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TITLE: Development of an Oxygen Carrier for Use in Hemorrhagic Shock Resuscitation

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CONTRACTING ORGANIZATION: Medical Technology Enterprise Consortium (MTEC)

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TABLE OF CONTENTS

Annual Technical Report

Annual Business Report

Annual Technical Status Report for

Development of An Oxygen Carrier for Use in Hemorrhagic Shock Resuscitation

Research Project No. 2019-453-001

EGS Number: MT17008.138

Reporting Period: Effective Date – 25 JUL '21

MTEC Research Project Awardee
Research Project Lead: W. Richard Light, PhD, CSO

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Submitted: August 26, 2021

1. **Project Status: Y1-Y2 (Year 1 first reported in the Annual Report submitted on 25JUL20)**

   a. **Accomplishments**

Administrative: Year 1

Product Name Update: The name of the product had been changed from the early development phase using VIR-IV1 to VTB's new trademarked product name, OxyBridge™.

Provisional Patent: Based on the findings previously reported on a potential synergistic effect between plasma products and Oxybridge on coagulation during hemorrhagic shock, a provisional patent was filed in June 2020 with the United States Patent Office, New Provisional Patent Application No 63/043668 (Compositions and Methods for Treating Hemorrhagic Shock).

Administrative: Year 2

Peer-Reviewed Publication:

Song, BK, Light, WR, Vandegriff KD, Tucker J, Nugent WH. Systemic and microvascular comparison of Lactated Ringer's Solution, VIR-HBOC, and alpha-alpha crosslinked hemoglobin-based oxygen carrier in a rat 10% topload model. Artificial Cells, Nanomedicine, and Biotechnology, Volume 48:1, 2020. https://doi.org/10.1080/21691401.2020.1809441

Task 1a: Year 1 – Studies Completed – OxyBridge / plasma studies. Reported in Y1Q2 Progress Report OxyBridge is formulated in a modified Lactated Ringer's Solution (LRS), with potential for coagulopathy dilutional effects. As such, in vitro resuscitation studies were designed to study potential coagulopathies. These studies used OxyBridge manufactured at the benchtop-scale at VTB's laboratory. This first in vitro study was completed in a joint study between VTB and Song Biotechnologies, LLC and was funded by this grant. A second complimentary in vitro study was also completed in joint between VTB and Michael A. Meledeo, CIV USARMY MEDCOM AISR, with VTB supplying the necessary supplies. While this study was not directly funded by this grant, the results provided confirmation of the results from first study. There was no coagulopathy noted beyond the expected dilutional effect. Notably, there was apparent synergy between plasma and OxyBridge. Successful results from these coagulation studies provided the first milestone for Check Point 1 to move forward to Task 1c.

This work was to be presented at MHSRS 2020, but this meeting was cancelled due to COVID-19.

Task 1a: Year 2 – Ex Vivo Coagulation Impact Study; Completed:

An Abstract on the hemostasis study in rats was submitted to MHSRS for the 2021 meeting and invited for an oral presentation, but again the meeting was cancelled due to COVID-19.

Light WR et al. Impact of controlled and Uncontrolled hemorrhage (two-hit model) and resuscitation on coagulopathy and hemostasis in rats. MHSRS Meeting Abstract, 2021.

Task 1b – Year 1: FDA Pre-IND meeting (VirTech Bio, Biologics Consulting).

VTB engaged the services of Biologics Consulting, Virginia to prepare the Pre-IND FDA Briefing Package. As reported in Q3, Biologics Consulting had prepared a GAP analysis for VTB's development program and the pre-clinical, clinical and chemistry and manufacturing sections had been drafted and were in the process of being finalized when the COVID-19 pandemic shifted worldwide attention. Current military imperatives shifted to promote early resuscitation of wounded warriors, as well as reaction to the COVID-19 emergency. This led to a sense of urgency within the FDA encouraging cooperation with the DoD and early participation. As such, Biologics Consulting in discussions with the CSO and CEO of VTB decided to move beyond an Interact Meeting directly to a Pre-IND meeting.

Task 1b – Year 2: FDA Pre-IND Meeting; Completed:

The FDA is promoting studies as an immediate reaction to the COVID-19 emergency. In response to this call, Biologics Consulting and VTB finalized the FDA Pre-IND briefing document to include Pre-clinical, CMC (Chemistry Manufacturing and Controls) and Clinical Sections. VTB held the FDA Pre-IND meeting with an OxyBridge Briefing Document with an indication to treat Acute Respiratory Distress Syndrome (ARDS). However, it is recognized that the CMC and pre-clinical work applies equally to other indications such as Hemorrhagic Shock. The Pre-IND briefing document was completed in Y2Q1 and submitted to the Agency. The FDA provided a written preliminary response to the Briefing Document's questions. The FDA written responses and advice were sufficiently clear and complete to obviate the need for further discussion until more preliminary data can be provided. Of note, there were no additional requests from the FDA regarding their past concerns with previous generation products from other organizations and that VirTech Bio was already following the guidelines they did reference.

Task 1c – Year 1: Manufacturing Process Finalization (VirTech Bio).

- During Y1 Q2, VTB performed a sufficient number of OxyBridge pilot runs to implement standard statistical evaluations to assess process control in the laboratory and initiate Technology Transfer to the CMO (Fujifilm Diosynth Biotechnologies).
- These studies showed that the current OxyBridge pilot-scale production process is robust.
- VTB has transferred safety documents, and process documents to Fujifilm and have weekly videoconferences to coordinate future activities, including review of the process development plan to insure it meets their needs for tech transfer.

Task 1c – Year 2: Manufacturing Process Finalization (VirTech Bio); Ongoing:

- During Y1, VTB performed a sufficient number of OxyBridge pilot runs to implement standard statistical evaluations to assess process control in the laboratory and initiate Technology Transfer to the CMO (FUJIFILM Diosynth Biotechnologies).
- These studies showed that the current OxyBridge pilot-scale production process is robust, and VTB transferred safety and process documents to the CMO. To expedite this process, VTB and FUJIFILM held weekly videoconferences to coordinate future activities.
- In Y2Q1, budgetary negotiations were completed between VTB and the CMO (FUJIFILM). The result was a budgetary increase of $1M. VTB provided MTEC with funding options to stay on course. This necessitated a hold on CMO activities until a funding decision was reached. A call with MTEC regarding this funding occurred on NOV 6, 2020. Subsequently, there have been several follow up calls with MTEC and JPC6 to resolve a revised SOW submitted (JAN 29) to reflect fully funding FUJIFILM and delaying subsequent pre-clinical tasks. These issues were resolved first with M02 in which the period of performance was extended to July 31, 2024 (22 month no-cost extension) and the Estimated Cost and Cost Share clause of the Research Project Award is amended to reflect an increase in funding of $782,012. And additional increase of $234, 569 was awarded with amendment M03 on June 22.
- Initial non-GMP production runs at FUJIFILM are awaiting Fujifilm review of the updated SOW.

b. Reportable Outcomes

Year 1

- 15+ pilot lots were produced, and VTB continued production of OxyBridge at the VirTech Bio (VTB) Production Laboratory, with analytical characterization of the product at the VTB Analytical Laboratory.

- The analytical documentation system was uploaded to the MediaLab, and documents remain under review in-house. Documentation and quality standards to the level appropriate for support of pre-clinical studies have advanced.
- OxyBridge manufacturing and characterization were performed that allowed in vitro coagulation studies as described above under Task 1.a.
- Two in vitro coagulation studies at separate laboratories were performed and demonstrate only minor dilutional impact with OxyBridge.
- Manufacturing process details were transferred to FujiFilm. There were budget negotiations between FUJIFILM, VTB, and MTEC.
- Regulatory documents for a Pre-IND FDA meeting for treatment of ARDS were submitted to the Agency, accepted, and a teleconference between VTB and FDA was held 26OCT20.

Paper Accepted for Publication:

- Song, BK, Light, WR, Vandegriff, KD, Tucker, J, and Nugent, WH. Systemic and microvascular comparison of Lactated Ringer's Solution, VIR-HBOC, and alpha-alpha crosslinked Hemoglobin-Based Oxygen Carrier in a rat 10% topload model. Art Cells, Nanomed, Biotech, accepted for publication, 2020.

New discoveries, inventions, or patent disclosures, and specific applications.

- OxyBridge + plasma provisional patent
- Indication for use in Acute Respiratory Distress Syndrome (ARDS), non-infectious or infection

Year 2 – Presentation at Conferences

During Y2Q1, two abstracts on the coagulation properties of OxyBridge were submitted to Military Health Systems Research Symposium (MHSRS). However, MHSRS 2020 cancelled. But VTB Abstract has been accepted for an oral presentation for MHSRS 2021, but again the meeting was cancelled due to COVID-19).[1]

New Specific Applications

Year 1

1. Indication for use of OxyBridge in Acute Respiratory Distress Syndrome (ARDS), non-infectious or infection, based on HBOC facilitated diffusion during O_2 uptake in infected lung and damaged tissue and O_2 extraction in in hypoxic tissues. An FDA Pre-IND Meeting request response letter regarding this indication has provided VTB a well outlined roadmap to advance this indication for regulatory submission.

Year 2

We are exploring an indication to achieve hemostasis during hemorrhage using a combination of OxyBridge plus spray dried plasma to treat hypovolemic shock through three mechanisms to increase survival through: 1) oxygen transport and blood volume maintenance (OxyBridge), 2) effective coagulation (plasma) to limit ongoing hemorrhage, and 3) treat hemorrhagic endothelialopathy. To address supplemental funding for this application with OxyBridge plus plasma, VTB has submitted a Full Proposal for DoD Funding Opportunity Number: W81XWH-21-DMRDP-BRISCC on 06APR21 and DARPA Broad Agency Announcement Fieldable Solutions for

[1] Light WR, Nugent W, Tucker J, Vandegriff K, Macko A, Song BK. Impact of Controlled and Uncontrolled Hemorrhage (Two-Hit Model) and Resuscitation on Coagulopathy and Hemostasis in Rats. MHSRS Meeting Abstract, 2020.

Hemorrhage with bio-Artificial Resuscitation Products (FSHARP) BIOLOGICAL TECHNOLOGIES OFFICE HR001121S0027.

c. Progress Detail (Y2, Annual Q1-Q4)

Task 1a: Ex Vivo Coagulation Impact Study – Originally Reported in Y1Q3 Progress Report

- Results were analyzed from the in vitro coagulation impact study performed with Michael A. Meledeo, CIV USARMY MEDCOM AISR (Coagulation and Blood Research Department, US Army Institute of Surgical Research) in collaboration with VTB. This study was not funded by this grant, but the data were provided in support for Check Point 1.
- Solutions tested included whole blood as control (n=6) and test articles, including 1) crystalloid (LRS), 2) OxyBridge, 3) fresh frozen plasma (FFP), 4) plasma and OxyBridge, and included a complete arm with dilution with PlasmaLyte as comparators.
- As noted previously, there was no coagulopathy noted beyond dilution.
- There was an apparent synergy between plasma and OxyBridge.

Results

- No coagulopathy was observed beyond expected dilutional effects.
- There was no pattern of statistical difference between the HBOC treatments, LRS, or appropriate dilutional control as noted by hematocrits (HCT, %).
- No adverse effects on platelet count [(PT (s)].
- No evidence of induced red blood cell lysis.
- No evidence of impact on fibrinogen.
- VIR-HBOC increased the total hemoglobin concentration whereas LRS decreased it.
- There were instances in which an apparent synergistic effect was observed between FFP and HBOC.

Conclusions:

- No unexpected coagulopathy observed in vitro or in vivo.
- Dilutional coagulopathy mitigated with plasma (FFP or Fresh).
- VIR- HBOC + Plasma may be more effective than either alone in achieving hemostasis after severe active bleed.

Year 2 Annual Conclusions:

- OxyBridge has been shown to extend survival in lethal hemorrhagic animal models, but an ongoing concern is that, without coagulation factors, there may be potential limits in the product's effectiveness during uncontrolled hemorrhage. Accordingly, as reported earlier, an in vitro study was performed in collaboration with Michael A. Meledeo, PhD, of the United States Army Institute of Surgical Research, to measure the impact on coagulation of OxyBridge and thawed fresh frozen plasma in a simulation of hemorrhage, dilution, and resuscitation. There were no negative effects attributed to OxyBridge alone beyond the expected dilutional effects, and there were some potential positive effects with reconstituted Plasma. This work completed **Task 1a**.

Task 1b – Year 1 Summary: FDA Pre-IND meeting (VirTech Bio, Biologics Consulting). VTB had engaged the services of Biologics Consulting, Virginia to prepare our Pre-IND FDA Briefing Package to a meeting. Previously as reported in Q3, Biologics Consulting had prepared a GAP analysis for VTB's development program and the pre-clinical, clinical and chemistry and manufacturing sections had been drafted and

were in the process of being finalized when the COVID-19 pandemic shifted worldwide attention. Current military imperatives are promoting early resuscitation of wounded warriors, as well as reaction to the COVID-19 emergency. This has led to a sense of urgency within the FDA encouraging cooperation with the DoD and early participation. As such, Biologics Consulting in discussions with the CSO and CEO of VTB decided to move beyond an Interact Meeting directly to a Pre-IND meeting. Biologics Consulting and VTB finalized the Pre-IND briefing document.

Task 1b. Year 2 Summary: FDA Pre-IND Briefing Document

- Current military imperatives are promoting early resuscitation of wounded warriors. But in addition, the FDA is also now promoting studies as an immediate reaction to the COVID-19 emergency. This latter indication has led to a sense of urgency within the FDA, encouraging cooperation with the DoD and early participation. In response to this call, VTB finalized a Pre-IND briefing document with Biologics Consulting (BC). In discussions between the CSO and CEO of VTB with BC, we decided to move beyond an Interact Meeting and move directly to a Pre-IND meeting. The Pre-IND briefing document was completed in Y2Q1 and submitted to the Agency. The virtual Pre-IND meeting with VTB and the FDA was scheduled for 26-OCT-20. The FDA provided a written response to the briefing document's questions. These written responses and advice were sufficiently clear and complete to obviate the need for further discussion.

- This FDA document introduced OxyBridge for an indication to treat Acute Respiratory Distress Syndrome (ARDS), non-infectious or infectious, based on HBOC facilitated diffusion during O_2 uptake in infected or damaged lung tissue and O_2 extraction in hypoxic tissues. The Briefing Document described pre-clinical development and manufacturing (CMC) plan that is indication independent and does apply for the indication of hemorrhagic shock as well. FDA review of the Pre-IND Meeting Briefing Document was completed 23OCT20. The FDA response provided a well outlined roadmap that follows closely what VTB had planned for regulatory allowance.

- The Pre-IND briefing document was completed in Y2Q1 and submitted to the Agency. The virtual Pre-IND meeting with VTB and the FDA was scheduled for 26-OCT-20. The FDA provided a written response to the briefing document's questions. The written response and advice were sufficiently clear and complete to obviate the need for further discussion, so at the intimation of the FDA and on the advice of our consultants, Biologics Consulting, VTB canceled the October 26, 2020 call. The FDA response provides a well outlined roadmap that follows closely what VTB had originally planned. VTB is encouraged and is taking advantage of this clear and complete guidance to finalize our program plans.

Task 1c – Year 1 Summary: Manufacturing Process Finalization (VirTech Bio).

- During Y1 Q2, VTB performed a sufficient number of OxyBridge pilot runs to implement standard statistical evaluations to assess process control in the laboratory and initiate Technology Transfer to the CMO (Fujifilm Diosynth Biotechnologies).
- These studies showed that the current OxyBridge pilot-scale production process is robust.
- VTB transferred safety documents, and process documents to Fujifilm and had weekly videoconferences to coordinate activities, including review of the process development plan to insure it met their needs for tech transfer.

Task 1c – Year 2 Summary: Manufacturing Process Transferred (VirTech Bio to FUJIFILM).

- VTB has transferred safety documents, process documents, and analytical methods to the CMO that addressed detailed questions such that FUJIFILM could develop manufacturing plan. This also allowed FUJIFILM to develop a more accurate budget.
- These studies showed that the current OxyBridge pilot-scale production process is robust, and VTB transferred safety and process documents to the CMO. To expedite this process, VTB and FUJIFILM and have had weekly videoconferences to coordinate future activities.
- In Y2Q1, budgetary negotiations were completed between VTB and the CMO (FUJIFILM). The result was a budgetary increase of $1M. VTB provided MTEC with funding options to stay on course. This has necessitated a hold on CMO activities until funding decisions are reached. A call with MTEC regarding this funding occurred on Nov. 6, 2020.
- JPC6, MTEC and VTB agreed on a revised SOW to reflect fully funding FUJIFILM.

2. Future Plans

The timeline has shifted for reasons discussed in Section 3.

Table 1

Task	Milestone/Deliverables	Former Timeline	Current Timeline
Base Agreement	***1a**. Ex Vivo Coagulation Impact Study*.	Y1Q1	Completed
	***1b**. FDA Pre-IND meeting booklet*.	Y1Q1	Completed
	***1c**. Manufacturing Process Finalization*. Scale-up, and statistical process control in laboratory. Support for tech transfer.	Y1Q1-Y1Q2	Completed
	***1d**. CMO Engineering Runs*. Process transferred to a CMO and pre-cGMP material produced for animal studies. Initiate stability study.	Y1Q2-Y1Q4	Y3Q1-Y3Q4
	***1e**. Safety and Coagulation Profile in Rat and Swine Models (4 studies)*	Y2Q1-Y2Q4	Y3Q1-Y3Q4

Task 1d Milestones – Ongoing *(Manufacturing Process Finalization)*

- This Milestone required CMO review of pilot-scale manufacturing processes to assure that the process development plan meets the CMO's needs for technology transfer.
- Technology Transfer to CMO for pilot scale Manufacturing has been completed.
- We now need to initiate production of pre-cGMP material for animal studies.
- Initiate stability study.
- Based on delays due to COVID-19 on the priorities of the CMO contracts, VTB obtained from MTEC supplemental funding required by the CMO to initiate and complete this Task, with CMO providing pre-cGMP product to be used in all upcoming pre-clinical studies.

Task 1e (Y3Q1-Q4) Milestones *(Safety and Coagulation Profile in Rat and Swine Models)*

- Safety and Coagulation Profile in Rat and Swine Models (4 studies).
- To advance the in vitro coagulation studies (see **Task 1a**) into in vivo work for **Task 1e**, we have completed preliminary evaluations of the OxyBridge resuscitant impact on coagulopathy and

hemostasis in an in vivo rat model (n=4 per group) designed to simulate limited resuscitation availability in austere combat theaters. While neither OxyBridge nor Plasma resuscitation alone provided successful hemostasis, a combination of the two (OxyBridge + Plasma) provided hemostasis in 100% of test animals (4/4 animals). The positive synergistic effect on hemostasis through the combination of OxyBridge and Plasma is under further investigation, and we are pursuing additional funding under DoD Funding Opportunity Number W81XWH-21-DMRDP-BRISCC to supplement and advance this research beyond the ex vivo **Task a** goal into in vivo rat and swine hemorrhage models with a statically significant number of experimental subjects.

Light WR et al. Impact of controlled and Uncontrolled hemorrhage (two-hit model) and resuscitation on coagulopathy and hemostasis in rats. MHSRS Meeting Abstract, 2020.

3. Problems / Issues

a. Current Problems / Issues

Provide a description of current problems or issues that may impede performance or progress of this project along with proposed corrective action. This may include administrative, technical, and/or logistical issues.

- Technology transfer to the Contract Manufacturing Organization (CMO) (FujiFilm) got off to a slow start due to logistical issues on the CMO's part and time/logistical constraints at VTB. Uncertainty with the COVID-19 pandemic has also hampered efforts substantially. Equipment previously assigned to this project from FujiFilm had to be reallocated to vaccine production. However, in Y1Q3, activity increased with regular teleconferences occurring, and while there has been a delay, there is confidence of achieving the goals. The schedule has been updated in Tables 1 and 2 below to clarify. The long-lasting effect of the pandemic is still unclear, but the tables reflect best faith estimates.
- The delay in manufacturing pre-clinical material has delayed the pre-clinical studies but some lost time will be made up for. Schedule updates are reflected in Tables 1 and 2.
- The long-lasting effect of the pandemic is still not clear but the tables reflect best faith estimates.

Table 1. Major Task Summary and Updated Timeline Comparison

Task	Milestone/Deliverables	Former Timeline	Current Timeline
Base Agreement	*1A. Ex Vivo Coagulation Impact Study*.	Y1Q1	Completed
	1B. FDA Pre-IND meeting booklet.	Y1Q1	Completed
	1C. Manufacturing Process Finalization. Scale-up, and statistical process control in laboratory. Support for tech transfer.	Y1Q1-Y1Q2	Completed
	1D. CMO Engineering Runs. Process transferred to a CMO and pre-cGMP material produced for animal studies. Initiate stability study	Y1Q2-Y1Q4	Y3Q1-Y3Q4
	1E. Safety and Coagulation Profile in Rat and Swine Models (4 studies)	Y2Q1-Y2Q4	Y3Q2-Y3Q4

b. Anticipated Problems / Issues

We believe that the revised schedule detailed in **Table 1** will be successful. It should be noted that the long-lasting effects of the pandemic is still unclear, but the Table(s) reflects best-faith estimates.

- Technology transfer to the CMO (FUJIFILM) got off to a slow start due to logistical issues on the CMO's part and time/logistical constraints at VTB. Uncertainty with the COVID-19 pandemic has also hampered efforts substantially. However, in Y1Q3, activity increased with regular teleconferences occurring, and while there has been a delay, there is confidence of achieving the goals outlined. The schedule has been updated in **Table 1** to clarify the ongoing issue. The long-lasting effect of the pandemic is still unclear, but the Tables reflect best faith estimates.

4. Financial Health

The total contract expenses to date are significantly down versus expected due to the Contract Manufacturing delays that have been previously reported. Through two years, the total contract expenses have been ~$1.6M as compared to a two year projected amount of $3.59M. The largest differentials can be assigned to Other Direct Costs and Subcontractors. Those categories are down ~$1.1M and ~$1M respectively due to delays. As a result of those delays, the supply expenses are down $275k and G&A is down $496k. There were no equipment or travel expenses in the past year due to COVID.

5. Personnel Effort

Personnel – Current Staff	Role	Percent Effort
William Richard Light, CSO	Principal Investigator	43
Joseph Tucker, President	Quality Assurance	33
Kim D. Vandegriff, VP R&D	Scientific Advisor	41
Ashok Malavalli	R&D Manager	16
Andres Benitez	Lab manager	39
Wayne Dion	Lab Technician	38
Bill Light	Lab Technician	67
Phil Farabaugh	Project Coordinator	33

6. Protocol and Activity Status

a. Human Use Regulatory Protocols

TOTAL PROTOCOLS:

No human subjects will be performed to complete this first phase (2 years) of the Statement of work.

One human protocol is planned for OPTION 2 (Year 4): CLINICAL PHASE 1 PROGRAM. FDA approval has not yet been obtained. In Y1Q4 we plan to have a Pre-IND meeting with the FDA to discuss the entire program continuing to a Phase 1 clinical program to assess safety in humans.

c. Animal Use Regulatory Protocols

TOTAL PROTOCOLS:

Six animal-use research protocols will be required to complete the Statement of Work. They are scheduled to begin in Y2. To date, the IACUC/ACURO process is underway.

PROTOCOLS:

Study 1 (Y2 Q1): Uncontrolled Hemorrhage, Hemostasis and Coagulopathy (Rat) – (Song Biotechnologies, LLC)
Unconscious rats will be subjected to a severe pressure and volume-controlled hemorrhage (primary hit to allow for addition of test material), hypovolemic resuscitation with test material, severing of tail (uncontrolled hemorrhage; secondary hit to test for hemostasis and coagulopathy),

and observed for six-hour survival. The primary hit (i.e. controlled bleed) in combination with fluid resuscitation is intended to uniformly create the acute injury consistent with what is seen in severe trauma patients (e.g. metabolic insult, altered coagulation function, hemodilution), while the secondary hit is intended to simulate a clinically important re-bleed scenario. Hence, the consistency and severity of the primary injury will allow for true in vivo assessment of coagulation function and hemostasis following an uncontrolled hemorrhage and allow for a lower N value to be relevant. Four test groups are being studied. LRS is the standard control, plasma is the colloid control, product, and then product and plasma are being explored for potential synergy.

Treatment Groups (N = 8 per group): 1) LRS, 2) OxyBridge, 3) Plasma, and 4) OxyBridge / Plasma Mixture
Measurements: 1) Cardiovascular, 2) Blood Gasses (ABL90 Flex), and 3) ROTEM and Stago (Coag)

Study 2 (Y2 Q2): Topload Safety/inflammatory/Coag (Swine) – (Song Biotechnologies, LLC)
Toxicity, PK/PD, inflammatory marker studies, coag, histopathology, and hypertension (MAP) studies will be performed with swine during a 10% top-load protocol that is sensitive for hypertensive and toxic effects. Swine responses are translatable to human responses and will support Phase 1 clinical trials. Swine will be subjected to a 10% total blood volume bolus (topload) with test article and observed for 24 hours. Blood sampling and measurements will occur at baseline, and post-infusion time points: 0, 1, 2, 4, 8, 16, and 24 Hour. Animals will be anesthetized and MAP monitored during the infusion and plus 2 hours post infusion, then the animals will regain consciousness. As a safety study, only 1 arm is being budgeted for this proposal, OxyBridge, but VTB may elect to add a second arm as additional cost share, Plasma.
Treatment Groups (N = 6 per group): 1) OxyBridge, and VTB Potential 2) Plasma.
Measurements: 1) Cardiovascular, 2) Blood Gasses (ABL90 Flex), 3) ROTEM and Stago (Coag), 4) blood sampling for inflammatory markers, 5) Histopathology

Study 3 (Y2 Q3): Survival following Pre-hospital Care Model (Rats) – (Song Biotechnologies, LLC)
Hypovolemic shock followed by hypovolemic resuscitation in in vivo models simulate far-forward combat theater without immediate medical evacuation and/or restorative surgical capacity. Furthermore, in this austere pre-hospital trauma environment, animals are limited (single bolus) in product, and follow the TCCC guidelines of resuscitation volumes. In addition to characterizing the systemic (cardiovascular and blood gasses) impact of test articles, this study will also look for minimal dose required for resuscitation. Pre-ported rats will be subjected to a two-step 60% total blood volume (TBV) withdrawal and resuscitated with treatment solutions at different percentages of total blood volume (hypovolemic resuscitation) when mean arterial pressure drops below 25 mmHg and seconds from demise. To emulate follow on care, following one hour of point of injury rats will be given as much fluid as possible to maintain a pressor response (MAP) above 60 mmHg. Hence, resuscitant will be titrated to MAP for as much fluid as required for up to 4 hours (versus the single dose used in 'Study 1'), and then animals will be placed in a cage for observation for up to 72 hours. Five test groups are being studied. LRS is the standard control, plasma is the colloid control, product at the same volume as the controls, product at half the volume as the controls to test for potency, and then product and plasma are being explored for potential synergy.

Treatment Groups (N = 8 per group): 1) LRS 20% TBV, 2) Plasma 20% TBV, 3) OxyBridge – 20% TBV, 4) OxyBridge – 10% TBV, 5) Plasma + OxyBridge mixture 20% TBV
Measurements: 1) Cardiovascular, and 2) blood gas (ABL90 Flex)

Study 4 (Y2 Q4): Uncontrolled Hemorrhage, Hemostasis and Coagulopathy (Swine) – (Song Biotechnologies, LLC)

Pre-ported and anesthetized swine will be subjected to a 50% TBV controlled (variable to blood pressure), severe, and otherwise lethal blood withdrawal, and then resuscitated with a hypovolemic 20% TBV infusion during the uncompromised state (near demise). Symptoms of acute coagulopathy of trauma (ACOT) will be evident through measurements such as clot firmness (ROTEM) and prothrombin (Stago). Coagulation will be further affected by the type of resuscitation fluid, and the direct impact on hemostasis will be tested immediately after resuscitation through a dermal incision injury. The secondary injury is not meant to be lethal, but effective enough to display differences in blood volume loss and time to hemostasis between treatment groups. An hour after the dermal injury, the swine will be given as much fluid as possible to maintain a pressor response (MAP) above 60 mmHg for up to 6 hours. Animals will then be brought back to consciousness and be observed for up to 72 hours. Only 3 arms are being budgeted for this proposal, LRS (standard control), OxyBridge (product), and plasma (colloid control), but based on results of study 1, VTB may elect to add a fourth arm as additional cost share, OxyBridge with Plasma.

Treatment Groups (N = 8 per group): 1) LRS, 2) OxyBridge, 3) Plasma, and VTB Potential 4) OxyBridge /
Plasma Mixture Measurement: 1) Cardiovascular, 2) Blood Gasses (ABL90 Flex), and 3) ROTEM and Stago (Coag)

Studies 5 and 6 – Safety Toxicology Studies (Y2 Q3-4): MULTIPLE-DOSE STUDIES (SoBran)
Two studies will be conducted using 1) mixed gender Sprague Dawley rats (250-275g) and 2) male, out-bred swine (50-55 kg).
Animals will be administered a bolus dose of 3.3, 6.6, or 10 ml/kg OxyBridge or LRS volume control via IV injection (at a set volume of 10 mL/kg) once daily for 7 days. Following a 14-day recovery phase after treatment stops, animals will be sampled again and then sacrificed. In-life evaluations (i.e., signs of pain, distress, changes in body weight, food or water consumption, feces, vomiting, etc.) will be conducted daily for all animals throughout the experimental time course. Four treatment groups (control, low, medium, and high dose OxyBridge) of 10-15 animals each will be used. Unscheduled sacrifices or deaths in this study necessitate necropsy.

Multi-Dose PK/TK Studies Detail:
During treatment days, rats within treatment groups will be sub-grouped into sampling time points (i.e., Control Sub-group 1 will be sampled at 2, 6, and 12 hours, while Control Sub-group 2 will be sampled at 1, 4, and 8 hours) to minimize blood draws while providing sufficient data points.

Measurements will include (Cmax), dose-normalized Cmax, time to peak concentration (Tmax), AUC0-t, dose normalized AUC0-t, T1/2, and hematological and biochemical parameters.

STATUS:

- **A protocol for large animal species that will form the basis for future Topload / Toxicology studies (Study 2, 5, and 6) in swine has been developed for initial testing in canine with laboratory grade material.**
- **Our Regulatory Status has been expedited to bypass the Interact meeting with FDA and move directly into a Pre-IND meeting with FDA for planning for Phase 1 Safety trials in human volunteers.**
- **Our interactions with our current CMO (FujiFilm) are on-track, and they are working with us to expedite material manufacturing to meet our FDA schedules to begin a Phase 1 trial.**

Annual Business Status Report for

Development of An Oxygen Carrier for Use in Hemorrhagic Shock Resuscitation

Research Project No. 2019-453-001

EGS Number: MT17008.138

Reporting Period: Effective Date – 25 JUL '21

MTEC Research Project Awardee

Research Project Lead: W. Richard Light, PhD, CSO

Other Research Project Team Member(s): Joseph Tucker, Bjorn K. Song, Kim D. Vandegriff, Ashok Malavalli, Wayne Dion, Andres Benitez, Bill Light

Research Project Business POC:
Joseph Tucker
27 Strathmore Rd
Natick, MA 01760
Phone Number: 508-627-0485
Email address: joe.tucker@virtechbio.com

Submitted: August 26, 2021

1. Current Staff

Personnel – Current Staff	Role	Percent Effort
William Richard Light, CSO	Principal Investigator	43
Joseph Tucker, President	Quality Assurance	33
Kim D. Vandegriff, VP R&D	Scientific Advisor	41
Ashok Malavalli	R&D Manager	16
Andres Benitez	Lab manager	39
Wayne Dion	Lab Technician	38
Bill Light	Lab Technician	67
Phil Farabaugh	Project Coordinator	33

2. CURRENT EXPENDITURES

A. Cost Reimbursable Contract:

Contract Expenditures	Current ANNUAL Expenditures	*Cumulative To Date Expenditures*
Labor (Personnel and Fringe)	$666,879.93	$996,856.32
Supplies/Materials	$48,282.33	$95,184.44
Travel	$0.00	$3,626.19
Equipment	$4,901.05	$4,901.05
Subcontractors and Consultants	$81,128.43	$134,013.98
Other Direct Costs	$22,331.88	$123,702.64
Indirect Costs	$172,363.49	$284,288.95
Total	**$995,887.11**	**$1,642,573.57**

B. Fixed Priced Contracts: Complete only if your contract is Fixed Priced.

MTEC Milestone Number	Milestone Description	Due Date	Government Funds
1		1/15/20	$1.00
2		2/15/20	$1.00
	Total Expenditures		$2.00 (Should reflect what has been invoiced for)

C. Cost Share Contributions: Complete only if you're reporting Cost Share:

Funding Source (Cash)	This Period	Cumulative to Date
Cash	$0.00	$0.00
Labor Dollars	$88,289.99	$131,976.43
Indirect Labor Rates (Overhead/Fringe Benefits)	$80,972.91	$121,038.82
Travel	$0.00	$920.37
General & Administrative Services	$43,748.12	$72,156.28
Equipment (New)	$1,243.95	$1,243.95
Material	$12,254.69	$24,159.06
Other Direct Costs	$5,668.12	$31,397.36
Consultants	$20,591.46	$34,014.51
Sub-Total	$252,769.24	$416,906.78
Funding Source (In-Kind)	**This Period**	**Cumulative to Date**
Use of Existing Equipment (Estimated fair market value)	$0.00	$0.00
Use of Existing Software (Estimated fair market value)	$0.00	$0.00
Intellectual Property (Estimated fair market Value)	$0.00	$0.00
Space (Land or buildings)	$0.00	$0.00
Sub-Total	$0.00	$0.00
Cost Share Total	$252,769.24	$416,906.78

3. STATUS OF MILESTONES – FILL OUT FOR ALL CONTRACT TYPES (all project milestones are to be included)

MTEC Milestone Number	Milestone Description	Due Date	% Completed this Reporting Period	Cumulative % Complete
1	**Process Study Design, Contact with FDA, in vitro coag initiation**	**8/30/19**	**0**	**100%**
2	**Process Robust Studies, conc, pH, FDA Pre-IND package, in vitro coag execution**	**9/30/19**	**0**	**100%**
4	**Process Robust Studies, glut, temp, time, FDA Pre-IND Meeting, in vitro coag report**	**10/31/19**	**0**	**100%**
5	**Process Robust std, hold, RX time, Boro**	**12/2/19**	**25%**	**95%**
6	**Transfer Product Information, specs (CMO)**	**12/31/19**	**0**	**100%**
8	**Transfer Manufacturing, Analytical Protocols, CMO**	**1/31/20**	**25%**	**95%**

9	**Review Method, Draft Protocols (CMO)**	**03/02/20**	**65%%**	**95%**
10	**Develop Protocols (CMO)**	**03/30/20**	**10%**	**10%**

4. DEVIATION FROM PROJECT PLAN

As we mentioned previously, the delay in the tech transfer process at Fujifilm has been a significant challenge and one we are still dealing with. Additional equipment required by Fuji has been discussed with MTEC and additional funding was allocated. VirTech also received a No Cost Extension in May of 2021 to continue our R&D efforts. We continue to work with FujiFilm to expedite our work despite the issues presented by Covid-19 including significant supply chain challenges related to supplies and equipment. We have previously provided MTEC with an accelerated plan that, with additional funding, would get us back on track with our milestone schedule.