A Phase 2 Randomized, Double-Blind, Placebo–Controlled Study of the Safety and Efficacy of Talactoferrin in Patients With Severe Sepsis*

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Objectives: Lactoferrin is a glycoprotein with anti-infective and anti-inflammatory properties found in secretions and immune cells. Talactoferrin alfa, a recombinant form of human lactoferrin, has similar properties and plays an important role in maintaining the gastrointestinal mucosal barrier integrity. In experimental animal models, administration of talactoferrin reduces translocation of bacteria from the gut into the systemic circulation and mortality from sepsis. Our objective was to determine if talactoferrin could reduce 28-day all-cause mortality in patients with severe sepsis and to assess its safety.

Design: Prospective, randomized, double-blind, placebo-controlled, multicenter phase 2 trial.

Setting: Adult ICUs and emergency departments in the United States.

Patients: One hundred ninety-four adults within 24 hrs of the onset of severe sepsis.

Interventions: Enterally administered talactoferrin 1.5 g or placebo every 8 hrs for up to 28 days or until discharge from the ICU.

Measurements and Main Results: Modified intention-to-treat analysis was used to assess the primary (28-day all-cause mortality) and secondary endpoints. The all-cause mortality at 28 days was 26.9% in the placebo group and 14.4% in the talactoferrin group (two-sided $p = 0.052$), representing a 12.5% absolute and a 46.5% relative reduction in mortality, meeting the protocol-specified primary endpoint. Reduction in all cause mortality was sustained at 6 months ($p = 0.039$). These reductions in mortality were observed across a wide spectrum of subgroups. The drug was well tolerated with a safety profile similar to that of placebo.

Conclusions: Enteral administration of talactoferrin reduced 28-day all-cause mortality in patients with severe sepsis. This reduction in mortality was sustained at 6 months. Talactoferrin was very well tolerated. (*Crit Care Med* 2013; 41:706–716)

Key Words: lactoferrin; septic shock; severe sepsis; talactoferrin alfa

***See also p. 908.**

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The TLF LF-0801 Investigator Group is listed in **Appendix 1**.

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Sepsis is a clinical syndrome defined by the presence of both infection and a systemic inflammatory response. Severe sepsis is defined as sepsis with the addition of one or more organ dysfunctions (1, 2). Approximately 750,000 cases of severe sepsis occur each year in the United States (3). The conventional management of sepsis consists of eradicating the underlying infection and providing supportive care for associated organ dysfunction (4, 5). Despite the advances in treatment, severe sepsis has a mortality of approximately 30%, leading to the loss of about 220,000 lives each year while adding $16 billion in healthcare costs (3).

Lactoferrins are members of the transferrin family of nonheme iron binding proteins (6). They are normally found in serum and in exocrine secretions such as milk, seminal fluid, intestinal secretions, tears, sweat, saliva, and nasal secretions in mammals (7–9) and in secretory granules of neutrophils (10). They are synthesized in epithelial cells and polymorphonuclear cell precursors (10).

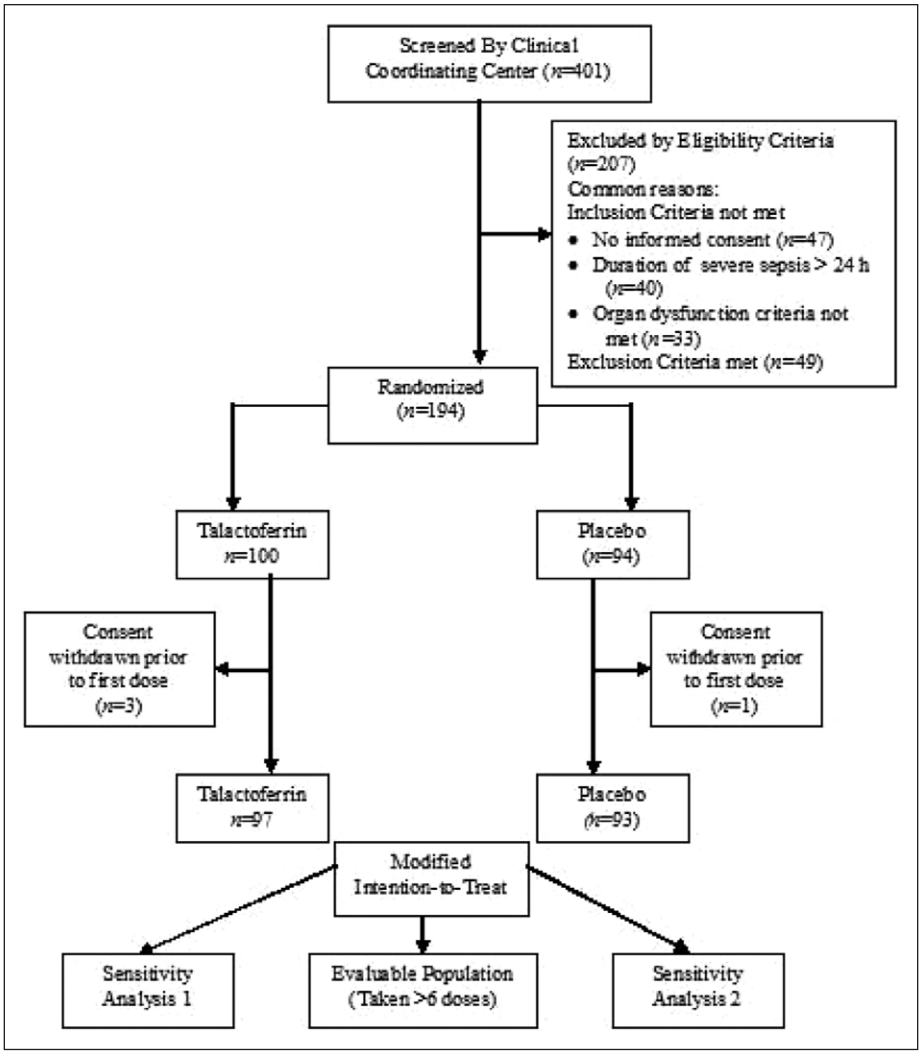

Figure 1. Flow diagram for study participants. About one half of the patients were excluded by the clinical coordinating center based on inclusion or exclusion criteria. Four patients were subsequently excluded because consent was withdrawn before the first dose of study drug.

Talactoferrin alfa (TLF) is a recombinant human form of lactoferrin produced in *Aspergillus niger var awamori* (11). Talactoferrin is equivalent to native lactoferrin from human milk in 3D structure, molecular weight, biological activity, and other physicochemical properties, differing only in the nature of glycosylation (12). Like the native protein, TLF displays anti-infective (11, 13–15) and anti-inflammatory (16–18) properties, which have been demonstrated in in vitro and preclinical studies. Talactoferrin has also been shown to attenuate indomethacin-induced enteropathy in healthy volunteers, suggesting that it helps maintain the barrier properties of gastrointestinal (GI) mucosa (19). In animal models of sepsis, enterally administered talactoferrin protects against mortality induced by bacteria (15, 20) and endotoxin administration (Agennix data on file).

Oral TLF acts locally at the level of the intestinal enterocytes and the gut-associated lymphoid tissue (GALT). Talactoferrin has been observed to protect against gut damage in both preclinical (21) and clinical studies (19), and to reduce translocation of bacteria across the gut mucosa. These effects are consistent with the demonstrated ability of lactoferrin and oral TLF to reduce mortality in bacterial (22, 23) and lipopolysaccharide (Agennix data on file) models of sepsis, respectively. The safety and efficacy of TLF has also been evaluated in other clinical trials in humans (24–27), enabling this study to explore TLF effects in human sepsis. This phase 2 trial was carried out to extend the preclinical sepsis results and to evaluate the safety and efficacy of TLF in severe sepsis in humans.

MATERIALS AND METHODS

This was a double-blind, placebo-controlled study of enteral TLF in patients with severe sepsis. The protocol was approved by the Institutional Review Boards of each of the 24 participating U.S. centers.

A total of 194 patients met the eligibility criteria as determined by an independent clinical coordinating center (Ocean State Clinical Coordinating Center, Providence, RI). Patients were randomized at a ratio of 1:1 to receive either TLF or placebo for up to 28 days or until discharge from the ICU, whichever occurred first. A TLF dose of 4.5 g/day (1.5 g every 8 hrs) was chosen based on extrapolations from the dosage regimens used in animal models of sepsis. This dose was well tolerated in phase 1 studies, and higher doses did not result in any apparent increase in activity (e.g., anti-tumor) in prior clinical studies (28, 29). Of the 194 patients enrolled, 100 were randomized to TLF and 94 were randomized to placebo. A total of 190 were treated, 97 in the TLF arm and 93 in the placebo arm (**Fig. 1**). Treatment arms were stratified by study center and by the presence or absence of cardiovascular (CV) dysfunction (vasopressor-dependent shock) due to sepsis at the time of randomization.

The study enrolled patients who had a diagnosis of sepsis and at least one acute organ dysfunction due to sepsis. Patients received standard care for severe sepsis, allowing drotrecogin alfa (activated) at the discretion of the primary physician. The first dose of study drug was administered within 4 hrs of randomization.

Safety was monitored daily in the ICU, and a final safety evaluation was performed 4 wk after the last dose of study drug. Patients were contacted at 28 days, 3 months, and 6 months postrandomization to determine survival status. An independent data safety monitoring board (DSMB) evaluated the safety and efficacy of talactoferrin after enrolling 40

patients followed by two other evaluations prior to study completion. During the early phase of the trial, a disproportionate number of patients were being enrolled with severe sepsis from a urinary tract source. Therefore, a decision was made to cap enrollment of such patients at 15% (30).

Participants

The inclusion criteria were as follows: age ≥ 18 yr; onset of severe sepsis within the previous 24 hrs; and the ability to take medication by enteral route (oral or via a feeding tube). Severe sepsis was defined as the presence of at least two of the four criteria of the systemic inflammatory response syndrome due to known or suspected infection in association with at least one of the following sepsis-induced organ dysfunctions: CV (shock), respiratory, renal, hematological, and metabolic (2) (**Appendix 2**).

The exclusion criteria were as follows: severe congestive heart failure (e.g., New York Heart Association Class IV); known HIV infection with CD4 < 200 cells/mm^3; presence of third degree burns involving > 20% body surface area; receiving immunosuppressants, to include prednisone 20 mg/day or equivalent for ≥ 2 wk immediately prior to evaluation for enrollment; patient is moribund; severe hypoxic encephalopathy (e.g., postcardiopulmonary resuscitation) or persistent vegetative state; Child-Pugh Class C liver disease; and lack of commitment to full, aggressive, life support.

Study Drug

The labeled study drug was shipped in cartons containing 1 wk's supply.

During the study, the sponsor's quality control process identified mislabeled study drug provided by the contract research organization responsible for drug labeling. The mislabeling impacted the study drug received by some patients. The study sponsor retrospectively traced exactly which carton(s) were administered to each patient and determined that the labeling and subsequent randomization errors occurred randomly. Because vials of study drug were dispensed in cartons containing 1 wk of drug supply, all patients enrolled in the study received the same study drug for the first week on study, whether correctly assigned by randomization or due to a randomization error as a result of mislabeling. The vial mislabeling incident was reported to the study DSMB and the FDA and an independent investigation was performed. The DSMB reviewed the details of the incident and reanalyzed the previous DSMB meeting data conservatively so as to not overestimate the efficacy or safety of TLF. The DSMB noted that these errors were random, and as such were not associated with the introduction of any bias. They concluded that no safety issues arose from the randomization errors and that enrollment should continue without modification.

Statistics

In this exploratory study, a sample size of 95 patients per arm provided 80% power to detect a ≥ 43% reduction in 28-day mortality (from 30% to 17%) in the talactoferrin arm with an a priori selected one-sided p value of < 0.1 (two-sided p value of < 0.2). All data are presented with two-sided p values. The primary analysis consisted of logistical regression with CV dysfunction (vasopressor-dependent shock) included in the model. The primary efficacy population and the modified intention-to-treat population (MITT) included all patients based on the treatment they received during the first week.

In the MITT population, the 45 patients who received incorrect treatment as a result of the mislabeling were assigned as follows. For the purpose of efficacy, the 23 patients randomized to placebo or TLF but who received the other study drug only were included in the arm of the study drug they actually received. The 22 patients who received a combination of both placebo and TLF (at least one dose of both) during the study were included in the arm of the study treatment they received during the first week. This was done because treatments administered within the first week after diagnosis of sepsis are thought to be most important in determining outcome (5, 31, 32). Furthermore, the average duration of study drug administration in the talactoferrin and placebo group was 8.7 and 7.1 days, respectively.

Two sensitivity analysis populations and an evaluable population were subsets of the MITT population. Sensitivity analysis 1 was performed by excluding the 22 patients who received both talactoferrin and placebo. Sensitivity analysis 2 was performed by excluding the 45 patients who received the study drug to which they were not randomized. Those patients in the MITT population who received at least six doses of the study drug were included in the evaluable patient population. The ITT-as-randomized population included patients as they were randomized.

For safety analysis, data from the 22 patients who received at least one dose of TLF and placebo were analyzed as follows. If the patient received TLF first, the patient was included in the TLF group only and all safety data for that patient were included in the TLF group. If the patient received placebo first, safety data for that patient were included in the placebo group until the time the patient received TLF; from that point, all subsequent safety data for that patient were included in the TLF group.

Analyses were conducted to evaluate the effects of the drug over a wide range of baseline characteristics such as the prior location of patient, time of first organ dysfunction, source of infection, comorbidities, severity of illness by Sequential Organ Failure Assessment or Acute Physiology and Chronic Health Evaluation (APACHE) II score, organ failure, race, gender, and age. Secondary endpoints were also analyzed, including the number of ICU days for survivors, the proportion of shock-, ventilator-, dialysis-, and organ dysfunction-free days in the ICU, duration of vasopressor use, time to death, and the incidence of new infections in the ICU.

RESULTS

A total of 401 patients were screened by the clinical coordinating center. Figure 1 illustrates the flow diagram for study participants. About one half of the patients were excluded by the clinical coordinating center based on the study protocol inclusion or exclusion criteria. Four patients were subsequently

TABLE 1. Baseline Characteristics of Patients-Modified Intention-to-Treat Population

Characteristic	Placebo *n* = 93	Talactoferrin *n* = 97
Age (mean), yr	61	58
Gender (% Male)	50.5%	53.6%
Race		
White	77.4%	77.3%
Black	12.9%	17.5%
Asian	3.2%	2.1%
APACHE II score (mean, SD)	25.4 (7.5)	24.1 (7.5)
APACHE II score <25, *n* (%)	47 (51%)	46 (47%)
Sequential Organ Failure Assessment score (mean)	9.0	8.7
Time from first organ dysfunction to randomization (hrs – mean)	18.0	17.6
Baseline lactate, mmol/L (mean, SD)	3.5 (3.6)	3.0 (2.7)
Number of organs with dysfunction, *n* (%)		
No of organs with dysfunction (mean)	2.1	1.9
2 or fewer organs	64 (68.8)	71 (73.2)
3 or higher organs	29 (31.2)	26 (26.8)
Type of organ dysfunction, *n* (%)[a]		
Cardiovascular shock	63 (67.7)	58 (59.8)
Respiratory	56 (60.2)	52 (53.6)
Renal	36 (38.7)	34 (35.1)
Metabolic	31 (33.3)	26 (26.8)
Hematologic	11 (11.8)	14 (14.4)
Concomitant interventions, *n* (%)[a]		
Corticosteroid use for septic shock	29 (31.2)	29 (29.9)
Type of vasopressor at baseline		
Dopamine	17 (18.3)	21 (21.6)
Norepinephrine	64 (68.0)	58 (59.8)
Vasopressin	18 (19.4)	16 (16.5)
Early goal-directed therapy	69 (74.2)	65 (67)
Drotrocogin alfa (activated)	5 (5.4)	10 (10.3)
Use of mechanical ventilation	73 (78.5)	73 (75.3)
Dialysis for chronic renal failure	10 (10.8)	11 (11.3)
Site of infection, *n* (%)[a]		
Lung	48 (51.6)	45 (46.4)
Urinary tract	21 (22.6)	20 (20.6)
Intra-abdominal	14 (15.1)	13 (13.4)
Skin or skin structure	14 (15.1)	14 (14.4)
Sites of positive cultures at baseline, *n* (%)[a]		
Any site	66 (71)	64 (66)

(*Continued*)

TABLE 1. (*Continued*) Baseline Characteristics of Patients-Modified Intention-to-Treat Population

Characteristic	Placebo *n* = 93	Talactoferrin *n* = 97
Blood	32 (34.4)	38 (39.2)
Urine	18 (19.4)	16 (16.5)
Respiratory secretions	25 (26.9)	16 (16.5)
Type of organism[a], *n* (%)		
Gram positive	52 (78.8)	47 (73.4)
Gram negative	29 (43.9)	23 (35.9)
Fungal	4 (6.1)	10 (15.6)
Polymicrobial	32 (34.4)	27 (27.8)

APACHE = Acute Physiology and Chronic Health Evaluation.
[a]Each patient may be counted in more than one category.

excluded because consent was withdrawn before receiving the first dose of study drug. The final MITT population consisted of 97 patients who received TLF and 93 patients who received placebo.

Baseline Characteristics

Baseline patient characteristics (**Table 1**) were balanced between the treatment groups with regard to age, gender, race, ethnicity, height, and weight. The mean (± SD) age was 61 ± 16 yr for the placebo group and 58 ± 17 yr for the TLF group. The ratio of males to females was approximately 1:1 in both groups. About 75% of the study participants in each group were white, and 15% African–American.

Each patient received an oral (or enteral, via a feeding tube) dose of 1.5 g TLF or placebo (1 vial, 15 mL) every 8 (± 2) hrs. In the TLF arm, 35.1% of patients received study drug only by mouth, 32.0% of patients received study drug only by a feeding tube, and 33.0% of patients received study drug by both routes. In the placebo arm, 26.9% of patients received study drug only by mouth, 48.4% of patients received study drug only by a feeding tube, and 24.7% of patients received study drug by both routes. 15.6% of TLF doses and 17.0% of placebo doses were delivered distally to the pylorus.

The mean number of organ dysfunctions was approximately two for each treatment group. CV dysfunction (vasopressor-dependant shock) was present in approximately 60% of patients. Concomitant therapeutic interventions were generally balanced between the respective placebo and TLF arms and included the use of drotrecogin alfa (activated) (5% and 10%), corticosteroids for refractory shock (31% and 30%), and site-reported adherence to early goal-directed therapy (74% and 67%). Nearly all patients were on systemic antibiotics before the first dose of study medication, and 92% and 96% of TLF and placebo patients, respectively, received antibiotics within 1 hr of meeting severe sepsis criteria. Adequacy of antibiotic therapy was assessed by asking if every microbial isolate identified from all baseline cultures was treated within 24 hrs of the culture being obtained, by an antibiotic to which it was sensitive. This assessment was made independently of the availability of culture results at the time of antibiotic initiation and independently of the role of the organism in the septic process. Based on this definition, the adequacy of antibiotic therapy (yes, no, and unknown) was determined for 58%, 19%, and 23% of TLF patients and 55%, 14%, and 32% of placebo patients, respectively.

TABLE 2. All-Cause Mortality-Modified Intention-to-Treat Population

	Overall Patient Population					
	Placebo *n* = 93 Deaths, *n* (%)	Talactoferrin *n* = 97 Deaths, *n* (%)	Absolute Reduction in Mortality	Relative Reduction in Mortality	Odds Ratio and 95% Confidence Interval	*p*[a] (two-sided)
28 days	25 (26.9)	14 (14.4)	12.5%	46.5%	0.48(0.23, 1.01)	0.052
3 months	27 (29.7)	17 (17.9)	11.8%	39.7%	0.54(0.27, 1.09)	0.085
6 months	32 (35.6)	20 (21.1)	14.5%	40.7%	0.5(0.26, 0.97)	0.039

[a]*p* value from two-sided logistic regression test with cardiovascular dysfunction (vasopressor-dependent shock) included in the model.

TABLE 3. Mortality in Patients With and Without Vasopressor-Dependent Septic Shock–Modified Intention-to-Treat Population

	Septic Shock Present				Septic Shock Absent			
	Placebo $n = 63$ Deaths, n (%)	Talacto-ferrin $n = 58$ Deaths, n (%)	Odds Ratio and 95% Confidence Interval	p^a (two-sided)	Placebo $n = 30$ Deaths, n (%)	Talacto-ferrin $n = 39$ Deaths, n (%)	Odds Ratio and 95% Confidence Interval	p^a (two-sided)
28 days	18 (28.6)	13 (22.4)	0.72 (0.32, 1.65)	0.439	7 (23.3)	1 (2.6)	0.09 (0.01, 0.75)	0.026
3 months	20 (32.8)	15 (26.3)	0.73 (0.33, 1.63)	0.443	7 (23.3)	2 (5.3)	0.18 (0.04, 0.96)	0.039
6 months	23 (38.3)	16 (28.1)	0.63 (0.29, 1.37)	0.241	9 (30)	4 (10.5)	0.28 (0.08, 1.01)	0.051

[a]p value from two-sided univariate logistic regression analysis.

The most common sites of infection were respiratory, followed by urinary tract, intra-abdominal, and skin. The incidence of positive cultures with Gram positive, Gram negative, or fungal organisms was similar between the groups. Isolation of Gram positive organisms was about twice as common as Gram negative organisms, and about a third of the cultures were polymicrobial. Blood cultures were positive in 34% of the placebo group and 39% of the TLF group. All cultures were negative in about a third of the patients in both groups.

All-Cause Mortality

The MITT analysis (using logistic regression with baseline CV dysfunction status [vasopressor-dependant shock]) demonstrated that treatment with TLF decreased mortality from 26.9% (placebo) to 14.4% (TLF) (**Table 2**). This decrease represents a 12.5% absolute and a 46.5% relative reduction in 28-day all-cause mortality. The primary analysis met the primary endpoint of 28-day all-cause mortality reduction (two-sided $p = 0.052$), with an odds ratio of 0.48 (Table 2). Univariate logistic regression analysis demonstrated an odds ratio of 0.46 and $p = 0.04$. The reduction in 28-day mortality was noted in patients with and without shock at baseline, although a greater decrease occurred in those without shock (**Table 3**). The reduction in all-cause mortality was sustained at 6 months in the overall population ($p = 0.039$; Table 2) and in those subgroups with or without vasopressor-dependent shock (Table 3). Kaplan–Meier survival curves (**Fig. 2**) also illustrate that the survival benefit of TLF at 28 days is sustained at 6 months.

Because of the mislabeling errors, two sensitivity analyses were performed to analyze potential impact on 28-day mortality; one analysis excluded the 22 patients who received a dose of both placebo and TLF and the second analysis excluded all 45 patients impacted by the mislabeling. As shown in **Table 4**, reductions in 28-day mortality in the TLF arms and odds ratios were similar in the MITT, ITT-as-randomized, sensitivity 1 and 2 populations, and the evaluable population (patients who received at least six doses of the study drug), suggesting that the mislabeling did not impact the study outcome.

Figure 3 shows the 28-day mortality according to baseline APACHE II scores in successive quartiles (0–19, 20–24, 25–30, and > 30). The numbers of patients

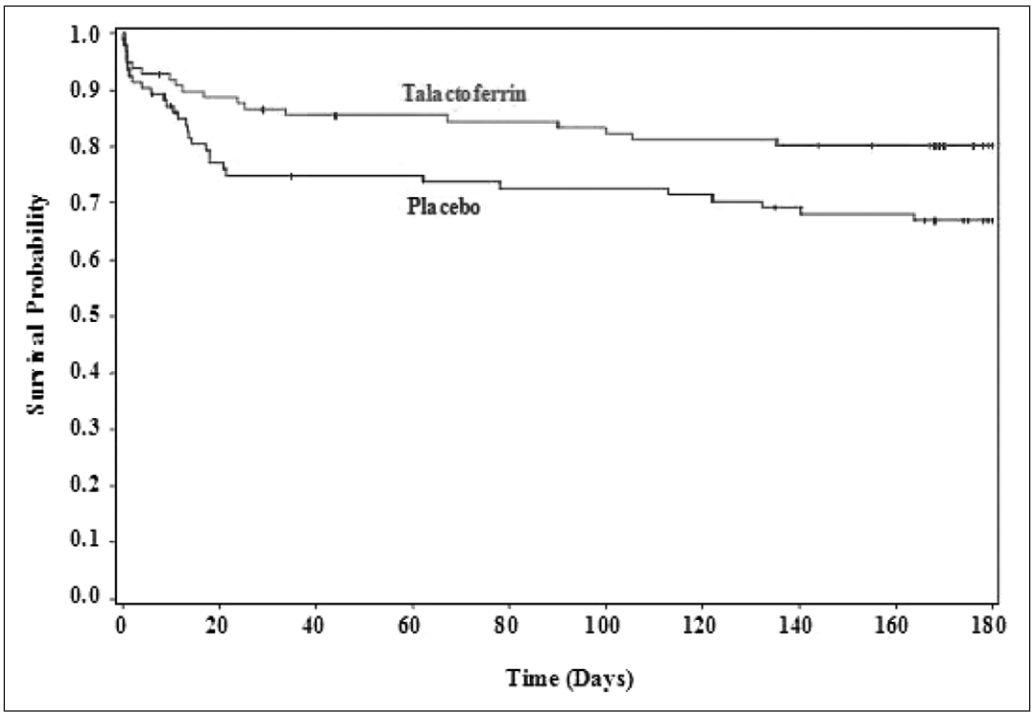

Figure 2. Kaplan–Meier survival-time curves for the modified intention-to-treat population. Treatment with talactoferrin, compared with placebo, resulted in a decrease in all-cause mortality at 28 days (two-tail $p = 0.052$) and at 3 ($p = 0.085$) and 6 months ($p = 0.039$).

TABLE 4. Analyses of 28-Day All-Cause Mortality in Different Populations

Population		*n*	Deaths *n* (%)	Absolute Reduction in Mortality	*p*[a] (two-sided)	Odds Ratio and 95% Confidence Interval
Modified ITT	Placebo	93	25 (26.9)	12.5%	0.052	0.48(0.23, 1.01)
	TLF	97	14 (14.4)			
ITT—randomized	Placebo	94	25 (27.2)	12.9%	0.023	0.42(0.20, 0.89)
	TLF	100	14 (14.3)			
Evaluable	Placebo	84	17 (20.2)	11.4%	0.053	0.41(0.16, 1.01)
	TLF	91	8 (8.8)			
Sensitivity analysis 1 (excludes 22 mixed)	Placebo	81	21 (25.9)	11.0%	0.116	0.53(0.24, 1.17)
	TLF	87	13 (14.9)			
Sensitivity analysis 2 (excludes 45 affected)	Placebo	70	20 (28.6)	12.6%	0.078	0.48(0.21, 1.09)
	TLF	75	12 (16.0)			

ITT = intention-to-treat; TLF = talactoferrin.

[a]Two-sided *p* values for treatment based on logistic regression analysis, including vasopressor-dependent shock in the model. Note: The analyses for each of these five populations met the a priori defined statistical endpoint of one-sided $p < 0.10$ (two-sided $p < 0.20$).

Modified ITT = all patients based on the treatment they received during the first week.

ITT as randomized = based on the randomized assignment.

Subsets of the modified ITT population: Evaluable = those who received at least six doses of the study drug. Sensitivity analysis 1 excluded those patients who received both TLF and placebo. Sensitivity analysis 2 excluded all patients who received the study drug to which they were not randomized.

in each group (placebo vs. TLF) were similar in each quartile. APACHE II scores were unknown for a single patient who was alive in the placebo group. Mortality increased with increasing APACHE II scores in both treatment groups. The decrease in mortality associated with TLF appeared to be greater for patients with higher APACHE II scores (Fig. 3).

Secondary Endpoints

No statistically significant differences were noted between treatment groups for the following secondary endpoints: the number of ICU days for survivors, the proportion of shock-, ventilator-, dialysis-, organ dysfunction-free days in the ICU, duration of vasopressor use, time to death, and the incidence of new infections in the ICU.

Subgroup Analysis

The Forest plot in **Figure 4** shows that the relative effect of TLF on 28-day all-cause mortality was consistent across the majority of subgroups. Exceptions were noted in three subgroups: female gender, metabolic organ dysfunction, and abdominal site of infection.

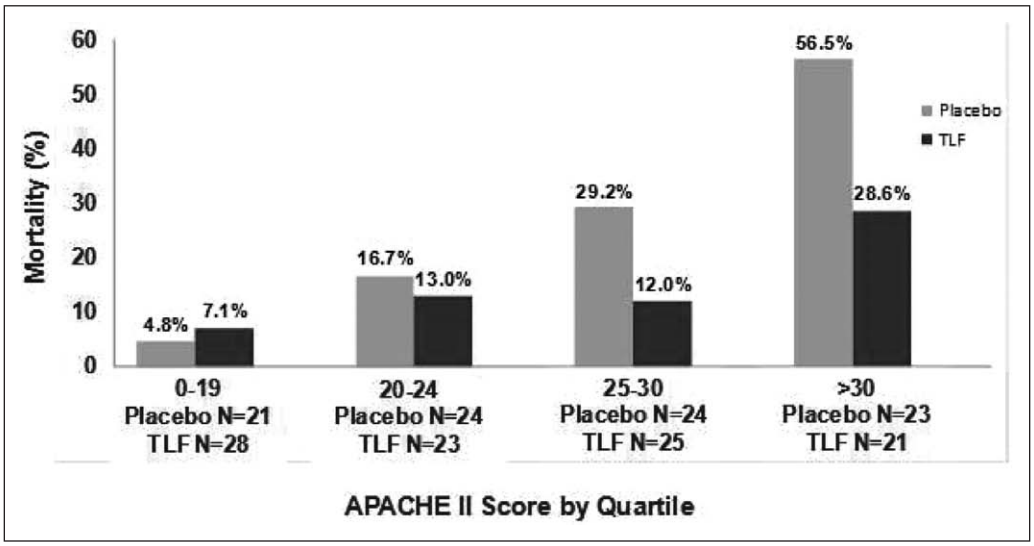

Figure 3. Overall 28-day all-cause mortality by Acute Physiology and Chronic Health Evaluation (APACHE) II quartiles. TLF = talactoferrin.

Safety Results

Talactoferrin was well tolerated. No clinically significant differences were noted between treatment groups in the incidence of adverse events (**Table 5**). The incidence of postbaseline infections was evaluated. These were defined as relapse or superinfection at the site of the original infection or as new infections. Relapse or superinfection was identified in 12.4% and 12.9% of TLF and placebo patients, respectively, and new infections were identified in 18.6% of TLF patients and 23.7% of placebo patients.

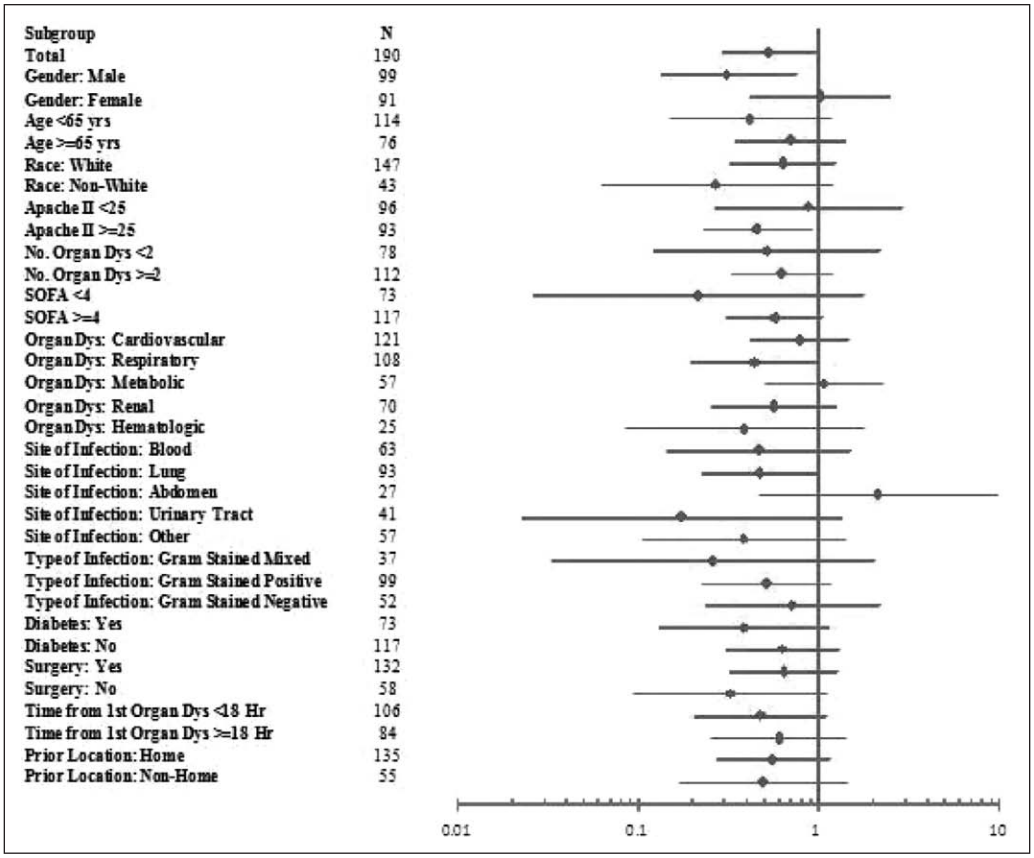

Figure 4. Effect of talactoferrin on 28 day all-cause mortality in various subgroups (relative risk and 95% confidence interval). APACHE = Acute Physiology and Chronic Health Evaluation; SOFA = Sequential Organ Failure Assessment.

DISCUSSION

The effect of talactoferrin alfa on 28-day all-cause mortality in patients with severe sepsis was evaluated in a randomized, double-blind, placebo-controlled phase 2 study. The trial achieved its primary endpoint of a reduction in 28-day all-cause mortality, from 26.9% in the placebo group to 14.4% in the talactoferrin group. This represents a 12.5% absolute and a 46.5% relative reduction in mortality. Furthermore, the effect of talactoferrin on reducing mortality was sustained at 6 months.

Lactoferrin is a naturally occurring 78 kDa iron-binding protein (33) present in mucosal surfaces and at the sites of inflammation (10). It is expressed throughout the body in immune cells and body surfaces exposed to the external environment. Although it is present in tears, saliva, and respiratory secretions, the highest concentrations are found in colostrum (34). Maternal milk plays a central role in helping to establish the immune system, including the GALT—the largest immune system in the body (15). In breast fed infants, enhancing the gut barrier to infection has been attributed at least in part to lactoferrin (35). Interestingly, the milk of mothers of premature infants has a much higher concentration of lactoferrin (> 5g/L) than that of the mothers of mature term infants (~ 1–5 g/L) (36). One recent study in very low birth weight and extremely low birth weight newborns showed that enteral administration of bovine lactoferrin decreased the occurrence of neonatal sepsis (37).

Lactoferrin binds to epithelial cells and modulates intracellular signaling pathways impacting cytokine secretion (e.g., nuclear factor-kB) (38), increases levels of key chemokines (e.g., CCL20) and cytokines (e.g., interferon-γ) derived from the GI tract, and decreases production of Th2-mediated cytokines (interleukin [IL]-4, IL-6, and IL-10). The biologic effects include a decrease in GI tract–induced systemic surges of proinflammatory cytokines that may contribute to the multiorgan dysfunction in sepsis. Lactoferrin appears to normalize gut permeability, decrease bacterial translocation across the gut, dampen the hyperimmune response to inflammatory stimuli, and potentially also impact the immunosuppressed state that follows the proinflammatory surge (39, 40). Sepsis is a severe clinical syndrome characterized by cytokine release, increased expression of adhesion molecules, release of reactive oxygen species, and expression of acute-phase proteins (41–43). These pathophysiological aspects of sepsis impact the gut, and this may be one area where talactoferrin acts. In 15 healthy human volunteers, talactoferrin reduced the indomethacin-mediated increase in gut permeability (19). In animal models of sepsis induced by bacteria (22) and endotoxin administration (Agennix data on file), enterally administered lactoferrin decreased mortality. The gut has been considered a "motor of multiorgan failure" in sepsis (44–46). The sepsis-induced increase in gut permeability results in increased bacterial translocation, which can further exacerbate the septic state. Both local and systemic sequelae are observed following the ischemia-reperfusion injury to the gut that is observed in sepsis (44–46). Oral talactoferrin, which acts locally on intestinal enterocytes and the GALT, may help stabilize the gut and interrupt this sequence of noxious events (19). Given the potential action of TLF in the gut, it is interesting to note that patients with abdominal infections tended to fair worse with TLF compared to placebo. This finding requires further evaluation. Direct infectious, ischemic or surgical insults to the gut may impact potentially protective mechanisms in the

TABLE 5. Incidence of ≥ Grade 3 Adverse Events

System Organ Class and Preferred Term	Placebo	Talactoferrin
Blood and lymphatic system disorders		
Anemia	6.5%	9.2%
Leukopenia	2.2%	0.0%
Cardiac disorders		
Atrial fibrillation	3.2%	0.0%
Gastrointestinal disorders		
Diarrhea	2.2%	0.0%
Ileus	4.3%	1.8%
General disorders and administration site conditions		
Multiorgan failure	7.5%	8.3%
Infections and infestations		
Pneumonia	1.1%	2.8%
Urinary tract infection	4.3%	1.8%
Wound infection	2.2%	0.0%
Sepsis	3.2%	3.7%
Catheter bacteremia	2.2%	0.9%
Metabolism and nutrition disorders		
Hyperglycemia	2.2%	0.9%
Hypoglycemia	1.1%	2.8%
Hypokalaemia	2.2%	1.8%
Hypophosphataemia	3.2%	1.8%
Hypoalbuminemia	3.2%	0.0%
Nervous system disorders		
Encephalopathy	3.2%	0.9%
Psychiatric disorder		
Confusional state	2.2%	0.0%
Renal and urinary disorders		
Renal failure	2.2%	0.9%
Acute renal failure	4.3%	1.8%
Respiratory, thoracic, and mediastinal disorders		
Hypoxia	6.5%	2.8%
Pleural effusion	4.3%	1.8%
Respiratory failure	3.2%	5.5%
Acute respiratory distress syndrome	4.3%	0.9%
Acute respiratory failure	2.2%	0.0%
Pulmonary embolism	2.2%	0.9%
Vascular disorders		
Hypertension	2.2%	0.0%
Hypotension	2.2%	2.8%

otherwise normal gut during sepsis. It is also possible that this outcome from a small subgroup in a small trial may be misleading.

The reduction in mortality from TLF administration in this phase 2 study is large compared to other sepsis trials. However, the use of concomitant medications (steroids, drotrecogin alfa) and the baseline characteristics, including the numbers of organ failure, sites of infection, types of organism, incidence of positive blood cultures, and APACHE II scores, were similar to other recently reported interventional sepsis studies (47, 48). The Kaplan–Meier survival curves begin to separate in the first few days, and their separation becomes more pronounced in the second and third week. Thereafter, the effect seems to be relatively constant. The reason behind this pattern of effect is unknown and will require additional investigation on the mechanism of action that may include effects on excessive inflammation as well as later hypoimmune phases of sepsis (49). A recent analysis of several immunomodulating therapies studied in severe sepsis has suggested a treatment-by-severity interaction, with greater benefit observed with increasing severity (50). Perhaps consistent with this observation, we observed that the greatest mortality reduction in the TLF-treated patients occurred in those with higher APACHE II scores.

The results of this study were scrutinized for sources of bias that could have occurred because of the study drug labeling error. An analysis of efficacy data performed only on patients without randomization errors provided results similar to those for the overall MITT population. In addition, results from analysis of the sensitivity population that excluded the 22 patients who received both TLF and placebo were also similar to those for the MITT population. Another analysis, which excluded the 45 patients who received study drug to which they were not randomized (sensitivity analysis 2), is the most conservative and in that sense, it could be argued, the most reliable. These sensitivity analyses provide further evidence that the errors in labeling of the study medication vials did not introduce bias nor impact the study outcome.

The findings from this phase 2 study, and the need for new therapies in severe sepsis, warrant further studies with talactoferrin. Recently, talactoferrin was evaluated globally in another phase 2/3 sepsis trial. The primary endpoint of this latter study was to determine the effect of oral talactoferrin alfa on 28-day all-cause mortality in patients with severe sepsis. Based on the review of available data, the DSMB for this second

study recommended that patient enrolment and treatment be stopped. The data from the phase 2/3 will be unblinded to better understand the results.

CONCLUSIONS

This study of talactoferrin in severe sepsis met the a priori determined outcome measure of reduction in 28-day all-cause mortality, which was sustained at 6 months. Furthermore, these effects were observed over a wide range of subgroups and talactoferrin had no significant side effects.

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