Open-Label, Dose Escalation Study of the Safety and Pharmacokinetic Profile of Tefibazumab in Healthy Volunteers

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Tefibazumab (Aurexis) is a humanized monoclonal antibody being evaluated as adjunctive therapy for the treatment of *Staphylococcus aureus* infections. This open-label, dose escalation study evaluated the safety and pharmacokinetics of tefibazumab in 19 healthy volunteers aged 18 to 69 years. Each subject received a single administration of tefibazumab at a dose of 2, 5, 10, or 20 mg/kg of body weight infused over 15 min. Plasma samples for pharmacokinetic assessments were obtained before infusion as well as 1, 6, 12, and 24 h and 3, 4, 7, 21, 28, 42, and 56 days after dosing. Plasma concentrations of tefibazumab were detected 1 h after the end of the infusion, with a mean maximum concentration of drug in serum ($C_{\max}$) of 59, 127, 252, and 492 $\mu\text{g/ml}$ following doses of 2, 5, 10, and 20 mg/kg, respectively. The median time to maximum concentration of drug in serum ($T_{\max}$) was 1.0 h for each dose. The mean elimination half-life ($t_{1/2}$) was approximately 22 days. The volume of distribution (V) was 4.7, 6.7, 7.2, and 7.2 liters after doses of 2, 5, 10, and 20 mg/kg, respectively. Clearance (CL) was 6.0, 9.2, 10.2, and 9.9 ml/hr, respectively. At the highest dose, plasma levels of tefibazumab were $>100 \mu\text{g/ml}$ for 21 days. On day 56, the mean plasma concentrations were 6.3, 10.0, 16.4, and 30.5 $\mu\text{g/ml}$ for the 2, 5, 10, and 20 mg/kg doses, respectively. Tefibazumab exhibited linear kinetics across doses of 5, 10, and 20 mg/kg. No anti-tefibazumab antibodies were detected after dosing in any subject. There were no serious adverse events, and tefibazumab was well tolerated over the entire dose range.

Infections due to *Staphylococcus aureus* continue to be a major medical and public health problem. Despite presently available antibiotics and infection control measures, mortality rates have not declined over the past 15 years (4). Factors that contribute to the growing problem of staphylococcal infections include an increased use of intravascular devices, increased incidence of antibiotic-resistant strains of *S. aureus* in both hospital and community-acquired infections, and limitations of present antibiotic therapies. Within the spectrum of numerous infections caused by *S. aureus*, bacteremia carries a significant burden of morbidity and mortality by causing septic shock or seeding secondary sites and/or medical devices.

While the introduction of new antibiotics for the treatment of methicillin-resistant *S. aureus* (MRSA) is important, this strategy has limitations. For example, shortly after the introduction of linezolid, a new antibiotic for the treatment of methicillin-resistant *S. aureus* and vancomycin-resistant enterococcal infections, strains of linezolid-resistant *S. aureus* were identified (10, 15). The ever-changing epidemiology and ecology of *S. aureus* and the continued morbidity and mortality associated with infections due to *S. aureus* highlight the need for novel therapies in addition to present antimicrobial approaches.

MSCRAMM (for “microbial surface components recognizing adhesive matrix molecules”) proteins are a family of cell surface adhesins that recognize and specifically bind to distinct extracellular matrix components within host tissues or to serum-conditioned implanted biomaterials such as catheters, ar-

tificial joints, and vascular grafts (2, 9). Once *S. aureus* successfully adheres to and colonizes host tissues, the expression of specific genes is altered, resulting in a phenotype that is more resistant to antibiotics (1). Therefore, interventions that impact early events in the infectious process may lead to an improved clinical outcome.

Clumping factor A (ClfA) is an MSCRAMM protein expressed by *S. aureus* that promotes binding of fibrinogen and fibrin to the bacterial cell surface (5, 6). The biological role of ClfA as a virulence factor and the therapeutic benefit of anti-ClfA antibodies have been evaluated in experimental animal models of septic arthritis and infective endocarditis (3, 8, 12, 13). These data indicate that ClfA is a valid target for the development of novel immunotherapeutic agents.

Tefibazumab is a humanized monoclonal antibody (immunoglobulin G1 kappa) with a variable antigen binding region composed of human ($>98\%$) and murine ($<2\%$) amino acid sequences. It specifically recognizes and has a high affinity for ClfA expressed by *S. aureus*.

We conducted a single-center, open-label, dose escalation, phase I study to evaluate the safety and pharmacokinetic profile of tefibazumab after single intravenous doses to healthy adults.

MATERIALS AND METHODS

Patients. Healthy men and nonpregnant women who were 18 to 70 years old, inclusive, were eligible to participate if they signed an informed consent, had a body mass index of 19 to 30 (inclusive), had a negative urine drug screen, and had negative blood screening results for hepatitis B surface antigen, hepatitis C antibody, and human immunodeficiency virus antibody. Subjects were excluded if they had an active medical condition that was under evaluation or treatment, had had major surgery within 4 weeks before dosing, or had a history of hypersensitivity to immune globulin preparations. All medications except oral contraceptives, nonprescription analgesics, or vitamins were discontinued at least 2

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TABLE 1. Summary of treatment-emergent adverse events by dose cohort

Event category	No. of adverse events in dose cohort				
	2 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg	Total
Possibly related adverse event					
Headache	1	1	0	1	3
Neutropenia	0	0	0	1	1
Gastroesophageal reflux disorder	1	0	0	0	1
Erythema	0	1	0	0	1
Severity					
Mild	11	9	7	3	30
Moderate	1	0	0	0	1

weeks before dosing. Subjects could not receive an investigational drug or donate blood within 30 days or consume alcohol within 72 h before dosing. The use of additional medications, immune globulins, blood products, or immunizations was avoided, where possible, for the 56-day course of the study. The protocol and written informed consent form were approved by the institutional ethics committee before the study was initiated.

Randomization. cohorts of four subjects were sequentially assigned to one of four escalating dose cohorts (2, 5, 10, or 20 mg of tefibazumab per kg of body weight).

Study drug. Tefibazumab for injection was supplied as a sterile, nonpyrogenic, phosphate-buffered solution at a concentration of 10 mg/ml. Each subject received a single dose by intravenous infusion over 15 min through a 0.22-micron-pore-size, low-protein-binding, in-line filter. Subjects were closely monitored, and the infusion was permanently discontinued for intravenous line extravasation or any signs of anaphylaxis. Subjects who did not receive a complete dose were replaced.

Safety monitoring. The first cohort received tefibazumab at a concentration of 2 mg/kg. Enrollment of sequential, dose-escalating cohorts proceeded following review of day 7 safety data from the prior cohort. Clinical adverse events (AE) were graded as mild, moderate, or severe-serious. Two additional subjects were added to a cohort if one of the four initial subjects experienced a moderate AE considered at least possibly related to tefibazumab. Dose escalation was halted, defining the maximum tolerated dose, if two or more subjects in a cohort experienced moderate AEs or if any subject experienced a serious adverse event that was possibly or definitely related to the study drug.

Screening evaluations included medical history, physical examination, clinical laboratory assessments, electrocardiogram, chest X ray, urine drug screening, hepatitis and human immunodeficiency virus screening, and pregnancy test, if applicable. Physical examinations were repeated on days 7 and 56, and pregnancy testing was repeated on day 56, for women of childbearing potential. Subjects were monitored during and for 24 h following the infusion of tefibazumab in a clinical research unit. Subjects returned to the unit for follow-up evaluations on study days 3, 4, 7, 14, 21, 28, 42, and 56.

Blood samples were obtained for analysis of anti-idiotypic antibodies to tefibazumab using an enzyme-linked immunosorbent assay at screening and on days 28 and 56. Anti-tefibazumab antibody titers were determined using twofold dilutions covering a range of 1:10 to 1:20,480. The assay included four positive and four negative controls. The cut-point value to identify positive anti-tefibazumab plasma samples was defined as the mean absorbance value of 1:10 dilutions of the negative controls and plate blanks plus three standard deviations.

Pharmacokinetic parameters. The plasma concentration of tefibazumab was determined using an enzyme-linked immunosorbent assay that was linear ($r^2 = 0.999$) between the lower (2 ng/ml) and upper (125 ng/ml) limits of quantitation. Three quality control samples (12.5, 37.5, and 93.8 ng/ml) were included in each run. The coefficient of variation was 3 to 16% over 3 days of analysis. If necessary, plasma samples were diluted with phosphate-buffered saline, 0.1% bovine serum albumin, and 0.05% Tween so that the measured concentrations were within the linear range.

Plasma concentration-time data were used to determine peak concentration of drug in serum (C_{max}), time to peak concentration of drug in serum (T_{max}), minimum concentration of drug in serum (C_{min}), area under the plasma concentration-time curve (AUC) from immediately prior to dosing (time 0) to the last sample [$AUC_{(0-last)}$] and extrapolated to infinity [$AUC_{(0-inf)}$], the apparent terminal elimination half-life ($t_{1/2}$), volume of distribution (V), and systemic clearance (CL). Actual sampling times were used for the calculation of pharmacokinetic parameters, and nominal sampling times were used for the creation of

mean concentration tables and mean concentration-time figures. All calculations were based on nonmissing plasma concentrations. Pharmacokinetic calculations were performed using a noncompartmental model (WinNonlin software program; Pharsight Corporation, Cary, N.C.). Dose proportionality was investigated by plotting the point estimate of the mean $AUC_{(0-last)}$ and C_{max} by dose. Formal statistical analysis was not performed, because the dose range and the number of subjects in each cohort were too small for meaningful analysis.

RESULTS

Enrollment. The planned study enrollment was 16 subjects divided into four cohorts. One subject in cohort 1 (dosage, 2 mg/kg of body weight) developed a headache of moderate intensity that was considered to be possibly related to the administration of the study drug. Two additional subjects were enrolled into cohort 1. One subject in cohort 2 experienced an extravasation of study drug and was not included in the pharmacokinetic assessments. An additional subject was enrolled as a replacement. Thus, 19 subjects participated in the study and were included in safety analyses. Eighteen subjects received complete doses and were included in pharmacokinetic analyses.

The study population consisted of 7 women and 12 men ranging in age from 18 to 69 years (mean $\pm$ standard deviation, 41.6 ± 17.9 years; median, 41 years). Sixteen subjects were Caucasian, 2 were African-American, and 1 was Hispanic-Caucasian. The mean body mass index was 25.9 ± 2.7 , with a range of 20.0 to 29.0 and a median of 26.0.

Safety results. With the exception of the infusion stopped due to extravasation, all infusions of study drug were completed without interruption, and all were well tolerated. Table 1 summarizes the distribution of adverse events by cohort for AEs that were at least possibly related to study drug administration and by severity grade for all AEs regardless of the relationship to the study drug.

Treatment-emergent AEs, regardless of the relationship to the study drug, were reported by 14 of 19 (73.7%) subjects. Of the 31 treatment-emergent AEs, 30 were mild and 1, that of a subject with a headache treated with acetaminophen, was moderate. Headache was reported by four subjects, one in each dose cohort, and back pain was reported by two subjects in cohort 3. No other treatment-emergent AE was reported by more than one subject.

A total of 6 of 31 (19.4%) treatment-emergent AEs were considered to be possibly related to the administration of the study drug. These AEs were reported by two subjects each in cohorts 1, 2, and 4 and included headache (three subjects),

FIG. 1. Mean plasma concentration of tefibazumab following single intravenous doses of 2 (●), 5 (○), 10 (■), or 20 (□) mg/kg of body weight to healthy subjects.

neutropenia, gastroesophageal reflux, and erythema (one subject each). No clinical laboratory findings were considered to be clinically significant or related to the study drug. The subject who experienced neutropenia had on-therapy values that were marginally lower than screening values and the lower limit of the normal range ($1.8 \times 10^9/\text{liter}$). This subject's absolute neutrophil count decreased from $3.0 \times 10^9/\text{liter}$ on day -15 to $1.8 \times 10^9/\text{liter}$ on day -7 and $1.5 \times 10^9/\text{liter}$ on day 6 before returning to normal on day 13 without intervention. Total white blood cell counts for this subject decreased from $5 \times 10^9/\text{liter}$ on day -15 to 3.7 and $3.0 \times 10^9/\text{liter}$ on day -7 and day 6, respectively, before increasing to within normal limits. Erythema was described as scattered pinprick red points on both cheeks that started 12.5 h after dosing and resolved within 3 days without intervention. Although the investigator indicated it was unlikely to be related to study drug, a causal relationship could not be ruled out definitively.

Pharmacokinetic evaluation. Plasma concentrations of tefibazumab were detected in all samples beginning 1 h after dosing (first postdose collection) through the final sample collection on day 56. For each dose, the mean plasma concentra-

tion was largest 1 h after dosing and gradually diminished throughout the sampling interval (Fig. 1). The last sampling times at which the mean plasma concentration of tefibazumab was $>100 \text{ g/ml}$ were 12, 168, and 336 h for the 5, 10, and 20 mg/kg doses, respectively. For the 2 mg/kg dose, the mean plasma concentration was $<100 \text{ µg/ml}$ at all sampling times.

The C_{max} and $\text{AUC}_{(0-\text{inf})}$ appeared to increase proportionally with tefibazumab doses of 5, 10, and 20 mg/kg, but the clearance and volume of distribution results were similar across all doses. The median T_{max} was 1.0 h for each dose, and the mean elimination $t_{1/2}$ was approximately 22 days (Table 2). The $t_{1/2}$ was calculated from the beta phase using the data points that describe the terminal log-linear decline in the regression. Only concentrations after C_{max} , with a minimum of three points, were used to describe the elimination phase. This occurred approximately between 7 and 56 days postdose.

All plasma samples collected on day 28 and day 56 were negative for anti-tefibazumab antibodies, indicating that the study subjects had no detectable immune response to tefibazumab.

TABLE 2. Mean pharmacokinetic parameters by dose

Parameter	Mean result $\pm$ SD for tefibazumab dose (mg/kg):			
	2 (n = 6)	5 (n = 4)	10 (n = 4)	20 (n = 4)
C_{max} (µg/ml)	59 ± 7	127 ± 10	252 ± 33	492 ± 79
T_{max} (h) ^a	1.0 (0.8–48.8)	1.0 (1.0–6.0)	1.0 (1.0–1.0)	1.0 (1.0–6.0)
$\text{AUC}_{(0-\text{inf})}$ (h · mg/ml)	25.5 ± 8.3	47.2 ± 2.7	82.5 ± 10.8	15.8 ± 13.5
Elimination $t_{1/2}$ (days)	23.5 ± 4.6	21.0 ± 3.7	20.7 ± 3.9	21.3 ± 2.8
CL (ml/h)	6.0 ± 1.9	9.2 ± 0.8	10.2 ± 1.4	9.9 ± 1.8
V (liter)	4.7 ± 1.2	6.7 ± 1.3	7.2 ± 1.0	7.2 ± 1.2

^a Median (minimum-maximum).

DISCUSSION

The increased incidence of antibiotic-resistant strains of *S. aureus* in both nosocomial and community-acquired infections and the significant morbidity and mortality associated with these infections highlight the need for novel therapies in addition to present antimicrobial approaches. The reduced infectivity of isogenic mutants unable to express ClfA in animal models of septic arthritis and infective endocarditis and protection against *S. aureus* septic arthritis and sepsis-induced death afforded by passive immunization of mice with anti-ClfA antibodies (3, 8, 12, 13) indicate that ClfA is a valid target for the development of novel immunotherapeutic agents.

Tefibazumab was well tolerated following administration of single intravenous doses of 2, 5, 10, and 20 mg/kg of body weight to healthy adults. All but one of the treatment-emergent AEs was classified as mild, and 25 of 31 treatment-emergent AEs were considered not to be related to the administration of the study drug. None of the subjects experienced dose-limiting toxicity; therefore, the maximum tolerated dose of tefibazumab was not established in this study.

There was no evidence of dose-related adverse events in this small study. The numbers of both treatment-emergent AEs and those considered to be at least possibly related to the study drug administration were distributed across the dose cohorts and did not appear to be associated with the dose of tefibazumab. No subject experienced a serious adverse event during the study. No clinically significant abnormalities were observed in clinical laboratory tests.

Dose-proportional increases in $C_{\max}$ and $AUC_{(0-\text{inf})}$ were observed following the administration of doses of 5, 10, and 20 mg/kg, but the V and CL were similar across all doses. These results indicate that tefibazumab exhibited linear pharmacokinetics across the dose range of 5 to 20 mg/kg. The relatively small V value may indicate that tefibazumab enters the interstitial fluid but does not cross the cell membrane into the intracellular fluids.

Plasma concentrations of tefibazumab were detected 1 h after dosing and remained detectable through day 56, the first and last collection points after dosing, respectively. The prolonged $t_{1/2}$ of tefibazumab is characteristic of immunoglobulin G monoclonal antibodies (7, 11, 14). The time during which the mean plasma concentration was $>100 \mu\text{g/ml}$ increased as the dose increased. This may prove to be an important consideration in dose selection. In the rabbit infectious endocarditis model, doses of tefibazumab that provided serum concentrations $>100 \mu\text{g/ml}$ at all times after dosing afforded significant reductions in bacteremia and the bacterial colony counts in the heart vegetation, kidney, and, in one study, the spleen (unpublished data).

The results of this phase I dose escalation study and the nonclinical data suggesting that tefibazumab may be effective

in preventing and treating staphylococcal infections indicate that further investigation of this novel immunotherapeutic agent is warranted.

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