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A multicenter, open-label, prospective, randomized, dose-ranging pharmacokinetic study of the anti-TNF-α antibody afelimomab in patients with sepsis syndrome

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Abstract *Objective:* To investigate the pharmacokinetics and safety of afelimomab, a murine antibody fragment against human tumor necrosis factor (TNF)-α in patients with sepsis.
Design: Multicenter, randomized, open-label, placebo-controlled phase I/II clinical trial.
Setting: Intensive care units of six academic medical centers in the United States.
Patients: Forty-eight patients with a clinical diagnosis of sepsis who received standard supportive care and antimicrobial therapy.
Interventions: Patients received 0.3, 1.0, or 3.0 mg/kg afelimomab or placebo intravenously over 20 min. Three patients in each dose group received single doses; the remaining nine patients in each group received multiple (nine) doses at 8-h intervals over 72 h.
Measurements and main results: Afelimomab appeared safe and well tolerated. Single- and multiple-dose kinetics were predictable and dose related. The elimination half-life was 44.7 h. Afelimomab treatment resulted in increased serum concentrations of TNF (includes TNF-antibody complexes) and decreased serum interleukin-6 concentrations, whereas no discernible trends were observed in placebo-treated patients. There was no significant treatment effect on 28-day mortality as was expected given the small number of patients. However, overall mortality was significantly ($p = 0.001$) associated with baseline interleukin-6 concentration. All patients experienced adverse events, but the vast majority were considered unrelated to the study drug and demonstrated no apparent relationship to afelimomab dose. Although 41 % of patients developed human anti-murine antibodies, there were no clinical sequelae.
Conclusions: Multidose therapy with afelimomab was safe, well tolerated, and had predictable linear kinetics. A large randomized trial comparing afelimomab to placebo in patients with well defined sepsis has recently been completed.

Keywords Afelimomab · MAK 195F · Anti-TNF-α antibody · Interleukin-6 · Sepsis · Septic shock

Introduction

Sepsis and septic shock remain major causes of morbidity and mortality in spite of the advances in antibiotic therapy and medical technology. The sepsis syndrome develops in more than 500,000 patients in the United States each year, and the incidence appears to be increasing despite a growing armamentarium of antibiotics and an enhanced knowledge of the pathophysiological processes involved [1]. Furthermore, surveys have shown a worrisome increase in resistance to important antibiotics [2, 3]. Based on analyses of large intensive care databases, the overall mortality for patients critically ill with sepsis ranges from 20 % to 60 % [4, 5, 6]. Many of the clinical sequelae and the high mortality rates associated with infection are due, at least in part, to the local and systemic inflammatory responses of the host triggered by the invading micro-organisms [7]. These host responses can result in the severe tissue damage and life-threatening physiological perturbations seen in sepsis and are not being adequately treated with current antibiotic therapy and general supportive care.

Experimental evidence suggests that the cytokines interleukin (IL)-1, tumor necrosis factor (TNF)-α, and IL-6 are the primary early mediators of these host responses [8, 9, 10, 11]. Elevated plasma TNF-α concentrations can be seen in cases of either Gram-negative or Gram-positive bacteremia, [10, 11], and several lines of experimental evidence have suggested that TNF-α is a particularly important mediator of sepsis. Following administration of live or heat-killed bacteria or endotoxin, TNF-α is present in the systemic circulation, [12, 13] and direct administration of TNF-α produces many of the physiological and laboratory changes associated with severe sepsis [14, 15, 16]. Antibodies against TNF-α have demonstrated a protective effect in animal models of severe sepsis [17, 18]. Recently Mira et al. [19] reported finding a polymorphism within the TNF-α gene promoter, associated with enhanced TNF-α production and negative outcome in some severe infections.

Because of the persuasive evidence that TNF-α is an important mediator of septic shock, therapy that includes TNF-α neutralization is a compelling approach. Several clinical trials in septic patients with antihuman TNF-α monoclonal antibodies or soluble TNF receptor constructs have recently been reported [20, 21, 22, 23, 24, 25]. Most trials have been unable to demonstrate a clinical benefit, but there has been some indication that anti-TNF-α therapy benefits those septic patients with the worst prognosis [21, 26]. In addition, no overt toxicity from anti-TNF-α monoclonal antibodies has been observed, and thus this anticytokine immunotherapeutic strategy remains promising.

The murine monoclonal $F(ab')_2$ antibody fragment afelimomab has been developed to avidly bind to and neutralize human TNF-α [27]. The fragment rather than the whole antibody was chosen to reduce potential immunogenicity, improve tissue penetration, and minimize interaction with Fc receptors. Afelimomab effectively blocks the biological effects of human TNF-α in vitro [27] and is highly effective in animal models of sepsis [18, 28]. A phase I study demonstrated the safety of repeated infusions of up to 3 mg/kg per dose in septic patients [29]. The primary objective of this phase I/II study was to determine the pharmacokinetics of intravenous afelimomab at three doses (0.3, 1.0, and 3.0 mg/kg) in patients with sepsis syndrome. Also evaluated were the safety, immunogenicity, serum TNF-α and IL-6 concentrations, and mortality in both afelimomab- and placebo-treated patients.

Materials and methods

This multicenter, open-label, randomized placebo-controlled study was conducted in six centers in the United States. The protocol was approved by the local ethics committees, and written informed consent was obtained for each patient from either the patient or a responsible relative. The study was conducted in accordance with the Helsinki Declaration as amended in Tokyo, Venice, and Hong Kong.

Male and nonpregnant female patients at least 18 years of age admitted to the intensive care unit with a clinical diagnosis of sepsis were enrolled in the study. Eligible patients had to fulfilled all five entry criteria within 24 h of one another: (a) clinical evidence of sepsis; (b) fever (≥38.0 °C) or hypothermia (≤35.6 °C); (c) tachycardia (≥90 beats/min) in the absence of β-blockade; (d) tachypnea (≥20 breaths/min) or respiratory distress requiring mechanical ventilation; (e) either hypotension (systolic blood pressure ≤90 mmHg or a sustained drop in systolic blood pressure ≥40 mmHg) in the absence of antihypertensive agents and vasopressors, or evidence of systemic toxicity or poor end-organ perfusion. Patients with systemic toxicity or poor end-organ perfusion were required to have two or more of the following: metabolic acidosis (pH ≤7.3), arterial hypoxia (pO_2 ≤75 mmHg) in those without overt pulmonary disease, plasma lactate concentration above the normal range of the testing laboratory, acute renal failure (oliguria with urine output ≤0.5 ml/kg per hour for 1 h or longer), unexplained coagulation abnormality (prothrombin time ≥1.2 or partial thromboplastin time ≥1.2× control value) within the prior 24 h, unexplained platelet depression (≤100,000 platelets/ml or a decrease by ≥50 % from baseline) within the prior 24 h, acute deterioration of mental status, and a cardiac index less than 4.0 l min^{-1} m^{-2} with a systemic vascular resistance greater than 800 dynes s^{-1} cm^{-5}. Patients were excluded if they had received an investigational agent within the prior 30 days, had previously received any murine monoclonal antibody, had received oral or parenteral steroids within the past 7 days, were HIV positive, or were septic following major burns or organ transplant. Patients could be withdrawn from the study for any reason but had to be withdrawn if they developed intolerable adverse experiences or any exclusion criteria.

Qualified patients were randomized to receive either placebo or one of 3 doses (0.3, 1.0, and 3.0 mg/kg) of the anti-TNF-α antibody afelimomab (also known as MAK 195F, Knoll, Ludwigshafen, Germany). The first three patients randomized to each of the four treatment groups received a single intravenous dose of study drug infused over 20 min. The subsequent nine patients in each

Table 1 Characteristics of multiple-dose patients at baseline (APACHE Acute Physiology and Chronic Health Evaluation, *TNF* tumor necrosis factor, *IL* interleukin)

Characteristic	Placebo ($n = 9$)	Afelimomab			All patients ($n = 36$)
		0.3 mg/kg ($n = 9$)	1.0 mg/kg ($n = 9$)	3.0 mg/kg ($n = 9$)	
Age (years)	67.2 ± 18.9	57.9 ± 15.8	60.2 ± 12.3	55.0 ± 21.4	60.1 ± 17.3
Sex (male/female)	5/4	6/3	4/5	3/6	18/18
Race (white/black)	8/1	6/3	9/0	8/1	31/5
Weight (kg)	72.2 ± 11.4	74.2 ± 14.6	79.6 ± 23.4	102.8 ± 40.3	82.2 ± 27.0
Severity of underlying disease (%)					
Nonfatal/none	77.8	55.6	66.7	77.8	69.4
Ultimately fatal	22.2	33.3	33.3	22.2	27.8
Rapidly fatal	0	11.1	0	0	2.8
Shock present (%)	88.8	66.7	100	66.7	80.6
Mechanically ventilated (%)	77.8	66.7	100	77.8	80.6
APACHE II score	29.3 ± 9.81	25.1 ± 7.47	27.4 ± 7.27	22.3 ± 10.17	26.0
TNF-α (pg/ml)					
Median	37.0	83.8	40.3	15.6	32.2
Range	≤15.6–392.0	≤15.6–1454.0	≤15.6–69.7	≤15.6–79.1	≤15.6–1454.0
IL-6 (pg/ml)					
Median	8599	3466	876	524	1537
Range	46–1.2×10^6	92–3.9×10^6	36–1.4×10^5	33–4.5×10^3	33–3.9×10^6

group received study drug every 8 h for a total of nine doses, each infused over 20 min. In addition, patients received antibiotic therapy as well as aggressive resuscitative, diagnostic, and supportive care as determined by their treating physicians.

Blood samples were drawn from each single-dose patient at 0, 5 min, and 0.5, 1, 6, 12, 24, 48, 72, 120, and 168 h postinfusion for determination of afelimomab concentrations. Multiple-dose patients had blood samples taken at 0, 5 min, and 0.5, 1, 6, and 12 h following the first infusion and at 1, 6, 12, 24, 48, 72, 120, and 168 h following the last infusion for determination of afelimomab concentrations. The serum concentration of afelimomab was determined by enzyme-linked immunosorbent assay (ELISA) with a 3.0 ng/ml lower limit of quantitation. The primary pharmacokinetic variables were the model-fitted parameters for systemic serum afelimomab concentrations, including the peak serum concentration (C_{max}), area under the serum concentration-time curve (AUC), clearance rate (CL), steady-state volume of distribution (V_{ss}), and elimination half-life ($t_{1/2}$). Area under the moment curve (AUMC) and mean residence time (MRT) were also determined.

Serum concentrations of TNF-α and IL-6 were determined in blood samples taken from multiple-dose patients at 0, 0.5, 1, 6, 12, 24, 48, 72, 120, and 168 h. Blood samples were taken just prior to afelimomab infusion. TNF-α and IL-6 concentrations were determined using the Medgenix TNF-α ELISA and IL-6 ELISA kits (BioSource Europe, Fleurus, Belgium), respectively, which had an estimated 15.6 pg/ml lower limit of quantitation for both cytokines.

Patients were followed for 28 days after the initial treatment. Patients were evaluated by the Acute Physiology and Chronic Health Evaluation (APACHE) II score on enrollment and on days 1, 2, and 3 of the study, and survival was assessed at 28 days after study initiation. All adverse clinical changes from the patient's pretreatment condition (including intercurrent illness) were recorded throughout the 28-day study. Safety parameters were collected throughout the study, including vital signs (temperature, blood pressure, and heart rate), respiratory parameters (respiratory rate, FIO_2, ventilatory mode, tidal volume, minute volume, and positive end-expiratory pressure/continuous positive airway pressure), arterial blood gases (pH, pCO_2, pO_2), urine output, and clinical hematology and biochemistry variables (hemoglobin, hematocrit, cell counts, prothrombin time, partial thromboplastin time, fibrinogen, electrolytes, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, glucose, albumin, C-reactive protein, lactate, uric acid, urea, and creatinine). Plasma samples were drawn at 0, 14, 21, and 28 days for qualitative assessment of human anti-mouse antibodies (HAMA) using the ImmuStrip HAMA IgG kit (Immunomedics, Morris Plains, N.J., USA). Twelve-lead electrocardiograms were taken at enrollment and on days 1 and 4 of the study.

Statistical analysis

All randomized patients who received trial medication were included in the analyses. Pharmacokinetic parameters for the single-dose patients were analyzed descriptively, and data from multiple-dose patients were analyzed separately.

Pharmacokinetic analyses were performed using WinNonlin Professional version 1.5, model 10 (two-compartment intravenous infusion input) or model 19 (three-compartment intravenous infusion input) selected by the best fit of the data. Actual (relative to start of infusion), rather than protocol-specified times of blood collection were used in all pharmacokinetic calculations unless unavailable. All skewed continuous variables were (natural log) transformed to better approximate a normal distribution. The relationships between AUC and dose and between log-transformed baseline TNF-α and IL-6 concentrations were assessed by regression analysis.

Efficacy and safety variables were analyzed descriptively. Because of the small number of patients in each treatment group, data were collapsed across centers and statistically significant differences were not expected. Testing was limited to efficacy variables and was intended to identify trends and generate hypotheses.

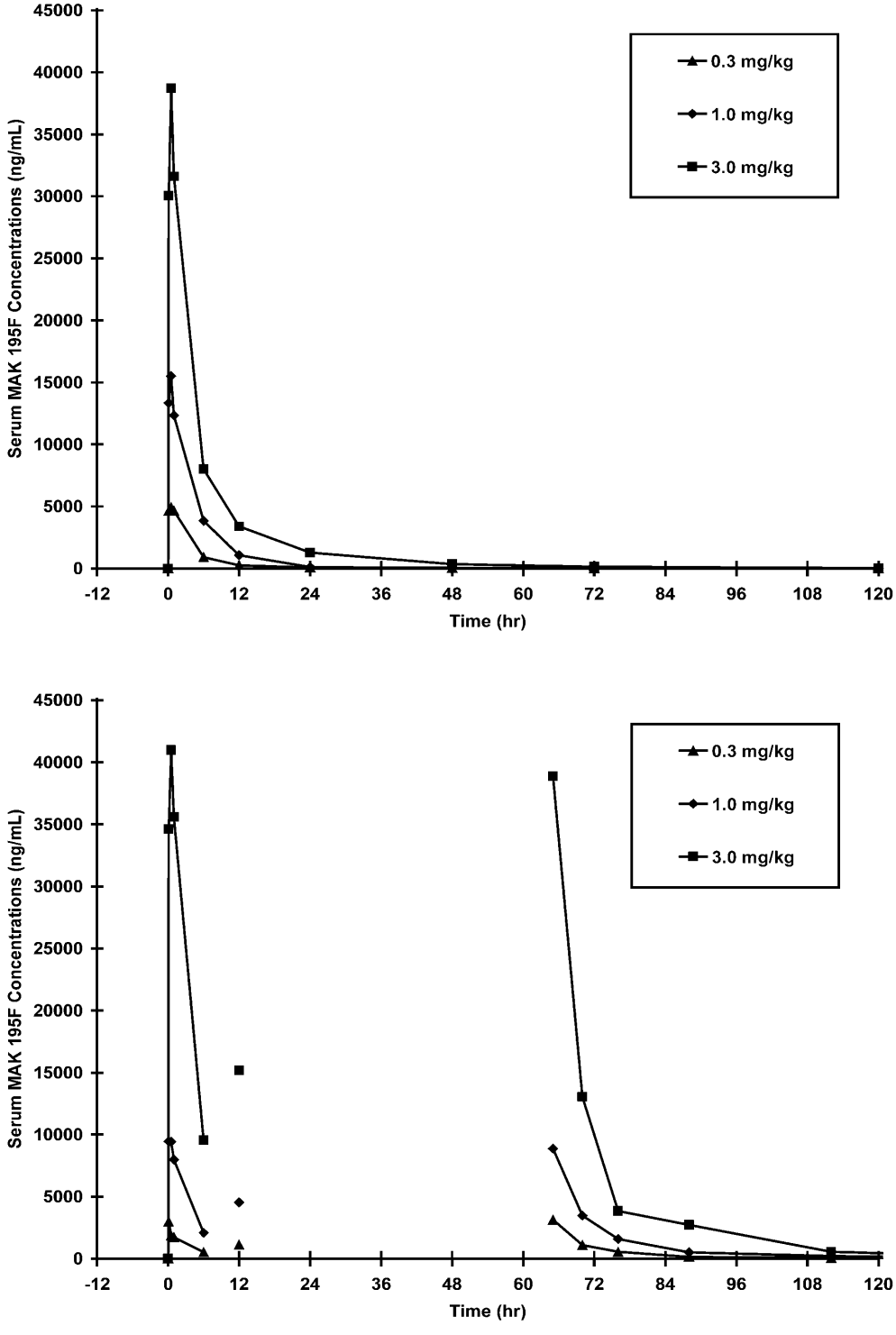

Fig. 1 Mean serum afelimomab profiles following single *(above)* and multiple *(below)* infusions

The analyses were performed using SAS version 6.08 (SAS Institute, Cary, N. C., USA).

The influence of baseline parameters on the probability of 28-day mortality was investigated with a logistic regression model. The dependent variable was mortality on day 28; treatment was included in the model a priori. Further selection among the factors considered clinically relevant was based on an initial univariate analysis. Factors included were those achieving a p value of 0.15 in a forward selection process. There were no α-corrections for multiple comparisons since all results were descriptive.

Table 2 Mean pharmacokinetic parameters for afelimomab (*AUC* area under the curve, *AUMC* area under the moment curve, C_{max} maximum serum concentration, *MRT* mean residence time, $t_{1/2}$ half life, *CL* clearance, V_{ss} volume of distribution at steady state)

Parameters	0.3 mg/kg ($n = 6$)	1.0 mg/kg ($n = 9$)	3.0 mg/kg ($n = 9$)
$AUC_0 \rightarrow \infty$ (ng ml^{-1} h^{-2})	16,039 ± 6,962	48,297 ± 19,289	169,671 ± 76,660
AUMC (ng ml^{-1} h^{-2})	213,629 ± 139,317	490,975 ± 397,213	1,454,965 ± 1,014,389
C_{max} (ng ml^{-1})	3,246 ± 922	12,817 ± 3,890	48,460 ± 19,154
CL (ml min^{-1} kg^{-1})	0.41 ± 0.30	0.40 ± 0.15	0.36 ± 0.19
V_{ss} (l kg–1)	0.259 ± 0.108	0.191 ± 0.073	0.182 ± 0.161
MRT (h)	12.2 ± 5.1	8.9 ± 4.3	8.3 ± 4.7
$t_{1/2}$, terminal (h)	63.6 ± 45.2	35.0 ± 14.0	56.3 ± 31.7

Results

A total of 48 patients were enrolled in the study, including 12 who received a single dose of study drug and 36 who received multiple doses. Multiple-dose patients across treatment groups were generally similar in their demographic characteristics and measures of disease severity, given the small number of patients in each group (Table 1). The population as a whole reflected the severity of illness of septic patients commonly seen in intensive care units. Cytokine (TNF-α and IL-6) concentrations at baseline varied widely among individuals and between treatment groups. All patients received multiple concomitant drugs including antimicrobials and other medications as a part of overall medical support during the study.

Mean serum afelimomab concentrations following either single (Fig. 1A) or multiple infusions (Fig. 1B) appeared to be dose-related. Pharmacokinetic parameters were determined from those multiple-dose patients who had sufficient numbers of blood samples for model fitting ($n = 24$). In multiple-dose patients, measures of systemic exposure (mean AUC, AUMC, and C_{max}) increased with increasing afelimomab dose (Table 2). The mean AUC appeared to be dose proportional across the three dose groups (0.3, 1.0, and 3.0 mg/kg; $r^2 = 0.675$). Measures of drug elimination (mean CL, Vss, MRT, and $t_{1/2}$) were comparable across dose groups with no apparent dependence on dose. The mean elimination $t_{1/2}$ for all afelimomab-treated patients was 44.7 h (range 16.5–150.9), with the high variability in part due to the wide range in final sampling times (120–504 h). However, CL and AUC estimations were not affected significantly by the variations in terminal $t_{1/2}$ as the contribution to the AUC from the serum levels beyond 120 h is minimal.

Serum TNF-α and IL-6 concentrations were highly variable both at baseline and throughout the study, and

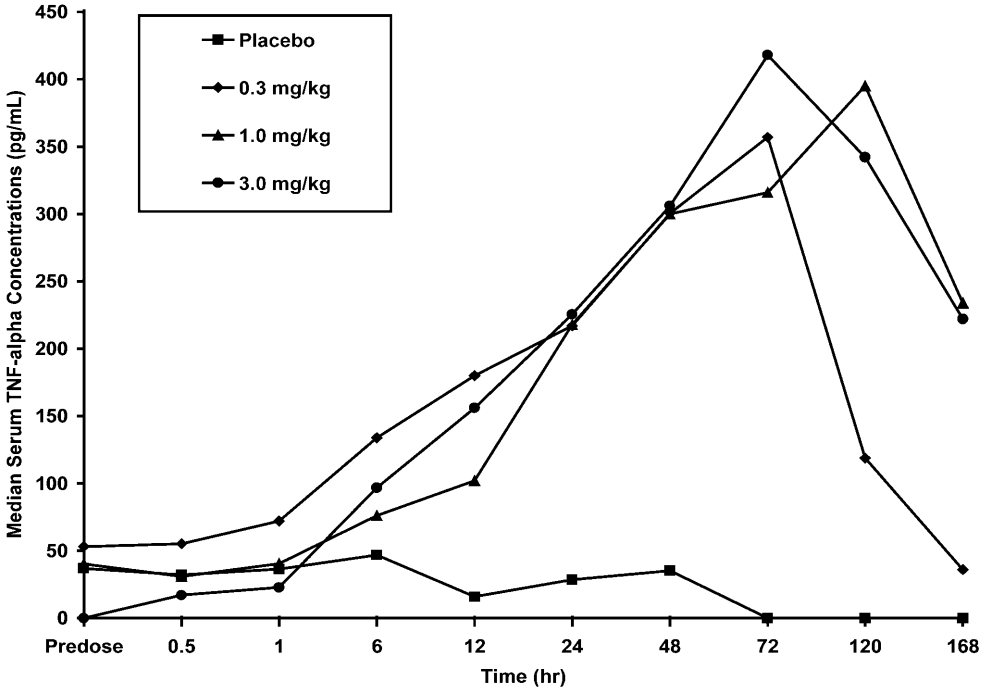

Fig. 2 Median serum TNF-α concentrations in patients receiving multiple doses of afelimomab or placebo

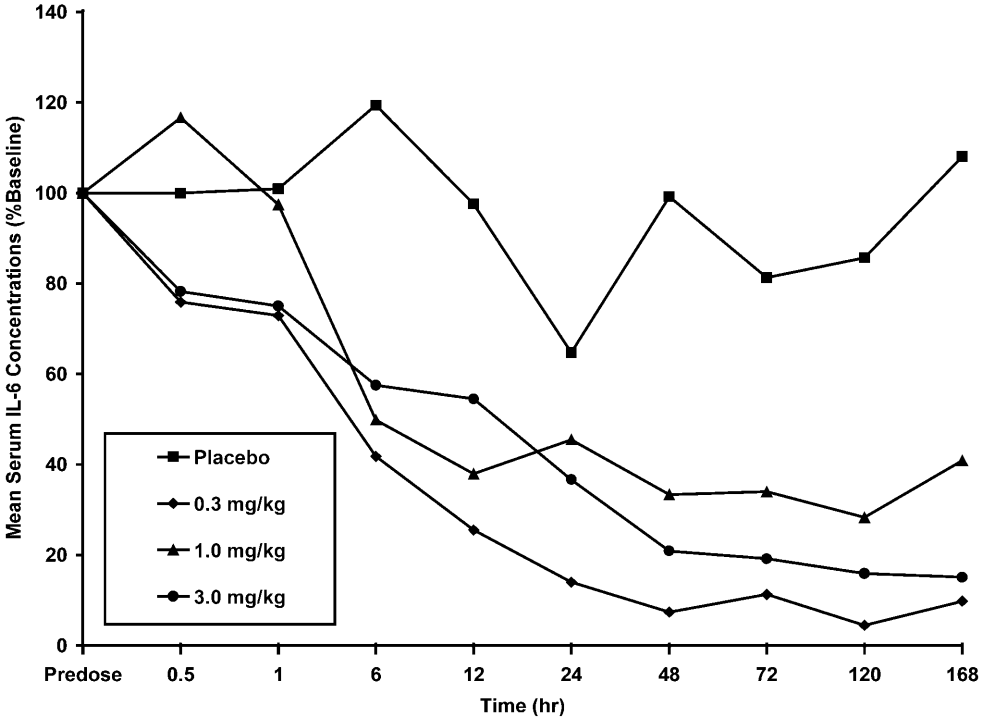

Fig. 3 Mean percentage of baseline serum IL-6 concentrations in patients receiving multiple doses of afelimomab or placebo

log transformation was used to compensate for the skewed distribution. The TNF-α concentration was below the limit of quantitation at baseline in 20 of the 48 (42 %) single and multiple-dose patients. However, in all patients with measurable TNF at baseline, there was a weak positive correlation between log transformed baseline TNF-α and IL-6 concentrations ($r^2 = 0.510$). The median serum TNF-α concentration for the placebo group changed little over the 168-h (7 day) observation period, whereas that for all three afelimomab groups increased, beginning at 6 h and continuing until 72–120 h after the first infusion, and declined thereafter (Fig. 2). Median peak TNF-α concentrations were 357, 395, and 418 pg/ml for the 0.3, 1.0, and 3.0 mg/kg dose groups, respectively. There was little difference in TNF-α concentrations between the three groups, with the exception of the return to baseline at 168 h in the lowest afelimomab dose group.

Contrary to TNF-α, median IL-6 concentrations in all treatment groups decreased with time and were less than 100 pg/ml in each group by 168 h. This occurred in the placebo group because of the early deaths (within 12 h) of three patients with the highest IL-6 concentrations (91–1200 μg/ml). Consequently, IL-6 concentrations were analyzed in only those patients with data available for at least 72 h ($n = 30$) and were expressed as percentage of baseline value because of the wide variability between patients. In those patients who received placebo there was no discernible trend in the IL-6 concentration over time (Fig. 3). However, in patients receiving afelimomab serum IL-6 concentrations decreased within the first 12–24 h to between 10 % and 40 % of baseline, with no apparent relationship to afelimomab dose.

Although this study was not designed or powered to assess the treatment effect on mortality, survival at 28 days following the first drug infusion was recorded for multiple-dose patients. All-cause mortality was highest in the placebo group (56 %) and less in the afelimomab-treated groups (33 %, 22 %, and 22 % for the 0.3, 1.0, and 3.0 mg/kg doses, respectively). When patients were stratified by baseline IL-6 concentrations greater than or less than 1000 pg/ml, a differential effect of afelimomab treatment was suggested. Survival curves from patients with baseline IL-6 less than 1000 pg/ml show few deaths in any treatment group (Fig. 4A). However, in patients with baseline IL-6 greater than 1000 pg/ml, mortality rates appeared different among treatment groups (Fig. 4B). All patients receiving placebo and 43 % of patients receiving 0.3 mg/kg afelimomab died, but no patients receiving 1.0 mg/kg and only 25 % of patients receiving 3.0 mg/kg afelimomab died.

Mortality rates for all multiple-dose patients appeared related to clinical measures of disease severity (i.e., organ failure, presence of shock, APACHE II score, underlying condition) and age. However, the small study population made it impossible to reach definitive conclusions in this regard. When analyzed using a logistic regression model, the log of the baseline IL-6

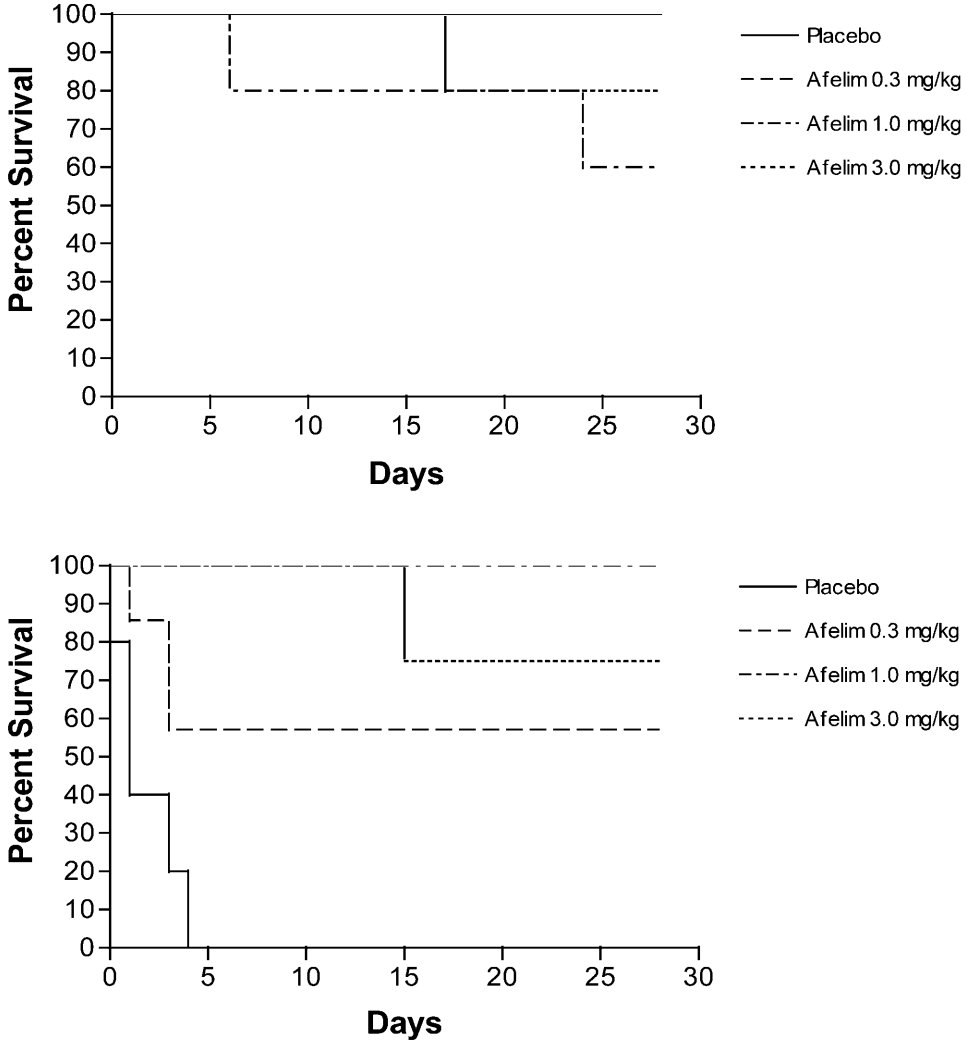

Fig. 4 Kaplan-Meier survival curves for patients receiving multiple doses of afelimomab or placebo stratified by baseline serum IL-6 concentrations < 1000 pg/ml *(above)* or ≥1000 pg/ml *(below)*

concentration was found to be significantly associated with mortality ($p = 0.001$, odds ratio = 1.72). This is reflected in the low 19% overall mortality in multiple-dose patients with baseline IL-6 concentration less than 1000 pg/ml ($n = 16$) and the 45% mortality in those with baseline IL-6 concentration greater than 1000 pg/ml ($n = 20$; Fig. 4).

Safety

All patients experienced adverse events. However, the vast majority of adverse events were judged by investigators to be unrelated to study medication, and no patient had treatment interrupted because of adverse events. The total number of adverse events reported for multiple-dose patients receiving afelimomab were 90, 129, and 92 for the 0.3, 1.0, and 3.0 mg/kg groups, respectively, and 48 for those receiving placebo.

The individual adverse events were of a wide variety expected in this patient population, and their incidence demonstrated no apparent relationship to afelimomab dose and no discernable pattern or relationship to a particular body system (Table 3). Afelimomab was well tolerated overall, and no clinical evidence of afelimomab-related toxicity (i.e., hypotension, arrhythmias, bronchospasm, skin rashes) was observed at any dose. HAMA developed in 11 of the 27 patients (41%) treated with afelimomab in a dose-dependent manner, but no sequelae attributable to the positive HAMA (including serum sicknesslike reactions, rashes, or evidence of hypersensitivity reactions) were demonstrated. The overall incidence of treatment-emergent electrocardiogram changes appeared to be greater in patients in the afelimomab dose groups, but their incidence was not dose dependent. In addition, there were no discernible differences between treatment groups in vital signs, treatment-emergent electrocardiographic changes or

Table 3 Most frequent adverse events (whether or not treatment-related) occurring in ≥20 % of patients

			Afelimomab							
	Placebo (n = 9)		0.3 mg/kg (n = 9)		1.0 mg/kg (n = 9)		3.0 mg/kg (n = 9)		All afelimomab (n = 27)	
Event	n	%	n	%	n	%	n	%	n	%
Hypotension	4	44.4	4	44.4	5	55.6	2	22.2	11	40.7
Cardiac arrest	3	33.3	2	22.2	2	22.2	1	11.1	5	18.5
Rash	2	22.2	1	11.1	2	22.2	3	33.3	6	22.2
Hypoxia	2	22.2	1	11.1	4	44.4	0	0	5	18.5
Vomiting	1	11.1	1	11.1	1	11.1	2	22.2	4	14.8
Dyspnea	1	11.1	1	11.1	1	11.1	2	22.2	4	14.8
Pneumothorax	1	11.1	0	0	2	22.2	0	0	2	7.4
Atrial flutter	0	0	0	0	2	22.2	0	0	2	7.4
Pharyngitis	0	0	2	22.2	0	0	0	0	2	7.4
Anxiety	0	0	1	11.1	0	0	2	22.2	3	11.1
Supraventricular extrasystoles	0	0	1	11.1	2	22.2	0	0	3	11.1
Congestive heart failure	0	0	1	11.1	2	22.2	0	0	3	11.1
Abdominal pain	0	0	2	22.2	0	0	1	11.1	3	11.1
Edema	0	0	1	11.1	2	22.2	1	11.1	4	14.8
Generalized edema	0	0	0	0	2	22.2	2	22.2	4	14.8
Peripheral edema	0	0	3	33.3	1	11.1	0	0	4	14.8
Fever	0	0	2	22.2	1	11.1	1	11.1	4	14.8
Nausea	0	0	2	22.2	0	0	2	22.2	4	14.8
Diarrhea	0	0	2	22.2	1	11.1	2	22.2	5	18.5
Ventricular extrasystoles	0	0	2	22.2	2	22.2	1	11.1	5	18.5
Atrial fibrillation	0	0	1	11.1	3	33.3	1	11.1	5	18.5
Supraventricular tachycardia	0	0	0	0	5	55.5	1	11.1	6	22.2

laboratory values. All deaths were ascribed to the ongoing course of the patient's clinical condition.

Discussion

Treatment with single and multiple doses of afelimomab up to 3.0 mg/kg appeared safe and well tolerated in this group of septic patients. Pharmacokinetic parameters that measure systemic exposure appeared to be dose dependent, and those parameters that measure the elimination of the agent had no apparent relationship to dose. There was no indication that the single-dose pharmacokinetics differed from those with multiple dosing, and the linear pharmacokinetics reported here confirm those found in a previous phase I study in septic patients [29].

As would be expected in this critically ill population, adverse events were observed in all patients in each treatment group. However, the majority of the adverse events were considered unrelated to treatment and showed no dose dependency. All deaths were attributable to the progression of sepsis. Although HAMA developed in 41 % of patients, there were no apparent clinical sequelae and no evidence of interference with afelimomab clearance or its ability to bind TNF-α. The development of anti-murine antibodies with multiple doses of afelimomab was considerably less frequent than has been reported with single-dose treatments of full-length murine monoclonal antibodies (76–100 % of patients) [26, 30]. These data confirm those from previously reported phase I and II studies that had also revealed no indications of intolerance to single- or multiple-doses of afelimomab [21, 29].

Afelimomab binds to a single TNF epitope, neutralizing TNF activity. There was no indication that the increase in total serum TNF observed in afelimomab-treated patients represented an increase in active cytokine. A possible explanation for the rise in TNF concentration was that the Medgenix ELISA recognized several epitopes on TNF-α and thus measured both free and antibody-bound TNF-α. Moreover, the TNF-antibody complex probably had a half-life similar to that of the antibody, rather than the shorter half-life of the cytokine, accounting for the apparent increase in concentration. Consistent with this hypothesis was the return to baseline value by 168 h in the lowest afelimomab-dose group, whereas in the higher dose groups the greater amount of antibody had not yet cleared. These pharmacodynamic findings suggest that under the conditions of the present study 1.0 mg/kg was the minimally effective dose relative to TNF neutralization. Similar increases in TNF concentration have been seen in studies with other anti-TNF antibodies or a TNF receptor construct using the same assay kit or similar methodology [20, 24, 31].

It has been postulated that high serum IL-6 concentrations are inversely correlated with survival [10, 32]. In this study there was a highly significant association between high baseline IL-6 concentration and mortality. Consistent with the role of TNF in stimulating IL-6 production, TNF-α neutralization resulted in a rapid reduction in serum IL-6 concentration regardless of baseline value or afelimomab dose, whereas treatment with placebo had no discernible effect.

Although the concept of TNF neutralization is promising and has been validated in animal models of sepsis, [17, 18] clinical trials with several anti-TNF agents have not yet convincingly demonstrated survival benefit [22, 23, 24]. These disappointing results might be due in part to the inability to accurately define a circumscribed patient population most likely to benefit from anti-TNF therapy [22, 33]. Indeed, the commonly used entry criteria for sepsis trials have been the highly nonspecific cluster of symptoms and signs comprising sepsis syndrome [1].

It is reasonable to speculate that patients with an inappropriate hyperinflammatory response to an infecting organism and elevated levels of TNF would be the group likely to benefit from anti-TNF therapy, whereas those with an appropriate response to the initial insult may not be helped. While concentrations of TNF-α in local tissues may be the best measure of the degree of the proinflammatory response, they are difficult to measure, and circulating TNF concentrations at the time of diagnosis have not been shown to be reliably correlated with disease severity [34]. Circulating IL-6 has been proposed as a marker for a hyperinflammatory response. IL-6, a circulating cytokine induced by TNFα, remains elevated for several days, and its level is correlated with disease severity and can be accurately measured [35, 36, 37].

Previous studies of monoclonal antibody against TNF have looked retrospectively for evidence of a beneficial treatment effect related to baseline plasma IL-6 levels greater than 1000 pg/ml, and data from two large trials did not demonstrate an important treatment effect in patients with these IL-6 levels [22, 32]. Similarly, neither baseline plasma IL-6 or TNF levels are predictive of response to p55 TNF receptor fusion protein [38]. However, in a small study, a retrospective analysis suggested that patients with a baseline IL-6 concentration above 1000 pg/ml derived survival benefit with afelimomab therapy [21].

In conclusion, the predictable pharmacokinetics, lack of overt immune reactions, and few treatment-related adverse events demonstrate that afelimomab is safe and well tolerated. The preliminary analysis of a recently completed randomized placebo-controlled trial ($n = 2634$) indicates that afelimomab treatment of septic patients significantly reduced risk-adjusted mortality (41.5 % versus 48.4 %, $p = 0.041$, for afelimomab and placebo, respectively) in patients with elevated baseline IL-6 levels.

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