoma) using a bone drill to access the epidural space via a craniotomy and then use a small bladder and fill with fluid. We would measure the ICP via the monitor and the ONS bilaterally using the U/S machine.

Once complete and patient is stable the veterinarian will administer the depolarizing paralytic. The pig will be ventilated utilizing intermittent positive pressure ventilation (IPPV) and peak airway pressure will be recorded. The AAT will then be placed on the pig according to the manufacturers instructions and the peak airway pressure will be measured once again. The AAT would then be removed and the paralytic allowed to metabolize for ten minutes before moving on to the wounding portion of the study.

Section 2. Application of the Tourniquet

When the mean arterial pressure is stable for a period of 10 minutes. The pig will be wounded utilizing the "Blade Lever Device" creating an injury from the left anterior superior iliac spine to the right ischial tuberosity. This injury model has been tested utilizing dead pig carcasses and should create a fatal wound that involves the iliac vessels proximal to the inguinal ligament. At this time the abdominal aortic tourniquet will be applied and inflated or the combat gauze packing (other FDA approved junctional tourniquets may be utilized in place of the combat gauze) will occur followed by 2 minutes of direct pressure. A five minute observation period will then occur with blood pressure obtained

each minute. Initial blood loss will be measured and recorded along with time to hemostasis. Following the observation period, 500 mL of Hetastarch will be bolus infused. The blood pressure will be maintained at a mean arterial pressure of 60 mmHg or above. If the mean arterial pressure drops below 60 mmHg normal saline fluid boluses will be given until the mean arterial pressure exceeds 65 mmHg. Blood pressure shall be recorded every 5 min. Following a total of 60 min. of observation following injury the animals will be euthanized and total blood loss measured.

V.2. Data Analysis:

This study will involve a within groups design, including up to 20 female pigs. (Groups will be six animals, additional animals are included for unexpected losses)

Dependent variables include:

1. Mean arterial pressure (taken every one minute for the first 5 minutes then every 5 minutes for a total of 17 times over the one hour procedure; analyzed of times)
2. Peak airway pressure (taken one time prior to wounding)
3. Blood loss at 5 and 60 minutes and recording of time of initial hemostasis
4. Optic Nerve Sheath size in mm (measured before and after fluid and/or mass is created)
5. Intracranial Pressure in mmHg (measured before and after fluid and/or mass is created)

Group sample sizes of $n = 6$ in the treatment group (AAT) and $n = 6$ in the control group (Combat gauze or other FDA approved junctional tourniquet) achieve 90% power to detect a difference between the group proportions of 0.80. The variable used to calculate power was survival from the previous studies. The proportion in the treatment group is assumed to be 0.10 under the null hypothesis and 0.90 under the alternative hypothesis. The proportion in the control group is 0.90. The test statistic used is the two-sided Z test with pooled variance. The significance level of the test was targeted at 0.05. The significance level actually achieved by this design is 0.0169.

V.3. Laboratory Animals Required and Justification:

V.3.1. No animal Alternatives Considered: We considered using means other than animals to demonstrate this technique but this is largely a study of physiology and it would not be feasible to replicate the infinite number of complex interactions between body systems with a simulation.

V.3.2. Animal Model and Species Justification: Pigs were chosen for this experiment because their size permits application of the device to their abdomen and is effective at replicating the experience in humans. Additionally, physiology is similar enough to humans to adequately model changes induced by hemorrhage and elevated ICP.

V.3.3. Laboratory Animals:

V.3.3.1. Genus & Species: Sus Scrofa

V.3.3.2. Strain/Stock: Yorkshire cross

V.3.3.3. Source/Vendor: Palmetto Research Swine; Reevesville, SC.

V.3.3.4. Age: under six months

V.3.3.5. Weight: 50 Kg

V.3.3.6. Sex: Either

V.3.3.7. Special Considerations: None

V.3.4. Number of Animals Required (By Species): 20 Pigs

V.3.5. Refinement, Reduction, Replacement:

V.3.5.1. Refinement: This technique is the first step to evaluate the utility of this device in controlling severe hemorrhage after having tested it on multiple swine cadavers. The relationship between ONS diameter and ICP has been observed in humans however, the nature of the relationship between the two (i.e. is it linear?) has not been described.

V.3.5.2. Reduction: The pigs utilized in this study will contribute to data for two different studies. Thereby reducing the total number of pigs needed if these studies were completed independently. The minimum amount of pigs will be used based on statistical power calculations with additional animals as required for pre-mature loss.

V.3.5.3. Replacement: No computer or other simulator can replicate hemorrhage and vascular control when evaluating a new device. Additionally no computer can effectively evaluate the effects of ICP on the ONS diameter. There are no other methods that can adequately model this complex physiologic events.

V.4. Technical Methods:

V.4.1. Pain/Distress Assessment:

V.4.1.1. APHIS Form 7023 Information:

V.4.1.1.1. Number of Animals: 20

V.4.1.1.1.1. Column C: 0

V.4.1.1.1.2. Column D: 20 Pigs, 100%

V.4.1.1.1.3. Column E: 0

V.4.1.2. Pain Relief/Prevention:

V.4.1.2.1. Anesthesia/Analgesia/Tranquilization: Animals will be heavily sedated with midazolam (0.5mg/kg) intramuscular injection. Catheter will be placed and animals will be induced with fentanyl (30-50mcg/kg) and propofol (0.8-1.6mg/kg) IV. General anesthesia will be facilitated by isoflurane delivered via an endotracheal tube. An arterial line will be placed to monitor BP. Anesthesia will be maintained with isoflurane and continuous rate infusion of fentanyl (50-100mcg/kg/hr) for pain control. Ketamine (2mcg/kg/min) will be used for pain control after completion of ONS experiments. Additional drugs such as dopamine (1-10mcg/kg/hr), lidocaine (50mcg/kg/min) and amiodarone (0.5-3.5mg/kg/hr) may be infused to support cardiac function. Epidurals will be utilized to facilitate pain control which will be a single dose of

morphine (0.1mg/kg) and bupivacaine (1mL/4.5kg).

V.4.1.2.2. Pre- and Post-procedural Provisions: Prior to anesthesia each pig will be weighed and examined to assess physical status. Up to 12 hours prior to the surgery, the animals will receive water, food, and enrichment as per the institution standard protocols. Food will be withheld at least 12 hours prior to anesthetic induction. Water will not be restricted. Prior to induction, pigs will be weighed to determine accurate dosage for all drugs. Catheters will be placed as needed for drug administration and fluid delivery and monitoring. This is a terminal procedure. Observation will be continuous by the attending veterinarian from the induction of anesthesia until the animal is sacrificed at the end of the procedure.

V.4.1.2.3. Paralytics:

Use of non-depolarizing paralytic agents is required to prevent patient motion during measuring of peak airway pressure. Use of paralytics will be performed under general anesthesia. Measuring peak airway pressure is essential to ensuring the device does not cause significant barotrauma after application.

Vecuronium (0.1mg/kg/hr) will be used. Its use will be employed once the following conditions have been met:

1. The animal has achieved a stable plane of anesthesia and has no change in vital parameters in the last five minutes.
2. The animal has received a LS epidural and no longer responds to superficial or deep pain stimulation.
3. The animal is receiving analgesia through multiple different mechanisms of action.

Once these conditions have been met it is at the discretion of the Veterinarian if the administration of Vecuronium can proceed.

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures:

This is a terminal procedure that will be done entirely under anesthesia. There are no other alternatives to test or hypothesis. See search result below.

V.4.1.3.1. Source(s) Searched: MEDLINE, PubMed, NIHReporter, DTIC, Agricola

V.4.1.3.2. Date of Search: 20 MAR 2013

V.4.1.3.3. Period of Search: All Time Periods

V.4.1.3.4. Key Words of Search: intracranial pressure, intracranial hypertension, optic nerve, myelin sheath, ONS, animal welfare, animal testing alternative, computer simulation, in vitro, virtual, cadaver, mannequin, abdominal aortic tourniquet, hemorrhage, bleeding, swine, sus, scrofa, femoral artery, tourniquets, hypotensive, iliac artery

V.4.1.3.5. Results of Search: One article discussed using a mannequin as a model for tourniquet application but it wouldn't allow for measurement of outcomes such as MAP (mean arterial pressure) which is going to be the indicator of whether or not this device will actually keep a human alive.

V.4.1.4. Unalleviated Painful Procedure Justification: N/A

V.4.2. Prolonged Restraint: N/A

V.4.3. Surgery:

V.4.3.1. Pre-surgical Provisions: The animals will be housed and sedated and anesthetized as described above prior to beginning the procedure.

V.4.3.2. Procedure:

Placement of the intraventricular catheter for monitoring of ICP and infusion of saline. The skin will be incised 2 cm anterior to the coronal suture in a parasagittal plane passing through the pupil of the ipsilateral eye. A twist drill hole will be made through the skull, and the dura will be nicked for catheter insertion. The catheter will be inserted 5–7 cm until CSF is obtained. The IVC will then be tunneled in a retrograde fashion to a distant skin exit site and is connected via a 3-way stopcock to an external pressure transducer and CSF drainage system. The catheter will be sutured in place and a nonocclusive dressing applied. The catheter will be connected to a transducer element to monitor pressure. 5-10mL of saline will be instilled and U/S of the eye will be completed to measure ONS diameter in relation to ICP.

Craniotomy. A midline incision from the level of lateral canthi to 4-7cm past the external occipital protuberance will be made. A sharp incision into the cutaneous coli muscles will be made. The cervioscutularis will be transected near midline, followed by sharp incision of the temporalis fascia. The temporalis musculature will be reflected ventrally by elevation to the side of the skull allowing access to bone. An oval defect approximately 10cm in diameter will be created using a high speed air drill. A periosteal elevator will be used to remove the bone flap off the skull. Rongeurs will be used to clean the edges and cautery to stop any bleeding. At this point a small bladder would be placed into the cranium and infused with 5-20mL of fluid to simulate an epidural mass.

Tourniquet Testing. The pig would be re-positioned in dorsal recumbency and aligned with the Blade Lever device. The pig would be strapped to the blade lever device and once the experiment is ready to begin the device would be applied to create the hemipelvectomy. Application will occur quickly in one swift motion from the lateral surface just cranial to the wing of the ilium to the caudal portion of the ischium. Once the hemipelvectomy is created the tourniquet would be rapidly applied to stop blood loss. The pig would remain anesthetized for another 60 minutes under monitoring before euthanasia.

V.4.3.3. Postoperative Provisions: This is a terminal procedure.

V.4.3.4. Location: Surgical Suite (Room 132) of building 38705 (DCI).

V.4.3.5. Surgeons:

Jake Lowry DVM: Attending Veterinarian, Eisenhower Army Medical Center. Dr. Jake Lowry is an experienced veterinary surgeon having 4 years of multi-species surgical experience and training while at the Colorado State University Veterinary Teaching Hospital, University of Wyoming and University of Texas Michael Keeling Center for Comparative Medicine & Surgery and during his tenure at DDEAMC.

Richard Schwartz MD: Chairman of Emergency Medicine. Dr Schwartz is an experienced physician with over 17 years experience with surgical experiments

with porcine models. He has published the results of these studies in respected peer reviewed journals.

Matt Lyon MD: Vice Chairman of Academic Programs. Dr. Lyon is an experienced physician and researcher with over 9 years experience with surgical experiments with porcine models. He has published the results of these studies in respected peer reviewed journals.

Brad Reynolds MD: Associate Residency Director. Dr. Reynolds is an experienced physician and researcher with over 8 years of experience with surgical experiments with porcine models. He has published the results of these studies in respected peer reviewed journals.

Steve Shiver MD: Residency Director. Dr. Shiver is dual trained in General Surgery and Emergency Medicine. He is an experienced physician and researcher with over 7 years experience with surgical experiments with porcine models. He has published the results of these studies in respected peer reviewed journals.

Steve Holsten MD: Trauma Surgeon. Dr. Holsten is a skilled trauma surgeon with the requisite surgical skills that make him a vital addition to the research team. He is also a gifted researcher and has published in respected peer reviewed journals.

V.4.3.6. Multiple Survival Surgery Procedures: N/A

V.4.4. Animal Manipulations:

V.4.4.1. Injections: All injections will be administered using an 18-27 gauge needle and appropriate volumes. Intramuscular injections will be given in the paralumbar, thigh, or neck/shoulder muscles as detailed above. Animals will be restrained either manually or using a restraint device when necessary to ensure the safety of both personnel and animals.

V.4.4.2. Biosamples: Blood samples for labs may be drawn pre-procedurally or during the procedure by veterinary staff.

V.4.4.3. Adjuvants: N/A.

V.4.4.4. Monoclonal Antibody (MAbs) Production: N/A

V.4.4.5. Animal Identification: Animals will be assigned an identification number after delivery. The animals will be marked with an indelible marker, or tag on their body or ear and have a cage card with their identification number. If the animals have previously been tagged or notched these methods may be implemented for identification purposes.

V.4.4.6. Behavioral Studies: None

V.4.4.7. Other procedures: See Section V.1.

V.4.4.8. Tissue Sharing: Tissue will be made available to other investigators after the completion of the study and euthanasia of the animals. Tissue will be held for

5 days prior to being released for tissue sharing.

V.4.5. Study Endpoint: The individual animals will be euthanized after the data collection from the tourniquet study is completed meaning the tourniquet had been applied for the sixty minutes and MAP and other variables recorded. This protocol will be concluded when group statistical power is achieved between control and treatment groups in that data from six animals was recorded to completion.

V.4.6. Euthanasia: The animal will be sacrificed following the procedure at the discretion of the attending veterinarian as specified by the AVMA guidelines.

V.5. Veterinary Care:

V.5.1. Husbandry Considerations: Swine for this protocol will be maintained in Building 38705 prior to the procedure. Rooms are maintained at appropriate temperature and humidity according to the Guide for Care and Use of Laboratory Animals. If possible, the animals will be group housed (2-3/run) prior to surgery. Animals will be fed standard Pig Chow prior to surgery. All animals will receive fresh drinking water ad libitum via the automatic watering system. A 12-hour light/dark cycle will be provided.

V.5.1.1. Study Room: Surgical Suite, BLDG 38705, Fort Gordon, GA.

V.5.1.2. Special Husbandry Provisions: None

V.5.1.3. Exceptions: None

V.5.1. Veterinary Medical Care:

V.5.2.1. Routine Veterinary Medical Care: Veterinary care will be under the supervision of the Attending Veterinarian. Any problems noted by the laboratory animal technicians are immediately reported to the senior technician and/or Attending Veterinarian and promptly dealt with. The Attending Veterinarian and PI will determine the course of treatment if medical issues arise in the animals. Ultimately the decision to euthanize a critically ill animal will be made by the Attending Veterinarian.

V.5.2.2. Emergency Veterinary Medical Care: Emergency care will be provided to treat any condition that is causing severe pain or distress and/or is life threatening. An attempt will be made to contact the PI prior to treatment. If the condition cannot be adequately treated, humane euthanasia will be performed. Animals are observed at least once daily by laboratory animal technicians including weekends. A veterinarian is always on call for response to emergencies.

V.5.3. Environmental Enrichment:

V.5.3.1. Enrichment Strategy: Pigs will receive enrichment IAW LASS SOP. A ball, or like item, will be placed in the cage to provide a form of activity for the animals. In addition, pre-approved food treats and human interaction will be provided. All pigs will be group housed.

V.5.3.3. Enrichment Restriction: None

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

All individuals will receive a safety orientation and occupational health and zoonotic disease from the Attending Veterinarian prior working in the vivarium the first time.

Additionally all individuals involved will complete the following training courses those training courses in addition to CV's will be uploaded into IRBNet.

1. Investigators, Staff and Students, Lab Animal Research
2. Working with Swine in Research Settings, Lab Animal Research

Richard Schwartz, MD: Dr. Schwartz is a 1989 graduate of Wayne State University School of Medicine. He completed his internship at William Beaumont Army Medical Center and then completed his Emergency Medicine Residency training at Madigan Army Medical Center in Tacoma, Washington. He has extensive military operational experience. He served with the 5th Special Forces Group (Airborne) during Operation Desert Shield and Desert Storm. He also served with the 1st Special Warfare Training Group (Airborne) and the Joint Special Operations Command (Airborne). Dr. Schwartz was Chief of Emergency Medicine at Eisenhower Army Medical Center before joining the MCG faculty and received numerous awards during his Army service including the bronze star and two meritorious service medals.

While at MCG, he has been instrumental in curriculum development. He was one of the original developers of the American Medical Association (AMA) National Disaster Life Support Courses. These innovative courses such as Basic and Advanced Disaster Life Support (BDLS and ADLS) bridge the gap between numerous military and civilian groups that may need to work together during major disasters. The courses have been taught in forty-eight states leading to the training of over one hundred thousand providers. More recently he has worked with the National Tactical Officers Association (NTOA) on the development of standardized training for tactical emergency medical support. He is very active in research pertaining to improving the methods employed in austere and disaster medical situations. His relevant publications include three Disaster Medicine textbooks (editor) and chapters in three additional texts. Dr. Schwartz has published extensively in the field with numerous abstracts and peer-reviewed articles pertaining to Disaster Medicine. Dr. Schwartz is an active participant in the Terrorism Injuries: Information Dissemination and Exchange (TIIDE) project with the Centers for Disease Control (CDC). This project has helped lead efforts for standardized disaster triage through the National Association for EMS Physician (NAEMSP). He is also a medical device innovator and has developed a unique video airway management system and he holds eight patents. His research areas of interest are in hemorrhage control, disaster medicine and airway.

James McPherson III, PhD: James McPherson III, PhD is the research biochemist at Dwight D. Eisenhower Army Medical Center Department of Clinical Investigations. He has been involved in animal model development for the past 30 years, mostly here at DDEAMC. He has published extensively over those years on very diverse sets of projects. He will function as the Site PI at DDEAMC coordinating the administrative and technical requirements for this project.

Matt Lyon, MD: Matthew Lyon, MD is the Director of the Section of Emergency and Clinical Ultrasound at GHSU and the Director of the MCG's emergency observation unit. Dr. Lyon is a board-certified emergency physician and has used ultrasound in his clinical practice for over 7 years. Dr. Lyon graduated from MCG's medical school and stayed at GHSU to complete his Emergency Medicine Residency.

Dr. Lyon has significant educational experience and has published over 30 peer-

reviewed articles on the use of ultrasound in clinical practice.

Brad Reynolds, MD: Dr. Reynolds is a 2000 graduate of The Medical College of Georgia (MCG). He completed his internship in Internal Medicine in 2001 at MCG then completed his Emergency Medicine Residency training at MCG in 2004. He was chief resident from 2003-2004. After a brief period in private practice, Dr. Reynolds returned to Georgia Health Sciences University.

While at MCG, he has been very involved in both residency and medical student education. He was appointed Assistant Residency Director in 2004 and Associate Residency Director in 2008. Dr. Reynolds is credentialed in Emergency Medicine ultrasound for both adult and pediatric hospitals at Georgia Health Sciences University and is involving in teaching ultrasound to both residents and medical students. His research interests include hemostatic agents, emergency airway, and ultrasound.

Steve Shiver, MD: Dr. Shiver is a 1997 graduate of the Medical College of Georgia School of Medicine. He completed General Surgery residency training at Wake Forest University Baptist Medical Center (1997-2002) and subsequently obtained board certification from the American College of Surgery. He completed additional training in Emergency Medicine at the Medical College of Georgia (2002-2005) and is board certified by the American Board of Emergency Medicine.

Dr. Shiver's primary focus is in the area of education. He became Assistant Residency Director in 2006 and assumed the role of Residency Director in 2008. He has received numerous awards, both institutional and departmental, for excellence in teaching. Niche areas of clinical expertise/interest include emergency ultrasound, airway management, and cardiology with a particular focus on EKG interpretation. Dr. Shiver is very active in clinical research and has published extensively in the field including numerous abstracts and peer-reviewed articles. His research has also been presented at many national conferences.

Steve Holsten MD: Dr. Holsten is a 1999 graduate of Jefferson Medical College. He completed his internship and residency at the Medical College of Georgia in Augusta, Georgia. He continued as teaching faculty at both at Georgia Health Sciences University Medical Center and the Charlie Norwood Veterans Administration Hospital, Augusta, Georgia. He recently completed a one year fellowship in Surgical Critical Care. His clinical work includes mission trips annually to Haiti where he performs humanitarian surgical work through International Allied Missions. His time was well spent at the Charlie Norwood VA where he worked primarily in surgical critical care, as well as gastrointestinal and oncological surgery. He developed the VISN (veterans integrated service network) 7 hernia clinic in 2006. His academic achievements include the development of a General Surgery Curriculum at Georgia Health Sciences University including basic science and cadaver lab practicum. More recently he has been integral in developing the Surgical Critical Care Curriculum, which includes institution of advanced journal club, interdisciplinary critical care conference and the establishment of an interdisciplinary critical care committee. Dr. Holsten's research covers post-traumatic stress disorder in civilian populations, education research (simulation and practicum effectiveness), effects of traumatic brain injury on quality markers, and documentation of resident proficiency. His most recent academic project is the development and editing of a case based approach to ventilator

management for ICU providers textbook.

Emergency Medicine Residents Involved:

Troy Akers DO (C Company DDEAMC)
James Black MD (Georgia Regents University)
Stefan Kazacos MD (C Company DDEAMC)
Charlie Moore MD (C Company DDEAMC)

VII. BIOHAZARD/SAFETY: No hazardous substances will be used in this protocol. All animals will be disposed in regulated medical waste.

VIII. ENCLOSURES: References.

ASSURANCES:

As the Primary Investigator on this protocol I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in, the manner described herein, unless a deviation is specifically approved by the IACUC.

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the "minimum number of animals needed for scientific validity are used."

D. Biohazard/Safety: I have taken into consideration, and I have made the proper coordination regarding all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, etc., in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R" which the DOD has embraced, namely, "Responsibility" for implementing animal use alternatives where feasible, and conducting humane and lawful research.

G. Scientific Review: The proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedures: *Include only if conducting research that will cause more than*

slight or momentary pain or distress (Column D or E by USDA classification). If so, the following statement must follow: I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals that WILL BE RELIEVED with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, using the methods and sources described in the protocol, I have determined that alternative procedures are not available to accomplish the objectives of the proposed experiment.

Richard Schwartz

(Type) Name of Principal Investigator

04 MAR 2013

Date

"This document has been electronically signed in accordance with all applicable regulations, and a copy is retained within our records."

X. Resource Requirements: *Begin PART VIII (Resource Requirements) on a new page.*

1. ANIMAL COSTS:

Cost Per Pig: \$270.00

Number Animals: 20

Total Cost: \$8100

2. PER DIEM COSTS (PER DAY): \$7.98

3. ESTIMATED NUMBER OF DAYS TO BE HOUSED (Including quarantine/conditioning period and study period): Average of 3 Days

4. PROJECTED ANNUAL PER DIEM COST: \$712

5. CONSUMABLE SUPPLIES: \$2,500, This includes drugs and non-reusable surgical supplies based on an average of \$100-150/per pig/per surgery

6. EQUIPMENT PURCHASE COSTS (Other than caging): None.

7. FACILITY MODIFICATION/CAGING COSTS/DEPARTMENT OVERHEAD: \$28,688.00

8. TOTAL COST OF ENTIRE STUDY: \$40,000.00

9. GRANTS, GIFTS AND LOANED EQUIPMENT: Grant

10. SOURCE OF GRANTS, GIFTS AND LOANED EQUIPMENT: USAMRMC PROPOSAL NUMBER 12096001, AWARD NUMBER W81XWH-12-1-0546 "Use of the Abdominal Aortic Tourniquet for Hemorrhage Control" \$189,526.26

11. VALUE OF GRANTS, GIFTS AND LOANED EQUIPMENT: \$44,188.00 is the amount being sent to the Geneva Foundation which includes the 20.94% overhead charge and will be available for DCI to expend on the project. Geneva has a cooperative agreement with the Army to allow DCI to execute dollars on behalf of Geneva to complete all work described in this protocol and provide the data collected to Geneva/GRU as completed products.

12. OTHER FUNDING: None

13. DCI SUPPORT: The signatures below indicate the availability of resources and the

ability of this department to support this protocol:

*Chief, Research Operations Service
Dept of Clinical Investigation, DDEAMC*

References

1. Mabry RL, Holcomb JB, Baker A, et al. US army rangers in Somalia: an analysis of combat casualties on an urban battlefield. *Journal of Trauma*. 2000;49:515–529.\
 2. Bellamy RF. Causes of death in conventional warfare: implications for combat casualty care research. *Mil Med*. 1984;149:55-62.
 3. Kelly JF, Ritenour AE, McLaughlin DF, et al. Injury severity and causes of death from Operation Iraqi Freedom and Operation Enduring Freedom: 2003-2004 versus 2006. *Journal of Trauma*. 2008;64:S21-S27.
 4. Holcomb JB, McMullin NR, Pearse L, et al. Causes of death in U.S. Special Operations Forces in the global war on terrorism: 2001–2004. *Annals of Surgery*. 2007;245:986–991.
 5. Rhee P, Brown C, et al. QuickClot Use in Trauma for Hemorrhage Control: Case Series of 103 Documented Uses. *J Trauma*. 2008;64:1093-1099.
 6. Wedmore I, McManus J, Pusateri A, Holcomb J. A special report on the chitosan-based hemostatic dressing: experience in current combat operations. *Journal of Trauma*. 2006;60(3):655-8.
 7. Blaivas M, Shiver S, Lyon M, Adhikari S. Control of hemorrhage in critical femoral or inguinal penetrating wounds--an ultrasound evaluation. *Prehosp Disaster Med*. 2006 Nov-Dec;21(6):379-82.
 8. Kragh JF Jr, Murphy C, Dubick MA, Baer DG, Johnson J, Blackbourne LH. New tourniquet device concepts for battlefield hemorrhage control. *US Army Med Dep J*. 2011 Apr-Jun:38-48.
-
1. Coordination between GRU and Fort Gordon investigators occurred.
 2. Protocol approved

**APPENDIX
STATEMENT OF WORK
TO
MASTER COOPERATIVE RESEARCH & DEVELOPMENT AGREEMENT or Collaborative
Agreement (DoD)
NO. MA-019**

A. IDENTIFICATION.

A.1. Subject Category: Medicine & Biology (Clinical Medicine), Code 57E, Title: "**Use of the Abdominal Aortic Tourniquet in a Hypovolemic, Hypotensive Porcine Model.**" Short Title: "**Abdominal Aortic Tourniquet.**"

A.2. The Clinical Investigation Regulatory Office (Federal Laboratory) and **The Geneva Foundation** (Collaborating Party) desire to collaborate in research and development and will cooperate in support of the clinical investigation protocol at **Dwight D. Eisenhower** Army Medical Center (EAMC) entitled, "**Use of the Abdominal Aortic Tourniquet in a Hypovolemic, Hypotensive Porcine Model.**" (the "Study") by **James C. McPherson, PhD** (Principal Investigator), serving at the **Dwight D. Eisenhower** Army Medical Center, **300 Hospital Road, FT Gordon, GA 30905**, acting under the guidance of the Federal Laboratory.

A.3. This Statement of Work (SOW) is executed under authority of the Stevenson-Wydler Technology Innovation Act of 1980 as amended by the Federal Technology Transfer Act (15 U.S.C. §§3701 et seq.) and the Master CRADA between the Parties, dated 25 February 1999 and revised 2 May 2006, and hereby incorporates all of the terms and provisions of the Master CRADA. Together, the Master CRADA and this SOW constitute the entire Agreement of the Parties. In the case of a conflict between the provisions of this SOW and the Master CRADA, the terms and provisions of the latter shall control.

B. PURPOSE.

B.1. Whereas, the Federal Laboratory and the Collaborating Party are entering into this Agreement for the mutual benefit of each Party. This joint research project will benefit the Collaborating Party by **accomplishing its mission to support and promote the advancement of military medicine and specifically military medical research.** The project will benefit the Federal Laboratory by providing valuable research experience for the medical residents of the teaching program involved. In addition, patients at **Dwight D. Eisenhower Army Medical Center** with **hemorrhage in non-compressible sites** will benefit through **the control of hemorrhage in these otherwise fatal injuries at the point of injury to allow time for transport to definitive care.**

C. MEDICAL OBJECTIVE.

C.1. **This study will utilize a porcine experimental model to evaluate the AAT technology with the following objectives:**

1. **Demonstrate the effectiveness of hemorrhage control of a 6mm femoral artery punch injury utilizing the AAT;**
2. **Measure the effect on blood pressure following application of the AAT and aortic occlusion in a hypotensive hypovolemic porcine model;**
3. **Identify thrombus in the deep veins of the lower extremities utilizing compression Doppler following 60 minutes of aortic occlusion; and**
4. **Measure the effect on peak airway pressure before and after the application of the AAT.**

D. DESCRIPTION OF WORK.

D.1. This protocol, "Use of the Abdominal Aortic Tourniquet in a Hypovolemic, Hypotensive Porcine Model," will focus on determining the efficacy of the abdominal aortic tourniquet to arrest hemorrhage in the setting of a standardized 6 mm femoral artery injury in a previously validated porcine model. The specific aims of the study are as follows:

Aim 1: To determine the degree of hemorrhage control when the abdominal aortic tourniquet is applied using a hypotensive, hypovolemic porcine model; and

Aim 2: To determine the presence of venous thrombus formation in the bilateral lower extremities following 60 minutes of aortic occlusion in a porcine model.

D.2. Specific tasks include: 1) Draft protocol for submission to the Institutional Animal Care and Use Committee (IACUC), 2) Submit protocol to the IACUC, 3) Obtain IACUC approval, 4) Initiate protocol, and 5) Complete protocol.

D.2.1. The methods for this study are as follows: A total of 20 pigs will be used in this experiment. Foley catheters will be placed following IM sedation. The animals will be intubated and undergo general anesthesia and paralysis utilizing a non-depolarizing paralytic. A carotid arterial line will be placed for hemodynamic monitoring along with an internal jugular line to be utilized as a resuscitation line. A 10 cm section of the femoral artery will be mobilized and the overlying abductor muscle will be excised. The femoral artery will be bathed in 2% lidocaine until the artery is dilated to greater than 1 cm in diameter. When the mean arterial pressure is stabilized at 65 mmHg or above (500 mL of Hextend followed by normal saline boluses may be utilized to stabilize blood pressure) a 6 mm vascular punch will be performed in the femoral artery and allowed to bleed continuously for 45 seconds. At this time the abdominal aortic tourniquet will be applied and inflated. Blood loss will be measured and recorded. Following application of the abdominal aortic tourniquet 500 mL of Hextend will be bolus infused. The blood pressure will be maintained at a mean arterial pressure of 60 mmHg or above. If the mean arterial pressure drops below 60 mmHg normal saline fluid boluses will be given until the mean arterial pressure exceeds 65 mmHg. Blood pressure and peak airway pressure shall be recorded every 5 min. Following 60 min. of aortic occlusion a vascular shunt shall be placed in the femoral artery to bypass the 6 mm femoral punch wound. The abdominal aortic tourniquet will then be released reestablishing blood flow to the lower extremities. Compression Doppler ultrasound of the bilateral femoral veins will be completed to determine the presence or absence of thrombus. Following the ultrasound study the animals will be euthanized.

D.3. All performance under this SOW will cease at either the completion of the study, exhaustion of funds, unilateral or mutual termination, or **29 September 2015**, whichever occurs first.

E. RESOURCES PROVIDED BY COLLABORATING PARTY.

E.1. The Collaborating Party will furnish the following research resources:

E.2. Investigational Drugs: **None**

E.3. Approved Drugs: **The Collaborating party is providing Hextend. This will be provided in-kind with an estimated market value of up to \$2,475.**

E.4. Approved Devices: **None**

E.5. Consumable Supplies: The Collaborating party will provide in-kind support for animals and animal per diem, euthanasia, anesthesia, surgical supplies, blade level apparatus, and consumable office supplies with an estimated market value of up to \$15,241.

E.6. Equipment: None

E.7. Assay Results: None

E.8. Services of Personnel: None

E.9. Loan of Equipment: None

E.10. Travel: None

E.11. Funds: None

E.12. Other: The Collaborating party will provide a subject matter expert for consultation and advice related to the protocol with an estimated market value of up to \$1,000. The Collaborating party will also reimburse the Department of Clinical Investigation (DCI), Dwight D. Eisenhower Army Medical Center for Technician monitoring services, Anesthesia monitoring services, Laboratory Cleaning Fees, and IACUC Review services for a total of \$5,633. Funds will be disbursed to the DCI by check payable to the U.S. Treasurer for reimbursement of study-related support by The Geneva Foundation.

E.13. The above are hereinafter referred to as "Resources." Information relating to them, including data generated under this Agreement, is hereinafter referred to as "Information." Dwight D. Eisenhower Army Medical Center agrees that the Resources and Information will be used for research and clinical purposes only as provided in this Agreement. The Resources shall not be sold, offered for sale, used for commercial purposes, or furnished to any other Party without advance written approval from the Collaborating Party.

E.14. Financial Obligation. The continued performance of research by Dwight D. Eisenhower Army Medical Center under this Agreement is conditioned on the payment of funds to Dwight D. Eisenhower Army Medical Center by the Collaborating Party as specified below. Dwight D. Eisenhower Army Medical Center will use the accounting procedures as required by applicable Army and Defense Finance and Accounting Service regulations for the handling of funds during the performance of the research. Dwight D. Eisenhower Army Medical Center shall not be obligated to perform any of the research specified herein or to take any other action required by this Agreement if the agreed-to funds are not transferred as required. The expiration/termination of this Agreement does not extinguish the obligation to pay any funds which have been earned by, or are due and owed at, the date of expiration/termination.

E.15. Payment Schedule. The Collaborating Party is offering goods and services with an estimated value of up to \$24,349 maximum to support study costs for subject matter expertise, animals and animal per diem, and consumable supplies.

E.16. Reimbursements. The Collaborating Party shall deposit funds in a distinct Department of the Army account under control of the Directorate of Resource Management, and managed by the Department of Clinical Investigation, Dwight D. Eisenhower Army Medical Center. The deposit shall be made in the form of a check made payable to the "Treasurer of the United States" as follows: Technician monitoring support services, Anesthesia monitoring support services, Lab cleaning fees, and EAMC DCI Fee provided by the Department of Clinical Investigation.

E.17. Other Funds. **None**

E.18. Accounting Records. When funds are provided to **Dwight D. Eisenhower Army Medical Center**, it shall maintain distinct accounts, records, and other evidence supporting expenditures under this Agreement. **Dwight D. Eisenhower Army Medical Center** shall provide the Collaborating Party an annual report accounting for the use of funds and a final fiscal report within four months after completing this Agreement or ending its research activities under this Agreement, if requested by the Collaborating Party. The financial accounts and records pertaining to this Agreement shall be available for reasonable inspection and copying by the Collaborating Party or its authorized representative(s).

E.19. The Payment Schedule is subject to modification by mutual consent of all Parties in the event unforeseen circumstances delay initiation of this project, including delays due to: insufficient volunteer enrollment, actions from responsible review or regulatory authorities, lack of equipment or malfunctions, or insufficient support personnel.

F. RESOURCES PROVIDED BY FEDERAL LABORATORY. **The Principal Investigator, Subinvestigators, and all other applicable parties will provide services for the research project as is feasible in accordance with the project requirements and their regular work schedules.**

G. REPORTS. **Dwight D. Eisenhower Army Medical Center** agrees to report in a timely manner the results of any research conducted with the Resources to the Collaborating Party. **Dwight D. Eisenhower Army Medical Center** agrees to provide all data supporting research results to the Collaborating Party. **Report requirements and submission dates to be determined between the Collaborating party and the Principal Investigator.**

H. PRINCIPAL INVESTIGATOR.

All notices required by this Agreement to be sent to the Principal Investigator will be sent to the following address:

James C. McPherson III, PhD
Dwight D. Eisenhower Army Medical Center
Bldg 38705
Ft. Gordon, Georgia 30905
Email: james.c.mcpherson4.civ@mail.mil
Phone Number: 706-787-0015
Fax Number: 706-787-2730

I. SIGNATORIES.

I.1. Clinical Investigation Regulatory Office (Federal Laboratory):

Colonel Donald A. Gagliano, Medical Corps (Date) _____
Director, Clinical Investigation Regulatory Office
U.S. Army Medical Research & Materiel Command(ATTN: MCMR-RPC)
1608 Stanley Road, Suite 2
Fort Sam Houston, TX 78234-5055
Phone Number: 210-221-2511

I.2. The Geneva Foundation (Collaborating Party)

Rachelle D. Mainard, JD, CRA
Grants and Contracts Director
The Geneva Foundation
917 Pacific Avenue, Suite 600
Tacoma, WA 98402-4446
Email: rmainard@genevausa.org
Phone Number: 253-682-3816
Fax Number: 253-284-1916

(Date)

**MASTER COOPERATIVE RESEARCH AND DEVELOPMENT
AGREEMENT**

**BETWEEN
DWIGHT DAVID EISENHOWER ARMY MEDICAL CENTER
DEPARTMENT OF CLINICAL INVESTIGATION
Fort Gordon, Georgia 30905**

AND

**GEORGIA REGENTS UNIVERSITY
Augusta, Georgia 30912**

The Dwight David Eisenhower Army Medical Center Department of Clinical Investigation, (hereinafter "Federal Laboratory"), Georgia Regents University (hereinafter "Collaborating Party") enter into this Master Cooperative Research and Development Agreement (CRADA) No. _____ for performing the medical Research, Development, Testing, and/or Evaluation (RDTE) work described in the Statement of Work (SOW) attached hereto as an Appendix, and agree as follows:

**Article 1
General**

1.1. Authority. This Master CRADA is entered into

pursuant to the Stevenson-Wydler Technology Innovation Act of 1980 as amended by the Federal Technology Transfer Act (Title 15, United States Code (U.S.C.) §§3701 et seq.), which permits directors of Federal laboratories to enter into cooperative research and development agreements and intellectual property licenses for intellectual property owned by or assigned to the United States Government. This is not a procurement contract, grant, or cooperative agreement as those terms are used in 31 U.S.C. §§6303, 6304, and 6305.

1.2. Entire Agreement. This Master CRADA includes the attached SOW (Appendix) and together they constitute a single, entire document hereinafter referred to as the "Agreement."

1.3. Purpose. The purpose of this Agreement is to expedite the transfer of federal technology from the Federal Laboratory to the private sector (represented by the Collaborating Party) through the sharing of resources and information towards the successful completion of the RDTE project (the "Study"). The medical objective of the Study is described in the SOW.

1.4. Statement of Work. The RDTE project, which is described in the SOW, will be conducted under a clinical research protocol which has been reviewed by the appropriate Institutional Review Board in accordance with Army Regulation 40-38, Clinical Investigation. The SOW incorporates all of the terms and provisions of these Articles by reference. In cases of apparent conflict between the terms and provisions of the SOW and these Articles, the terms and provisions of the Articles shall control.

1.5. Consideration. The Federal Laboratory and the Collaborating Party agree that they are entering into this Agreement for the mutual benefit of each Party. The Federal Laboratory and the Collaborating Party will cooperate in support of the clinical investigation protocol specified in the SOW.

1.6. Principal Investigator. The RDTE project conducted under the SOW will be supervised by a Principal Investigator named therein. The Principal Investigator may be changed for good cause by written notification to the other Party(ies).

1.7. Federal Laboratory Representative. The person

signing this Agreement on behalf of the Federal Laboratory represents that he or she has the authority to enter into this Agreement. Notwithstanding this authority, the Secretary of the Army has reserved to the Assistant Secretary of the Army (Research, Development, and Acquisition) the authority provided by 15 U.S.C. §3710a(c)(5)(A) to disapprove or require modification of this Agreement within 30 days of the date it is presented to the Assistant Secretary. If the Assistant Secretary disapproves this Agreement, the Agreement is null and void. If the Assistant Secretary requires modification of this Agreement, the Collaborating Party shall have 30 days from notification of such action to ratify the modification(s) or terminate this Agreement.

1.8. Collaborating Party Representative. The person signing this Agreement on behalf of the Collaborating Party represents that he or she has the authority to bind the Collaborating Party to the terms of this Agreement and the execution and delivery of this Agreement does not contravene any material provision of, or constitute a material default under, any material agreement binding on the Collaborating Party or any valid order of any court, any regulatory agency, or other body having authority to which the Collaborating Party is subject.

Article 2

Definitions

2.1. "Agreement" refers to the entire CRADA including the SOW.

2.2. "Adverse Drug Experience" means an adverse event as defined under 21 C.F.R. §310.305, Records and Reports Concerning Adverse Drug Experience, and other applicable Federal Regulations.

2.3. "Background inventions" mean inventions other than subject inventions.

2.4. "Clinical Brochure" means a document containing all the relevant information about a drug, including animal screening, preclinical toxicology, and detailed pharmaceutical data. Also included, if available, is a summary of current knowledge about pharmacology, mechanism of action, and a full description of the clinical toxicities.

2.5. "Collaborating Party" means the person(s), intermediary(ies), or entity(ies), including medical and pharmaceutical companies, sponsoring a research project pursuant to this Agreement.

2.6. "Computer software" or "software" means computer programs, source code, source code listings, object code listings, designs, details, algorithms, processes, flow charts, formulae, and related material that would enable the software to be reproduced, recreated, or recompiled. Computer software does not include computer databases or computer software documentation.

2.7. "FDA" means the Food and Drug Administration, Department of Health and Human Services.

2.8. "Federal Laboratory" means the Clinical Investigation Regulatory Office, U.S. Army Medical Department Center & School, Fort Sam Houston, which has been designated by the Secretary of Army as a Federal laboratory.

2.9. "Government" means the United States of America and the agencies thereof.

2.10. "Government purpose" means any activity in which the Government is a Party, including cooperative agreements with international or multinational defense organizations, or sales or transfers by the Government to foreign governments or international organizations, and competitive procurements. Government purpose does not include for commercial purposes.

2.11. "Invention" means any invention or discovery which is or may be patentable or otherwise protected under Title 35 of the United States Code or any novel variety of plant which is or may be protected under the Plant Variety Protection Act (7 U.S.C. §§2321 et seq.).

2.12. "Made" when used in conjunction with any invention means the conception or first actual reduction to practice of such invention.

2.13. "Party" or "Parties" refers to the Federal Laboratory, the Intermediary, or the Collaborating Party or all (in singular or plural usage as indicated by the context).

2.14. "Principal Investigator" means an individual who

actually conducts a clinical investigation (i.e. under whose immediate direction a drug is administered or dispensed to a subject). In the event an investigation is conducted by a team of individuals, the Principal Investigator is the responsible leader of the team. "Subinvestigator" includes any other individual member of that team (21 C.F.R. §312.3).

2.15. "Proprietary information" means information which embodies trade secrets or which is confidential technical, business, or financial information provided that such information:

- a. is not generally known, or is not available from other sources without obligations concerning its confidentiality;
- b. has not been made available by the owners to others without obligation concerning its confidentiality;
- c. is not described in an issued patent or a published copyrighted work or is not otherwise available to the public without obligation concerning its confidentiality;
- d. can be withheld from disclosure under 15 U.S.C. §3710a(c)(7)(A)&(B) and the Freedom of Information Act, 5 U.S.C. §552 et seq; and
- e. is identified as such by labels or markings designating the information as proprietary.

2.16. "Raw Data" means the primary quantitative and empirical data first collected by the intramural and extramural investigators from experiments and clinical trials conducted under the scope of this Agreement.

2.17. "Subject data" means any technical data first produced in the performance of work under this Agreement.

2.18. "Subject invention" means any invention conceived or first actually reduced to practice in the performance of work under this Agreement.

2.19. "Technical data" means recorded information, regardless of the form or method of the recording, of a scientific or technical nature (including computer software documentation and databases). The term does not include computer software or data incidental to the administration of this Agreement, such as financial or

management information.

2.20. "Master CRADA" means an agreement incorporating Articles 1 - 27, which forms the basis for repetitive cooperative research and development agreements between the same parties upon the subsequent execution of a SOW.

Article 3

Cooperative Research

3.1. Review of Work. Periodic conferences may be held between the Parties for the purpose of reviewing the progress of the cooperative effort. It is understood that the nature of this cooperative effort is such that completion within the period of performance specified or within the resources allotted cannot be guaranteed. Accordingly, it is agreed that all cooperative research and development activities performed by either Party are to be performed on a best efforts basis.

3.2. Changes. If at any time the Principal Investigator, a Collaborating Party, or the Federal Laboratory determines that the research data dictates a substantial change in the direction of the work, he or she shall promptly notify the Parties, and the Parties shall make a good faith effort to agree to any necessary changes to the SOW consistent with the basic scope of research.

3.3. Assignment of Personnel. If the SOW contemplates the assignment of one Party's personnel to the other Party's facilities, then the employees shall remain employees of the assigning Party and will not be considered as employees of the other Party for any purpose, including but not limited to any requirements to provide workers' compensation, payment of salary or other benefits, or withholding of taxes. Assigned personnel will observe the other Party's security, safety, health, and environmental facility regulations. Assigned personnel can be denied access or removed by the other Party from its facilities at its discretion. Collaborating Party personnel assigned to a Federal Laboratory will work under the direction of the Principal Investigator only. That direction will be limited to matters within the scope of the actual research and will not extend to any matters that are normally encompassed by the employer-employee relationship. For example, the Collaborating Party is responsible for determining the working hours of its assigned personnel.

Article 4

Reports

4.1. Progress Reports. As provided in the SOW, the Parties will prepare and exchange written reports, in a timely manner, on the progress of their work, results obtained, problems encountered, and recommendations for further research and development. To the extent reasonable, further detail concerning the contents of the reports shall be provided upon request, if necessary for the other Party to fully understand the results achieved. At a minimum, the Principal Investigator will submit annual progress reports to the Parties.

4.2. Final Report. As provided in the SOW, the Parties will prepare and exchange a final report at the completion of the cooperative effort performed under this Agreement, on the progress of their work, results obtained, problems encountered, and recommendations for further research and development. To the extent reasonable, further detail concerning the contents of the report(s) shall be provided upon request if necessary for the other Party to fully understand the results achieved.

4.3. Adverse Drug Experiences, Annual Reports, Other Investigational New Drug Data. The Federal Laboratory will provide the Collaborating Party with copies of all adverse drug experience reports. The Federal Laboratory shall establish and maintain records and make reports to the FDA for the following Adverse Drug Experiences: (1) all serious, unexpected adverse drug experiences, (2) any significant increase in the frequency of serious unexpected adverse drug experiences, and (3) any significant increase in the frequency of therapeutic failure.

Article 5

Transfer of Funds

5.1. Payment Schedule. There is no intention for the transfer of funds between the Collaborating Party and Federal Laboratory. In the event of cancellation or termination of a research project, the U.S. Government shall not reimburse the Collaborating Party for its expenditures prior to cancellation or termination of the research project.

5.2. Federal Laboratory. The Federal Laboratory shall not provide any Federal funds directly to the Collaborating Party. The Federal Laboratory shall contribute equipment, material and services toward the cooperative research and development effort as set forth in the SOW.

5.3. Collaborating Party. The Collaborating Party shall provide research services and personnel resources to the Federal Laboratory for the performance of research and development as set forth in the SOW.

5.4. Salaries and Travel. Unless otherwise provided in the SOW, each Party shall provide financial support to its respective personnel in the performance of this Agreement, including salary, reimbursement for travel, and other expenses as appropriate.

5.5. Accounting Records. The Federal Laboratory and the Collaborating Party shall each maintain separate and distinct current accounts, records, and other evidence supporting all its expenditures under this Agreement. The accounts and records of the Federal Laboratory which are relevant to the conduct of this project shall be available for reasonable inspection and copying by the Collaborating Party or its authorized representative.

Article 6

Personal and Real Property

6.1. Personal Property. Any tangible personal property provided by a Party during the performance of this Agreement shall remain the personal property of the Party providing it, unless otherwise agreed in the SOW. Property provided by a Party to another Party may only be used for the performance of the cooperative effort under this Agreement, unless otherwise agreed in the SOW. Government property may be repaired or modified by the Collaborating Party at its expense only after obtaining the written approval of the Federal Laboratory. Any repair or modification of the property shall not affect the title of the Government. The Federal Laboratory makes no warranty, express or implied, with respect to property contributed or loaned by it. Upon completion of the cooperative effort performed under this Agreement, each Party shall immediately account for the property in its possession and return, at its expense, all property

belonging to the other Party in the condition in which it was received, normal wear and tear excepted. Any disposal of property shall be in accordance with applicable Federal, State, and local environmental laws and regulations.

6.2. Real Property. Any real property made available for use by a Party to another Party for the performance of this Agreement shall remain the property of the Party providing it. Any use of such property shall be in accordance with all applicable Federal, State, and local laws and regulations to include environmental laws and regulations.

Article 7

Patents

7.1. Disclosure. Each Party shall promptly disclose in writing to the other Party subject inventions made by its employees or subcontractors in sufficient detail to enable someone with skill in the art to make and use the inventions.

7.2. Federal Laboratory Inventions. The Federal Laboratory, on behalf of the Government, shall retain title to each subject invention made solely by its employees. The Federal Laboratory may file patent applications on these subject inventions at its own expense. The Federal Laboratory grants to the Collaborating Party a royalty-free, nonexclusive, irrevocable license to practice or have practiced worldwide by or on behalf of the Collaborating Party subject inventions covered by any resultant patents. Such nonexclusive license(s) shall be evidenced by a confirmatory document prepared by the Federal Laboratory in a form satisfactory to the Collaborating Party.

7.3. Collaborating Party Inventions. The Collaborating Party shall retain title to each subject invention made solely by its employees. The Collaborating Party may file patent applications on these subject inventions at its own expense. The Collaborating Party grants to the Government a royalty-free, nonexclusive, irrevocable license to practice or have practiced worldwide by or on behalf of the Government for Government purposes subject inventions covered by any resultant patents. Such nonexclusive license(s) shall be evidenced by a confirmatory license agreement prepared by the Collaborating Party in a form

satisfactory to the Federal Laboratory. If the Collaborating Party transfers or releases the rights to employee inventions provided for by this paragraph, such transfer or release shall be subject to the Government purpose license granted to the Government.

7.4. Joint Inventions. Title to subject inventions made jointly by employees of the Federal Laboratory and the Collaborating Party shall be held jointly by the Government and the Collaborating Party. The Collaborating Party shall have the initial option to file patent applications on joint subject inventions at its own expense.

7.5. Filing of Patent Applications. The Party having the right to retain title and/or file patent applications on a specific subject invention may elect to file patent applications thereon provided it so advises the other Party within 120 days from the date of the report of the subject invention. In the event that the Party, having the right to file patent applications, fails to advise the other Party, within 120 days of the report of the subject invention, of its intent to file patent applications (and in which countries it intends to file), then the other Party may elect (but is not required) to file patent applications on such subject invention in those countries instead. If the other Party elects to file patent applications, the Party initially having the right to file patent applications on the subject invention agrees to assign to the other Party its rights, title, and interest in such subject invention in those countries in which the other Party elects to file, subject to the retention by the assigning Party of a royalty-free, nonexclusive, irrevocable license to practice or have practiced worldwide by or on behalf of that Party the subject invention covered by any resultant patents. The Party filing an application shall provide a copy thereof to the other Party. NOTE: Any patent application filed on any invention made under this Agreement shall include in the patent specification thereof the statement: "This invention was made in the performance of a Cooperative Research and Development Agreement with the Department of the Army. The invention may be manufactured and used by or for the Government of the United States for all government purposes without the payment of any royalty."

7.6. Patent Expenses and Cooperation. The expenses attendant to the filing of patent applications as specified above shall be borne by the Party filing the

patent application. Each Party shall provide the other Party with copies of patent applications it files in the U.S. Patent and Trademark Office or any foreign patent offices, along with the power to inspect and make copies of all documents retained in the official patent application file by the applicable patent office. The Party filing the patent application shall have the right to control the prosecution of the application. The Parties agree to cooperate with each other in the preparing and prosecution of patent applications.

7.7. Maintenance Fees. The fees payable to a patent office in order to maintain the patent's enforcement will be paid by the Party owning the patent. If that Party decides not to pay the maintenance fees, it shall promptly notify the other Party, who may pay the maintenance fees if it desires to maintain the enforcement of the patent.

7.8. Exclusive Licensing of Government Inventions. The Federal Laboratory, on behalf of the U.S. Government, agrees to grant, at the Collaborating Party's option, a limited-term, exclusive license in each Government invention (Federal Laboratory made and jointly made) subject to the reservation of a royalty-free, nonexclusive, paid-up license to practice and have practiced worldwide the subject invention by and on behalf of the U.S. Government for government purposes. The Federal Laboratory agrees to enter into negotiations with the Collaborating Party, as requested, for the exclusive licensing of Government inventions for any field of use at a fair and reasonable royalty rate to be negotiated in good faith. The Collaborating Party shall exercise the option to obtain an exclusive license by giving written notice thereof to the Federal Laboratory within three months after disclosure of the invention. The royalty rate and other terms and conditions of the license shall be set forth in a separate license agreement and shall be negotiated promptly after notice is given.

7.9. Assignment and Transfer. The Collaborating Party agrees that any nonexclusive license granted to the Collaborating Party by the Government pursuant to this Article may not be assigned, sublicensed, or otherwise disposed of except to a corporate affiliate of the Collaborating Party or to the successor of the Collaborating Party or its corporate affiliate. Exclusive licenses granted to the Collaborating Party pursuant to paragraph 7.8 may be sublicensed by the Collaborating Party.

7.10. Background Inventions. The Parties grant each other, to the extent that each has the authority to do so, expressed or implied, royalty-free, nonexclusive licenses to practice or have practiced on their behalf, background inventions necessary for the performance of work under this Agreement. However, this Agreement does not grant any implied licenses for practicing background inventions in the performance of work not being conducted under this Agreement.

7.11. Commercialization of Subject Inventions. The Collaborating Party agrees that with respect to any subject invention in which it has acquired title or an exclusive license under this Agreement, the Government has the right to require the Collaborating Party or an assignee or exclusive licensee of the subject invention to grant a nonexclusive license in any field of use to a responsible applicant or applicants upon terms that are reasonable under the circumstances, and if the Collaborating Party, assignee, or exclusive licensee refuses such request the Government has the right to grant such a license itself, if the Government determines that one or more of the following conditions exist:

7.11.1. Practical Application. Such action is necessary because the Collaborating Party, assignee, or licensee has not taken, or is not expected to take within a reasonable time, effective steps to achieve practical application of the subject invention. Practical application means to manufacture in the case of a composition or product; to practice in the case of a process or method; or to operate in the case of a machine or system; and, in each case, under conditions as to establish that the invention is being utilized and that its benefits are to the extent permitted by law or Government regulations available to the public on reasonable terms.

7.11.2. Health or Safety. Such action is necessary to alleviate health or safety needs which are not reasonably satisfied by the Collaborating Party, assignee, or licensee.

7.11.3. Public Use. Such action is necessary to meet requirements for public use specified by Federal regulation and such requirements are not reasonably satisfied by the Collaborating Party, assignee, or licensee.

7.12. Other Inventions. Inventions which are developed by either Party before or after the term of this Agreement remain the sole property of that Party.

Article 8

Copyrights

8.1. Works Created by Collaborating Party. Ownership to copyrights for original works of authorship created solely by employees of the Collaborating Party or for hire by the Collaborating Party in the course of performance of work under this Agreement are retained by the Collaborating Party. The Collaborating Party shall mark any such works with a copyright notice showing the Collaborating Party as an owner and shall have the option to register the copyright at the Collaborating Party's expense. The Collaborating Party grants to the Government a royalty-free, nonexclusive, irrevocable license to use, modify, prepare derivative works, reproduce, distribute, perform, and display worldwide such copyrighted works by or on behalf of the Government for Government purposes. The Collaborator will mark prominently each such copyrighted work with the words: "This work was created in the performance of a Cooperative Research and Development Agreement with the Department of the Army. The Government of the United States has a royalty-free government purpose license to use, duplicate or disclose the work, in whole or in part and in any manner, and to have or permit others to do so, for government purposes."

8.2. Joint Works. Ownership of copyrights for joint works prepared by employees of (or for hire by) the Federal Laboratory and the Collaborating Party in the course of performance of work under this Agreement are retained solely by the Collaborating Party. The Collaborating Party, however, grants to the Government a royalty-free, nonexclusive, irrevocable license to use, modify, prepare derivative works, reproduce, distribute, perform, and display worldwide such copyrighted works by or on behalf of the Government for Government purposes.

8.3. Software. The Party creating software in the course of the performance of work under this Agreement will provide the other Party with the source code, object code, and minimum support documentation needed by a competent user to use the software.

Article 9

Trademarks

9.1. Trademark Use. The Parties recognize that the Collaborating Party may seek to obtain trademark protection for goods developed under this Agreement which it subsequently commercially markets. The Parties agree that the Government may indicate on any similar goods produced by or for the Government that the goods are a Government version of the goods protected by the trademark. The Government shall also have the right to use the trademark in print or communications media.

9.2. Qualifying Notice. Prior to the use of the trademark by the Government, the Parties will negotiate any reasonable qualifying language that must accompany the trademark.

Article 10

Proprietary and Protected Information

10.1. Exchange of Data. The Parties agree to exchange all subject data produced in the course of the performance of this Agreement. All information or data exchanged between the Parties in the course of, or in contemplation of, this Agreement may be used and disseminated without restriction by the Parties for any purpose unless the data or information is proprietary or otherwise protected as provided in paragraph 10.2 or Article 8.

10.2. Proprietary and Protected Information.

10.2.1. Form. Proprietary or protected information may be disclosed to another Party orally, electronically, visually, in writing, or in any other tangible or intangible form. If it is initially disclosed in a nonfixed media, then the Party disclosing the data must furnish the other Party with the information in a fixed media with appropriate marking within ten days of its initial disclosure. Failure to furnish the fixed media within ten days or to prominently mark the information as proprietary or otherwise protected will not automatically result in the loss of the information's protected status, but will excuse any Party's unauthorized disclosure or use of the information caused by the failure to meet the ten-day suspense to properly mark the information.

10.2.2. Collaborating Party Background Information. The Collaborating Party shall place a proprietary legend on all proprietary information that it furnishes to the Federal Laboratory under this Agreement which was produced or obtained prior to this Agreement or during the term of this Agreement, but not in the course of the performance of this Agreement. The legend shall prominently and explicitly identify which material is proprietary and which material is not proprietary. Any such marked proprietary information furnished by the Collaborating Party to the Federal Laboratory under this Agreement, or in contemplation of this Agreement, shall be used by the Federal Laboratory only for the purpose of carrying out this Agreement and for Government administrative and oversight purposes. Such marked proprietary information, as long as it maintains its proprietary status, shall not be disclosed or otherwise made available outside the Government without the consent of the Collaborating Party.

10.2.3. Federal Laboratory Background Information. The Federal Laboratory shall place a nondisclosure legend on all protected information it produced or obtained prior to this Agreement or during the term of this Agreement, but not in the course of the performance of this Agreement, that it furnishes to the Collaborating Party under this Agreement and that it asserts is protected. The legend shall prominently and explicitly identify which material is protected and which material is not protected. Any such marked protected information furnished by the Federal Laboratory to the Collaborating Party under this Agreement, or in contemplation of this Agreement, shall be used by the Collaborating Party only for the purpose of carrying out this Agreement. Such marked protected information, as long as it maintains its protected status, shall not be disclosed or otherwise made available to anyone other than the Collaborating Party without the consent of the Federal Laboratory.

10.2.4. Subject Data. Subject data produced by employees of either Party or jointly by employees of the Parties may be designated as protected material by either Party if such information would be proprietary information if obtained from a non-Federal Party. Unless a lesser period of time is set forth in the SOW, the Federal Laboratory will provide appropriate protection against dissemination of such information, including exemption from 5 U.S.C. Chapter 5, Subchapter II, for five years after the data is developed, unless the

information loses its protected status earlier. The Federal Laboratory shall have the right to use subject data for government purposes. The Collaborating Party may use subject data for any purpose. Protected subject data must contain a prominent legend stating: (1) it is protected, (2) the rights to use of the Parties, and (3) the date the protected status is due to expire.

10.2.5. Other Proprietary or Protected Information. Proprietary or protected information other than subject data or background information that is furnished by the Collaborating Party to the Federal Laboratory under this Agreement and which is marked proprietary or protected shall be used by the Federal Laboratory only for the purpose of carrying out this Agreement and for Government administrative and oversight purposes. Such marked proprietary or protected information, as long as it maintains its proprietary or protected status, shall not be disclosed or otherwise made available outside the Government without the consent of the Collaborating Party. Proprietary or protected information other than subject data or background information that is furnished by the Federal Laboratory to the Collaborating Party under this Agreement and which is marked proprietary or protected shall be used by the Collaborating Party only for the purpose of carrying out this Agreement. Such marked proprietary or protected information, as long as it maintains its proprietary or protected status, shall not be disclosed or otherwise made available to anyone other than the Collaborating Party without the consent of the Federal Laboratory.

10.2.6. FDA Documents. If this Agreement involves a product regulated by the FDA, then the Collaborating Party or the Federal Laboratory, as appropriate, may file any required documentation with the FDA. In addition, the Parties authorize and consent to allow each other or its contractor or agent access to, or to cross-reference, any documents filed with the FDA related to the product.

10.2.7. Standard of Care. Each Party is obligated to use reasonable care in the protection of proprietary and protected information.

Article 11

Prepublication Review

11.1. Publication. The Parties anticipate that their

employees may wish to publish technical developments and/or research findings made under this Agreement. Each Party shall submit to the other Party prior to publication or other public disclosure, any proposed publication or disclosure pertaining to work under this Agreement. The other Party shall provide a written response within 30 days either objecting or not objecting to the proposed publication or public disclosure. The proposed publication or public disclosure shall not be deemed objectionable unless the proposed publication contains proprietary information, protected information, or material that would create potential statutory bars to the filing of U.S. or corresponding foreign patent applications, or for any other reasonable basis.

11.2. Protection of Proprietary Rights. If requested in writing by either Party, the Collaborating Party, the Principal Investigator, and/or the Federal Laboratory shall withhold such submission for publication an additional 60 days to allow for filing a patent application or taking such measures as the requester deems appropriate to establish and preserve its proprietary rights in the information in the manuscript or disclosure.

Article 12

Export Control

12.1. Compliance. The Parties understand that information and technology resulting from the performance of this Agreement may be subject to export control laws and regulations, and each Party is responsible for its own compliance with such laws and regulations. Nothing in this Agreement waives any such statutory or regulatory requirement.

Article 13

U.S. Competitiveness

13.1. Manufacture. The Parties agree that a purpose of this Agreement is to provide substantial benefit to the U.S. economy. To the extent feasible, the Parties agree to exercise reasonable efforts to manufacture substantially in the United States, products embodying intellectual property developed under this Agreement.

Article 14

Liability

14.1. **NO WARRANTY. EXCEPT AS SPECIFICALLY STATED ELSEWHERE IN THIS AGREEMENT OR THE SOW, THE PARTIES MAKE NO EXPRESS OR IMPLIED WARRANTY AS TO THE CONDITIONS OF THE RESEARCH, INVENTIONS, OR TECHNICAL DATA, OR PRODUCTS EXCHANGED, MADE, OR DEVELOPED UNDER THIS AGREEMENT, OR THE OWNERSHIP, MERCHANTABILITY, OR FITNESS FOR A PARTICULAR PURPOSE, TECHNICAL FEASIBILITY, OR FREEDOM FROM INFRINGEMENT OF INTELLECTUAL PROPERTY RIGHTS OF THE RESEARCH, INVENTIONS, TECHNICAL DATA, OR PRODUCTS. NEITHER PARTY SHALL BE LIABLE FOR LOST PROFITS, LOST SAVINGS, SPECIAL, CONSEQUENTIAL, INCIDENTAL, OR OTHER INDIRECT DAMAGES, EVEN IF SUCH PARTY IS MADE AWARE OF THE POSSIBILITY THEREOF.**

14.2. Products Liability. To the extent not specifically prohibited by applicable State or local law, the Collaborating Party agrees to indemnify and hold harmless the Government for any loss, claim, damage, expense, or liability of any kind occurring as a result of the making, using, or selling of a product, process, or service by or on behalf of the Collaborating Party, its assignees and licensees, which was derived from work performed under this Agreement. In respect to this provision, the Government shall not be considered an assignee or licensee of the Collaborating Party as a result of reserved Government rights under this Agreement. The Government's liability for losses, claims, damages, or expenses of the Collaborating Party occurring as a result of the making or using of a product, process, or service by or on behalf of the Government shall be governed by the provisions of the Federal Tort Claims Act.

14.3. Parties' Employees. To the extent not specifically prohibited by applicable State or local law, the Collaborating Party shall indemnify and hold harmless the Government for any loss, claim, damage, expense, or liability of any kind involving an employee or independent contractor of the Collaborating Party arising in connection with the performance of work under this Agreement, except to the extent that such loss, claim, damage, or liability arises from the negligence of the Federal Laboratory or its employees. The Government's liability for the loss of property, personal injury or death, or otherwise arising out of any negligent act or omission of its employees in connection with the

performance of work under this Agreement shall be governed by the Federal Tort Claims Act.

14.4. Notice and Assistance. The indemnification provisions of this Article shall apply only if the Party upon which the claim or lawsuit is asserted gives the other Party prompt notice of the claim or lawsuit and allows that Party to participate in the defense/adjudication of the claim or lawsuit as is permitted by applicable laws and Government regulations.

Article 15

Force Majeure

15.1. Force Majeure Events. Neither Party shall be liable for any unforeseen event beyond its reasonable control not caused by the fault or negligence of such Party, which causes such Party to be unable to perform its obligations under this Agreement and which it has been unable to overcome by the exercise of due diligence. Such unforeseen events include, but are not limited to, fire, storm, flood, earthquake or other natural catastrophes, accidents, acts of civil disturbance or disobedience, war, rebellion, insurrection, labor strikes or disputes, compliance with any laws, requirements, rules, regulations, or orders of any governmental authority or instrumentality thereof, sabotage, invasion, quarantine, and embargoes.

15.2. Best Efforts. The excused Party shall use its best efforts to resume performance as quickly as possible and shall suspend performance for only such period as is reasonably necessary as a result of the force majeure event.

Article 16

Severability

16.1. Contrary to Law. Any provision of this Agreement, to include the SOW, that is prohibited by applicable law is void, but the remaining provisions shall survive.

Article 17

Termination

17.1. Mutual Consent. The Parties may elect to

terminate this Agreement, or portions thereof, at any time by mutual consent.

17.2. Unilateral Action. Either Party may unilaterally terminate this Agreement at any time by giving the other Party written notice, not less than 30 days prior to the desired termination date.

17.3. Termination Costs. Unless otherwise specifically provided in this Agreement, each Party shall be responsible for all of the costs for which it bears responsibility under this Agreement which have been incurred through the effective date of termination. Each Party shall be solely responsible for any costs it incurs after the effective date of termination.

17.4. Continuing Obligations. In the event of termination, the Parties shall specify the disposition of all property, patents, and other results of work accomplished or in progress under this Agreement, when such disposition is not otherwise specified in this Agreement. All obligations under this Agreement to safeguard proprietary and other protected information and relating to rights in intellectual property or technical data shall survive any termination of this Agreement. The termination of this Agreement shall not affect the rights and obligations of the Parties accrued prior to the effective date of termination.

Article 18

Disputes

18.1. Resolution Procedures. The Parties recognize that disputes arising under this Agreement are best resolved at the working level. Both Parties are encouraged to be imaginative in designing mechanisms and procedures to resolve disputes at the lowest level possible as soon as practicable. The Parties agree to use their best efforts to resolve any dispute amongst themselves. Any dispute arising under this Agreement which is not disposed of by agreement of the Parties at the working level shall be submitted jointly to the signatories of this Agreement or their successors or their designees for resolution. Although the Parties may agree to use alternate disputes resolution (ADR) techniques to resolve disputes, nothing in this Agreement precludes either Party from pursuing resolution of a dispute using other legal review available by law.

18.2. ADR Process and Costs. If the Parties decide by mutual consent on an appropriate ADR method (to include the choice of mediator, judge, or panel members), they shall bear the costs of the ADR process equally.

18.3. Obligations. Pending the resolution of a dispute pursuant to this Article, the Parties agree to diligently continue performing all obligations in accordance with the SOW.

Article 19 Modifications

19.1. Modifications. If either Party desires to modify this Agreement, the Parties, upon reasonable notice of the proposed modification by the Party desiring the change, shall confer in good faith to determine the desirability of such modification. Any resulting modification shall not be effective until a written amendment is signed by the duly authorized representatives of the Parties. Any material modification of this Agreement is subject to the authority of the Assistant Secretary of the Army (Research, Development, and Acquisition) as provided in paragraph 1.7 of this Agreement to disapprove or require modification within 30 days of the date it is presented to the Assistant Secretary.

Article 20 Interpretation

20.1. Entire Agreement. This Agreement includes Articles 1 - 27 and the SOW (Appendix). Together, they constitute the entire agreement between the Parties with respect to the subject matter hereof and all prior representations or agreements relating hereto have been merged into the documents and are superseded in totality by this Agreement.

20.2. Precedence. In the event of a conflict between the terms and provisions of the SOW and the terms and provisions in the Articles, the terms and provisions in the Articles shall control.

Article 21

Notices

21.1. Notices. All notices, pertaining to or required by this Agreement, shall be in writing and shall be delivered by hand or sent by certified mail, return receipt requested, express mail, or private delivery service addressed as specified below. Any Party may change such address by written notice given to the other Party in the manner set forth.

Mailing Address of Federal Laboratory:

Mr. Larry Thompson (for Dr. Jeremy L.
Goodin)
Department of Clinical Investigation
ATTN: MCHF-CI
Dwight David Eisenhower Army Medical
Center
Building 38711, 7th Alley
Fort Gordon, GA 30905-5650
Phone: (706) 787-4826 FAX: (706) 787-
8123
E-mail: larry.thompson@us.army.mil
Mailing Address of Collaborating Party:

Collaborator Institutional POC:

Christopher D. McKinney
Associate Vice President
Technology Transfer & Economic Development
Georgia Regents University
Augusta, Georgia USA
706-721-4062 (office)
706-721-2917 (fax)

Scientist POC:

Richard B. Schwartz, M.D.
Chairman, Department of Emergency Medicine
Medical College of Georgia
Georgia Regents University
AF-2044
1120 15th Street
Augusta, GA 30912-2800
Phone: (706) 721-4910
Fax: (706) 721-0374
Email: rschwartz@gru.edu

21.2 Waiver. None of the provisions of this Agreement

shall be considered waived by any Party unless such waiver is given in writing to the other Party. The failure of a Party to insist upon strict performance of any of the terms and conditions hereof, or failure or delay to exercise any rights provided herein or by law, shall not be deemed a waiver of any right of any Party hereto.

Article 22

Nonassignment

22.1. Nonassignment. This Agreement may not be assigned or otherwise transferred by either Party without the prior written consent of the other Party.

Article 23

Officials Not To Benefit

23.1. Officials Not to Benefit. No member of Congress shall be admitted to any share or part of this Agreement, or to any benefit that may arise therefrom; but this provision shall not be construed to extend to this Agreement if made with a corporation for its general benefit.

Article 24

Endorsements

24.1. No Endorsements. By entering into this Agreement, the Federal Laboratory does not directly or indirectly endorse any product or service provided by the Collaborating Party, its successors, assignees, or licensees. The Collaborating Party shall not in any way imply this Agreement is an endorsement by the Government of any such product or service.

24.2. Use of Name. The Collaborating Party may use, refer to, and disseminate reprints of scientific, medical, and other published articles which disclose the name of the Federal Laboratory consistent with U.S. copyright laws, provided such use does not constitute, or imply, an endorsement of any commercial product or service by the U.S. Government. The Collaborating Party shall take every step possible to ensure that references to the articles are accurate, and shall explicitly state

that any such reference does not claim, infer, or imply an endorsement or recommendation of the product or service by Government investigators, the Federal Laboratory, or the U.S. Government. The Collaborating Party shall not use the name of the Principal Investigator or the Federal Laboratory in any advertising, packaging, or promotional material in connection with a product or service. The Principal Investigator and the Federal Laboratory shall not use the name of the Collaborating Party in any publication or presentation regarding the Study except with the written permission of the Collaborating Party or as may be required by law.

Article 25

Governing Law

25.1. The construction, validity, performance, and effect of this Agreement for all purposes shall be governed by the laws applicable to the Government of the United States.

Article 26

Duration of Agreement

26.1. Effective Date]. This Agreement will be effective upon the date that the last Party signs this Agreement.

26.2. Duration. It is mutually recognized by the Parties that the objectives to be attained by this Agreement cannot be rigidly defined in advance and that projected milestones are subject to adjustment by mutual agreement of the Parties. Notwithstanding, this Agreement will not extend beyond the latest period of either ten years following the date of the last signature to this Agreement or the latest period of time stated in the SOW executed under this Agreement.

26.3. Continuing Obligations. All obligations under this Agreement to safeguard proprietary and other protected information and relating to publication, liability, rights in intellectual property or technical data existing at the termination or expiration of this Agreement shall survive the termination/expiration of this Agreement.

Article 27
HIPAA Compliance
PRIVACY OF PROTECTED HEALTH INFORMATION

27.1. *Definitions.* As used in this clause:
Individual has the same meaning as the term “individual” in 45 CFR 164.501 and shall include a person who qualifies as a personal representative in accordance with 45 CFR 164.502(g).

Privacy Rule means the Standards for Privacy of Individually Identifiable Health Information at 45 CFR part 160 and part 164, subparts A and E.

Protected Health Information has the same meaning as the term “protected health information” in 45 CFR 164.501, limited to the information created or received by the Collaborating Party from or on behalf of the Government.

Required by Law has the same meaning as the term “required by law” in 45 CFR 164.501.

Secretary means the Secretary of the Department of Health and Human Services or his/her designee.

Terms used, but not otherwise defined, in this Agreement shall have the same meaning as those terms in 45 CFR 160.103 and 164.501.

27.1.1. The Collaborating Party agrees to not use or further disclose Protected Health Information other than as permitted or required by the Agreement or as Required by Law.

27.1.2. The Collaborating Party agrees to use appropriate safeguards to prevent use or disclosure of the Protected Health Information other than as provided for by this Agreement.

27.1.3. The Collaborating Party agrees to mitigate, to the extent practicable, any harmful effect that is known to the Collaborating Party of a use or disclosure of Protected Health Information by the Collaborating Party in violation of the requirements of this Agreement.

27.1.4. The Collaborating Party agrees to report to the Government any use or disclosure of the Protected Health Information not provided for by this Agreement.

27.1.5. The Collaborating Party agrees to ensure that any agent, including a subcontractor, to whom it provides Protected Health Information received from, or created or received by the Collaborating Party on behalf of the Government agrees to the same restrictions and conditions that apply through this Agreement to the Collaborating Party with respect to such information.

27.1.6. The Collaborating Party agrees to provide access, at the request of the Government, and in the time and manner designated by the Government to Protected Health Information in a Designated Record Set, to the Government or, as directed by the Government, to an Individual in order to meet the requirements under 45 CFR 164.524.

27.1.7. The Collaborating Party agrees to make any amendment(s) to Protected Health Information in a Designated Record Set that the Government directs or agrees to pursuant to 45 CFR 164.526 at the request of the Government or an Individual, and in the time and manner designated by the Government.

27.1.8. The Collaborating Party agrees to make internal practices, books, and records relating to the use and disclosure of Protected Health Information received from, or created or received by the Collaborating Party on behalf of, the Government, available to the Government, or at the request of the Government to the Secretary, in a time and manner designated by the Government or the Secretary, for purposes of the Secretary determining the Government's compliance with the Privacy Rule.

27.1.9. The Collaborating Party agrees to document such disclosures of Protected Health Information and information related to such disclosures as would be required for the Government to respond to a request by an Individual for an accounting of disclosures of Protected Health Information in accordance with 45 CFR 164.528.

27.1.10. The Collaborating Party agrees to provide to the Government or an Individual, in time and manner designated by the Government, information collected in accordance with this Clause of the Agreement, to permit the Government to respond to a request by an Individual for an accounting of disclosures of Protected Health Information in accordance with 45 CFR 164.528.

27.2. General Use and Disclosure Provisions.

27.2.1. Except as otherwise limited in this Agreement, the Collaborating Party may use or disclose Protected Health Information on behalf of, or to provide services to, the Government for the following purposes, if such use or disclosure of Protected Health Information would not violate the Privacy Rule or the Department of Defense Health Information Privacy Regulation if done by the Government to carryout the purposes of this Cooperative Research and Development Agreement as stated in the Statement of Work.

27.3. Specific Use and Disclosure Provisions.

24.3.1. Except as otherwise limited in this Agreement, the Collaborating Party may use Protected Health Information for the proper management and administration of the Collaborating Party or to carry out the legal responsibilities of the Collaborating Party.

27.3.2. Except as otherwise limited in this Agreement, the Collaborating Party may disclose Protected Health Information for the proper management and administration of the Collaborating Party, provided that disclosures are required by law, or the Collaborating Party obtains reasonable assurances from the person to whom the information is disclosed that it will remain confidential and used or further disclosed only as required by law or for the purpose for which it was disclosed to the person, and the person notifies the Collaborating Party of any instances of which it is aware in which the confidentiality of the information has been breached.

27.3.3. Except as otherwise limited in this Agreement, the Collaborating Party may use Protected Health Information to provide Data Aggregation services to the Government as permitted by 45 CFR 164.504(e)(2)(i)(B).

27.3.4. Collaborating Party may use Protected Health Information to report violations of law to appropriate Federal and State authorities, consistent with 45 CFR 164.502(j)(1).

27.4. Obligations of the Government. Provisions for the Government to Inform the Collaborating Party of Privacy Practices and Restrictions.

27.4.1. Upon request, the Government shall provide the Collaborating Party with the notice of privacy practices that the Government produces in accordance with 45 CFR 164.520, as well as any changes to such notice.

27.4.2. The Government shall provide the Collaborating Party with any changes in, or revocation of, permission by Individual to use or disclose Protected Health Information, if such changes affect the Collaborating Party's permitted or required uses and disclosures.

27.4.3. The Government shall notify the Collaborating Party of any restriction to the use or disclosure of Protected Health Information that the Government has agreed to in accordance with 45 CFR 164.522.

27.5. Permissible Requests by the Government.

27.5.1. The Government shall not request the Collaborating Party to use or disclose Protected Health Information in any manner that would not be permissible under the Privacy Rule if done by the Government, except for providing Data Aggregation services to the Government and for management and administrative activities of the Collaborating Party as otherwise permitted by this clause.

27.6. Termination.

27.6.1. A breach by the Collaborating Party of this clause, may subject the Collaborating Party to termination under any applicable default or termination provision of this Agreement.

27.7. Effect of Termination.

27.7.1. If this Agreement has records management requirements, the records subject to the Clause should be handled in accordance with the records management requirements. If this Agreement does not have records management requirements, the records should be handled in accordance with paragraphs (2) and (3) below.

27.7.2. If this Agreement does not have records management requirements, except as provided in paragraph (3) of this section, upon termination of this Agreement, for any reason, the Collaborating Party shall return or destroy all Protected Health Information received from the Government, or created or received by the Collaborating Party on behalf of the

Government. This provision shall apply to Protected Health Information that is in the possession of subcontractors or agents of the Collaborating Party. The Collaborating Party shall retain no copies of the Protected Health Information.

27.7.3. If this Agreement does not have records management provisions and the Collaborating Party determines that returning or destroying the Protected Health Information is infeasible, the Collaborating Party shall provide to the Government notification of the conditions that make return or destruction infeasible. Upon mutual agreement of the Government and the Collaborating Party that return or destruction of Protected Health Information is infeasible, the Collaborating Party shall extend the protections of this Agreement to such Protected Health Information and limit further uses and disclosures of such Protected Health Information to those purposes that make the return or destruction infeasible, for so long as the Collaborating Party maintains such Protected Health Information.

27.8. Miscellaneous.

27.8.1. Regulatory References. A reference in this Clause to a section in the Privacy Rule means the section as in effect or as amended, and for which compliance is required.

27.8.2. Survival. The respective rights and obligations of Business Associate under the "Effect of Termination" provision of this Clause shall survive the termination of this Agreement.

27.8.3. Interpretation. Any ambiguity in this Clause shall be resolved in favor of a meaning that permits the Government to comply with the Privacy Rule.

IN WITNESS WHEREOF, the Parties have caused this Agreement to be executed by their duly authorized representatives as follows:

For the U.S. Government (Federal Laboratory):

BY: _____

DATE: _____

BG William B. Gamble, Medical Corps
Commander, Dwight David Eisenhower Army Medical
Center
Phone Number: (706) 787-0300

For Georgia Regents University (Collaborating Party):

BY: _____

DATE: _____

Christopher D. McKinney
Associate Vice President
Technology Transfer & Economic Development
Georgia Regents University
Augusta, Georgia USA
706-721-4062 (office)
706-721-2917 (fax)

APPENDIX STATEMENT OF WORK

A. IDENTIFICATION.

A.1. Subject Category: Medicine & Biology (Clinical Medicine), Code 57E, Title: "The Molecular Basis for Acute Mountain Sickness: Evaluating the Role of Angiopoietin-Related Protein 4 Isoforms." Short Title: "AMS ARP-4 Project."

A.2. The Clinical Investigation Regulatory Office (Federal Laboratory) and James Madison University(Collaborating Party) desire to collaborate in research and development and will cooperate in support of the clinical investigation protocol at Dwight David Eisenhower Army Medical Center (DDEAMC) entitled, " The Molecular Basis for Acute Mountain Sickness: Evaluating the Role of Angiopoietin-Related Protein 4 Isoforms," (the "Study") by MAJ Jeremy L. Goodin (Principal Investigator), serving at the Dwight David Eisenhower

Army Medical Center, Fort Gordon GA, acting under the guidance of the Federal Laboratory.

A.3. This Statement of Work (SOW) is executed under authority of the Stevenson-Wydler Technology Innovation Act of 1980 as amended by the Federal Technology Transfer Act (15 U.S.C. §§3701 et seq.) and the Master CRADA between the Parties hereby incorporates all of the terms and provisions of the Master CRADA. Together, the Master CRADA and this SOW constitute the entire Agreement of the Parties. In the case of a conflict between the provisions of this SOW and the Master CRADA, the terms and provisions of the latter shall control.

B. PURPOSE.

B.1. Whereas, the Federal Laboratory and the Collaborating Party are entering into this Agreement for the mutual benefit of each Party. This joint research project will benefit the Collaborating Party by allowing for research to occur at James Madison University that will support the University's research, education and training programs. The project will benefit the Federal Laboratory by providing valuable research experience for the military research scientists and medical residents of the teaching program involved. In addition, U.S. military Warfighters that may be deployed to mountainous environments will benefit through enhanced understanding of the patho-physiological mechanisms of acute mountain sickness and improved diagnostics for acute mountain sickness susceptibility.

C. MEDICAL OBJECTIVE.

C.1. We hypothesize that Angiopoietin-Related Protein 4 (ARP-4) plays a significant role in mitigating high altitude stress facilitating acclimatization. Furthermore, we believe that naturally occurring variation within the ARP-4 protein sequence results in decreased functional efficiency of ARP-4, particularly with regard to inhibition of vascular leakiness, resulting in an increased susceptibility to AMS. We will specifically evaluate the correlation between ARP-4 protein sequence isoforms and the regulation of signal transduction events that modulate vascular permeability. In addition, we plan to evaluate the role of ARP-4 in the inhibition of hypoxia induced

vascular leakiness in mice.
Specific Aims

D. DESCRIPTION OF WORK.

D.1. Project Title: The Molecular Basis for Acute Mountain Sickness: Evaluating the Role of Angiopoietin-Related Protein 4 Isoforms

Contemporary United States geopolitical interests and on-going overseas contingency operations frequently require military action in mountainous environments at moderate (1500m to 3500m) to high altitudes (3500m to 5500m). Acute mountain sickness (AMS) is an illness that affects many individuals at altitudes above 2,400 m (8,000 ft) resulting in decreased cognitive and physical performance with the more severe, life-threatening condition presenting as cyanosis, coughing, confusion, disorientation and pulmonary or cerebral edema. Currently, there are no known susceptibility markers other than prior history of AMS.

In order to better elucidate the genetic basis for altitude response, our lab, in collaboration with the U.S. Army Research Institute of Environmental Medicine, has employed quantitative real time PCR (qRT-PCR) gene array analysis to identify changes in gene expression in a U.S. Army Warfighter population. As a result of this preliminary work, we have identified several genes that are differentially expressed in response to high altitude. Of particular interest, we have identified the angiopoietin-related protein 4 (ARP-4) as a significantly up-regulated gene in response to altitude/hypoxia. ARP-4 has been well characterized as a pleiotrophic protein with functions in lipid metabolism, glucose homeostasis, inhibition of angiogenesis and vascular permeability. ARP-4 is known to be up-regulated in response to Dexamethasone, a treatment for AMS and high altitude cerebral edema (HACE). However, the role of ARP-4 in high altitude response has not been explored. Population based resequencing of the gene has identified functionally significant polymorphisms in amino acids 40 and 266 (E40K and T266M) with documented phenotypic effects. Specific effects attributed to E40K and/or T266M variants include increased plasma triglyceride levels (through impaired lipoprotein lipase inhibition) and increased risk of coronary heart disease. Evaluation of

E40K and T266M variants relative to ARP-4 mediated vascular permeability and hypoxia response has not been performed.

We hypothesize that ARP-4 plays a significant role in mitigating high altitude stress facilitating acclimatization. Furthermore, we believe that naturally occurring variation within the ARP-4 protein sequence results in decreased functional efficiency of ARP-4, particularly with regard to inhibition of vascular leakiness, resulting in an increased susceptibility to AMS. We will specifically evaluate the correlation between ARP-4 protein sequence isoforms and the regulation of signal transduction events that modulate vascular permeability. In addition, we plan to evaluate the role of ARP-4 in the inhibition of hypoxia induced vascular leakiness in mice.

D.2. Specific Aims

1. Evaluate the role of ARP-4 as a significantly up-regulated gene in response to high altitude, hypoxia response and acclimation to altitude. Assess the capacity for ARP-4 variants to suppress Raf/MEK/ERK mediated signal transduction cascades in endothelial cells. The key signaling ERK1/2 MAP kinase cascade has been implicated in angiogenesis and physiological processes leading to increased vascular permeability. Naturally occurring genetic variation in ARP-4 protein sequence may attenuate Raf/MEK/ERK inhibition by ARP-4.

2. Evaluate the role of ARP-4 in the inhibition of hypoxia induced vascular leakiness in vivo. Vascular leakiness may contribute to AMS by producing a mild cerebral edema prior to the development of more severe high altitude cerebral edema. Assess the functional efficiency of purified ARP-4 variants (E40K and T266M) and modulators of ARP-4 gene expression (Dexamethasone and Fenofibrate) for inhibition of cerebral and peripheral vascular leakiness in vivo using a rodent model.

Approach/Methods

- I. Experiments in support of specific aim #1. Role of ARP4 as a significantly up-regulated gene in response to altitude and hypoxia.

- A. Expression, purification and characterization of recombinant ARP4 protein isoforms. Purpose: to provide purified recombinant ARP4 wild-type, E44K and T266M

proteins for subsequent in vitro analysis and animal studies. The coding region of ARP4 wild-type, E44K and T266M variants will be fused at the COOH terminus to the FLAG epitope and subcloned into the pCEP4 episomal mammalian expression vector for purification from HEK293 cells. Conditioned medium, containing secreted ARP4-FLAG variants, will be collected and filtered. To purify ARP4-FLAG fusion protein variants, cell culture media (contain secreted ARP-4 variants) will be transferred to an anti-FLAG antibody (M2) affinity gel, washed with PBS, eluted with Gly-HCl (pH 3.0) and immediately neutralized with Tris-HCl (pH 8.0). Purified protein will be visualized by SDS-PAGE with Coomassie Brilliant Blue Staining. Protein identity will be confirmed by Western blot analysis.

B. Analysis of ARP-4 mediated suppression of Raf/MEK/ERK signal transduction by immunoprecipitation and western blotting. Purpose: to better define the molecular pathways by which wild-type ARP4 suppresses vascular leakiness and to evaluate the effect of ARP4 polymorphisms on protein function. The Carboxy terminal portion of wild-type ARP-4 has been show to be sufficient to suppress bFGF-induced phosphorylation of Raf-1 and MEK1/2, but not activation of Ras and auto-phosphorylation of FGF receptor-1 (FGFR1). We will evaluate the effect of ARP-4 wild-type, E44K and T266M amino acid substitutions on ARP-4 mediated suppression of the key Raf/MEK/ERK signaling pathway. HUVEC cells will be treated with purified ARP-4 variants (5ug/ml) for 30 min, followed by stimulation with growth factors (bFGF or VEGF) for various periods. Cell lysates will be subjected to immunoblot analysis for determination of phospho- and total ERK1/2, phospho- and total MEK1/2, and phospho- and total Raf-1. Immunoprecipitated FGFR1 or VEGFR-2 receptors will be evaluated for phosphorylation state by immunoblotting with antiphospho receptor-specific antibody.

II. Experiments in support of specific aim #2. Role of ARP-4 in the inhibition of hypoxia induced vascular leakiness in vivo using a rodent model.

A. Analysis of cerebral and peripheral blood vessel permeability using Miles and fluorescein assays. Purpose: to evaluate the effect of ARP-4 polymorphisms on peripheral and cerebral vascular permeability in mice. The Miles assay uses intradermal injection of test substances and intravascular injection of Evans blue dye

(which binds to endogenous serum albumin) as a tracer to assay permeability in peripheral vessels. As an alternative, sodium fluorescein (MW 376.3) is a fluorescent tracer that does not cross an intact blood-brain barrier and has been used to assay vascular permeability of brain vessels. Vascular permeability will be induced in male 8-week-old BALB/c mice by either exposure to exogenous VEGF or 24 hr exposure to 8-12% O₂ in a hypoxia chamber (see Table 1 in statement of work for study design).

B. Evaluation of pharmacological modulators of ARP-4 gene expression as inhibitors of hypoxia induced vascular leakiness in vivo. Purpose: to evaluate drugs that enhance ARP-4 protein production as treatments for AMS. All three classes of PPAR agonists, alpha, beta/delta and gamma have been shown to increase ARP-4 expression through the gene's PPAR element.

We intend to evaluate the specific PPAR agonist Fenofibrate in comparison to Dexamethasone relative to vascular permeability and subsequent vulnerability to AMS in mice. Cerebral and vascular permeability will be assessed using the Miles and fluorescein assays.

D.3. All performance under this SOW will cease at either the completion of the study, exhaustion of funds, unilateral or mutual termination, or 01 October 2020 , whichever occurs first.

E. RESOURCES PROVIDED BY COLLABORATING PARTY.

E.1. The Collaborating Party will furnish the following research resources:

E.2. Investigational Drugs: N/A

E.3. Approved Drugs: N/A

E.4. Approved Devices: N/A

E.5. Consumable Supplies: Consumable supplies necessary for standard molecular biology and protein analysis as described D.2.

E.6. Equipment: Contemporary laboratory equipment necessary for completion of the project as described in D.2.

E.7. Assay Results: Results of protein expression, purification and characterization studies. In addition, results of molecular signal transduction analysis as described in D.2.

E.8. Services of Personnel: Personnel capable of performing experiments in support of Specific Aim #1 as described in D.2.

E.9. Loan of Equipment: N/A

E.10. Travel: N/A

E.11. Funds: Funds will not be transferred between either Party.

E.12. Other: None.

E.13. The above are hereinafter referred to as "Resources." Information relating to them, including data generated under this Agreement, is hereinafter referred to as "Information." DDEAMC agrees that the Resources and Information will be used for research and clinical purposes only as provided in this Agreement. The Resources shall not be sold, offered for sale, used for commercial purposes, or furnished to any other Party without advance written approval from the Collaborating Party.

E.14. Financial Obligation. Funds will not be transferred between either Party.

F. RESOURCES PROVIDED BY FEDERAL LABORATORY. DDEAMC Principle Investigator Dr. Jeremy L. Goodin will work closely with James Madison University to facilitate the molecular analysis of ARP-4 protein isoforms and evaluate the role of ARP-4 in acute mountain sickness susceptibility. DDEAMC will provide the Collaborator with reagents, materials and information necessary to facilitate protein purification and characterization as described in D.2 Specific Aim #1. Role of ARP4 as a significantly up-regulated gene in response to altitude and hypoxia. DDEAMC will provide all personnel and resources necessary to complete experiments in support of Specific Aim #2. Role of ARP-4 in the inhibition of hypoxia induced vascular leakiness in vivo using a rodent model.

G. REPORTS. Both JMU-ISAT and DDEAMC agree to report in a timely manner the results of any research conducted with the Resources to each Collaborating Party. JMU-ISAT and DDEAMC agree to provide all data supporting research results to each Collaborating Party. JMU-ISAT and DDEAMC will provide a summary of all research results in a final report at the conclusion of the study.

H. PRINCIPAL INVESTIGATOR.

H.1. The Principal Investigator (Dr. Jeremy L. Goodin) may be assisted by others designated as Subinvestigators. Subinvestigators for this project are designated below:

Dr. Balakrishna Prasad
Dr. Joseph Wood

H.2. All notices required by this Agreement to be sent to the Principal Investigator will be sent to the following address:

Goodin)	Mr. Larry Thompson (for Dr. Jeremy L.
	Department of Clinical Investigation
	ATTN: MCHF-CI
Center	Dwight David Eisenhower Army Medical
	Building 38711, 7th Alley
	Fort Gordon, GA 30905-5650
8123	Phone: (706) 787-4826 FAX: (706) 787-
	E-mail: larry.thompson@us.army.mil

I. SIGNATORIES.

I.1. For the U.S. Government (Federal Laboratory):

BY: _____ DATE: _____

BG William B. Gamble, Medical Corps
Commander, Dwight David Eisenhower Army Medical
Center
Phone Number: (706) 787-0300

I.2. Georgia Regents University (Collaborating Party):

BY: _____ DATE: _____

Christopher D. McKinney
Associate Vice President
Technology Transfer & Economic Development
Georgia Regents University
Augusta, Georgia USA
706-721-4062 (office)
706-721-2917 (fax)

2. Budget Status

Provide financial information requested below. Add additional row for subaward greater than one and include the name of the organization receiving the subaward.

	This Quarter	Cumulative
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Personnel	0.00	62,166.66
Fringe Benefits	0.00	9,555.24
Travel		
Equipment		
Supplies	0.00	1,094.07
Other		
Subaward - Organization		
Subtotal	0.00	72,815.97
Indirect Costs	0.00	36,408.00
Fees		
TOTAL	0.00	109,223.97

3. Protocol Status

Human Use Regulatory Protocols

TOTAL PROTOCOLS:

State the total number of human use protocols required to complete this project.

. None

PROTOCOLS:

List all human use protocols to be performed to complete the project, include approved target number for clinical significance, followed by type of submission and type of approval with associated dates, and include performance status relating to recruitment number, enrollment number, and issues that may impact performance or progress of the study (e.g. slow enrollment or large dropouts).

Protocol 1 Title:

Target required for clinical significance:

Target approved for clinical significance:

Submitted to and Approved by:

-

Status:

-

Animal Use Regulatory Protocols

TOTAL PROTOCOLS:

State the total number of animal use protocols required to complete this project.

One

PROTOCOLS:

List all animal use protocols to be performed to complete the project, include approved target number for statistical significance, followed by type of submission and type of approval with associated dates, and include any administrative, technical, or logistical issues that may impact performance or progress of the study (e.g. animal use protocol need revision to minimize animal suffering, animal protocol modification to include additional staff).

In Progress

Protocol 1 Title:

Target required for clinical significance:

Target approved for clinical significance:

Submitted to and Approved by:

-

Status:

-