A Rapid, Device-Based Therapy for Treatment of Ebola and Marburg Infections

The Seraph® Microbind®
Affinity Blood Filter

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Introduction

The world is facing the largest Ebola outbreak since Ebola was first identified in 1976. There are, however, no available anti-viral therapeutics or vaccines with proven efficacy against Ebola. Even if a new pharmaceutical cure is found, there is little chance that it can be provided in volume in time to meet the needs of the current crisis. Today the only option is supportive care. **Here we propose a new strategy to treat Ebola by rapid clearance of viremia with an extracorporeal device.**

Executive Summary

ExThera Medical Corporation of Berkeley, CA has developed a whole-blood sorption hemoperfusion device that should quickly reduce the circulating concentration of Ebola virus in blood during a short dialysis-like extracorporeal treatment. This reduction is expected to significantly improve survival rates based on the following:

1. Ebola and Marburg viruses bind to heparan sulfate and heparin (1)
2. Our device uses heparin as the primary ligand for pathogen binding and removal
3. Our device has demonstrated ability to remove viruses from whole blood:
    a. CMV,
    b. HSV-1
    c. HSV-2
4. Reduction of blood-borne Ebola concentration significantly improves survival in validated non-human primate models
    a. 10^4 PCU/mL (10^8 RNA copies) is considered the 'survival threshold' (2)

This rationale is supported by several relevant publications referenced in this document.

Our device adds nothing to the blood, it is already developed, and clinical units are being routinely manufactured on a pilot-scale. Feasibility testing on Ebola and manufacturing scale up can begin immediately.

The Problem

Filoviruses, including Ebola, Marburg, Lassa, and Crimean-Congo can cause severe hemorrhagic fever in humans. Before March 2014, there were a total of 1849 confirmed Ebola hemorrhagic fever (EHF) cases leading to 1288 deaths since 1976. (3) As of September 17th, there have been 5,357 confirmed cases and 2,630 deaths just since the beginning of the current outbreak. (4) A September 8th Situational Awareness issued by the World Health Organization warns affected countries to prepare for exponential increases in cases, and states that non-conventional interventions are needed to combat Ebola. (5) The CDC now estimates that up to 1.4 million people will have been infected by the end of January, 2015. (6) This crisis has also had a major impact on healthcare workers in West Africa. The WHO reported on August 26 that 240 physicians, nurses, and other healthcare workers had contracted the disease and more than 120 have died. (7)

There are four species of Ebola filoviruses, including Zaire ebolavirus (ZEBOV), Sudan ebolavirus (SEBOV), Ivory Coast ebolavirus (ICEEBOV), and Reston ebolavirus (REBOV). The species identified in the Guinea, Liberia, Nigeria, and Sierra Leone is ZEBOV with a typical mortality near 50%. Initial symptoms are nonspecific and include fever, vomiting, and severe diarrhea. Additionally, visible hemorrhage will occur in roughly half of the cases. The incubation period ranges between 2 and 21 days. As the disease progresses, immunosuppression occurs along with increased vascular permeability and impaired coagulation. Due to this Ebola-induced immunosuppression, the immune system is unable to respond to the high concentration of Ebola virus in blood, and the body is quickly overwhelmed.

The only current medical intervention that appears to be working to improve patient outcome is supportive care to allow time for a person's own immune system to respond to the infection. Primary damage from Ebola occurs within the peripheral endothelium and liver cells. However, circulating viremia acts as a 'reservoir' of viral particles for the propagation of the disease. A study published by Towner *et.al*. (2) concluded that the bloodborne viral load is a strong indicator of patient survival. They clearly showed that viral RNA titer, as measured by reverse transcription polymerase chain reaction (RT-PCR), averaged 3.4×10^{9}/mL for non-survivors as compared to 4.3×10^{7}/mL for survivors. Based on this data, they suggest that a circulating titer of 10^{8} RNA copies/mL (≡10,000 plaque forming units (PFU)/mL) is considered to be a concentration threshold that predicts a fatal outcome with a positive predictive capability of >90%. Among experts, it is believed that if the PFU concentration can be kept below this level for 14 days, the patient *will* survive. This indicates that circulating viremia should be the primary therapeutic target to address when designing Ebola countermeasures. This approach is supported by the current practice of discharging Ebola patients from healthcare centers after three consecutive days with negative blood cultures.

Significant research is underway to develop antiviral drugs and vaccines, however there are no approved or proven remedies to address the *current* crisis, and most drugs are in an early stage of development. (8) This summer Mapp Biopharmaceuticals provided *all* of their available experimental ZMapp serum for emergency use, but it was only enough to treat 7 patients. Three of the US patients that received the experimental serum recovered, however it is unknown whether ZMapp or improved supportive care was responsible. While the results *look* promising, a controlled clinical trial is required to prove safety and efficacy

for humans. Unfortunately Mapp may be unable to respond quickly to develop additional serum for testing. Another pharmaceutical company, Tekmira Pharmaceuticals of Canada, had been performing a safety study in humans, however it was placed on hold in July after safety concerns. However, the FDA is modifying its decision and the study may still move forward.

These efforts demonstrate the difficulty of quickly developing new vaccines and drugs to combat pathogens, especially during an international crisis. Drugs can be difficult to manufacture and extensive trials are often required to prove safety and efficacy. Alternative antiviral strategies must be considered, especially if they can be scaled up quickly to respond to the global need. ExThera Medical has a device-based therapy that is ready now. It is *very* likely that our ***Seraph® Microbind® Affinity Blood Filter*** will be able to remove the circulating Ebola virus from blood and maintain the concentration well below the 'threshold concentration' of 10,000 PFU/mL for an extended period. This would give time for the Ebola patient's immune system to mobilize and combat the infection.

The Solution

ExThera Medical Corporation proposes that a biomimetic device, based on affinity adsorption, can quickly reduce Ebola viremia and maintain the concentration below the 'survival threshold' of 10,000 PFU/mL. Many disease-causing microorganisms use cell-surface heparan sulfate (HS) for cell attachment and to subvert the host immune response. (9) **A recent study by Salvador *et. al*. demonstrated that Filoviruses, including Ebola and Marburg target cell-surface heparan sulfate for their attachment to cells.** (1) **They also demonstrated that Ebola will bind to both surface-bound (immobilized) *and* to soluble heparin**. ExThera has developed a strategy to remove HS-binding microorganisms and toxins from *whole* blood by attaching heparin (an analogue to HS) to a high-surface-area adsorption media that mimics the binding sites found on the endothelium. Our first product, the **Seraph® Microbind® Affinity Blood Filter (Seraph) uses our whole blood purification technology based on the unique binding capability of immobilized heparin** molecules. Seraph is unlike other blood purification methods that capture molecular adsorbates (e.g., cytokine) using 'size exclusion', a non-specific separation process. Instead, **Seraph uses the activity of naturally-occurring heparin molecules to achieve results, through a *specific* binding process.**

Heparin molecules can be used as an effective binding site for Ebola or Marburg when they are covalently bound on a solid surface by so-called end-point attachment (by a single covalent chemical bond). This exposes 'all' the binding sites on the heparin molecule to the flowing blood. When blood is passed through a disposable Seraph adsorption column the pathogen is diverted from the blood to the surface-bound heparin, and effectively removed.

ExThera has already demonstrated that Seraph can remove many pathogens from whole blood. These include Cytomegalovirus (CMV), *S. aureus* (SA), MRSA, (10), Enterobacteriaceae, Carbapenem Resistant Enterobacteriaceae ('CRE Superbugs'), Extended Spectrum Beta lactamase Enterobacteriaceae, *A. baumannii*, *Candida albicans,* HSV-1 and HSV-2, and several other pathogens and toxins. Other important pathogens

that will likely bind to ExThera's technology include dengue virus and Plasmodium falciparum.

Our work with CMV, HSV-1 and HSV-2 viruses is highly relevant to the Ebola indication. In the CMV experiment defibrinated horse was spiked with the live virus. The average starting concentration was $7.95x10^4$ PFU/mL and the final concentration averaged $1.41x10^4$ PFU/mL, an 82% reduction. Note that during a typical four-hour therapeutic session the patient's entire circulating blood volume may pass through Seraph 10-15 times, possibly reducing viral load to undetectable levels. Quantitative capacity data from our CMV study shows that a single Seraph device is capable of removing a total of $3.5x10^7$ viral particles. A typical CMV reactivation patient has a titer of 1000 PFU/mL in 5 L of blood. Using these figures, **a single Seraph device has a 7X overcapacity as a treatment for CMV**.

Additional virus capture was demonstrated in a study with Herpes simplex in which HSV-1 and HSV-2 were removed from human blood, serum, and buffered saline (Table 1). In

Table 1. HSV-1 and HSV-2 reduction using Seraph's heparin-functional media

Experiments				
Matrix Volume	**Virus**	**Media**	**Input Challenge**	**% Reduction**
1 mL	Herpes HSV-1	Buffered NaCl	10^{11} Particles	94.5
1 mL		Hum Serum	10^{11} Particles	97.6
1 mL		Human Blood	10^{11} Particles	99.1
1 mL	Herpes HSV-2	Buffered NaCl	10^{10} Particles	88.3
1 mL		Human Blood	10^{11} Particles	99.8

this experiment, 1 mL of human blood with 10^{11}/mL of radiolabeled HSV-1 or HSV-2 virus particles were passed through a column packed with 1 mL of Seraph™ adsorption media. It was demonstrated during this (non-optimized) experiment that 99.1% of HSV-1 and 99.8% of HSV-2 were removed from whole blood in a single pass. This data suggests that ExThera's heparin-functional media has both rapid binding kinetics *and* huge binding capacity, to significantly reduce viremia intensity. **Experiments will begin shortly testing the removal of Ebola from whole blood at the Texas Biomedical Research Institute**, which operates a BSL-4 lab for Ebola studies. Note also that the inherent safety of end-point-attached heparin as a blood-contacting surface is well known from its use in oxygenators, dialyzers and vascular grafts.

Rapid reduction of viremia using ExThera's Seraph device could have at least two potential benefits Ebola victims:

1. By quickly reducing the viral load to below a 'fatality threshold', it should be possible to provide additional time for treatment with supportive care while preventing further damage to endothelial cells. The replication cycle for Ebola is approximately 24 hours. Based on our work with other viruses, we believe that a 4-hour Seraph treatment, once a day or less should be effective at keeping circulating viremia well below 10,000 PFU/ml.

2. The rapid reduction of viral titer could also amplify the effects of any new antiviral drugs. With a lower circulating titer to consume the drug, the bioavailability of the drug may increase, to better target infected tissues. Recent studies with vaccines have shown that they must be administered very early (24-48 hours of infection), otherwise the efficacy greatly decreases. Additionally, this approach could reduce the total concentration of drug needed for efficacy. This might reduce the potential for drug toxicity or side effects, and reduce overall expense when drugs are otherwise cost prohibitive for widespread use, or in very low supply.

Technology Readiness

We believe that Seraph may be the only therapeutic technology available today that can be scaled up in time to address the *current* Ebola Crisis. **Every pathogen that ExThera has tested, which has been reported in the literature to bind to heparin or heparan sulfate, has shown high affinity to our surface-bound heparin adsorption media**. Pre-clinical work includes completion of a GLP porcine safety study and ISO 10993 biocompatibility testing. Devices are routinely manufactured under ISO 13485 by DSM Biomedical in Berkeley, CA. which can scale up the manufacturing under GMP guidelines. Sterilization and packaging validation is currently taking place.

During a recent teleconference with FDA's Office of Emergency Preparedness/Operations and Medical Counter Measures group within CDRH ExThera discussed its technology and possible regulatory options regarding the Ebola indication. The FDA recommended that ExThera prepare an Emergency Use Exemption pre-submission document as our next step.

Conclusion

Alternative, device-based strategies to combat severe viral infections, such as Ebola, must not be ignored. Therapies for diseases in which high viremia correlates with high mortality must focus on reducing the blood borne viral load. Conventional therapeutics, such as drugs and vaccines are often considered as the only solution. However unconventional technologies, such as the *Seraph® Microbind® Affinity Blood Filter*, could be more effective

with less patient risk. Significant published research indicates that reducing the concentration of viremia and shortening the duration of viremia would be the most effective method to treat Ebola. ExThera proposes that its Seraph® Filter could be a new therapy for quickly and safely reducing the circulating viral load. The next steps toward product development for the Ebola indication involve *in vitro* feasibility studies showing reduction of viral load and animal studies. With proper funding, these studies can begin immediately.

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This device is not cleared by the FDA for distribution in the United States