Sigyn Therapy™, an Emerging Candidate to Address Endotoxemia, Sepsis, and Drug-Resistant Viral & Bacterial Infections

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Introductory Note from the Author

When we designed Sigyn Therapy™, we envisioned a medical device that could overcome the limitations of previous blood purification technologies to treat life-threatening conditions that are beyond the reach of drugs. Along with the efforts of our dedicated team, contributions from science advisors, collaborators and shareholders have helped to advance our vision toward reality.

Consider that in the midst of the COVID-19 pandemic, we progressed Sigyn Therapy™ from concept through initial product development and then completed a series of five invitro study programs that validated the ability of Sigyn Therapy™ to extract twelve relevant therapeutic targets from human blood plasma. Subsequently, we completed first-in-mammal studies at the University of Michigan and then leveraged dialysis industry relationships to establish the treatment protocol, clinical site locations, and principal investigators for first-in-human studies of Sigyn Therapy™.

In parallel with these achievements, we designed ChemoPrep™, a device to improve the delivery of cancer chemotherapy, ChemoPure™ to reduce chemotherapy toxicity, and we then introduced the ImmunePrep™ platform to expand the use and enhance the performance of immunotherapeutic antibodies, which account for 9 of the top 15 selling cancer drugs. While these therapeutic candidates will be the subject of future communications, the focus of this paper is Sigyn Therapy™. Beyond our mission to save lives, I believe we have created a foundation for value creation.

Sincerely, Jim

Jim Joyce is co-founder and CEO of Sigyn Therapeutics and can be reached at jj@sigyntherapeutics.com.

Introduction

Sigyn Therapy™ is a novel hemoadsorption technology designed to extract pathogen sources of life-threatening inflammation from the bloodstream in concert with dampening down dysregulated immune responses that are pathogen induced. Our candidate treatment indications are not addressed with drug therapies and include endotoxemia, sepsis, and drug-resistant viral and bacterial infections.

Subsequent to our completion of pre-clinical *invitro* and first-in-mammal studies, we established the treatment protocol and identified clinical site locations to support first-in-human feasibility studies of Sigyn Therapy™ in dialysis dependent end-stage renal disease (ESRD) patients with endotoxemia. Endotoxemia is associated with cardiovascular disease, infection, and sepsis. These are the three leading causes of early death in the ESRD patient population. The successful completion of our feasibility study would set the stage for pivotal efficacy studies to seek potential

market clearance for Sigyn Therapy™ to treat endotoxemia and other candidate treatment indications.

Sigyn Therapy™ Notable Features

- Broad-spectrum extraction of viral pathogens, bacterial toxins, hepatic toxins, inflammatory cytokines, and other mediators of inflammation from the bloodstream.

- Highly efficient mechanism. Sigyn Therapy™ processes the entire bloodstream ~15 times during a four-hour treatment.

- Substantial capture capacity. Sigyn Therapy™ incorporates a formulation of adsorbent components with 200,000+ square meters (~50 acres) of surface area on which to capture and remove therapeutic targets from the bloodstream.

- Sigyn Therapy™ is delivered for use on dialysis and continuous renal replacement machines already located in hospitals and clinics around the world.

- To optimize potential scalability, Sigyn Therapy™ is comprised of non-biological components that are readily available from established industry vendors.

- Early clinical opportunities in ESRD as Sigyn Therapy™ can be administered in series with normally schedule dialysis treatments to conveniently treat conditions that shorten the lives of ESRD patients.

The Team Behind Sigyn Therapy™

With the exception of our CFO, the members of our Sigyn Therapeutics team worked with me at Aethlon Medical. While at Aethlon, we advanced the Aethlon Hemopurifier™ from concept to becoming the first medical device to receive two "Breakthrough Device" designations from FDA and it was also the first blood purification therapy to receive FDA "Emergency Use Authorization" (EAU) approval to treat a life-threatening virus. During our tenure working together, Time Magazine named the Hemopurifier a "Top 25 Invention" and one of the "11 Most Remarkable Advances in Healthcare."

Overcoming the Limitations of Previous Blood Purification Therapies

Sigyn Therapy™ was designed to be a best in category product with expansive therapeutic opportunities. To appreciate the capabilities of Sigyn Therapy™, consider the mechanism of two blood purification technologies that have been broadly commercialized outside of the United States.

The Toraymyxin™ device was developed by Toray Industries and is licensed to Spectral Medical in North America. The device has a high specificity to remove endotoxin from the bloodstream and is currently being evaluated as a candidate treatment for sepsis in a promising FDA-cleared

clinical study. Outside of the United States, Toraymyxin™ has been administered to more than 340,000 patients. While the device is highly selective in its ability to address endotoxin, it does not address viral pathogens, gram-positive bacterial toxins, or pro-inflammatory cytokines.

The CytoSorb™ device from CytoSorbents Corporation pioneered the use of an adsorbent component to eliminate pro-inflammatory cytokines from circulation as a strategy to combat sepsis and other life-threatening inflammatory disorders. Outside of the United States, more than 228,000 CytoSorb™ treatments have been administered. While the device is effective in extracting inflammatory cytokines from the bloodstream, it does not address viral pathogens or bacterial toxins, including endotoxin.

Our Candidate Treatment Indications

Candidate treatment indications for Sigyn Therapy™ include endotoxemia, sepsis, and drug-resistant viral and bacterial infections. Each of these therapeutic opportunities represent an unmet need in global health.

Endotoxemia

First-in-human feasibility studies of Sigyn Therapy™ are designed to enroll dialysis dependent end-stage renal disease (ESRD) patients with endotoxemia, defined as the presence of endotoxin in the bloodstream. Endotoxin (lipopolysaccharide, LPS) is a major component of the outer membrane of gram-negative bacteria and is a potent activator of sepsis and other life-threatening inflammatory conditions when it enters the bloodstream. It is notable that mortality rates associated with bacterial infections are 50 times higher in ESRD patients as compared to the general population. An effective strategy to treat endotoxemia in ESRD and non-ESRD patient populations could reduce the incidence of sepsis, which is a leading cause of hospital deaths and a major financial burden to the U.S. healthcare system.

The gut microbiota is the primary natural reservoir for endotoxins, which are shed in significant quantities as bacteria die. As an example, one gram-negative Escherichia Coli bacteria contains ~2 million endotoxin molecules. Researchers report the human intestine to contain more than a thousand times more endotoxin than a lethal dose if injected into the bloodstream. While a healthy gut will maintain endotoxin within the intestine, inflammation can increase the permeability of the gastro-intestinal barrier and allow for endotoxin to translocate into the bloodstream. Thus, establishing a continuous feedback loop of endotoxin-induced inflammation that promotes intestinal permeability that allows for the further passage of endotoxin into the bloodstream.

According to the United States Renal Data System ("USRDS"), more than 550,000 individuals suffer from ESRD, resulting in approximately 85 million kidney dialysis treatments being administered in the United States each year. Endotoxemia and concurrent inflammation are common, yet untreatable conditions that contribute to an annual mortality of ~20% of the ESRD patient population. Studies report the incidence of endotoxemia to be 60-90% in those who have been on dialysis for periods ranging from 30-72 months.

Endotoxemia induces systemic inflammation, a hallmark feature of ESRD as reflected by the excessive production of inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6). Sigyn Therapy™ establishes a candidate strategy to improve the health and extend the life of ESRD patients, which could be of considerable value to the dialysis industry. Beyond extracting endotoxin, TNF-α, IL-1β, and IL-6 from the bloodstream, Sigyn Therapy™ can be administered in series with an ESRD patient's regularly scheduled dialysis treatment to combat infection, reduce the incidence of sepsis, and curb the inflammation associated with cardiovascular disease. These are the three leading causes of early death in the ESRD patient population.

Sepsis

Sepsis is a dysregulated life-threatening immune response primarily induced by endotoxin and other bloodstream pathogens. According to the Centers for Disease Control and Prevention (CDC), at least 1.7 million adults in the U.S. develop sepsis each year and nearly 270,000 will die as a result. Sepsis is a leading cause of hospital deaths and a notable financial burden to healthcare systems around the world. The Centers for Medicare and Medicaid Services (CMS) and the Agency for Healthcare Research & Quality estimate that the US healthcare system spends about $38 billion annually on sepsis, with costs rising about 8% a year and most hospitals losing money on each sepsis incident.

As with each of our therapeutic targets, there are no market cleared drugs to treat sepsis. To date, more than 100 human studies have been conducted to evaluate the safety and efficacy of candidate drugs to treat sepsis. With one brief exception (Xigris, Eli Lilly), none of these studies resulted in a market cleared therapy.

In the absence of drug therapies, the broad-spectrum mechanism of Sigyn Therapy™ allows for pathogen and inflammatory targets underlying sepsis to be extracted from the bloodstream.

Drug-Resistant Viral Pathogens

Of the ~270 viruses known to be infectious to humans, a vast majority are not addressed with an antiviral drug or vaccine. Furthermore, three to four new viral species are being discovered each year and by nature will remain untreatable until a corresponding drug or vaccine is developed and demonstrated to be safe and effective in human studies.

In the absence of an approved drug or vaccine, extracorporeal blood purification therapies have been considered first-line treatment countermeasures. As an example, the first therapies cleared by FDA under Emergency-Use Authorization (EAU) to treat COVID-19 were blood purification therapies. In connection with these authorizations, FDA published the following statement:

Blood purification devices may be effective at treating certain patients with confirmed COVID-19 by reducing various pathogens, cytokines, and other inflammatory mediators, that is, small active proteins in the bloodstream that control a cell's immune response by filtering the blood and returning the filtered blood to the patient. The proteins that are removed are typically elevated during infections and can be associated with a "cytokine storm" that may occur in some COVID-

19 patients, potentially leading to severe inflammation, rapidly progressive shock, respiratory failure, organ failure and death.

Sigyn Therapy™ is designed to extract viral pathogens, viral proteins, proinflammatory cytokines and other mediators of inflammation from the bloodstream. Such a mechanism may be of crucial importance as the emergence of new viral pathogens is increasingly being fueled by a confluence of global warming, urban crowding, and intercontinental travel.

Sigyn Therapy also aligns with initiatives established through the US Public Health Emergency Medical Countermeasure Enterprise ("PHEMCE") to support the development of broad-spectrum medical countermeasures that offer to mitigate the impact of an emerging bioterror or pandemic threat, yet also have potential viability to treat known infectious agents.

Drug-Resistant Bacterial Infections

According to the U.S. Centers for Disease Control and Prevention ("CDC"), nearly three million individuals are infected with multi-drug resistant bacterial infections in the U.S. each year, which results in more than 35,000 deaths. The United Nations reported approximately 5 million deaths in 2019 were associated with antimicrobial drug resistance and projects the annual death toll could increase to 10 million by 2050. In the absence of an antibacterial drug, Sigyn Therapy™ offers a strategy that provides for the simultaneous clearance of bacterial toxins and their resulting inflammatory mediators from the bloodstream.

First-in-Human Feasibility Studies

To support first-in-human feasibility studies of Sigyn Therapy™, we collaborated with dialysis industry executives to establish our treatment protocol, clinical site locations, and principal investigators. Our study plan calls for a total enrollment of 12-15 end-stage renal disease (ESRD) subjects with endotoxemia at three dialysis centers. To support the advancement of these studies, we have drafted an Investigational Device Exemption (IDE) for submission to the U.S. Food and Drug Administration ("FDA"). As per our IDE study protocol, Sigyn Therapy™ will be administered in series with the regularly scheduled dialysis treatments of enrolled subjects. In this regard, it should be appreciated that optimal blood flow rates of Sigyn Therapy™ correspond with those of hemodialysis. Aside from the significant incidence of endotoxemia in ESRD subjects, our clinical strategy also benefits from the fact that ESRD patients have established blood access and a demonstrated ability to tolerate extracorporeal blood purification therapy.

The primary objective of our study will be to evaluate the safety of Sigyn Therapy™ in health compromised ESRD patients. A secondary objective will be to quantify changes in circulating levels of endotoxin, tumor necrosis factor-αlpha (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and other inflammatory markers that result from Sigyn Therapy™ administration. The successful completion of our feasibility study would position Sigyn Therapy™ for potential pivotal efficacy studies necessary to seek market clearance to treat endotoxemia and other candidate treatment indications.

Potential to Augment Dialysis Industry Revenues

If we are successful in completing our first-in-human study, future studies of Sigyn Therapy™ will aim to collect efficacy data outcomes that might be expected to extend the lives of ESRD patients. A clinical strategy that extended ESRD patient lives should be of considerable value to the dialysis industry. Especially, when considering that ~20% of dialysis patients die each year.

In North American, the dialysis industry is dominated by Fresenius Medical Care and DaVita Kidney Care. Based on the total number of ESRD patients being treated by Fresenius in North America alone, one month of extended life is worth approximately ~$1.2 billion in added revenues. Each month of extended life for DaVita's network of ESRD patients is worth ~$1 billion in added revenues.

Furthermore, when ESRD patients are hospitalized, Fresenius and DaVita lose revenues as their patients are out of network and receive in-hospital dialysis. On average, dialysis patients spend 11.2 days in the hospital each year. For Fresenius, just one week of reduced hospitalization is worth ~$300 million in recouped revenues. Whereas one week of reduced hospitalization for DaVita's network of ESRD patients is worth ~$250 million in recouped revenues. Beyond a potential to enhance dialysis industry revenues, Sigyn Therapy™ provides a pathway into significant non-dialysis market opportunities.

A Potential Bridge to Kidney Transplantation

In the absence of a kidney transplant, ESRD patients rely on dialysis for survival. Unfortunately, many ESRD patients will die while waiting for a transplantable kidney. Every day, ~17 individuals die while waiting for an organ transplant and kidneys are in shortest supply. Of the 550,000+ individuals living with ESRD in the United States, more than 89,000 are on the national transplant waiting list, yet only 17,000 kidney transplants are performed each year. At present, the average wait time for a matched transplantable kidney is ~5 years.

By treating the conditions that shorten life, we hope to increase the likelihood that an ESRD patient will live long enough to find a matched transplantable kidney.

Previous *Invitro* Study Programs

Sigyn Therapy™ has been evaluated in five *in vitro* blood purification programs that have modeled its utility to extract relevant therapeutic targets from the bloodstream. The insight resulting from these studies reinforced the rationale of Sigyn Therapy™ being a candidate to treat endotoxemia, sepsis, and drug-resistant viral and bacterial infections.

Each *in vitro* study program was designed to evaluate pediatric versions of Sigyn Therapy™ to extract pathogenic and inflammatory molecules from human blood plasma. The studies were conducted on our behalf by a third-party organization that specializes in the development and testing of extracorporeal blood purification devices.

The studies validated the clearance of viral pathogens (including SARS-CoV-2); endotoxin (gram-negative bacterial toxin); peptidoglycan and lipoteichoic acid (gram-positive bacterial toxins); hepatic toxins (ammonia, bile acid, and bilirubin); and tumor necrosis factor alpha (TNF alpha), interleukin-1 beta (IL-1b), and interleukin 6 (IL-6), which are pro-inflammatory cytokines whose dysregulated production (the cytokine storm) play a prominent role in each of our therapeutic opportunities.

The adsorbent components incorporated within Sigyn Therapy™ were also validated to extract 104 nanometer liposomes from human blood plasma. Based on similar size and surface characteristics, our liposome extraction study served as a model for the potential clearance of extracellular vesicles that transport inflammatory cargos (cytovesicles) throughout the bloodstream.

Completion of First-in-Mammal Studies

Subsequent to the completion of our *in vitro* studies, we initiated and completed first-in-mammal studies at the University of Michigan, which demonstrated Sigyn Therapy™ to be safe and well tolerated. In the studies, Sigyn Therapy was administered via standard dialysis machines utilizing conventional blood-tubing sets, for periods of up to six hours in eight (8) porcine (pig) subjects, each weighing approximately 40-45 kilograms. The study was comprised of a pilot phase (two subjects), which evaluated the feasibility of the study protocol in the first-in-mammal use of Sigyn Therapy™; and an expansion phase (six subjects) to further assess treatment safety and refine pre-treatment set-up and operating procedures. Of the eight treatments, seven were administered for the entire six-hour treatment period. One treatment was halted early due to the observation of a clot in the device, which was believed to be the result of a procedural deviation in the pre-treatment set-up. Overall, Sigyn Therapy™ was well tolerated by all eight animal subjects and no serious adverse events were reported in any treated animal. Important criteria for treatment safety – including hemodynamic parameters, serum chemistries and hematologic measurements – were stable across all subjects. The animal study protocol was reviewed and approved by the University of Michigan Institutional Animal Care and Use Committee (IACUC) and designed to correspond with FDA's best practice guidance on demonstrating reasonable feasibility and performance of a medical device prior to human study consideration. It should be noted that porcine animal studies are a generally accepted model for the study of extracorporeal blood purification devices that are intended to treat infectious disease and inflammatory disorders in humans.

Sigyn Therapy™ Manufacturing

To optimize scalability and minimize manufacturing costs, Sigyn Therapy™ is comprised of non-biological adsorbents that are integrated within already commercialized plasma filtration devices. At present, each component that comprises Sigyn Therapy is readily available from established industry vendors. Additionally, our management team has experience in establishing "Quality Systems" and "current Good Manufacturing Practices" (cGMP) necessary to support market clearance of medical device technologies in the United States and abroad.

Sigyn Therapy™ Delivery Infrastructure

To support widespread implementation, Sigyn Therapy™ is a single-use disposable device designed for use on hemodialysis and continuous renal replacement therapy (CRRT) machines that are already located in hospitals and clinics around the world. During first-in-mammal studies, Sigyn Therapy™ was deployed for use on hemodialysis machines manufactured by Fresenius Medical Care, a global leader in the dialysis industry.

As blood flow rates of Sigyn Therapy™ correspond with those of hemodialysis, Sigyn Therapy™ can be placed in series with normally schedule dialysis treatments that are administered to ESRD patients. Thus, establishing early clinical and commercialization opportunities to conveniently treat conditions that shorten the lives of ESRD patients.

Mechanistic Details of Sigyn Therapy™

Broad-Spectrum Extraction of Therapeutic Targets

Incorporated within Sigyn Therapy™ is a "cocktail" of adsorbent components formulated to optimize the broad-spectrum reduction of therapeutic targets from the bloodstream. In the medical field, the term "cocktail" is often a reference to the simultaneous administration of multiple drugs with differing mechanisms of actions. While drug cocktails have emerged to be potential mechanisms to treat cancer, they are proven life-saving countermeasures to treat HIV and Hepatitis-C infections. However, dosing of multi-drug agent cocktails is limited by organ toxicity and adverse events that can result from deleterious drug interactions.

Sigyn Therapy™ is not constrained by such limitations as active adsorbent components are maintained within Sigyn Therapy™ and not introduced into the body. As a result, we are able to incorporate a substantial quantity of adsorbent components to extract therapeutic targets outside of the body as they circulate through Sigyn Therapy™. Each adsorbent component has differing capture characteristics that contribute to optimizing the potential of Sigyn Therapy™ to reduce the circulating presence of pathogenic and inflammatory targets that are associated with each of our therapeutic opportunities.

Expansive Capacity and Efficient Bloodstream Processing

The adsorbent components incorporated within Sigyn Therapy™ provide more than 200,000 square meters (~50 acres) of surface area on which to adsorb, bind, or sequester deleterious factors, including circulating viruses, viral proteins, bacterial toxins, hepatic toxins, proinflammatory cytokines and other inflammatory mediators. Beyond an expansive capacity to extract therapeutic targets from the bloodstream, Sigyn Therapy™ has a highly efficient mechanism of action. Based on optimized blood flow rates of 350ml/min, a patient's entire bloodstream can be processed through Sigyn Therapy more than fifteen times during a single four-hour treatment period.

Technical Mechanism of Action

From a technical perspective, Sigyn Therapy™ is a 325mm long polycarbonate column that internally contains polyethersulphone hollow fibers that have porous walls with a median pore size of ~200 nanometers (nm). As blood flows into Sigyn Therapy™, plasma and therapeutic targets below 200nm in diameter travel through the porous walls as a result of blood-side pressure created by optimized blood flow rates through the device. As the hollow fibers within Sigyn Therapy™ create resistance to blood flow, a pressure drop is created along the length of the device such that the blood-side pressure is higher at the blood inlet and lower at the blood outlet. This allows for plasma and therapeutic targets to flow through the fiber walls into the extra-lumen space (inside the polycarbonate shell, yet outside the hollow-fiber bundle) to interact with Sigyn Therapy™ adsorbent components in a low shear force environment to optimize target extraction and eliminate blood cell interactions. In the distal third of the fiber bundle, the pressure gradient is reversed, which allows for plasma to flow back through the fiber walls to be reconvened with the bloodstream and returned to the patient without the presence of therapeutic targets that were retained in the extra-lumen space of Sigyn Therapy™.

Conclusion

Sigyn Therapy™ is designed to overcome the limitations of previous blood purification devices to treat life-threatening conditions that are not addressed with drugs. Candidate treatment indications include endotoxemia, sepsis, and drug-resistant viral and bacterial infections.

To date, Sigyn Therapy has been demonstrated to extract viral pathogens, bacterial toxins, hepatic toxins, and inflammatory cytokines from human blood plasma. It is deployed for use on dialysis and continuous renal replacement machines that are already located in hospitals and clinics. Sigyn Therapy has an immense capacity to extract therapeutic targets and its highly efficient as the entire bloodstream of a patient can pass through the device ~15 times during a four-hour treatment.

First-in-human feasibility studies of Sigyn Therapy plan to enroll dialysis dependent end-stage renal disease (ESRD) patients with endotoxemia. The successful completion of this study would set the stage for pivotal efficacy studies to seek potential market clearance for Sigyn Therapy to treat endotoxemia and other candidate treatment indications.

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Sigyn Therapy™ in Action During First-In-Mammal Studies

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