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14. ABSTRACT

The first ErythroMer prototype (EM-V1) was structurally stable and toroidal, with diameter ~ 1/50th that of RBCs and amenable to lyophilization and rapid reconstitution. In addition, EM p50 (with novel pseudo-Bohr effect), NO sequestration and vasoactivity were equivalent to RBCs – establishing POC for the bio-inspired design. We also developed a rabbit hemorrhagic shock-resuscitation model (40% BV removal; outcomes: mean arterial pressure, lactic acidosis and liver pO_2); in this model, EM was non-inferior to shed blood and superior to 5% Albumin. To further optimize biocompatibility, circulation time, and Hb payload density and retention, we developed EM-V2. In prior progress reports, we included data on V2 biophysical and functional characterization, which recapitulates RBC physiology similarly (to V1). PK testing with EM-V2 revealed extended elimination $t_{1/2}$=4.5hrs in rabbits (n=4). In rabbit acute hemorrhagic shock – resuscitation studies, EMV2 also demonstrated superiority to 5% Albumin and non-inferiority to shed blood (N=6/group). Of note, just prior to the end of project Y2, Dr. Doctor was recruited from Washington University (WUSM) to the University of Maryland (UMB), to serve as founding director for the Center for Blood Oxygen Transport and Hemostasis (CBOTH), a major resource that will accelerate EM development. Related to this transition, there were changes with other key personnel, which are detailed below, including transition of KaloCyte from the St Louis Cortex District to the Baltimore Biopark (KC lab is now embedded in CBOTH, facilitating collaboration). Resulting from these changes, the timing of contract transition from WUSM to UMB, and from COVID19 related lab shutdowns, the only project work in Y3, was performed by KaloCyte; information is provided in detail below. As such, an NCE was requested and approved, enabling the team to resume progress (NCE-Y4) towards completing project goals; a time-adjusted SOW is included in this report.

15. SUBJECT TERMS

ErythroMer (EM), Artificial Red Blood Cell (RBC), Prolonged-Field Care (PFC), PFC models, Resuscitation, Oxygenation, Hemorrhagic Shock, Pharmacokinetics, and Biocompatibility

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1. INTRODUCTION:

The overall goal of this project is to optimize a red blood cell substitute, ErythroMer (EM) for resuscitation of casualties with hemorrhagic shock. This will be accomplished by developing EM prototypes with optimal oxygen (O_2) binding affinity that allows for O_2 capture in the lungs and O_2 release in other tissues as well as optimizing formulation and dosing to achieve stable circulation suitable for PFC. EM will also be tested for compatibility with Thrombosomes and other hemostatic adjuncts to prevent dilutional coagulopathy via co-administration with EM. Finally, we will establish EM's efficacy and safety in resuscitation of a hemorrhagic shock model with/without Thrombosomes and/or hemostatic adjuncts.

2. KEYWORDS:

ErythroMer (EM), Artificial Red Blood Cell (RBC), Prolonged-Field Care (PFC), PFC models, Resuscitation, Oxygenation, Hemorrhagic Shock, Pharmacokinetics, and Biocompatibility

3. ACCOMPLISHMENTS:

What were the major goals of the project?

See Revised SOW for adjusted timeline, anticipated completion dates listed below.

Major Task 1: Select & Fabricate ErythroMer (EM) prototypes with high/low O_2 affinities.
Milestone #1: Select EM prototypes meeting high/low P50 targets (completed 04/2018).
Milestone #2: Fabricate selected EM prototypes for *in vivo* testing (completed 10/2018).

Major Task 2: Test efficacy *in vivo*.
Milestone #3: Obtain IACUC/ACURO approval (completed 01/2018 at WUSM; 10/2020 at UMB).
Milestone #4: Establish O_2 delivery benefit conferred by EM prototypes with high/low O_2 affinities (defined as 20% improvement in tissue pO_2 relative to current prototype). (completed 04/2019)

Major Task 3: Measure EM pharmacokinetics (PK).
Milestone #5: Calculate EM PK as a function of Blood Volume (BV)% replacement. (80% complete, target 03/2021). Added PK with MPS depletion (by clodronate), this simulates MPS saturation by massive transfusion, as expected in PFC (20% complete, target 04/2021)

Major Task 4: Develop EM PFC dosing.
Milestone #6: Confirm EM dosing strategy for rabbit PFC models; will be informed by MPS depletion model. (70% complete, target 06/2021).

Major Task 5: Determine EM:HA (hemostatic adjunct) compatibilities *ex vivo*.
Milestone #7: Obtain IRB/HRPO approval (completed 01/2018 at WUSM; 10/2020 at UMB).
Milestone #8: Confirm EM:HA *ex vivo* compatibility. (completed 01/2018).

Major Task 6: Develop goal-directed HA algorithm for EM-based dilutional coagulopathy (DC).
Milestone #9: Develop goal directed HA algorithm for EM-induced DC suitable for in vivo testing (rabbits). (completed 10/2018).

Major Task 7: Pilot PFC Scenarios.
Milestone #10: Obtain IRB/HRPO approval (completed 01/2018 at WUSM; 10/2020 at UMB).
Milestone #11: Pilot & Optimize PFC Scenarios (A, B, C) to achieve 50% 48h mortality for colloid resuscitation controls. (50% completed, target 04/2021).

NB. Major Tasks 8-10 run concurrently, efficiently using the same rabbits for multiple tasks.

Major Task 8: Establish EM efficacy *in vivo.*
Milestone #12: Establish EM efficacy in comparison to shed blood (O_2 delivery non-inferiority yes/no) and colloid resuscitation (mortality superiority yes/no). (25% completed, target 09/2021).

Major Task 9: Optimize PFC HA Algorithms *in vivo*.
Milestone #13: Optimize goal directed HA algorithm for DC and TIC during resuscitation in PFC Scenario B (uncontrolled hemorrhage, with dilutional coagulopathy) and PFC Scenario C (controlled hemorrhage + polytrauma, with TIC). Identify differences required (amongst colloid, blood and EM-based resuscitation) for HA administration. (25% completed, target 09/2021).

Major Task 10: Screen EM safety *in vivo*.
Milestone #14: Identify laboratory and histologic evidence of EM toxicity during resuscitation from PFC Scenarios A-C, in comparison to that observed in blood re-infusion and colloid resuscitation groups. (25% completed, target 09/2021).

What was accomplished under these goals?

What was accomplished under these goals?

1. **Select & Fabricate ErythroMer (EM) prototypes with high/low O_2 affinities.**
   - Y1: Testing of the first EM prototype (EM-V1) completed
     - Identified EM-V1 with O_2 affinities that match RBCs or are >30% and <20% that of RBCs. (MT 1, Milestones 1 and 2).
     - The prototype with optimal O_2 affinity was tested *in vivo* in both PK and in oxygenation studies following controlled hemorrhage (MT 2; Milestones 3 and 4).
   - Y2: Optimized design w/r/t biocompatibility, improved payload retention, and improved lyophilization/reconstitution, yielding EM-V2.
     - Two complimentary proposals have been awarded to support additional optimization of the EM formulation:
       - DoD BA190035 (FOA USA-MRMC-BAA-2018-W81XWH18SBAA1) "Optimized Formulation, Delivery & Dosing for ErythroMer (Artificial Red Cell)"
       - NIH/NHLBI SBIR Phase I H193-004-0067 "Rapid Reconstitution of a Lyophilized, Bio-inspired, Artificial Red Blood Cell."
   - Y3: Optimization of ErythroMer components and processes, including fabrication and assembly; cleanup; and lyophilization/reconstitution (details in narrative, below).
2. **Biocompatibility**
   Hemostasis
   - Y1-2 Biocompatibility was evaluated in *ex vivo* ROTEM analyses
     - EM-V1 and EM-V2 particles had no effect upon (*ex vivo*) hemostasis other than a correctable dilution effect, indicating need for co-administration of plasma and platelets when transfusion exceeds ~ 50% BV (e.g. massive transfusion) (see Y1 report). (MT 5, Milestone 7)
     - Generation of a goal-directed HA algorithm to optimize co-administration testing of hemostatic adjuncts to maintain hemostasis during resuscitation (see Y1 report). (MT 6, Milestone 8)
     - Extensive *ex vivo* analysis of EM biocompatibility with Thrombosomes (see Y2Q1 & Y2Q3 reports). (MT 5, Milestone 7)
       - EM-V2 (empty shells [focused study of surface biocompatibility] and Hb-loaded EM-V2) had no effect on coagulation studies (size distribution, aggregometry, surface marker expression, thrombin generation) with Thrombosomes (desired outcome).
     - Submission of two complimentary proposals to fund additional work exploring EM biocompatibility and co-administration with:
       - Freeze dried plasma: DoD PR190685 (FOA W81XWH-19-PRMRP-TTDA) "Freeze-Dried Hemostatic O_2 Carrier for Damage Control Resuscitation) (awarded, in partnership with Haima Therapeutics)
       - Synthetic platelets: DoD/DHA SBIR Phase I H193-004-0067 "Nanoformulated Dried Whole Blood Surrogate for Hemostatic Resuscitation". (not awarded)
   - Y3 – due to move from St Louis → Baltimore and pandemic lockdown, no work was performed on this task in Y3.

3. **Pharmacokinetics**
   - Y1: Top-loading (10% BV replacement) PK studies were completed with both EM-V1 and EM-V2. Analysis of EM-V2 in rabbits indicated a $t_{1/2}$ of ~4.5h. (MT 3, Milestone 5)
   - Y2: Confirmation of findings, in the context of 20 & 40% BV replacement (data in Y2Q3 report). We anticipate that PK in the setting of higher EM dosing (>40% BV replacement) may exhibit complex multi-phase elimination due to saturation of the mononuclear phagocytic system (MPS), the principal route of elimination for EM. We have designed experiments to test this hypothesis, employing an established liposomal clodronate model for MPS depletion (see Narrative below).
   - Y3: due to move from St Louis → Baltimore and pandemic lockdown, no work was performed on this task in Y3.
   - Y4: We will confirm anticipated $t_{1/2}$ extension (following MPS depletion) of labeled liposomes (controls) and then of EM in this model and with BV replacement > 40%. These results will influence dosing in our PFC models (below).
4. **Safety/Toxicity**
   - Y2: Key exploratory work evaluating rheologic impact of "Nanocrit" (BV comprised by EM particles) in relation to Hematocrit (BV comprised by RBCs).
     - Circulating concentrations of EM (50, 75, 125, 150, 300 x 10^9 particles/mL) were tested in both murine and rabbit models. Findings indicates a slight impairment of O_2 transport (liver pO_2, lactate) when 'NanoCrit' exceeded 150 x 10^9 particles/mL (data in Y2Q1, Q2 and Q3 reports).
     - This is important safety information and given [Hb]/particle, this issue will not limit our ability to provide adequate O_2 carrying capacity during resuscitation, since the circulating 'NanoCrit" can be maintained below this level and still achieve adequate circulating [Hb] to support O2 delivery.
     - Note: Given the differences in flow dynamics and vessel caliber, we anticipate that the therapeutic window (for elevated NanoCrit) will be broader in humans, allowing for greater concentrations/higher particle abundance to be tolerated.
   - Y3: due to move from St Louis → Baltimore and pandemic lockdown, no work was performed on this task in Y3.
   - Y4: We will continue to evaluate the effect of 'NanoCrit' in the efficacy experiments planned in Y4 and report on the basic safety – toxicity screen embedded in our efficacy protocols (basic metabolic profile, liver enzymes, renal function panel).
5. **Efficacy**
   - Y2: Rabbit hemorrhagic shock model pilot (MT 7, Milestone 10)
   - Initial shock studies reliably demonstrated non-inferiority of EM-based resuscitation to re-infused shed blood and superiority of EM- to Colloid-based resuscitation (for MAP, lactate, tissue pO_2), (Data reported in Y2Q1-3).
   - Several features have been evaluated to optimize our shock model and establish appropriate conditions for further PK analysis with volume replacement.
     - Initial studies were performed using ventilation with 100% oxygen. However, to realistically simulate hemorrhagic shock under field conditions, we reduced FiO_2 to room air.
     - Additionally, our team was previously concerned about potential auto-resuscitation (release of sequestered red blood cells) due to splenic contraction. Further studies (+/- splenectomy) suggest that the responses we observed are minimally, if at all, associated with splenic contraction. As such, we fine-tuned the model so splenectomy is not necessary, which more accurately models hemorrhagic shock in field.
     - We have now completed initial pilot work to optimize our hemorrhagic shock + polytrauma model (with pseudofracture & quadricep crush injury and liver laceration).
   - Y3: Due to move from St Louis → Baltimore and pandemic lockdown, no work was performed on this task in Y3. "Dry lab" working included preparation for PFC resuscitation scenarios (acute bleed, uncontrolled hemorrhage, acute bleed with polytrauma) – this required design, purchase and installation of state of the art telemetry system (secured with Dr. Doctor seed fund) to enable 48h FPC model.
   - Y4: We expect to complete efficacy experiments remaining in the SOW (see attached revised SOW).
     - Work stopped on this grant at WUSM on 6/7/2019.
     - Due to personnel turnovers at the sponsoring agency, the transition of this grant was

6. Team Transition to Baltimore

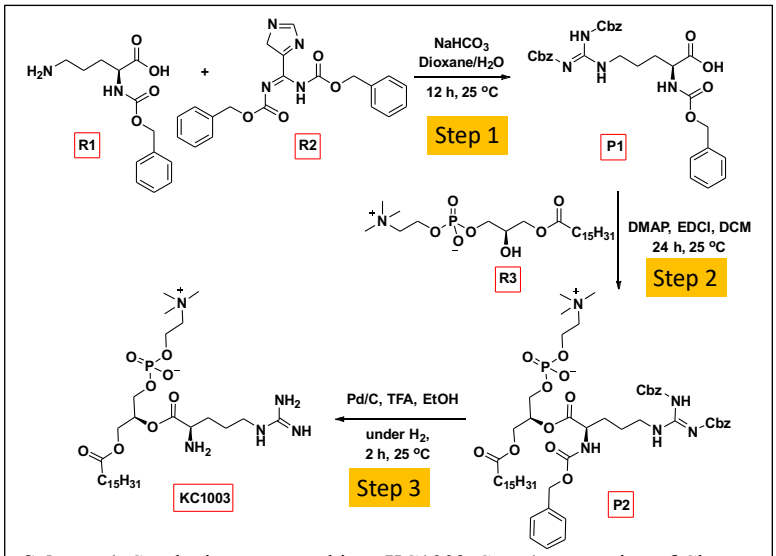
- Y2: Drs. Doctor, Pan and the KaloCyte Team transitioned from WU, UIUC and the St. Louis Cortex District (respectively) to join the Center for Blood Oxygen Transport and Hemostasis (CBOTH) at the University of Maryland, Baltimore – which Dr. Doctor directs. CBOTH is located in the new Health Sciences Facility (HSF) III and includes resources that will accelerate project task completion (10,000 sf labs, with six core labs: RBC and Hematology; Nanofabrication and Characterization; Imaging; Small Animal Surgery and Physiology; Analytical Chemistry; and Biospecimen Repository and Clinical Research.
    - Work stopped on this grant at WUSM on 6/7/2019.
    - Due to personnel turnovers at the sponsoring agency, transition of this grant was delayed.
- Y3: The grant was successfully transferred from WUSM to UMB on 6/19/20, though funds were not activated for the Doctor Lab until 10/30/20. The grant was amended, effective date 20-Aug-2020, extending the period of performance to 29-Sept-2021.

Y3 Project Narrative: Here, we provide a brief summary of work performed by KaloCyte during Y3. As noted elsewhere in this report, the UMB CBOTH laboratory was shutdown during the pandemic; however, KaloCyte was able to use temporary wet lab space in the UMB BioPark, made available to small commercial affiliates and so was able to maintain operations during the pandemic shutdown. This work was limited to additional optimization and scaling of EM source materials and fabrication, to better prepare for an accelerated period of in vivo testing that will commence in the NCE Y4.

1. Hb Purification
    a. A formal, GLP level SOP for Hb purification, with quality control procedures and release specifications was drafted, optimized and approved.
    b. Significant improvement was made in improving efficiency and scale for improving purity of Hb preparations – specifically, with regard to removing residual phospholipid (from the RBC membrane). The following options were tested: washing Hb with various organic solvents, passing Hb over resins designed to bind phospholipids, ultracentrifugation and tangential flow filtration, and solid phase extraction (Empore SDB-RPS discs). A final protocol including the above (other than use of resins) was implemented, with ability to reduce residual phospholipid contamination < 3 ug/gm Hb, well below FDA expectation and standard for other HBOC formulations.

2. **EM Shell Precursor:** Major improvements were made with regard to synthesis, purification and in particular, scaling for the synthetic peptidic lipid that comprises the precursor element to the ErythroMer shell. This is the highly novel, most important element of the EM source material – that enables the unique geometry and functional properties (efficacy and safety) of the fully assembled EM artificial RBC. The optimized sequence is briefly summarized below.

Step 1 – Protection: The reactant 1 **(R1)** (L(+)-N-Cbz-ornithine, MW 266.30, 1 eq) is mixed with sodium bicarbonate ($NaHCO_3$) solution (1.25 eq) in dioxane and water followed by addition of reactant 2 **(R2)** (MW 378.39, 0.774 eq) for 12 h at 25°C. The solvent is removed under vacuum. The resulting residue is dissolved in ethyl acetate, washed with diluted hydrochloric acid (HCl) (1 M) and brine. The product is dried by lyophilization. The product **(P1)** (MW 576.61) is confirmed by NMR and LC-MS (purity, yield, quantity), and as a colorless gum. The crude product was subjected to multiple solubilization and precipitation cycles and used for the next step.



Scheme 1. Synthetic steps to achieve KC1003. Step 1: protection of Cbz-ornithine; Step 2: esterification of Lyso-PC and Step 3: deprotection of the Cbz protection group by Pd/C catalyzed hydrogenation.

Step 2 – Esterification: The reactant 3 **(R3)** (Lyso-PC, MW 495.64, 1 eq) and the product 1 **(P1)** from Step 1 (MW 576.61, 0.86 eq) is mixed with 4-Dimethylaminopyridine (DMAP) (2.5 eq) and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (2.5 eq) in dichloromethane (DCM) at 25°C for 24 h. The mixture is filtered and then concentrated. The resulting lipid-peptidic regioisomers will be analyzed using reverse phase HPLC (Waters). A C8 HPLC column (Waters) will be used with column heating at 60 °C with a gradient from 15 % acetonitrile in 0.1 % TFA to 95 % acetonitrile in 0.1 % TFA over 15 minutes with 0.5 mL/min flow rate. The product 2 **(P2)** (MW 1054.23) is obtained as a brown semi solid. It is confirmed by LC-MS. The optimization of the reaction in terms of product yield, duration of the reaction, and purity will be conducted to improve the scale up the product.

Step 3 – Hydrogenation (Deprotection): The tri-Cbz intermediate product **(P2)** from Step 2 (MW 1054.23, 1 eq), trifluoroacetic acid (TFA) (5 eq) and Pd/C (20% w/w) are allowed to stir in ethanol (EtOH) under H_2 (15 psi) at 25°C for 2 h. The reaction mixture is filtered, and concentrated under vacuum at 35-40°C. The product is diluted with EtOH/H_2O, then solvent is removed by lyophilization and was subjected to multiple solubilization and precipitation cycle.

The final product, **KC1003**, (MW 651.83) is obtained as a white solid. It is confirmed by LC-MS, HR-MS, ^{1}H-NMR, ^{15}P-NMR, and ^{9}F-NMR. This is the source material that is used for EM particle assembly (with the payload elements: Hb, RSR13, leucomethalyne blue).

Sourcing for this EM component: Currently, Wuxi has supplied us with approximately 10g total and another 5g is anticipated in the near future. This is all with >99% purity. Through a competitive process, KaloCyte has selected Avanti Polar Lipids to scale-up the precursor manufacturing. The amount of KC1003 currently in hand is more than sufficient to fabricate all EM needed to complete all experiments planned for the remainder of this Award.

3. **EM Fabrication/Assembly from precursor and payload elements.** This process was optimized for efficiency and to reduce product variability. The resulting SOP is briefly summarized below:

   A thin film is prepared from a mixture of KC1003 precursor (80.66 mM), cholesterol (17 mM) and PEG2000-PE (2.34 mM [of 25 mg/mL]) by first dissolving these components in anhydrous chloroform to a concentration of (8 mg/mL), in a round bottom flask (RBF). Chloroform is then slowly evaporated in a flask under reduced pressure by rotary evaporator at 50°C to generate a thin lipid film and then dried in a vacuum oven (12h, RT). Purified Hb and other payload elements (RSR13 and leucomethylene blue) is transferred to the dried film via micropipette. The round bottom flask is gently swirled until all lipid film is in suspension. Probe sonication is performed with the RBF in ice water and the following settings are used for the sonication: pulse mode (on 2s, off 1s) process time, 5 minutes, for 10 cycles. After sonication, free Hb is removed by dialysis. To remove unencapsulated payload elements, assembled EM suspensions are then dialyzed against PBS that includes Chelating Sepharose Fast Flow gel (5 grams of wet gel in 2L of PBS) for 3-4 hours at 4°C while stirring.

4. **Lyophilization and Reconstitution** This process was optimized for efficiency and to reduce product variability. The lyophilization cycle, which incorporates. trehalose as a cryoprotectant, is briefly summarized in the table below. Reconstitution time, in sterile water for injection, is complete within 1 minute – with reconstituted EM matching pre-lyophilized EM biophysical specifications with less than 10% variation.

Step #	Step Action	Type of action	Time (min)	Temperature (°C)	Vacuum (mTorr)
1	Freezing	Ramp	180	-45	-
2	Freezing	Hold	240	-45	-
3	Primary Drying	Ramp	30	-35	120
4	Primary Drying	Hold	1620	-35	120
5	Secondary Drying	Ramp	300	5	100
6	Secondary Drying	Hold	240	5	100

What opportunities for training and professional development has the project provided?

Multiple team members attended and presented at the American Society of Hematology (ASH) and Symposium on Cell Therapies & Transfusion in Trauma & Critical Care conferences – the latter meeting, in particular, included many presentations related to support of patients with hemorrhagic shock and resuscitation as well as opportunity to engage with leaders in the field in small workshops and informal settings. Multiple team members are at various stages in their research training; this project has greatly enhanced their education.

How were the results disseminated to communities of interest?

Our Team presented data from this project at the following seminars during Y3:

"ErythroMer: Nano Fabricated Red Cells: Bio-Inspired Design and Performance Data". Plenary Presentation. International Symposium on Blood Substitutes & Oxygen Therapeutics, Nara, Japan, 2019

"Bio-Inspired Artificial Red Blood Cell: Design, Pre-Clinical Results and Novel Indications". Special Seminar. American Society of Hematology (ASH) Annual Meeting, Orlando FL, 2019.

"Encapsulated HBOCs to Artificial & Engineered Red Cells: Update and Preclinical Results" Symposium on Cell Therapies & Transfusion in Trauma & Critical Care, San Diego, 2019

"Artificial Red Blood Cell (ErythroMer): Optimizing Safety and Efficacy Through Bio-Inspired Design". Invited Speaker for FDA OBRR-OTAT Seminar Series; Silver Spring, MD, 2020

"Bio-Inspired Artificial Red Blood Cell: Design, Pre-Clinical Results and Novel Indications"
George Mason University, Department of Bioengineering, Invited Research Seminar. Virtual. 2020.

What do you plan to do during the next reporting period to accomplish the goals?

As outlined in our modified SOW, further PK and oxygenation/acute shock studies are planned for Y4 in our PFC (48h survival models) – this work will be the focus of Y4 activity. This set of PFC models will be exploited to further our understanding of dosing (PK), biocompatibility, safety and efficacy. We also plan to use this set of experiments to optimize our hemostasis/oxygenation algorithm in concert with Cellphire's platelet derived product, to determine compatibility of these products in *in vivo*.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

1. We have developed a unique rabbit model for precise evaluation of acute hemorrhagic shock and simulation of pragmatic in-field resuscitation; this model (uniquely) includes specific readouts for tissue oxygen tension as well as ability to independently evaluate impact of dilutional and trauma-induced coagulopathy.

2. We have identified a new parameter that helps determine maximum tolerated dosing for artificial RBCs or any encapsulated hemoglobin based oxygen carrier (HBOC) – we term this parameter the 'NanoCrit' and is the nanoparticle (encapsulated HBOC) correlate for the Hematocrit (which represents the %age of blood volume occupied by red blood cells. The combination of the NanoCrit and Hematocrit determine blood viscosity, which if increased beyond tolerance, may impair blood flow and oxygen delivery.

What was the impact on other disciplines?

1. As noted above, our hemorrhagic shock and resuscitation models enable independent evaluation of the two major causes for coagulopathy encountered in resuscitation of combat casualties (dilutional and trauma-induced coagulopathy); we have used these models in related projects to evaluate efficacy of hemostatic resuscitation (RDCR) – with plasma and platelets.

What was the impact on technology transfer?

This project involves significant partnership with KaloCyte, Inc. – a startup created to commercialize ErythroMer. During this project period, KaloCyte has made significant progress in advancing EM to a commercial product:

1. Raised additional significant private funding.
2. Relocated from the St Louis Cortex District to the UMB Biopark; the KaloCyte space (offices and lab) is embedded in the UM School of Medicine Center for Blood Oxygen Transport and Hemostasis.
3. KaloCyte has filed two additional provisional patents related to EM development.
4. KaloCyte has completed a strategic plan for EM development, with specific targets set for preIND (Q1, 2022), IND (Q3, 2022) and FIM testing (Q4, 2022).

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

There have been no changes to the defined Aims, Major Goals or Tasks with regard to content. However, just prior to the end of project Y2, Dr. Doctor was recruited from Washington University (WUSM) to the University of Maryland (UMB), to serve as founding director for the Center for Blood Oxygen Transport and Hemostasis (CBOTH), a major resource that will accelerate EM development. Related to this transition, there were changes with other key personnel, which are detailed below, including transition of KaloCyte from the St Louis Cortex District to the Baltimore Biopark (KC lab is now embedded in CBOTH, facilitating collaboration). Resulting from these changes, the timing of contract transition from WUSM to UMB, and from COVID19 related lab shutdowns, the only project work in Y3, was performed by KaloCyte; information is provided in detail below. As such, an NCE was requested and approved, enabling the team to resume progress (NCE-Y4) towards completing project goals; a time-adjusted SOW is included in this report.

A brief summary of the major changes (scientific team and administrative activity) is provided below:

Summary of Major Changes for Scientific Team

WUSM: Washington University in St Louis, School of Medicine
UMB: University of Maryland, Baltimore
UIUC: University of Illinois, Urbana Champaign
FDA CBER: Food & Drug Administration, Center for Biologics Evaluation & Research
CHOP: Children's Hospital of Philadelphia

1. Dr. Doctor moves from WUSM to UMB.
2. Dr. Pan moves from UIUC to UMB.
3. Dr. Doctor opens Center for Blood Oxygen Transport and Hemostasis (CBOTH) at UMB.
4. Dr. Buehler moves from FDA CBER to UMB CBOTH (expertise is in HBOC toxicology and pharmacokinetics).
    a. Dr. Buehler assumes responsibility for project pharmacokinetic modeling from Dr. Zuppa (CHOP/University of Pennsylvania). Zuppa/CHOP/UPenn no longer on project.
5. Experimental work at UMB ends (Bochicchio Lab) just prior to end of Y2
    a. Bochicchio Lab was originally responsible for SA4 (rabbit PFC scenarios) Major Tasks 7-10.
    b. This work transitioned to the Small Animal Surgery and Physiology (SASP) Core at CBOTH, under the direction of Dr. Doctor (PI) and Dr. Wang (SASP Core Director).
    c. Ability to initiate this work was delayed by UMB campus-wide lab closures & personnel access restrictions related to the COVID-19 Pandemic.
6. Dr. Spinella (part of WUSM team) remains in St. Louis and assumes role as site PI for WUSM (now Site 3).
    a. WUSM had been prime awardee; with Dr. Doctor transition to UMB, UMB became prime awardee and WUSM became Site 3.
7. KaloCyte moves from St Louis Cortex to University of Maryland BioPark.
    a. Dr Richards (original site PI) resigns from KaloCyte and remains in St. Louis.
    b. Dr. Mittal (new site PI) joins KaloCyte.
    c. KaloCyte remained active in the University of Maryland BioPark during the UMB Campus shut-down.
8. No changes to Site 4 (Cellphire, Thrombosome supplier, Dr. Fitzpatrick)
9. No changes to Site 5 (American University, Biostatistics, Dr. Gill).

Summary of Major Administrative Changes

1. WUSM closes DoD contract (release letter 06/07/2019); this event occurs during project Y2 (09/30/2018 – 09/29/2019).
2. UMB requests grant transfer (acceptance letter request 06/07/2019)
3. UMB changes subaward sites (see above SOW major changes) and budget from original. UMB secures UMB IACUC & DoD ACURO approval (04/04/2020) and UMB IRB approvals (original 10/21/2019, but all IRB protocols suspended due to COVID pandemic, modification approved 11/22/2019, additional modifications approved 10/02/20 and 10/22/2020 (addition of staff, change to consent requested by DoD HRPO), with DoD HRPO approval on 10/30/2020. Human protocol resumption allowed by UMB campus (Phase II COVID return to research) 11/05/2020.
4. DoD approves contract transfer to UMB (approval letter 08/20/2020).
5. UMB budget start date 09/30/20 (NCE Year, Project Y4, which ends 09/29/2021)
6. WUSM, Cellphire and American University subaward start dates are 09/20/20; these sites stopped work when WUSM closed the DoD contract (06/07/19) and resumed work with resumption of UMB work and Y4 budget start on 09/30/20, since their work required UMB labs to be open. These subawardees therefore experienced budget interruption between 06/08/19 and 09/30/20.
7. KaloCyte resumed work on 09/29/2019 (onset of Grant Y3) since KaloCyte did not experience lab shutdown and their tasks did not require UMB labs to be open. Kalocyte experienced budget interruption from 06/08/2019 – 09/29/2019.

Therefore, the only work occurring during project Y3 was performed by Kalocyte.

Actual or anticipated problems or delays and actions or plans to resolve them

Major delays resulted from: (1) team transition from St Louis to Baltimore and (2) administrative delays in grant transfer from Washington University in St Louis to the University of Maryland and (3) the COVID-19 pandemic and response.

1. Though WUSM closed the DoD contract on 06/07/2019 (during Y2), DoD approval of the contract transfer from WUSM to UMB was delayed until 08/2020 and funds were not available at UMB until 09/30/2020.
2. Work at WUSM, Cellphire and American University was paused when WUSM closed the DoD contract, since their work was dependent upon activities at the UMB labs. Work on these subawards resumed on 9/30/2020, with the beginning of UMB, Project Y4. The subawardees experienced budget interruption from 06/08/2019- 09/30/20.
3. KaloCyte resumed work on 09/29/2019 (onset of Y3) since KaloCyte's labs were not shutdown and their tasks did not require that UMB labs be open. Related to the grant transfer process (WUSM to UMB), KaloCyte experienced a budget interruption from 06/08/2019- 09/29/2019.
4. CBOTH laboratories were shut down as part of UMB's response to the COVID-19 pandemic. CBOTH returned to limited laboratory capacity and function in 06/2020 (work allowed only on COVID19 related projects); activity was increased in a phased fashion beginning in Fall, 2020 (Phased increase in lab staff return to work) – allowing resumption of activity related to this project at the onset of the NCE (Y4) period (10/01/20).

Changes that had a significant impact on expenditures

As mentioned above, the only expenditures during Y3 were for work performed by KaloCyte. Funds were not available at UMB or the subaward sites from 06/08/2019 (Y2) until 09/30/2020 (beginning of Y4). A No-Cost Extension was granted through a grant amendment, with the period of performance extended to 09/29/2021.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

As noted elsewhere in this report the Animal and Human research components of this project transitioned from Washington University School of Medicine (WUSOM) to the University of Maryland, School of Medicine (UMSOM). As such, all protocols for this project that had been approved at WUSM, were reviewed and approved at UMSOM.

1. UMB IACUC Approval & DoD ACURO Approval (04/04/2020)

Significant changes in use or care of human subjects

UMB IRB and DoD HRPO approvals were granted in Y3 for the transfer of the grant from WUSM to UMB. We are still approved to enroll 50 Healthy Adults. There have been no "significant deviations" or protocol violations for human subjects.

1. UMB IRB approval (originally approved 10/21/2019, but all IRB protocols were suspended due to the COVID-19 pandemic). Modifications were approved on 11/22/2019, 10/02/2020 and 10/22/2020, to add staff and change consent (as requested by DoD HRPO).
2. DoD HRPO approval 10/30/2020.
3. Human protocol resumption allowed by UMB (Phase II COVID return to research) 11/05/2020.
4. Human Research for this project is now complete and the Human Subjects protocol will be closed in this NCE Year.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Multiple manuscripts are in preparation/submission, as indicated above.

Books or other non-periodical, one-time publications.

A new book is in preparation, a chapter will be devoted to ErythroMer: Title: Blood Substitutes and Oxygen Therapeutics, Ed: Jonathan Jahr, Chapter 22: ErythroMer. Allan Doctor

Other publications, conference papers and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

KaloCyte, Inc maintains a website that provided updated information on company activity and press releases: https://www.kalocyte.com/

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

1. During Y1, an exclusive license was obtained by KaloCyte from Washington University for all relevant patent rights for EM-V1: Pan D, **Doctor A**, Spinella PC and Lanza GM. Blood Substitute Composition and Method of Use. U.S. Patents # 9,486,508; 9,655,952 & 9,750,241. 2016. Also filed in: Europe, Australia, Canada, Japan and South Africa.

2. A provisional patent application has been filed by KaloCyte for its development of the novel composition (EM-V2). Pan D, Spinella PC and **Doctor A.** Self-Assembling Oxygen Carrier Compositions. US Patent (Provisional) # 63,014,665. 2020.

3. A provisional patent application has been filed jointly by KaloCyte and UMB for a novel system to prevent interference by ErythroMer of laboratory instrumentation, in blood of trauma victims who received EM. Pan D, Mittal N and **Doctor A**. Compositions and Methods for Removing Bio-Synthetic Nanoparticles from Bodily Fluids. US Patent (Provisional) # 63,159,547. 2021.

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

The table below indicates the various changes in institutional participation and roles with transfer of this award from Washington University in St Louis to the University of Maryland, Baltimore (UMB). Dr. Doctor (PI) transitioned from WUSM to UMB and also recruited Dr. Pan (UI) to join him at the new Center for Blood Oxygen Transport and Hemostasis. The collaboration with Dr. Bochicchio (WUSM), a trauma surgeon who supported our animal models was transitioned to Dr. Wang (UMB) a small animal surgeon and Director of the CBOTH Small Animal Surgery and Physiology Core who will support our animal models. Dr. Spinella (WUSM), who advised regarding our hemostasis assays, remained at WUSM, without change in effort. Dr. Gill moved to American University; his role is unchanged. Dr. Zuppa (CHOP) who supported our pharmacokinetic analyses will be replaced by Dr. Buehler (UMB), a pharmacologist specializing in HBOC evaluation recruited to CBOTH from FDA CBER. KaloCyte – also moved from St. Louis to Baltimore and with this move Dr. Richards was replaced with Dr. Mittal and Dr. Wang was replaced with Dr. Yildiz. There are no changes with regard to Cellphire.

Investigator Changes

Institution	Original Award		After Transfer of Award	
	Investigator	**Role**	**Investigator**	**Continued Role or Change**
Washington University (WUSM)	Doctor	PI		Moved to UMB
	Bochicchio	CoI – surg model		Replaced by Wang (UMB)
	Spinella	CoI - hemostasis		CoI – hemostasis (remains at WUSM)
	Gill	CoI - biostats		Moved to AU
University of Maryland, Baltimore			Doctor	PI, Moved from WUSM
			Pan	CoI, Moved from UIUC
			Buehler	CoI, replaces Zuppa (CHOP)
			Wang	CoI, new – surgical model Replaces Bochicchio
			Rogers	CoI, moved from WUSM EM benchmarking
University of Illinois (UI)	Pan	CoI – bioengineer		Moved to UMB
Children's Hosp Philadelphia (CHOP)	Zuppa	CoI – pharmacology		Replaced by Buehler (UMB)
American University			Gill	CoI - biostats Moved from WUSM
KaloCyte, Inc	Richards	CoI – ErythroMer	Mittal	replaces Richards
	Wang	CoI – ErythroMer	Yildiz	replaces Wang
Cellphire, Inc	Fitzpatrick	CoI – Thrombsomes	Fitzpatrick	No change

Changes to Personnel (Lab Staff)

Institution	Original Award		After Transfer of Award	
	Personnel	**Role**	**Personnel**	**Continued Role or Change**
Washington University (WUSM)	Xue Lin	Doctor Lab staff		No longer on project
	Jose Aldana	Bochicchio Lab Staff		No longer on project
	Rohit Rasane	Bochicchio Lab Staff		No longer on project
	Sarbani Ghosh	Bochicchio Lab Staff		No longer on project
	Anja Fuchs	Bochicchio Lab Staff		No longer on project
University of Maryland, Baltimore (UMB)			Mary Brummet	Doctor Lab Manager
			Alex Lander	Doctor Lab Technician
			Tori Boyer	Doctor Lab Research Coordinator
			Parikshit Moitra	Pan Lab Postdoctoral Fellow
University of Illinois (UI)	Dinabandhu Sar			No longer on project
	Maha Alafeef			No longer on project
Children's Hosp Philadelphia (CHOP)				
American University				
KaloCyte, Inc	Richards	CoI – ErythroMer	Nivesh Mittal	CoI (replaces Richards)
	Wang	CoI – ErythroMer	Tugba Yildiz	Formulation Scientist (replaces Wang
			Shannon Dougherty	KaloCyte Lab Manager
			Darci Bartlett	KaloCyte Program Manager
Cellphire, Inc				No changes

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

none

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: *Attached separately*

An updated SOW is attached.