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Efficacy and Safety of AFN-1252, the First *Staphylococcus*-Specific Antibacterial Agent, in the Treatment of Acute Bacterial Skin and Skin Structure Infections, Including Those in Patients with Significant Comorbidities

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This open-label noncontrolled, phase II multicenter trial was designed to evaluate the safety, tolerability, and efficacy of 200 mg of AFN-1252, a selective inhibitor of *Staphylococcus aureus* enoyl-acyl carrier protein reductase (FabI), given by mouth twice daily in the treatment of acute bacterial skin and skin structure infections (ABSSSI) due to staphylococci. Important aspects of the current study included a comparison of early response efficacy endpoints with end-of-treatment and follow-up endpoints. Many patients in the intent-to-treat population ($n = 103$) had significant comorbidities. The overall early response rate at day 3 was 97.3% (wound, 100%; abscess, 96.6%; cellulitis, 94.4%) in the microbiologically evaluable (ME) population. Within the ME population, 82.9% of patients had a ≥20% decrease in the area of erythema, and 77.9% of patients had a ≥20% decrease in the area of induration, on day 3. *S. aureus* was detected in 97.7% of patients ($n = 37$ patients with methicillin-resistant *S. aureus* [MRSA], and $n = 39$ with methicillin-sensitive *S. aureus* [MSSA]). No isolates had increased AFN-1252 MICs posttreatment. Microbiologic eradication rates for *S. aureus* were 93.2% at short-term follow-up (STFU) and 91.9% at long-term follow-up (LTFU) in the ME population. Eradication rates for MRSA and MSSA were 91.9% and 92.3%, respectively, at STFU and 91.9% and 89.7%, respectively, at LTFU. The most frequently reported drug-related adverse events, which were mostly mild or moderate, were headache (26.2%) and nausea (21.4%). These studies demonstrate that AFN-1252 is generally well tolerated and effective in the treatment of ABSSSI due to *S. aureus*, including MRSA. (This study has been registered at ClinicalTrials.gov under registration no. NCT01519492.)

Widespread overuse of broad-spectrum antibiotics over the past 20 years has been associated with significant rates of antibiotic resistance, with multidrug resistance prevalent among a broad range of bacterial pathogens (1), including *Staphylococcus aureus*. Many experts have advocated the development of species-targeted agents which reduce off-target selection pressures on the human microbiome and could potentially decrease such conditions as antibiotic-induced colitis and candidiasis (2). Antiinfectives that specifically target *Staphylococcus* spp. may be less likely to lead to the development of resistant enterococcal, pneumococcal or other common bacterial pathogens.

FabI catalyzes the last step in the essential bacterial fatty acid biosynthetic pathway and is the sole form of enoyl-acyl carrier protein (ACP) reductase present in *S. aureus*, *Staphylococcus epidermidis*, and other staphylococci (3, 4, 5, 6). Other enoyl-ACP reductase enzymes are present in many bacterial species, but these are sufficiently different to enable species-specific selectivity (7). AFN-1252, a FabI inhibitor, is the result of a program tasked with finding a new antimicrobial agent specifically active against *Staphylococcus* spp., including methicillin-resistant *S. aureus* (MRSA) (8).

The objective of the current proof-of-concept study was to investigate the efficacy and safety of orally administered AFN-1252 in patients with acute bacterial skin and skin structure infection (ABSSSI) due to staphylococci. The study design followed recent FDA guidance (9) on the development of drugs for the treatment of ABSSSI. The inclusion criteria were designed to identify patients with a high likelihood of having a proven staphylococcal infection to ensure a high number of microbiologically evaluable patients.

MATERIALS AND METHODS

Antibacterial agent. AFN-1252 (free base, AFN-12520000) was formulated as immediate-release (IR) tablets.

Study design. This noncontrolled, open-label, phase II trial was designed as a proof-of-concept study to evaluate the safety, tolerability, and efficacy of 200 mg of AFN-1252 given orally twice daily in the treatment of ABSSSI due to staphylococci. Patients with clinically documented diagnoses of ABSSSI (abscess, wound infections, and cellulitis) were screened at 15 sites throughout North America using a Gram stain of lesion samples to identify approximately 100 patients with infections likely to be due to staphylococci. Investigators had the option of adding a nonstaphylococcal active agent which would cover *Streptococcus* spp. if they deemed it necessary. The study consisted of a screening period, which included the baseline visit (day 1); a maximum treatment period of 14 days (28 doses), including 3 visits (day 3, day 5, and end-of-treatment (EOT); and a fol-

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low-up period, which included a short-term follow-up (STFU) visit (4 to 10 days after EOT) and a long-term follow-up (LTFU) visit (11 to 17 days after the STFU). A test-of-cure (TOC) evaluation was conducted at the STFU visit. The EOT visit could be conducted as early as after 5 days of treatment (10 doses of treatment) or after a clinically appropriate duration of treatment (up to 28 doses, depending upon the clinical condition and clinical response). For those patients who had an outcome of clinical cure at TOC, the durability of response was determined at the LTFU.

Main criteria for inclusion. Eligible patients were male or female, were 18 to 70 years of age, and had moderate or severe ABSSSI with a lesion characterized as a wound infection, an abscess, an acutely infected burn, or cellulitis. Additionally, patients had to demonstrate at least two standard defined symptoms of systemic inflammation or complicating factors. The primary lesion had to be 75 cm^2 or greater in area and had to have at least 3 of the following: purulent or semipurulent drainage or discharge, erythema, fluctuance, induration or edema, and heat or localized warmth. Patients had to have a Gram stain with Gram-positive cocci in clusters or a PCR result indicating an infection with staphylococci. Microbiological samples were obtained from purulent wound exudates, skin lesion biopsy samples, tissue samples, or aspirates of abscess cavities.

Although the study was designed with this enrichment of patients who had staphylococcal infections to ensure a large microbiologically evaluable patient population, the protocol was amended such that an antistreptolysin O (ASO) titer was performed on the last 35 patients enrolled into the study. Patients who had failed previous treatment were eligible if cultures were still positive at the time of the baseline visit.

Patient populations. Five patient populations were included in the analyses: the intent-to-treat (ITT) comprised all patients who received one or more doses of study drug; the modified intent-to-treat (MITT) comprised all ITT patients who had a staphylococcal isolate at baseline; the per-protocol (PP) population included all patients who met eligibility criteria, completed the study, and had a positive baseline culture for *Staphylococcus* spp.; the clinically evaluable (CE) population was defined as all ITT patients who had completed at least 5 days (10 doses) of treatment as a success or at least 2 days (4 doses) of treatment if classified as a failure, had taken at least 80% of prescribed doses, did not require any prohibited antistaphylococcal antibiotics, and had completed the STFU visit; and the microbiologically evaluable (ME) population comprised all CE patients who had staphylococci plus any other bacterial pathogen isolated at baseline. The MITT and ME populations were of particular importance in this study due to the emphasis on determining efficacy in patients with confirmed staphylococcal infections.

Efficacy. Efficacy was evaluated on day 3 and at the EOT, STFU, and LTFU visits. The primary efficacy endpoints were the number of patients with staphylococci isolated at baseline with an early response of the lesion to treatment on day 3 and the number of patients determined to be clinical failures.

Lesion size was determined by direct measurements of the investigators and by tracing the lesion size on acetate sheets and sending the acetate sheets to a central lab for analysis. Digital photography and thermography were also performed to corroborate the investigator measurements and diagnoses. Early response was defined by the resolution or absence of fever ($<$38.0°C) at 3 consecutive recordings within the previous 24 h or, initially, by a decrease, or at least no increase, in the lesion measurement of total area, the size of erythema and edema/induration, and/or the amount of purulent drainage on day 3. Our criteria for early response were later modified in line with the FDA guidance, in which early response is defined as a $\geq$20% reduction of lesion size.

Clinical failures were patients who received at least 4 doses of study drug and met any 1 of the following criteria: (i) died from any cause after the start of treatment; (ii) required incision and drainage of the ABSSSI site that was not anticipated and completed within the first 48 h of treatment or was not specified in the protocol; (iii) demonstrated lack of clinical response, defined as persistent or increasing purulent drainage, increasing area of erythema, persistent fever, progression of the area of induration, or pain that continued without evidence of improvement relative to baseline, at day 5; (iv) required rescue antibacterial medication or initiation of nontrial antibacterial medications for treatment of the primary ABSSSI lesion due to staphylococci; (v) required non-protocol-specified antibacterial medications for treatment of any other infection, unless there was laboratory documentation that the antibacterial medication added to the study drug did not have demonstrable antibacterial activity in the treatment of staphylococci associated with the ABSSSI; or (vi) were assessed by the investigators as a clinical failure at the STFU visit.

Secondary efficacy endpoints included investigator assessments of the clinical outcome at EOT, STFU, and LTFU, the composite assessment of clinical outcome (CACO), the microbiological outcome at STFU and LTFU, the time to resolution of the primary lesion, and the durability of clinical response.

The investigator assessment of clinical outcomes was defined as follows: (i) cure: evidence of at least partial response of the skin infection at 48, 72, and 96 h of treatment and resolution of the infection at EOT, STFU, and LTFU; (ii) improved: some symptoms remain, but patient improved to an extent that no additional antibiotic treatment was necessary. (Improved responses were considered failures for purposes of the primary analysis.); (iii) failure: additional, nonstudy, or rescue antibiotics required because of lack of efficacy after $\geq$4 doses of study medication or because of treatment-related AEs, or because antibiotic therapy was required for longer than 28 doses, and/or the need for unplanned surgical intervention $>$48 h after study entry; or (iv) indeterminate: response could not be assigned, because an assessment was not completed at the EOT, STFU, and LTFU visits. Indeterminate responses were considered failures for purposes of the primary analysis.

The CACO by infection type was defined as a combined outcome using the early response of the lesion to treatment at day 3 and the investigator assessment of clinical outcome at the STFU visit.

Microbiological outcomes were determined at the STFU and LTFU visits for all patients who had isolated *S. aureus* at baseline, by certain virulence factors (e.g., Panton-Valentine leukocidin [PVL]) and by susceptibility testing. Microbiological outcomes were defined as follows: (i) eradicated: baseline pathogen absent in follow-up cultures of the original site of infection; (ii) presumed eradicated: no material available for culture, and patient had clinical response of cure; (iii) persisted: baseline pathogen present in follow-up cultures of the original site of infection; (iv) presumed persisted: no material available for culture, or no culture obtained, and patient had clinical response of failure; (v) superinfection: new pathogen cultured from original site of infection during therapy, in the presence of signs and/or symptoms of infection; or (vi) new infection: new pathogen cultured from original site of infection after therapy in the presence of signs and/or symptoms of infection.

Durability of clinical response was determined at the LTFU assessment for those patients achieving clinical success at STFU/TOC. The rates of relapse, recurrence, and superinfection were also reported. Patients defined as clinical responders at the early efficacy time points (days 3 and 5) who then required rescue therapy after these assessments were counted as clinical failures at the later time points (EOT, STFU, and LTFU).

Safety. Safety was assessed from adverse events (AEs), clinical laboratory tests, vital sign measurements, electrocardiograms (ECGs), physical examination findings, and concomitant medications. Each treatment-emergent AE (TEAE), using standard criteria, was judged, to be related, possibly related, or unrelated to the study treatment and graded in severity to be mild, moderate, or severe. All AEs were followed until resolution occurred or until the investigator assessment that the patient status had returned to normal. A serious AE (SAE) was defined as any untoward medical occurrence that, at any dose, resulted in death, required inpatient hospitalization, or contributed to persistent or significant disability/incapacity.

Ethical conduct. The clinical study protocol, protocol amendment, and informed consent form were reviewed and approved by the institutional review boards prior to study initiation. The study was conducted in

TABLE 1 Patient demographics and baseline characteristics in the ITT, MITT, and ME populations

Characteristic	Value by population		
	ITT ($n = 103$)	MITT ($n = 87$)	ME ($n = 76$)
Age (y)			
Mean (SD[a])	40.6 (12.6)	40.2 (12.7)	40.6 (13.0)
Minimum, maximum	19, 64	19, 64	19, 64
Male	66 (64.1)	58 (66.7)	49 (64.5)
Mean (SD) duration of infection (days)	5.6 (3.4)	5.5 (3.5)	5.5 (3.6)
No. (%) of patients by primary infection type			
Wound infection	36 (35.0)	32 (36.8)	27 (35.5)
Cutaneous abscess	40 (38.8)	31 (35.6)	30 (39.5)
Cellulitis	28 (27.2)	25 (28.7)	20 (26.3)
No. (%) of patients by severity of infection			
Mild	3 (2.9)	1 (1.1)	0 (0.0)
Moderate	77 (74.8)	66 (75.9)	57 (75.0)
Severe	23 (22.3)	20 (23.0)	19 (25.0)
No. (%) of patients with complicating factor			
Illicit drug user	56 (54)		
HIV infection	3 (2.9)		
Hepatitis C infection	26 (25.2)		
Hepatitis B infection	2 (1.9)		
Past or present tuberculosis	4 (3.9)		
Mean (SD) baseline assessments			
Investigator measurement (cm^2)			
Area of erythema	259.7 (200.9)	264.3 (213.9)	263.4 (211.8)
Area of induration	125.0 (123.9)	126.3 (124.2)	129.7 (129.9)
Area of swelling and/or edema	205.3 (233.4)	210.8 (248.95)	212.1 (260.76)
Acetate tracing sheet assessment (cm^2)			
Area of erythema	202.6 (154.2)	205.7 (163.5)	208.4 (166.0)
Area of induration[b]	97.2 (92.5)	98.6 (92.8)	101.2 (96.9)
Area of abscess[c]	9.9 (15.1)	8.9 (10.5)	9.2 (11.0)

[a] SD, standard deviation.
[b] Values for the mean area of induration were 101, 86, and 76 cm^2 in the ITT, MITT, and ME populations, respectively.
[c] Values for the mean area of abscess were 66, 53, and 47 cm^2 in the ITT, MITT, and ME populations, respectively.

accordance with the protocol and the following current applicable regulations: U.S. 21 Code of Federal Regulations, current International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, guidelines on Good Clinical Practice, and local ethical and legal requirements. Written informed consent was obtained from each patient prior to performing any study-related assessments.

RESULTS

Patient populations. The ITT population comprised 103 treated patients (Table 1), of whom 89 (86.4%) received ≥10 doses of study drug and completed the study. Reasons for noncompletion included noncompliance, loss to follow-up, protocol violation, patient-requested withdrawals, and adverse events. A total of 56 (54.4%) patients in the ITT population reported opiate, opioid, heroin, narcotic, or intravenous (i.v.) drug use. Three patients were reported to have HIV infection. A total of 26 patients (25.2%) reported active hepatitis C virus infection, and 2 patients (1.9%) reported active hepatitis B virus infection. Infection with tuberculosis was reported in 4 patients. Other medical history included (but was not limited to) the following: alcohol abuse, ongoing opiate withdrawal syndromes, diabetes, hypertension, asthma, and hyperlipidemia. Ampicillin or amoxicillin was given in combination with AFN-1252 to 10 (9.7%) patients.

In the MITT population ($n = 87$), the mean age was 40.2 years, and the majority of patients were white (74.7%) and male (66.7%) (Table 1). The majority of infections were moderate (75.9%) or severe (23.0%), with a mean duration of infection of 5.5 days before enrollment into the study. The types of infections were wound infection (36.8%), cutaneous abscess (35.6%), and cellulitis (28.7%). There were no patients with burn infections. The average area of skin lesions exceeded 200 cm^2. Baseline investigator measurements of mean areas of erythema and induration were greater (approximately 25%) than those determined using acetate tracing sheet assessments. Similarly, the investigator baseline measurements of swelling and/or edema were also greater than the mean areas of abscess based on acetate tracing sheet measurements.

Primary efficacy analyses. The primary efficacy endpoint of early response in all populations at day 3 (based on temperature and the investigator measurements of the lesion with decrease, or at least no increase, in the lesion size) was 100% for wound infection and cutaneous abscess; the early response for cellulitis was

TABLE 2 Early response rates on day 3 based on temperature and acetate tracing sheets or investigators' measurements

Population wound infection type	Acetate tracing sheets			Investigator measurements		
	N′[a]	Response rate[b], no. (%[c])	95% CI[d]	N′[a]	Response rate[b], no. (%[c])	95% CI[d]
ITT population						
Overall	92	89 (96.7)	90.8–99.3	93	92 (98.9)	94.2–100.0
Wound infection	32	32 (100.0)	89.1–100.0	32	32 (100.0)	89.1–100.0
Abscess	36	35 (97.2)	85.5–99.9	37	37 (100.0)	90.5–100.0
Cellulitis	24	22 (91.7)	73.0–99.0	24	23 (95.8)	78.9–99.9
MITT population						
Overall	80	78 (97.5)	91.3–99.7	80	80 (100.0)	95.5–100.0
Wound infection	29	29 (100.0)	88.1–100.0	29	29 (100.0)	88.1–100.0
Abscess	29	28 (96.6)	82.2–99.9	29	29 (100.0)	88.1–100.0
Cellulitis	22	21 (95.5)	77.2–99.9	22	22 (100.0)	84.6–100.0
ME population						
Overall	73	71 (97.3)	90.5–99.7	73	73 (100.0)	95.1–100.0
Wound infection	26	26 (100.0)	86.8–100.0	26	26 (100.0)	86.8–100.0
Abscess	29	28 (96.6)	82.2–99.9	29	29 (100.0)	88.1–100.0
Cellulitis	18	17 (94.4)	72.7–99.9	18	18 (100.0)	81.5–100.0

[a] N′, the number of patients with the specified infection type who had valid body temperature and lesion assessments at baseline and at the specified time point.
[b] Response rate was defined as the no. (%) of patients with an early response on day 3, as defined by resolution or absence of fever (<38°C) at 3 consecutive recordings within the previous 24 h and cessation of spread of lesion from baseline based on acetate tracing sheets or investigator measurements.
[c] Percentages were calculated using N′ as the denominator.
[d] CI, confidence interval. 95% CIs are exact binomial confidence intervals.

95.8% in the ITT population (Table 2) and was 95.0% in the CE population. Wound infection response rates were 100% in all populations based on either acetate tracing sheets or investigator measurements. However, early response rates as determined by acetate tracing sheets were lower for abscesses and cellulitis in the ITT (97.2% and 91.7%, respectively), MITT (96.6% and 95.5%), and ME (96.6% and 94.4%) populations. In a further analysis of the ME population, 82.9% of patients had a ≥20% decrease in the area of erythema on day 3, and 77.9% of patients had a ≥20% decrease in the area of induration on day 3.

Clinical failure rates were 20.8%, 19.3%, and 18.4% for the ITT, MITT, and ME populations, respectively. For each population, the use of antibacterials for other infections was the most frequent reason for clinical failure and was reported in 13.5%, 12.0%, and 10.5% of the ITT, MITT, and ME populations, respectively.

Secondary efficacy criteria. Clinical success rates were 93.2% and 93.4% at EOT for the CE and ME populations, respectively, and ranged from 93.2% to 93.4% at STFU and 93.1% to 93.3% at LTFU (Table 3). In a composited analysis evaluating early response rates and later success rates, 77 (95.1%) patients with an early response on day 3 were also categorized as cured at STFU. Of the 7 patients not exhibiting an early response (within 72 h of treatment onset), 5 were cured at STFU. The CACO analysis also evaluated early response rates at day 3 and clinical success at the later STFU time point. This analysis demonstrated that, in the ME population, 89.5% of patients responded at both day 3 and STFU. There were no patients who failed to respond at both the early and the STFU time points. The CACO success rates in the ME population were highest (96.3%) for wound infections and lowest (75.5%) for cellulitis.

None of the patients who were considered cured at EOT or STFU had relapse or recurrence. Complete resolution of symptoms of erythema, edema, abscess, and induration were observed in a median 14 to 15 days, and complete healing of the lesion was seen at a median of 10 days. Fevers resolved in 2 days. Leukocytosis resolved in a median of 5 days, and patients resumed normal activity in a median of 7 days.

Microbiological outcomes. *S. aureus* was detected in 97.7% of patients, with similar numbers of methicillin-sensitive *S. aureus* (MSSA) ($n = 39$) and MRSA ($n = 37$) in the MITT population. In addition, 5 staphylococcal isolates were identified as *Staphylococcus capitis* ($n = 1$), *S. epidermidis* ($n = 2$), *Staphylococcus haemolyticus* ($n = 1$), or *Staphylococcus simulans* ($n = 1$). One isolate each of *Streptococcus constellatus*, *Streptococcus dysgalactiae*, *Streptococcus viridans* group, and *Enterobacter cloacae* was also detected. All *S. aureus* isolates were susceptible to daptomycin, doxycycline, linezolid, and vancomycin. For AFN-1252, the MIC_{50} for *S. aureus* (including MRSA and MSSA) was 0.008 μg/ml, and the MIC_{90} for *S. aureus* (including MRSA and MSSA) was 0.015 μg/ml.

Microbiologic eradication rates for *S. aureus* were 93.2% at STFU and 91.9% at LTFU in the ME population. Corresponding eradication rates at STFU were 91.9% for MRSA and 92.3% for MSSA. At LTFU, eradication rates were 91.9% for MRSA and 89.7% for MSSA. PVL virulence had no obvious effect upon eradication rates. The presumed persistence rates at STFU and LTFU were 8.1% and 7.7% for MRSA and MSSA, respectively, but no identified isolates were found to have increased AFN-1252 MICs posttreatment. None of the patients who were cured at EOT had a relapse, recurrence, and/or superinfection after EOT.

ASO titers were performed on the last 35 patients admitted to the study. Seven patients (20.0%) had convalescent titers >200 units/ml, which were strongly suggestive of acute streptococcal infection. Three of these patients received either amoxicillin or ampicillin. The other 4 did not receive additional antibiotics but were considered improved at EOT and cured at STFU.

Safety. Overall, 69 (67.0%) patients experienced at least 1 TEAE, and 52 (50.5%) patients experienced a drug-related TEAE

TABLE 3 Investigator assessment of clinical outcome

Assessment by time point	CE population ($n = 88$)	ME population ($n = 76$)
End of treatment		
No. (%[a]) response		
Cure	79 (89.8)	69 (90.8)
Improved	3 (3.4)	2 (2.6)
Failure	6 (6.8)	5 (6.6)
Indeterminate	0 (0.0)	0 (0.0)
Missing	0 (0.0)	0 (0.0)
Clinical success rate[b]: no./N′[c] (%[d])[e]	82/88 (93.2)	71/76 (93.4)
95% CI[f]	85.7, 97.5	85.3, 97.8
Short-term follow-up		
No. (%[a]) response		
Cure	82 (93.2)	71 (93.4)
Failure	6 (6.8)	5 (6.6)
Indeterminate	0 (0.0)	0 (0.0)
Missing	0 (0.0)	0 (0.0)
Clinical success rate[b]: no./N′[c] (%[d])	82/88 (93.2)	71/76 (93.4)
95% CI[f]	85.7, 97.5	85.3, 97.8
Long-term follow-up		
No. (%[a]) response		
Cure	81 (92.0)	70 (92.1)
Failure	6 (6.8)	5 (6.6)
Indeterminate	0 (0.0)	0 (0.0)
Missing	1 (1.1)	1 (1.3)
Clinical success rate[b]: no./N′[c] (%[d])	81/87 (93.1)	70/75 (93.3)
95% CI[f]	85.6, 97.4	85.1, 97.8

[a] Percentages for cure, improved, failure, and indeterminate responses were based on use of the number in the column heading as the denominator.
[b] Clinical success rate was defined as (no. of cures)/(no. of cures + no. of failures) × 100.
[c] N′, the number of patients with clinical outcome of cure or failure at the specified time point.
[d] Percentages for the clinical success rate were calculated using N′ as the denominator.
[e] For the end-of-treatment clinical success rate calculation, patients who had clinical outcomes of improved were classified as cures.
[f] CI, confidence interval. 95% CIs are exact binomial confidence intervals.

TABLE 4 Overview of TEAEs in the ITT population

Variable	No. (%) of patients (total $n = 103$)
Patients with at least 1 TEAE	69 (67.0)
Patients with at least 1 drug-related TEAE	52 (50.5)
Patients with TEAEs by maximum severity	
Mild	36 (35.0)
Moderate	28 (27.2)
Severe	5 (4.9)
Patients with drug-related TEAEs by maximum severity	
Mild	31 (30.1)
Moderate	17 (16.5)
Severe	4 (3.9)
Deaths	0 (0.0)
Patients with at least 1 treatment-emergent SAE	4 (3.9)
Patients with at least 1 drug-related treatment-emergent SAE	0 (0.0)
Patients who discontinued due to a TEAE	4 (3.9)[a]
Patients who discontinued due to a drug-related TEAE	2 (1.9)

[a] In the analysis of patient disposition, 1 of these 4 patients was considered a per-protocol completer due to the number of doses completed.

(Table 4). The most frequently reported TEAEs were headache (31.1%) and nausea (25.2%), followed by vomiting (7.8%), skin infection (6.8%), and pruritus (5.8%).

The majority of patients with TEAEs (64 of 69; 92.7%) experienced mild or moderate TEAEs. Five patients had severe TEAEs, of which 4 ($n = 2$, headache; $n = 1$, abdominal pain; and $n = 1$, thrombocytopenia) were reported as possibly drug related (Table 5). The one episode of thrombocytopenia occurred 2 days after the start of study drug in a 53-year-old white male smoker with a history of skin infections, liver disease, active hepatitis C virus infection, osteoarthritis, osteoporosis, rheumatoid arthritis, i.v. drug abuse, asthma, seasonal allergies, year-round allergies, headaches, and incision/drainage. His lowest platelet count was $67 \times 10^3/\mu l$ (normal range, 150 to $350 \times 10^3/\mu l$) on day 3 and returned to normal on day 5 while the study drug was continued. Study drug was continued through day 7.

One patient discontinued treatment due to the severe TEAEs of vomiting and headache, and another patient discontinued treatment due to severe headache. Both headache-associated severe TEAEs resolved within a day. Only 4 patients (3.9%), of whom 2 received concomitant antibiotics, suffered diarrhea. There were no deaths.

Four patients had treatment-emergent SAEs, all of which were considered moderate in severity and unrelated to study drug. The SAEs included drug withdrawal syndrome (due to heroin withdrawal), abscess in the neck (due to ongoing heroin injections), and cellulitis in 2 patients. Study drug was withdrawn, and other treatment was instituted for both cases of cellulitis.

Laboratory parameters were examined by shift analyses from baseline to the worst postbaseline observation. Overall, shifts in liver function tests were infrequent and generally associated with patients who had significant concurrent health problems, including hepatitis C virus infection, ongoing i.v. drug abuse, or alcohol abuse. The largest numbers of hematology shifts were seen with hematocrit, including 20 shifts from normal to high hematocrit concentrations. Twelve shifts from high to normal white blood cell counts were observed. Similarly, with neutrophil and absolute neutrophil counts, there were a number of shifts from high to normal (12 and 8 shifts, respectively); however, there also were 15 shifts from normal to high neutrophil counts and 3 shifts from normal to high absolute neutrophil counts.

Mean changes in vital signs were generally small. There was no apparent relationship observed between AFN-1252 plasma concentrations and QTc interval using Bazett's formula (QTcB) or Fridericia's formula (QTcF), and no apparent relationship was observed between predicted C_{max} at steady-state and QTcB or QTcF intervals.

DISCUSSION

This study evaluated the clinical efficacy and safety of an agent specifically developed to target only *Staphylococcus* spp., and we believe it is the first such study to do so. *Staphylococcus* is one of several major bacterial pathogens that warrant such a focus due to its involvement in a wide range of infections and rapidly emerging resistance to many antibiotics. The agent, AFN-1252, is a novel

TABLE 5 Drug-related TEAEs in ≥2% of patients (ITT population)

Preferred term by SOC[a]	No. (%) of patients with events[b] (total $n = 103$)
Blood and lymphatic system disorders	6 (5.8)
Leukopenia	4 (3.9)
Thrombocytopenia	3 (2.9)
Cardiac disorders	4 (3.9)
Tachycardia	3 (2.9)
Gastrointestinal disorders	28(27.2)
Diarrhea	4 (3.9)
Nausea	26 (25.2)
Vomiting	8 (7.8)
Infections and infestations	3 (2.9)
Fungal infection	3 (2.9)
Investigations	10 (9.7)
Alanine aminotransferase increased	4 (3.9)
Asparate aminotransferase increased	5 (4.9)
Nervous system disorders	36 (35.0)
Dizziness	5 (4.9)
Headache	32 (31.1)
Skin and subcutaneous tissue disorders	11 (10.7)
Pruritus	6 (5.8)

[a] SOC, system organ class.
[b] Although a patient may have had 2 or more TEAEs, the patient was counted only once within an SOC category. The same patient may have contributed to 2 or more preferred terms in the same SOC category.

inhibitor of the fatty acid biosynthesis pathway, specifically inhibiting the essential staphylococcal FabI enoyl-ACP reductase (8).

Current treatment options for serious staphylococcal infections, particularly where MRSA may be involved, include several established classes of anti-Gram-positive agents, but each class has limitations due to resistance, safety, or lack of an oral treatment option. Hence, there is a need not only for new agents against staphylococci but also for agents which avoid existing bacterial resistance mechanisms and offer other clinical benefits, including an improved safety profile, minimal impact on the human microbiome, and flexibility in administration formulations.

Two important aspects of the current study design included the use of newly defined early response efficacy endpoints at day 3 and screening of patients to confirm staphylococcal infection prior to enrollment to ensure a large microbiologically evaluable patient population. The study population included many with diabetes and obesity and a substantial (54.4%) number of patients who reported ongoing opiate, opioid, heroin, narcotic, or i.v. drug abuse in addition to a variety of concomitant illnesses, including HIV, hepatitis C virus, and hepatitis B virus. It is not surprising that such a population may allow their infections to be quite advanced, as evidenced by the average area of skin lesions exceeding 200 cm^2. Despite the potential compliance difficulties inherent in a population with serious comorbidities, 90 (87.4%) completed treatment, and 89 (86.4%) of the 103 patients completed the study.

Interestingly, although the early response criteria for clinical cure were developed by the FDA from data dealing with group A streptococcal infections, the approach does seem to apply to the natural history of staphylococcal skin disease as well. Although investigator measurements of the lesion size tended to be larger than those determined using acetate tracing sheets, a high degree of agreement in early response determinations at day 3 was noted. Both methods of lesion size measurement were highly predictive of clinical outcome using conventional and composite assessments at EOT, STFU, and LTFU. As such, early response measurements may have considerable potential in clinical trials of new agents with Gram-positive pathogens.

The overall response rates were high (>90%) regardless of infection type, population, and method of evaluation utilized in this study. Clinical success rates based on the investigator assessments of clinical outcome at EOT, STFU, and LTFU visits were approximately 93% overall for each population, with response rates of 100% for wound infections, 93.3% for cutaneous abscesses, and 80.0% for cellulitis at each time point. As cellulitis can be complicated by the presence of multiple organisms, the success rates for cellulitis were expected to be lower than those of other infection types. The response rate in patients with cellulitis is consistent with the rate of positive ASO titers taken in the last 35 patients enrolled into the study, with ~20% ASO titers >200 units/ml. Microbiologic eradication rates were high for *S. aureus* overall (93.2% at STFU and 91.9% at LTFU in the ME population) and within specific taxonomic groups, i.e., for MRSA and by PVL status. Response rates for AFN-1252 compare favorably with results reported for ceftaroline (10), linezolid, and tedizolid (11).

Despite the diversity of patients and the high incidence of comorbidities, AFN-1252 was generally well tolerated, but headache, nausea, and vomiting were reported. Headaches were generally considered to be mild or moderate and were transient, typically lasting 1 day. In all but 4 patients, there was no request for acetaminophen or other pain medication as treatment for discomfort. Four individuals identified headache as severe and withdrew from the study. The low incidence of loose stools or diarrhea suggests minimal impact on the gastrointestinal microbiome. Four patients had SAEs, none of which were considered to be drug related.

In conclusion, this study has shown that AFN-1252 is well tolerated and highly effective in the treatment of ABSSSI due to staphylococci, including MRSA. Early response to treatment is evident at day 3 and highly predictive of clinical outcome. These results, coupled with novel mode of action, targeted spectrum, low propensity for resistance development, and ongoing clinical development of novel oral and i.v. formulations, further support the potential for AFN-1252 as the agent of choice for the treatment of confirmed staphylococcal infections.

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