Executive Summary: rhMFG-E8 for Sepsis Indication

Company Profile:

TheraSource LLC is a highly research-orientated biopharmaceutical company with specialization of new drug discovery and development in inflammatory diseases, including sepsis, hemorrhage, transplantation (ischemia/reperfusion-induced injury), stroke, inflammatory bowel disease, acute alcohol injury, and acute radiation syndrome. TheraSource was founded in 2004 in New York and our R&D unit resides in Long Island, New York, USA, with a state-of-the art animal facility. Our team is consisted of industry experts in different aspects, including protein chemistry, cell biology, molecular biology, immunology, and clinical medicine. Since its foundation, TheraSource has received Small Business Innovation Research (SBIR) awards from the National Institutes of Health (NIH) to conduct pre-clinical Phase I and Phase II studies to develop the MFG-E8 technology platform for sepsis. The company has been issued a patent in treating sepsis indication.

Why Sepsis?

Sepsis is a disease defined as a systemic inflammatory response associated with a proven or suspected infection, which eventually progresses to multiple organ dysfunction and intractable hypotension, leading to lethality. More than 750,000 patients develop severe sepsis each year with an overall mortality rate of 28.6% in the USA alone. The average costs per case are $22,100, with annual total costs of more than $16 billion nationally. Recently, the only FDA-approved anti-sepsis medicine Xigris (Activated Protein C) has recently been voluntarily withdrawn by Eli Lilly. Thus, it is urgent to identify and develop a new class of therapeutic agents against sepsis. Undoubtedly, if a novel anti-sepsis biological drug is successfully developed and marketed, it has high commercial benefits with significant global market potential.

What is MFG-E8?

The full name of MFG-E8 is milk fat globule epidermal growth factor-factor VIII, also known as lactadherin. MFG-E8 is abundant in human breast milk as a major glycoprotein in the human milk fat globule membrane. The main biological activity of MFG-E8 is to help phagocytes to recognize dying or apoptotic cells and then engulf these cells. Due to this character, MFG-E8 is able to effectively lower the over-reacted inflammatory response, subsequently leading to organ damage, by cleaning up the dying cells accumulated in the injured or infected body. In addition, MFG-E8 can directly inhibit macrophages from releasing proinflammatory cytokines and block activated neutrophils from infiltrating to the organs that cause tissue damage.

Preclinical Data:

Our R&D team started to investigate MFG-E8 in 2005. So far, we have published 13 papers related to this protein in sepsis disease models. The preclinical model of sepsis is induced in mice or rats by cecal ligation and puncture (CLP), a well-described clinically relevant model of polymicrobial sepsis. We first identified the reduction of MFG-E8 levels in septic animals, indicating the need of MFG-E8 replenishment. By administering MFG-E8 in different formats, starting from MFG-E8 in isolated dendritic cell-derived exosomes to recombinant murine MFG-E8, and then recombinant human (rhMFG-E8), we have demonstrated a significant reduction of apoptotic cells, proinflammatory cytokine levels, organ damage index, and mortality rate in comparison to the septic animals alone without treatment. Based on these encouraging results, we have recently performed a dose-response analysis of rhMFG-E8.
(20, 40, 80, and 160 µg/kg body weight) in a 7-day survival study of septic animals. With the treatment of rhMFG-E8, survival rate of septic rats was significantly improved from 36% to 68-72% (Fig. 1). The requirement of MFG-E8 in protecting animals from sepsis-induced mortality is further confirmed by demonstrating a significantly lower survival rate of MFG-E8 deficient mice in comparison to that of wild-type mice (Fig. 2). Taken together, our preclinical studies strongly indicate that rhMFG-E8 is a potential therapeutic agent for treating sepsis and warrant to further development in clinical setting. In addition, we have completed the pharmacokinetics and safety/toxicity analysis of rhMFG-E8 in healthy and sepsis animals.

**Production of rhMFG-E8:**

*Expression and purification of rhMFG-E8.* The mature form of human MFG-E8 (NM_005928.2; Leu24-Cys387) with histidine-tag at the N-terminal has been produced by recombinant protein expression system. The lysate has been subjected to chromatography and precipitation procedures to purify a >99% purity of rhMFG-E8 (Fig. 3, SDS-PAGE; lane 1, 1 µg; lane 2, 0.5 µg; lane 3, marker; lane 4, total lysate) with validation by mass spectrum analysis at the Rockefeller University, NY, USA. Endotoxin content is far below the industry standard.

*Quality control of rhMFG-E8 production.* Two methods have been developed and applied to monitor the quality of purified rhMFG-E8. The first one is a cell-based phagocytosis assay to measure the capability of rhMFG-E8 in enhancing the number of apoptotic cells engulfed by macrophages, an indication of biological activity. The second one is an ELISA-based cell-free assay to measure the capability of rhMFG-E8 in binding to phosphatidylserine, an indication of structural integrity.

**Milestones in 5 Years:**

- Produce GLP/GMP-grade rhMFG-E8.
- Demonstrate a beneficial effect of rhMFG-E8 on a large animal model of sepsis.
- File an Investigational New Drug (IND) application in USA.
- Complete Phase I Clinical Trial to evaluate the safety and dose ranging of rhMFG-E8.
- Initiate Phase II Clinical Trial to evaluate the efficacy of rhMFG-E8 in sepsis patients.

**Patents:**


Publications:


