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The Prevalence of Inflammation and Endotoxemia in End-Stage Renal Disease (ESRD)

- >550,000 ESRD patients receive ~85 million dialysis treatments annually (US)
- Prominent inflammatory biomarkers IL-1 β, IL-6, and TNF-α are elevated in up to 60% (~330,000) of patients, with nearly 30% of subjects having all 3 biomarkers elevated (~165,000)
- Endotoxemia occurs in 60-90% of ESRD patients (>330,000)

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Background

The United States Renal Data System (USRDS) reports the prevalence of End-Stage Renal Disease (ESRD) to exceed 550,000 individuals in the US, who cumulatively receive ~85 million dialysis treatments each year.[1] Excessive inflammation and/or endotoxemia are common, yet untreatable conditions that contribute to an annual mortality of ~20% of the ESRD patient population.

To address this unmet medical need, Sigyn Therapeutics (the Company) is advancing a clinical strategy to conduct first-in-human studies of Sigyn Therapy™ in dialysis-dependent ESRD subjects. Sigyn Therapy is a broad-spectrum blood purification technology designed to treat pathogen-associated inflammatory disorders. Beyond ESRD inflammation and/or endotoxemia, candidate treatment indications for Sigyn Therapy include sepsis (leading cause of hospital deaths), community acquired pneumonia (a leading cause of death among infectious diseases), and emerging pandemic threats. Mechanistically, Sigyn Therapy extracts pathogen sources of life-threatening inflammation in concert with dampening down the dysregulated overproduction of inflammatory cytokines, which often play a prominent role in each of these treatment indications.

Aside from high rates of inflammation and/or endotoxemia, it is anticipated that ESRD subjects may be efficiently enrolled as the Company's clinical study protocol provides for Sigyn Therapy to be administered in series with normally scheduled dialysis treatments. A clinical strategy that benefits from the fact that ESRD patients have established blood access and a demonstrated ability to tolerate extracorporeal blood purification therapy.

The intent of this paper is to summarize the published scientific knowledge related to the prevalence of inflammation and/or endotoxemia in the ESRD patient population. A clinical strategy that improved or potentially extended the lives of ESRD patients would fulfill an unmet need in healthcare and have a positive impact on the dialysis industry, whose global market exceeded $116 billion in 2022.

Inflammation in End Stage Renal Disease

Inflammation is a hallmark comorbidity of chronic kidney disease. Clinically significant inflammation increases with declining kidney function, and generally presents together with uremia, the accumulation of toxic metabolites and proinflammatory molecules in the bloodstream.[2-3] A chronic syndrome of uremia and inflammation among ESRD patients contributes to and predicts overall mortality.[3] While the expected survival of dialysis dependent patients has gradually increased over the past 2 decades, the urgent need of new treatment options to augment dialysis is underscored by 60-70% foreshortened life expectancy among hemodialysis patients, as compared to the general US population.[1] In addition, the causes of death attributable to inflammation associated diseases comprise nearly 65% of all-cause mortality in this population. Quality of life declines, and for many patients, early mortality follows from cardiovascular disease, infectious complications, and other causes. [4-7]

Standard dialysis is insufficient to remove most uremic toxins and inflammatory mediators from the circulation, leading to an ever-increasing excess over time. Inflammation typically worsens according to patient hemodialysis vintage, the time elapsed since the initiation of hemodialysis therapy.[2] This uremic - inflammatory syndrome appears to be linked to a state of acquired immune dysfunction, osteoporosis, depression, and metabolic and nutritional derangements that often promote a general condition of frailty, protein energy wasting, and premature aging – so called 'inflammaging'. [3,8]

A significant body of clinical evidence suggests that the pathophysiology leading to excess inflammation in ESRD has many contributing factors. Several of these stem directly from kidney failure and dialysis dependence, including the incomplete removal of uremic toxins by dialysis, hypoxia and fluid overload, oxidative stress, and others.[9-14] In addition, microbial factors include endotoxemia, translocation of gut bacteria and other bacterial products across the endothelial barrier into the circulation, long term vascular access, and gut dysbiosis increase inflammation or the risk of serious infection.[15-16] Together, these contributors are believed to interact with individual patient genetic predispositions, comorbidities, concurrent illnesses, and treatments, including dialysis, to drive the progression and outcomes associated with the inflammation inherent to ESRD.[3]

Key Biomarkers of Inflammation in ESRD

Because of the relative contributions of this host of factors, the specific inflammatory markers observed in individual patients vary widely. In general, the circulating concentrations of proinflammatory biomarkers increase with progression of kidney failure and dialysis vintage.[2-3] Clinical studies, including the large Chronic Renal Insufficiency Cohort study, have demonstrated that many endogenous proinflammatory mediators, including IL-1β, IL-1, IL-1 RA, IL-6, TNF-α, CRP, hs-CRP, fibrinogen, and others become elevated in ESRD patients receiving dialysis. [2,17-19] An elevated cytokine is one for which the measured circulating concentration is greater than the laboratory reference value that defines the upper limit of normal.

ESRD patients who exhibit elevated proinflammatory cytokines, including IL-1 β, IL-6, and TNF-α, have been demonstrated to suffer increased mortality.[17] Several clinical studies suggest that IL-

6 may be the most robust predictor of comorbidities, including cardiovascular disease, and all-cause mortality in these patients.[5,20] Elevated TNF-α has also been demonstrated to be predictive of all-cause mortality in ESRD.[5]

While the prevalence of elevated inflammatory biomarkers and endotoxemia in clinical studies of ESRD are generally not reported directly, data from over 4,000 patients in the studies referenced suggest, conservatively, that 60% of dialysis dependent ESRD patients exhibit elevated circulating levels of one or more of the key clinical proinflammatory biomarkers IL-1 β, IL-6, or TNF-α. A robust clinical study showed that 28% of dialysis dependent ESRD patients had significantly elevated levels of all 3 biomarkers. [17]

Extrapolating from these study results, roughly 330,000 ESRD patients in the US would be anticipated to have elevated levels of one or more of these biomarkers. This estimated population receives up to 45 million dialysis treatments each year. Up to 150,000 potentially have elevated levels of all 3 key proinflammatory biomarkers, IL-1 β, IL-6, *and* TNF-α. These patients bear the greatest risk of early mortality and life-threatening complications among all ESRD patients.

Endotoxemia: A Pathogen Associated Inflammatory Biomarker

Endotoxemia is another characteristic comorbidity among ESRD patients. It is defined by the presence of Gram-negative bacterial lipopolysaccharide (LPS) in the circulation. LPS is not removed by dialysis, and on the contrary, dialysis components and long-term vascular access are well recognized as potential sources of LPS contamination and routes for bacterial infection. The circulating concentration of LPS varies from patient to patient, but endotoxemia has been demonstrated to generally increase with decreasing kidney function.[21-22] In patients with ESRD, endotoxin levels increase sharply after the initiation of hemodialysis, and correlate with both cardiac injury and mortality.[21, 23-24] Endotoxemia has recently been demonstrated simultaneously with elevated levels of IL-1 β, IL-6, and TNF-α, supporting the clinical view that LPS induces inflammation in ESRD patients.[25]

Measurement of LPS levels has been challenging historically, with wide variability between studies, depending on the LPS assay used, and within studies, based on significant interpatient variability. However, data including approximately 500 hemodialysis dependent ESRD patients in 4 separate studies, measured endotoxemia in 60-90% of patients with dialysis vintage averaging between 30 -76 months .[21-22, 26-27.]

Taking the large data variability into account, a conservate estimate of the prevalence of endotoxemia in established hemodialysis patients is 40%. Extrapolating from this number, roughly 220,000 ESRD dialysis dependent patients in the US are anticipated to have endotoxemia. This population is estimated to receive up to 34 million dialysis treatments each year.

Proinflammatory Biomarkers as Therapeutic Targets in ESRD and Other Pathogen Associated Inflammatory Diseases

As with ESRD, inflammation and endotoxemia are of critical clinical importance in many other life-threatening, pathogen associated inflammatory diseases. While the specific pattern of

biomarkers in individual patients varies with the indication, patient factors, and course of progression, IL-1β, IL-6, TNF-α, and LPS are key biomarkers of excess inflammation. Moreover, they are pivotal in their individual and collective contributions to the morbidity and mortality of major acute inflammatory diseases, including sepsis, pneumonia, acute respiratory distress, and others.

A growing body of clinical evidence supports the view that in addition to their utility as biomarkers, proinflammatory mediators represent an important class of therapeutic targets that has largely eluded pharmacologic medicine. Based on their roles as major drivers of the pathophysiology of many inflammatory disorders, the strategy of rapidly reducing excess circulating levels of IL-1 β, IL-6, and TNF-α, and LPS, using a broad-spectrum extracorporeal blood purification device such as Sigyn Therapy would be expected to blunt their most acute inflammatory effects. If this mode of action bears out clinically, a major therapeutic goal of Sigyn Therapy will be to augment the current standard of care by mitigating, and perhaps preventing severe tissue and organ injury associated with inflammatory diseases, thereby helping patients to normalize immune system function, and in severe instances, to regain immune homeostasis.

This goal aligns with the recent FDA position, in the context of the COVID-19 pandemic, that based on the scientific evidence available, it believes that the removal of proinflammatory cytokines from the blood by medical devices may ameliorate cytokine storm due to the over-abundance of proinflammatory cytokines and, in turn, provide clinical benefit.[28-31]

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Eric Lynam is the Director of Clinical Affairs of Sigyn Therapeutics. He has 30-years of experience in clinical product development, playing key roles in the development, contracting and execution of over 190 clinical trials. Mr. Lynam will oversee clinical studies of Sigyn Therapy™ in the United States and abroad.

Previously, Mr. Lynam was the Director of Scientific and Medical Affairs for Pharmatech, Inc., until its acquisition by Caris Life Sciences. While at Pharmatech, Mr. Lynam and his team pioneered Just-In-Time, a high efficiency, patient centered clinical trials system, providing on demand, trial-based treatments for patients in a network of 1,500 clinical investigators. The Just-In-Time system has become the gold standard methodology for precision oncology and clinical trials of other rare cancers. It is used by NCI, cooperative groups, CROs, molecular diagnostic laboratories, and institutional review boards across the United States.

Jim Joyce is the co-founder and CEO of Sigyn Therapeutics. He has 30+ years of diverse public market experience, which includes two decades of public company CEO and corporate board leadership roles. He is the inventor of ChemoPrep™ and ChemoPure™ and co-inventor of Sigyn Therapy™, an extracorporeal blood purification technology being advanced to treat pathogen-associated inflammatory disorders.

Previously, Mr. Joyce was the founder, former Chairman and CEO of Aethlon Medical, a therapeutic technology company that he navigated from single shareholder start-up to Nasdaq-traded Company with 8000+ shareholders. During his tenure, Mr. Joyce oversaw the development of the Aethlon Hemopurifier®, a first-in-class blood purification technology to address life-threatening viruses and immunosuppressive exosomes secreted by cancer. Under his leadership, the Hemopurifier® became the first therapeutic candidate to be awarded two "Breakthrough Device" designations from the U.S. Food and Drug Administration (FDA) and received Emergency-Use Authorization from FDA, Health Canada and the German government to treat Ebola virus. Time Magazine named the Hemopurifier® one of the "11 Most Remarkable Advances in Healthcare" and designated the device to its "Top 25 Best Inventions" award list. Readers Digest Magazine named the Hemopurifier® a "Top 10 Medical Breakthrough."

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