A Novel Drug for Treatment of Necrotizing Soft-Tissue Infections A Randomized Clinical Trial

ARTICLE *in* JAMA SURGERY · APRIL 2014

Impact Factor: 3.94 · DOI: 10.1001/jamasurg.2013.4841 · Source: PubMed

17 AUTHORS, INCLUDING:

Martin A Schreiber
Oregon Health and Science University

Bruce Ham
Oregon Health and Science University

Steven M Opal
Brown University

Anat Shirvan

Original Investigation

A Novel Drug for Treatment of Necrotizing Soft-Tissue Infections
A Randomized Clinical Trial

Eileen M. Bulger, MD; Ronald V. Maier, MD; Jason Sperry, MD; Manjari Joshi, MD; Sharon Henry, MD; Frederick A. Moore, MD; Lyle L. Moldawer, PhD; Demetrios Demetriades, MD; Peep Talving, MD; Martin Schreiber, MD; Bruce Ham, MD; Mitchell Cohen, MD; Steven Opal, MD; Irit Segalovich, BS; Greg Maislin, MSc; Raymond Kaempfer, PhD; Anat Shirvan, PhD

IMPORTANCE Necrotizing soft-tissue infections (NSTI) have high morbidity and mortality rates despite aggressive surgical debridement and antibiotic therapy. AB103 is a peptide mimetic of the T-lymphocyte receptor, CD28. We hypothesized that AB103 will limit inflammatory responses to bacterial toxins and decrease the incidence of organ failure.

OBJECTIVES To establish the safety of AB103 in patients with NSTI and evaluate the potential effects on clinically meaningful parameters related to the disease.

DESIGN, SETTING, AND PARTICIPANTS A prospective, randomized, placebo-controlled, double-blinded study was performed in 6 academic medical centers in the United States. Participants included adults with NSTI. Of 345 patients screened, 43 were enrolled for the intent-to-treat analysis, and 40 met criteria for the modified intent-to-treat analysis; 15 patients each were included in the high-dose and low-dose treatment arms, and 10 in the placebo arm.

INTERVENTION Single intravenous dose of AB103 (0.5 or 0.25 mg/kg) within 6 hours after diagnosis of NSTI.

MAIN OUTCOMES AND MEASURES Change in the Sequential Organ Failure Assessment score within 28 days, intensive care unit–free and ventilator-free days, number and timing of debridements, plasma and tissue cytokine levels at 0 to 72 hours, and adverse events.

RESULTS Baseline characteristics were comparable in the treatment groups. The Sequential Organ Failure Assessment score improved from baseline in both treatment groups compared with the placebo group at 14 days (change from baseline score, −2.8 in the high-dose, −2 in the low-dose, and +1.3 in the placebo groups; $P = .04$). AB103-treated patients had a similar number of debridements (mean [SD], 2.2 [1.1] for the high-dose, 2.3 [1.2] for the low-dose, and 2.8 [2.1] for the placebo groups; $P = .56$). There were no statistically significant differences in intensive care unit–free and ventilator-free days or in plasma and tissue cytokine levels. No drug-related adverse events were detected.

CONCLUSIONS AND RELEVANCE AB103 is a safe, promising new agent for modulation of inflammation after NSTI. Further study is warranted to establish efficacy.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT01417780

Invited Commentary

Supplemental content at jamasurgery.com

JAMA Surg. doi:10.1001/jamasurg.2013.4841
Published online April 16, 2014.

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Eileen M. Bulger, MD, Department of Surgery, Harborview Medical Center, University of Washington, 325 Ninth Ave, PO Box 358796, Seattle, WA 98104 (ebulger@u.washington.edu).

Necrotizing soft-tissue infections (NSTI) represent a spectrum of severe skin and soft-tissue infections that result in tissue necrosis and systemic signs of sepsis. Patient management focuses on rapid and extensive surgical debridement of necrotic tissue and broad-spectrum antibiotics. β-Hemolytic streptococci, clostridial species, and methicillin-resistant *Staphylococcus aureus* have been implicated in monomicrobial infections, and some patients present with polymicrobial infections, which include additional gram-negative aerobic and anaerobic organisms.[1,2] Some staphylococcal and streptococcal strains can elaborate exotoxins, including superantigens, which bypass the normal immune response for bacterial antigens.[3] In a conventional immune response, 0.01% of T cells interact with antigens to orchestrate a limited, highly specific immune response, whereas superantigens engage 20% to 30% of T cells, regardless of antigen specificity, leading to polyclonal expansion and release of proinflammatory cytokines. This excessive activation of the host response can result in septic shock and multiple organ failure. Despite optimal therapy, patients with NSTI have significant morbidity rates owing to serial debridements and organ failure. Mortality average 16% to 35%.[4-6]

AB103 (originally p2TA) is a novel synthetic CD28 mimetic octapeptide that selectively inhibits the direct binding of superantigen exotoxins to the CD28 costimulatory receptor on T-helper 1 lymphocytes.[7] Preclinical studies demonstrated that AB103 and related superantigen mimetic peptides are associated with improved survival in animal models of toxic shock and sepsis.[7-11] A phase 1 study identified no drug-related toxic effects in healthy subjects (clinicaltrials.gov identifier: NCT01166984). We hypothesized that AB103 could be administered safely in patients presenting with NSTI and would modulate the immune response to reduce the development or progression of organ failure.

Methods

Study Design

This is a phase 2a multicenter, randomized, double-blinded, placebo-controlled trial to evaluate treatment arms of AB103 (0.25 or 0.5 mg/kg) given as a single dose vs placebo. The doses were selected based on optimal response in animal models. The primary purpose of this study was to establish the drug's safety and pharmacokinetics in a population of patients with NSTI. Clinical end points were assessed for potential efficacy. Institutional review board approval was obtained at all sites.

Patient Selection

Inclusion was based on a clinical diagnosis of NSTI due to bacterial infection (eg, necrotizing fasciitis, group A *Streptococcus* toxic shock, Fournier gangrene, clostridial gangrene or myonecrosis, and synergistic necrotizing cellulitis) and a decision to perform urgent surgical exploration and debridement. Exclusion criteria were as follows: age less than 18 years; weight greater than 150 kg (owing to logistics related to drug preparation); pregnancy or lactation; prior curative tissue debridement; human immunodeficiency virus infection (CD4 count <200 cells/mm^3 or <14% of lymphocytes); immunosuppression; diabetes mellitus with below-ankle infection; overt peripheral vascular disease in the involved area; refractory hemodynamic instability, coagulopathy, or hypoxia; cardiac arrest within the past 30 days; expected survival less than 30 days because of underlying medical condition; and burn wounds.

Randomization and Informed Consent

All patients with suspected NSTI were screened for eligibility on arrival to the emergency department. Patients or legal next of kin provided written informed consent. Patients were randomized by a computer-generated system to receive placebo or a low or high dose of AB103 in a 10:15:15 ratio. All investigators and care providers remained masked throughout the study.

Baseline Assessment

Baseline data were collected before drug administration. This included laboratory and clinical data to determine the baseline Acute Physiology and Chronic Health Evaluation II (APACHE II), Sequential Organ Failure Assessment (SOFA), Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC), and Anaya scores. The APACHE II score provides an overall assessment of critical illness and is a predictor of intensive care unit (ICU) mortality.[12] The SOFA score summarizes organ dysfunction across 6 organ systems.[13] *Organ failure* was defined as a SOFA score above 2, *organ dysfunction* as a SOFA score of 1 or 2, and *no organ dysfunction* as a SOFA score of 0. The LRINEC score predicts the likelihood of NSTI, and the Anaya score is correlated with mortality rates in an NSTI population.[14,15]

Intervention

The study drug was administered as soon as feasible after enrollment, but no longer than 6 hours after the diagnosis of NSTI, with scheduled surgical debridement. As a result, patients could receive the infusion before, during, or after surgery. Eligibility criteria were reassessed immediately before drug administration. If the drug was given during or after surgery, a surgical confirmation of NSTI was required. Patients did not receive the intervention if they consented to the study but either did not have confirmed NSTI at the time of surgery or met an exclusion criterion before drug administration. Drug was administered via infusion pump for 10 minutes. Serial blood samples were obtained for pharmacokinetic determinations.

Antibiotic management was not standardized, but all patients were treated with broad-spectrum coverage on admission. The most common regimen was a combination of vancomycin, clindamycin, penicillin or piperacillin-tazobactam, and ciprofloxacin or gentamicin. Operative debridement was prioritized. One patient received hyperbaric oxygen treatment, and another received intravenous immunoglobulin.

Systemic and Tissue Cytokine Analysis

Blood samples were obtained before drug administration (time 0) and 4, 24, 48, and 72 hours after drug administration. Plasma was stored at −70°C for future analysis. All samples were shipped to a core laboratory, where they were analyzed with a Luminex assay (Luminex Corp) for a panel of cytokines, including interleukin 1 (IL-1α), IL-1β, IL-8, IL-12(p70), IL-17, and IL-23; interferon γ; and tumor necrosis factor (TNF). Data are presented using a mixed-model repeated-measures approach adjusted for baseline values. Values below the lowest level of detection were imputed as the midpoint between the lowest detectable value and zero.

Tissue samples were collected at the initial operation and subsequent debridements within 48 hours. Two samples were taken: one near the most active site of tissue infection (epicenter), excluding overtly necrotic tissue, and the other from the lesion margin. Samples were frozen immediately and stored at −80°C. Samples were cut to 1 mg of tissue and homogenized in the cold with 10× phosphate-buffered saline with protease inhibitors. Homogenates were clarified by centrifugation at 15 000 *g* for 8 minutes. Cytokine concentrations were measured on the supernatant by Luminex assay and adjusted for the amount of starting material.

Clinical End Points

The primary end point was to establish the safety and pharmacokinetics of AB103 in patients with NSTI. Clinical data were collected throughout the hospital stay to assess for adverse events. Exploratory efficacy end points included resolution of organ dysfunction over time (based on SOFA scores); number of debridements through day 7; hospital length of stay; and ICU-free, vasopressor-free, and ventilator-free days within the first 28 days. *Debridement* was defined as removal of necrotic tissue.

Statistical Analysis

All patients receiving the study drug were in an intent-to-treat (ITT) population and included in all safety analyses. Because drug administration may have started before definitive surgical diagnosis of NSTI, a modified ITT population was defined as patients in the ITT analysis who were properly randomized, treated (received AB103 or placebo), and evaluable (definitive surgical diagnosis of NSTI).

This phase 2a study was considered exploratory, and thus no formal hypothesis testing was proposed. The study was not powered to determine definitive efficacy. The numbers and percentages of adverse events were compared between groups. A safety review committee reviewed the data after the first 20 patients and approved continuation of the trial.

The SOFA scores were calculated on days 0 (before drug administration), 1 (first 24 hours after drug administration), 2, 3, 7, 14, and 28. To account for missing data, the change in SOFA score over time was assessed as both an observed SOFA score and as a last-observation-carried-forward analysis. The change in SOFA score over time and the proportion of patients with organ dysfunction were assessed. The preplanned sample size for this study was 40 patients (10 received placebo, 15 received 0.25 mg/kg of AB103, and 15 received 0.5 mg/kg of AB103).

The Wilcoxon rank sum and Fisher exact tests were used to compare groups. Mixed-model repeated-measures analysis of covariance models were used to assess dose group differences for SOFA scores and biomarkers.

Results

Patient Enrollment and Treatment Allocation

From December 15, 2011, to August 7, 2012, a total of 345 patients were screened, 62 provided consent, 43 were randomized (ITT), and 40 met criteria for modified ITT analysis (**Figure 1**). The 3 patients in the ITT population who were excluded from the modified ITT population included 1 who did not meet the clinical diagnosis of NSTI, 1 subsequently found to have a CD4 count less than 200 cells/mm^3, and 1 who received a higher-than-planned dose. These exclusions were made before unmasking, and all patients in the ITT analysis were included in the safety analysis. The baseline characteristics and the timing of drug administration are shown in **Table 1**. The culture results for type of infection are shown in eTable 1 [Supplement]. The antibiotic coverage was reviewed relative to the culture results by 2 independent microbiologists and deemed appropriate in all but 1 patient who was in the placebo group.

Safety Analysis

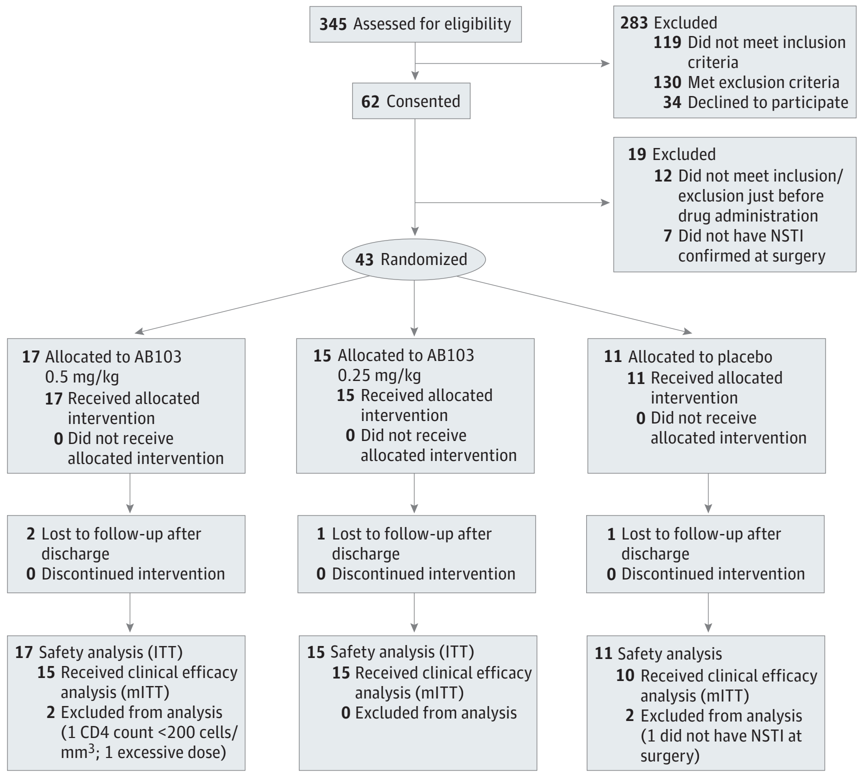
A summary of the adverse events observed is shown in **Table 2**. None of the adverse events were judged by the independent medical monitor to be drug related. Fifteen of the 43 patients experienced an adverse event before drug administration (day 0), including 9 in the high-dose arm (53%), 4 in the low-dose arm (27%), and 2 in the placebo arm (18%).

After drug administration, 39 of the 43 patients had at least 1 adverse event, including 16 of 17 (94%) in the high-dose arm, 14 of 15 (93%) in the low-dose arm, and 9 of 11 (82%) in the placebo arm. There were no statistically significant differences in summary adverse event rates. Thirty-nine adverse events were classified as serious. The distribution of patients with serious adverse events after drug administration was 5 (29%) in the high-dose, 8 (53%) in the low-dose, and 3 (27%) in the placebo group. Four deaths occurred during the study, 2 in the placebo group and 1 in each treatment arm, resulting in mortality of 6%, 7%, and 18% in the high-dose, low-dose, and placebo groups, respectively.

Resolution of Organ Dysfunction

The progression and resolution of organ dysfunction was tracked over time using the SOFA score (**Figure 2**). After an initial increase between day 0 (screening) and day 1 (time of drug administration), which can be explained by worsening of disease state and the first surgery, SOFA scores decreased gradually in all groups until day 7. After day 7, organ function started to worsen among patients in the placebo group but continued to improve among those in the treated groups. The maximal difference between groups was observed at day 14, when mean SOFA total scores were 0.7, 1.1, and 2.7 for the high-dose, low-dose, and placebo groups, respectively ($P = .02$). At 14 days, the SOFA scores had improved from baseline in both treatment groups compared with placebo (change in score, −2.8 for high-dose, −2 for low-dose, and +1.3 for placebo group; $P = .04$). The proportion of patients with organ failure during

Figure 1. Patient Enrollment and Treatment Allocation



ITT indicates intent-to-treat analysis; mITT, modified intent-to-treat analysis; and NSTI, necrotizing soft-tissue infection.

Table 1. Baseline Characteristics and Timing of Drug Administration and Surgery

Patient Characteristic and Treatment Timing	AB103 0.5 mg/kg (n = 15)	AB103 0.25 mg/kg (n = 15)	Placebo (n=10)	*P* Value
Sex, No. male/female	9/6	11/4	6/4	.76
Age, mean (range), y	52 (28-85)	46 (27-73)	56 (25-88)	.29
Weight at day 0, mean (range), kg	100 (54-141)	95.2 (68-140)	93.5 (66-120)	.78
Medical conditions, No. (%)				
Diabetes mellitus	7 (47)	7 (47)	3 (30)	.78
Hypertension	8 (53)	7 (47)	6 (60)	.92
Chronic kidney disease	1 (7)	2 (13)	1 (10)	.99
Coronary artery disease	2 (13)	2 (13)	0	.65
COPD/asthma	1 (7)	0	2 (10)	.27
Obstructive sleep apnea	1 (7)	2 (13)	1 (10)	.99
Congestive heart failure	0	1 (7)	1 (10)	.71
History of cancer	2 (13)	2 (13)	1 (10)	.99
Baseline scores, mean (SD)				
APACHE II	8.3 (4.5)	7.5 (5.0)	9.6 (4.7)	.55
Admission SOFA	3.5 (2.5)	2.9 (2.7)	3.1 (2.0)	.80
LRINEC score	7.5 (2.1)	7.1 (2.8)	7.4 (3.9)	.70
Anaya score	2.3 (2.0)	1.7 (1.5)	2.6 (1.4)	.41
Time to drug administration, mean (SD), h	4.38 (1.1)	3.57 (0.77)	4.26 (1.0)	.06
Time to 1st debridement, mean (SD), h	2.49 (1.5)	2.04 (1.36)	2.06 (1.62)	.66
Vasopressor treatment in 1st 24 h, No. (%)	8 (53)	2 (13)	3 (30)	.06

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; COPD, chronic obstructive pulmonary disease; LRINEC, Laboratory Risk Indicator for Necrotizing Fasciitis; SOFA, Sequential Organ Failure Assessment.

Table 2. Most Common Adverse Events (Intent-to-Treat Analysis)

Event	Patients, No. (%) AB103 0.50 mg/kg (n = 17)	Patients, No. (%) AB103 0.25 mg/kg (n = 15)	Patients, No. (%) Placebo (n = 11)
Electrolyte/laboratory abnormalities			
Hypokalemia	8 (47)	6 (40)	4 (36)
Hypophosphatemia	7 (41)	7 (47)	2 (18)
Hypomagnesemia	7 (41)	5 (33)	5 (45)
Hypocalcemia	3 (18)	1 (7)	1 (9)
Hyperkalemia	1 (6)	1 (7)	2 (18)
Hyperglycemia	5 (29)	2 (13)	2 (18)
Anemia/thrombocytopenia	3 (18)	7 (47)	5 (45)
Metabolic acidosis	2 (12)	0	3 (27)
Organ failure/dysfunction			
Respiratory failure/ALI or ARDS	4 (24)	4 (27)	3 (27)
Acute renal failure	2 (12)	1 (7)	2 (18)
Hepatic failure	1 (6)	0	0
Delirium/agitation	2 (12)	3 (20)	1 (9)
Cardiovascular events			
Fluid overload/generalized edema	0	0	3 (27)
Peripheral edema	0	1 (7)	2 (18)
Cardiac arrhythmia	2 (12)	0	5 (45)
Hypotension	3 (18)	3 (20)	4 (36)
QTc prolongation	2 (12)	0	0
Nosocomial infection[a]	1 (6)	1 (7)	1 (9)
Death	1 (6)	1 (7)	2 (18)

Abbreviations: ALI, acute lung injury; ARDS, acute respiratory distress syndrome; QTc, corrected QT interval.

[a] Including pneumonia, urinary tract infection, and fungemia.

the first 14 days (SOFA score, >2) was higher in the placebo group than in the treatment groups. In particular, 4 of 10 patients (40%) in the placebo group still had organ failure at day 14, compared with 1 of 14 (7%) in the high-dose group (*P* = .12) and 2 of 14 (14%) in the low-dose group (*P* = .19). The changes in the organ system components of the SOFA score are shown in eTable 2 [Supplement].

Number of Debridements

The mean (SD) number of debridements was 2.2 (1.1) in the high-dose, 2.3 (1.2) in the low-dose, and 2.8 (2.1) in the placebo groups. Two patients (13%) in the high-dose group required 4 or more debridements (*P* = .36 for comparison with placebo group), compared with 3 (20%) in the low-dose group (*P* = .65 for comparison with placebo group) and 3 in the placebo group (30%).

Critical Care End Points

Hospital and ICU length of stay and duration of mechanical ventilation tended to be longer in the placebo group than in the treatment arms, but the differences did not reach statistical significance. The mean (SD) values were as follows for the high-dose, low-dose, and placebo groups: ventilator-free days: 24.1 (6.3), 23.5 (8.8), and 20.8 (9.1); ICU-free days: 21.3 (6.2), 21.7 (8.5), and 17.1 (9.9); and total hospital stay: 17.4 (9.0), 17.1 (8.0), and 20 (7.0) days. There was no difference between groups in vasopressor-free days.

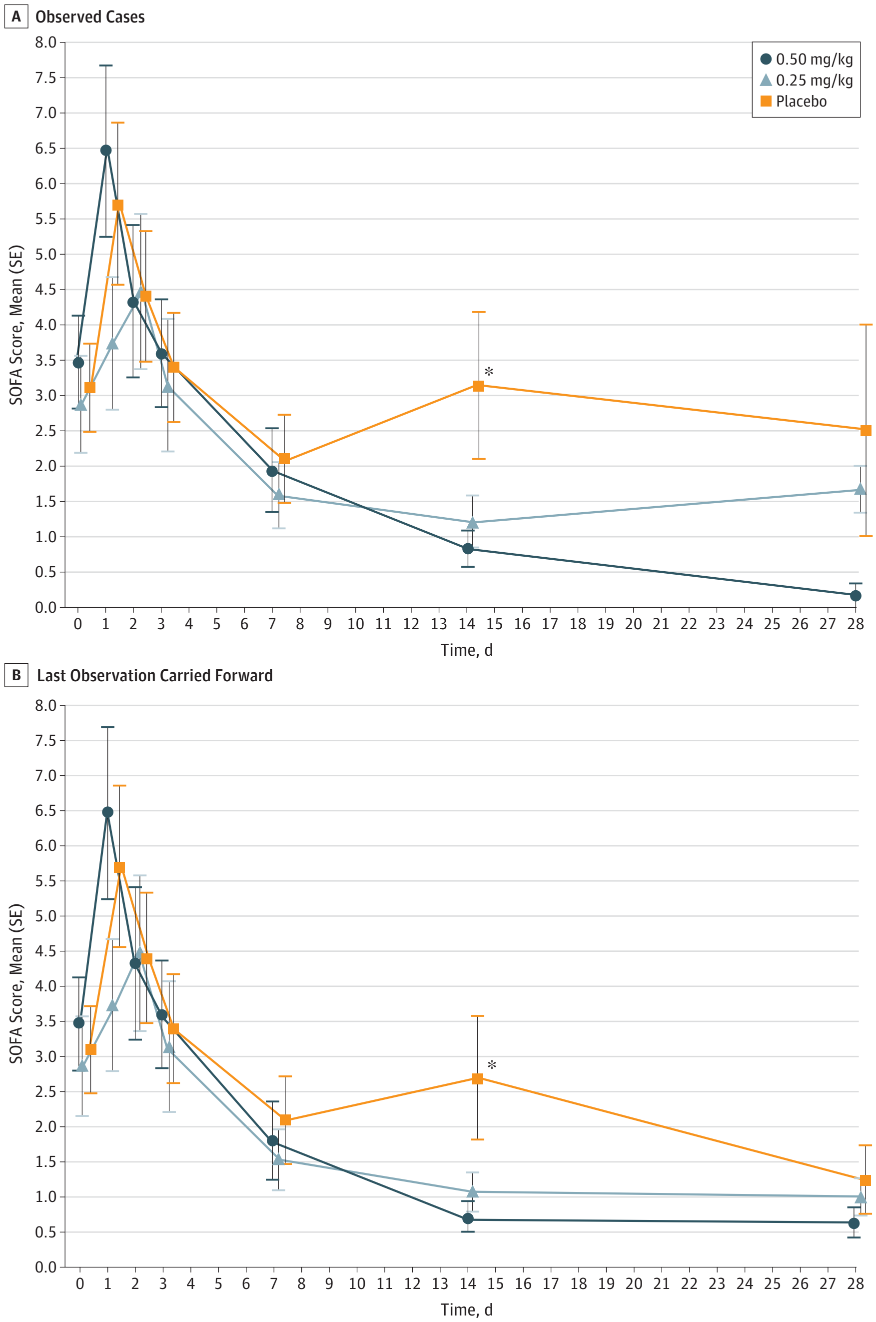
Plasma Cytokine Levels

Five of the 10 cytokines measured had levels above the lowest level of detection: IL-6, IL-8, IL-10, monocyte chemoattractant protein 1, and TNF (**Figure 3**). Patients treated with the higher dose exhibited lower levels of cytokines (except IL-10) than patients given placebo, with levels in most patients starting to diverge from those in the placebo group 24 hours after administration. Responses in the low-dose group were higher than those in the placebo group for some levels (TNF and IL-10). Overall, drug-related changes in systemic cytokine response were detected in several proinflammatory and chemoattractant biomarkers but not in the anti-inflammatory biomarker IL-10. These changes did not reach statistical significance.

Tissue Cytokine Levels

Adequate tissue samples were available for 5 patients per group. The concentrations of many inflammatory cytokines could be detected from tissues at the epicenter of the infection but with no consistent drug effect on cytokine expression over time (data not shown). In contrast, in the margins of the wound, high-dose AB103 was associated with reduced tissue concentrations of IL-1 (α and β), IL-8, IL-12, IL-23, and TNF. Only a single cytokine (IL-8) had reduced levels in response to the high dose of AB103 in both the epicenter and the margins of the inflamed tissue. Interleukin 1α, TNF, and IL-8 levels were increased in the low-dose group. Owing to the small sample sizes,

Figure 2. Progression of Sequential Organ Failure Assessment (SOFA) Score Over Time by Treatment Arm



Day 0 was the baseline score on admission; day 1 represents the worst score in the first 24 hours after drug administration. A, Observed SOFA scores, which reflect missing data at later time points owing to death or discharge. B, Analysis using a last-observation-carried-forward approach. *$P < .05$ for comparison at day 14 between placebo and treatment groups.

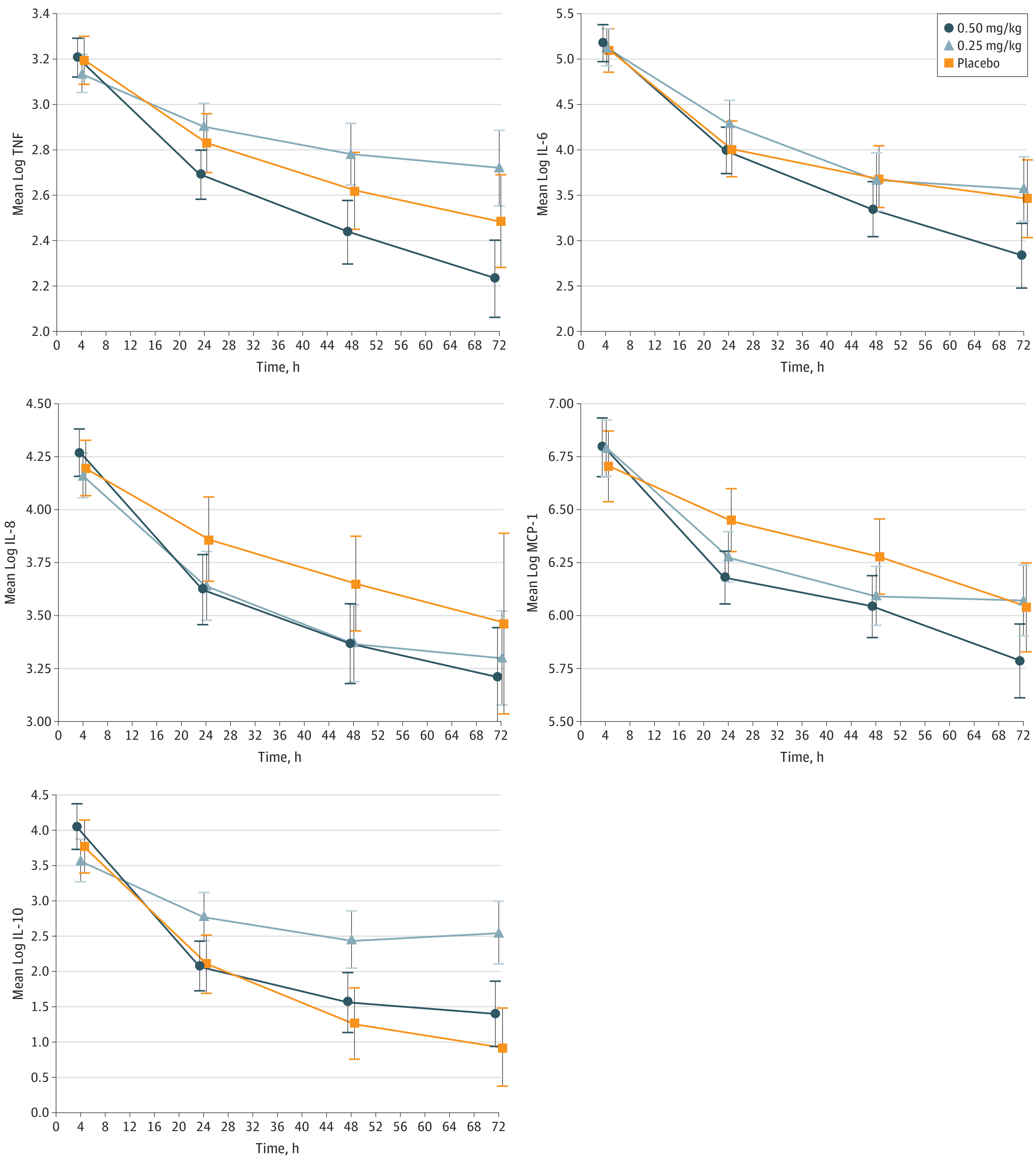
these differences did not reach statistical significance (eFigure [Supplement]).

Discussion

To our knowledge, this is the first study of a novel investigational drug, AB103, as an adjuvant to the treatment of NSTI. This phase 2a study establishes the safety of the drug in this patient population, with no drug-related adverse events and no significant difference between the treatment arms for adverse events. This study was not powered to establish efficacy, but several consistent trends demonstrate improvement across multiple clinical parameters. One dose given within 6 hours of clinical diagnosis was sufficient to demonstrate more rapid resolution of organ dysfunction in the treatment arms.

AB103 has a dual mechanism of action, modulating the innate immune response to exotoxins and endotoxins. It interferes with superantigen exotoxin ability to bind and activate the CD28 receptor on T-helper 1 lymphocytes, resulting in downregulation of the excessive host inflammatory cytokine

Figure 3. Plasma Cytokine Production by Treatment Arm During the First 72 Hours



Data are presented as mean ratios of log values at 0, 4 (±1), 24 (±6), 48 (±8), and 72 (±8) hours based on the summary statistics, with the 95% CIs for the mean ratios for the 3 individual treatment groups. Data were analyzed using a mixed-model repeated-measures approach, and group differences over time were adjusted for baseline values (time 0). There were no statistically significant differences. IL-6 indicates interleukin 6; IL-8, interleukin 8; IL-10, interleukin 10; MCP, monocyte chemoattractant protein; and TNF, tumor necrosis factor.

response[7] that can lead to organ dysfunction and failure. This mechanism is particularly relevant in treating patients with NSTI because these patients frequently experience the effects of tissue-invasive strains of *S aureus* and *Streptococcus pyogenes*, which can express more than 2 dozen superantigen exotoxins, leading to systemic signs of toxic shock syndrome.[16-18] There is also evidence that gram-negative infections result in the overproduction of T-helper 1 proinflammatory cytokines, leading to septic shock.[19] Derived from the CD28 homodimer interface,[7] AB103 can attenuate CD28 signaling independent of superantigens and can therefore affect the downstream signaling of CD28 in cases of gram-negative infections.

Animal studies have demonstrated that AB103 protects mice from direct exposure to exotoxins[7,20] as well as live bacterial infections with gram-positive,[20] gram-negative, and polymicrobial pathogens. AB103 and related superantigen antagonist peptides protected against lethal doses of staphylococcal and streptococcal exotoxins in mice, with 100% survival if given intravenously 30 minutes before exposure and 50% survival if given 7 hours after exposure.[7-9] In a murine model of NSTI (thigh infection with *S pyogenes*), 100% survival was observed with attenuation of plasma cytokine levels and decreased necrosis at the site of infection.[20] Additional studies have demonstrated protection of mice from shock induced by lipopolysaccharide. Many patients with NSTI have mixed bacterial infections, so the ability to influence the response to several bacterial toxins is important.

Current therapy for NSTI relies on aggressive surgical debridement and administration of broad-spectrum antibiotics. The use of intravenous immunoglobulin has been reported, particularly in patients with streptococcal infections and toxic shock syndrome.[21,22] This treatment relies on adequate titers of neutralizing antibodies against streptococcal superantigens from pooled human serum. One small study (21 patients) suggested a potential reduction in mortality rate but did not reach statistical significance.[23] AB103 has the advantage of attenuating the host inflammatory response to a broad array of bacterial species. Although not statistically significant, the results of the systemic and local cytokine response suggest that the higher dose of the drug has effects consistent with its anti-inflammatory mechanism of action.[7] A lack of dose response was seen, however, for changes in circulating TNF and IL-10, with inconsistent results at the lower dose (0.25 mg/kg). A larger sample will be required to further delineate the effect on the inflammatory response. These results suggest that the next study should focus on the higher dose (0.5 mg/kg) for a more consistent effect.

Conclusions

In summary, this is the first clinical trial of AB103 in patients with NSTI and establishes the safety of this agent in these critically ill patients. In addition, to our knowledge, this is the first multicenter, randomized clinical trial of an early intervention for patients with NSTI. As such, it demonstrates the feasibility of studying novel interventions in this challenging patient population. The fact that this was a multicenter trial, representing the full spectrum of NSTI, lends to the generalizability of the results. The results of this trial are also important for the design of future trials in this patient population. Mortality rates for these patients have been declining in recent years and thus are not likely to be the optimal end point for clinical trials. End points that reflect the magnitude of the systemic inflammatory response, such as organ dysfunction, and the local inflammatory response, such as number of debridements, are clinically meaningful and a good reflection of the morbid effects of this disease.

The primary limitation to the study is the small sample size. As a phase 2a study, it has insufficient statistical power to allow assessment of efficacy. However, consistent trends across several important clinical end points suggest that this agent can reduce the progression of organ dysfunction. A larger trial is needed to definitively establish efficacy.

ARTICLE INFORMATION

Accepted for Publication: July 26, 2013.

Published Online: April 16, 2014. doi:10.1001/jamasurg.2013.4841.

Author Affiliations: Department of Surgery, Harborview Medical Center, University of Washington, Seattle (Bulger, Maier); University of Pittsburgh, Pittsburgh, Pennsylvania (Sperry); Department of Surgery and Medicine, University of Maryland, Baltimore (Joshi, Henry); Department of Surgery, University of Florida, Gainesville (Moore, Moldawer); Department of Surgery, University of Southern California, Los Angeles (Demetriades, Talving); Department of Surgery, Oregon Health & Science University, Portland (Schreiber, Ham); Department of Surgery, University of California, San Francisco (Cohen); Department of Medicine, Alpert Medical School of Brown University, Providence, Rhode Island (Opal); Atox Bio Ltd, Ness Ziona, Israel (Segalovich, Kaempfer, Shirvan); Biomedical Statistical Consulting, Wynnewood, Pennsylvania (Maislin); Department of Molecular Biology, Hebrew University Hadassah Medical School, Jerusalem, Israel (Kaempfer).

Author Contributions: Drs Bulger and Shirvan had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Bulger, Maier, Joshi, Cohen, Opal, Segalovich, Kaempfer, Shirvan.
Acquisition of data: Bulger, Joshi, Henry, Moore, Moldawer, Demetriades, Talving, Schreiber, Ham, Cohen.
Analysis and interpretation of data: Bulger, Maier, Sperry, Moore, Moldawer, Demetriades, Schreiber, Cohen, Opal, Segalovich, Maislin, Shirvan.
Drafting of the manuscript: Bulger, Demetriades, Ham, Maislin.
Critical revision of the manuscript for important intellectual content: Maier, Sperry, Joshi, Henry, Moore, Moldawer, Demetriades, Talving, Schreiber, Cohen, Opal, Segalovich, Kaempfer, Shirvan.
Statistical analysis: Maislin.
Obtained funding: Maier, Segalovich, Kaempfer, Shirvan.
Administrative, technical, or material support: Bulger, Maier, Henry, Moore, Moldawer, Talving, Schreiber, Cohen, Opal, Segalovich, Kaempfer, Shirvan.
Study supervision: Bulger, Joshi, Moore, Moldawer, Demetriades, Schreiber, Ham, Opal, Segalovich, Shirvan.

Conflict of Interest Disclosures: Dr Bulger currently serves as a consultant to Atox Bio Ltd for subsequent trial design but did not receive any direct support during the conduct of this trial. Dr Joshi was a paid consultant with Atox Bio before this study, starting to review information regarding the product research in clinical trial. Ms Segalovich and Dr Shirvan are employees of Atox Bio. Mr Maislin is principal biostatistician for Biomedical Statistical Consulting, Wynnewood, Pennsylvania, which provided biostatistical services for this project. Dr Kaempfer is an inventor on patents covering p2TA, is a scientific founder of Atox Bio, and serves as its chief scientist.

Funding/Support: Financial support and the drug for this trial was provided by the manufacturer, Atox Bio Ltd.

Role of the Sponsor: Atox Bio Ltd had input into the design of the study and review of the manuscript, for which authorship is identified (Ms Segalovich and Drs Kaempfer and Shirvan are employees of Atox Bio). The statistical analysis was performed by an independent statistician. Atox Bio was not involved in the conduct of the study; management and interpretation of the data; preparation or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We acknowledge the study coordinators at each site: Harborview

Medical Center, University of Washington, Seattle; University of Pittsburgh, Pittsburgh, Pennsylvania; University of Maryland, Baltimore; University of Florida, Gainesville; University of Southern California, Los Angeles; and Oregon Health & Science University, Portland.

REFERENCES

1. Elliott D, Kufera JA, Myers RA. The microbiology of necrotizing soft tissue infections. *Am J Surg*. 2000;179(5):361-366.

2. Anaya DA, McMahon K, Nathens AB, Sullivan SR, Foy H, Bulger E. Predictors of mortality and limb loss in necrotizing soft tissue infections. *Arch Surg*. 2005;140(2):151-158.

3. Herman A, Kappler JW, Marrack P, Pullen AM. Superantigens: mechanism of T-cell stimulation and role in immune responses. *Annu Rev Immunol*. 1991;9:745-772.

4. Anaya DA, Dellinger EP. Necrotizing soft-tissue infection: diagnosis and management. *Clin Infect Dis*. 2007;44(5):705-710.

5. Cuschieri J. Necrotizing soft tissue infection. *Surg Infect (Larchmt)*. 2008;9(6):559-562.

6. Wong CH, Chang HC, Pasupathy S, Khin LW, Tan JL, Low CO. Necrotizing fasciitis: clinical presentation, microbiology, and determinants of mortality. *J Bone Joint Surg Am*. 2003;85-A(8):1454-1460.

7. Arad G, Levy R, Nasie I, et al. Binding of superantigen toxins into the CD28 homodimer interface is essential for induction of cytokine genes that mediate lethal shock. *PLoS Biol*. 2011;9(9):e1001149.

8. Arad G, Levy R, Hillman D, Kaempfer R. Superantigen antagonist protects against lethal shock and defines a new domain for T-cell activation. *Nat Med*. 2000;6(4):414-421.

9. Arad G, Hillman D, Levy R, Kaempfer R. Superantigen antagonist blocks Th1 cytokine gene induction and lethal shock. *J Leukoc Biol*. 2001;69(6):921-927.

10. Arad G, Hillman D, Levy R, Kaempfer R. Broad-spectrum immunity against superantigens is elicited in mice protected from lethal shock by a superantigen antagonist peptide. *Immunol Lett*. 2004;91(2-3):141-145.

11. Kaempfer R, Arad G, Levy R, Hillman D. Defense against biologic warfare with superantigen toxins. *Isr Med Assoc J*. 2002;4(7):520-523.

12. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med*. 1985;13(10):818-829.

13. Dominguez TE, Portnoy JD. Scoring for multiple organ dysfunction: Multiple Organ Dysfunction Score, Logistic Organ Dysfunction, or Sequential Organ Failure Assessment. *Crit Care Med*. 2002;30(8):1913-1914.

14. Wong CH, Khin LW, Heng KS, Tan KC, Low CO. The LRINEC (Laboratory Risk Indicator for Necrotizing Fasciitis) score: a tool for distinguishing necrotizing fasciitis from other soft tissue infections. *Crit Care Med*. 2004;32(7):1535-1541.

15. Anaya DA, Bulger EM, Kwon YS, Kao LS, Evans H, Nathens AB. Predicting death in necrotizing soft tissue infections: a clinical score. *Surg Infect (Larchmt)*. 2009;10(6):517-522.

16. Hackett SP, Stevens DL. Superantigens associated with staphylococcal and streptococcal toxic shock syndrome are potent inducers of tumor necrosis factor-beta synthesis. *J Infect Dis*. 1993;168(1):232-235.

17. Marrack P, Blackman M, Kushnir E, Kappler J. The toxicity of staphylococcal enterotoxin B in mice is mediated by T cells. *J Exp Med*. 1990;171(2):455-464.

18. Marrack P, Kappler J. The staphylococcal enterotoxins and their relatives. *Science*. 1990;248(4956):705-711.

19. Opal SM, Palardy JE, Parejo NA, Creasey AA. The activity of tissue factor pathway inhibitor in experimental models of superantigen-induced shock and polymicrobial intra-abdominal sepsis. *Crit Care Med*. 2001;29(1):13-17.

20. Ramachandran G, Tulapurkar ME, Harris KM, et al. A peptide antagonist of CD28 signaling attenuates toxic shock and necrotizing soft-tissue infection induced by *Streptococcus pyogenes*. *J Infect Dis*. 2013;207(12):1869-1877.

21. Kaul R, McGeer A, Norrby-Teglund A, et al; The Canadian Streptococcal Study Group. Intravenous immunoglobulin therapy for streptococcal toxic shock syndrome: a comparative observational study. *Clin Infect Dis*. 1999;28(4):800-807.

22. Johansson L, Thulin P, Low DE, Norrby-Teglund A. Getting under the skin: the immunopathogenesis of *Streptococcus pyogenes* deep tissue infections. *Clin Infect Dis*. 2010;51(1):58-65.

23. Davies HD, McGeer A, Schwartz B, et al; Ontario Group A Streptococcal Study Group. Invasive group A streptococcal infections in Ontario, Canada. *N Engl J Med*. 1996;335(8):547-554.