Pharmacodynamic Profile of GSK2140944 against Methicillin-Resistant *Staphylococcus aureus* in a Murine Lung Infection Model

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GSK2140944 is a novel bacterial type II topoisomerase inhibitor with *in vitro* activity against key causative respiratory pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA). We described the pharmacodynamics of GSK2140944 against MRSA in the neutropenic murine lung infection model. MICs of GSK2140944 were determined by broth microdilution. Plasma and epithelial lining fluid (ELF) pharmacokinetics were evaluated to allow determination of pulmonary distribution. Six MRSA isolates were tested. GSK2140944 doses of 1.56 to 400 mg/kg of body weight every 6 h (q6h) were utilized. Efficacy as the change in $\log_{10}$ CFU at 24 h compared with 0 h controls and the area under the concentration-time curve for the free, unbound fraction of a drug (fAUC)/MIC required for various efficacy endpoints were determined. GSK2140944 MICs were 0.125 to 0.5 mg/liter against the six MRSA isolates. ELF penetration ratios ranged from 1.1 to 1.4. Observed maximal decreases were 1.1 to 3.1 $\log_{10}$ CFU in neutropenic mice. The mean fAUC/MIC ratios required for stasis and 1-log-unit decreases were 59.3 ± 34.6 and 148.4 ± 83.3, respectively. GSK2140944 displayed *in vitro* and *in vivo* activity against MRSA. The pharmacodynamic profile of GSK2140944, as determined, supports its further development as a potential treatment option for pulmonary infections, including those caused by MRSA.

While a recent CDC surveillance study reports a decrease in health care-associated invasive methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the United States from 2005 through 2011 (1), it remains a prevalent pathogen causing hospital-acquired (HAP) and ventilator-associated pneumonia (VAP) (2) and represents a major health threat with significant mortality rates (3, 4). Until lately, most cases of health care-associated pneumonia (HCAP), HAP, and VAP were thought to be caused by the hospital-acquired MRSA (HA-MRSA) strains, but a second variant of MRSA, community-acquired MRSA (CA-MRSA), has emerged in health care settings as a cause of severe pneumonia (2, 4). While the significance of this shift in epidemiology remains unknown (2), CA-MRSA has exhibited more rapid replication (5) and greater efficiency of transfer than HA-MRSA isolates (6). Additionally, virulence factors such as Panton-Valentine leukocidin (PVL), mostly associated with CA-MRSA strains, can cause severe necrotizing pneumonia even in young, otherwise healthy individuals (4).

To cope with the emergence of these hard-to-treat multidrug-resistant organisms, the development of new classes of antibacterial agents targeting type IIA topoisomerase was prompted in recent years (7). GSK2140944 is one such agent. GSK2140944 is a novel bacterial type II topoisomerase inhibitor with a mode of action distinguished from that of fluoroquinolones currently marketed or under development (8); instead of stabilizing the DNA double-strand breaks like fluoroquinolones, it stabilizes the precleavage topoisomerase-DNA complex prior to DNA cleavage and generates single-strand breaks (7, 9). The potent inhibition of supercoiling by DNA gyrase confers GSK2140944 *in vitro* activity against key causative respiratory pathogens, including MRSA (10).

In this study, we aimed to evaluate the pharmacokinetic and pharmacodynamic properties of GSK2140944 against diverse MRSA isolates using the murine lung infection model to guide its development as a potential treatment option for pulmonary infections caused by MRSA.

MATERIALS AND METHODS

Antimicrobial test agents. GSK2140944 mesylate (powder of batch 2140944E-B-01P; GlaxoSmithKline, Collegeville, PA, USA) was used throughout these experiments. For *in vitro* studies, GSK2140944 was first dissolved in dimethyl sulfoxide (DMSO) and further diluted in sterile water followed by cation-adjusted Muller-Hinton broth to yield DMSO concentrations of ≤0.5%, consistent with Clinical and Laboratory Standards Institute (CLSI) guidelines (11). In *in vivo* studies, the GSK2140944 powder was weighed in a quantity required to achieve the desired concentration and dissolved in 0.9% sterile saline. GSK2140944 was administered by subcutaneous (s.c.) injections of 0.2 ml.

For *in vitro* MIC studies, levofloxacin analytical standard powder (Sigma-Aldrich, St. Louis, MO) was utilized, while commercially available levofloxacin for injection (Akorn, Inc., Lake Forest, IL; 25-mg/ml stock) was utilized for *in vivo* analyses. Levofloxacin was administered by s.c. injections of 0.2 ml. Linezolid was prepared from analytical standard powder (Sigma-Aldrich, St. Louis, MO) in both *in vitro* MIC studies and *in vivo* efficacy studies and was administered by s.c. injections of 0.3 ml.

Bacterial isolates. *S. aureus* 508 (GSK WCUH29) and 509 (GSK PVL-2) were provided by GlaxoSmithKline (Collegeville, PA, USA), and four out of the six MRSA isolates were provided by the Center for Anti-

TABLE 1 *In vitro* activity of GSK2140944 and comparators against MRSA isolates utilized in the murine lung infection model

S. aureus isolate	Characteristic(s)[a]	MIC (mg/liter)		
		GSK2140944	Levofloxacin	Linezolid
456	VRSA	0.125	32	1
508	MRSA	0.25	0.125	4
F40-14	MRSA	0.25	>32	1
146	USA300, PVL(+), SCC*mec* IV, CA-MRSA	0.5	8	2
156	USA300, PVL(−), *spa* type 1, SCC*mec* IV, CA-MRSA	0.5	8	4
509	USA300, PVL(+), CA-MRSA	0.5	0.125–0.25	2–4

[a] VRSA, vancomycin-resistant *S. aureus*; MRSA, methicillin-resistant *S. aureus*; PVL(+), produces Panton-Valentine leukocidin; PVL(−), does not produce PVL; SCC*mec* IV, staphylococcal cassette chromosome *mec* type IV; CA-MRSA, community-acquired methicillin-resistant *S. aureus*.

Infective Research and Development (Hartford, CT, USA), which included two CA-MRSA isolates, two isolates producing PVL, and one vancomycin-resistant strain (Table 1).

MICs for GSK2140944, levofloxacin, and linezolid were determined in triplicates (three times using three replicate samples) by broth microdilution in accordance with Clinical and Laboratory Standards Institute guidelines (11). The modal MICs were reported. Isolates were stored in skim milk (BD Biosciences, Sparks, MD) at −80°C and were subcultured twice onto Trypticase soy agar with 5% sheep blood (BAP; BD Biosciences) within 48 h prior to use.

Neutropenic murine lung infection models. Specific-pathogen-free, female BALB/c mice 7 to 8 weeks old were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). The protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Hartford Hospital, and studies were conducted in accordance with GlaxoSmithKline policy on the care, welfare, and treatment of laboratory animals. Neutropenia was induced by intraperitoneal (i.p.) injections (0.2 ml in normal saline) of cyclophosphamide (Baxter Healthcare Corp., Deerfield, IL) at doses of 250 mg/kg of body weight 4 days prior to infection and 100 mg/kg 1 day prior to infection. Pneumonia was induced by instillation of 0.05 ml suspension in 3% hog gastric mucin in normal saline containing approximately 10^7 CFU/ml of the *S. aureus* isolate into the isoflurane-anesthetized animal's oral cavity while blocking the nares and holding the mouse in a vertical position until it aspirated the fluid inoculum (12); this took 2 to 4 inhalations, and the mouse was held in this position for 5 to 10 additional inhalations. After allowing the mice to fully recover from anesthesia in an oxygen-enriched chamber, they were randomized into treatment and control groups. For randomization, individual mice were arbitrarily selected from a box containing the group of inoculated animals such that each animal had an equal likelihood of receiving control or active therapies.

Pharmacokinetic studies. (i) Blood pharmacokinetic studies. For neutropenic pharmacokinetic studies, at 3 h postinoculation (0 h), groups of 48 infected mice were administered GSK2140944 s.c. in single doses of 6.25, 50, or 200 mg/kg. Blood samples were collected from groups of six mice at 5 min and 0.25, 0.5, 1, 1.5, 2, 3, and 4 h postdose for 6.25- or 50-mg/kg doses and 5 min and 0.25, 0.5, 1, 1.5, 2, 4, and 6 h postdose for the 200-mg/kg dose via cardiac puncture; animals were euthanized by CO_2 followed by cardiac puncture and ultimately cervical dislocation. All blood samples were collected in K_2 EDTA Microtainer tubes (BD, Franklin Lakes, NJ, USA) and centrifuged to obtain plasma, which was stored at −80°C until analysis.

(ii) Pulmonary distribution. Lavage fluid was collected from both neutropenic infected mice subsequent to terminal blood collection via bronchoalveolar lavage (BAL) at 0.5, 1, 2, and 3 h postdose for mice receiving 6.25 or 50 mg/kg GSK2140944 and at 0.5, 1, 2, and 4 h postdose for mice receiving 200 mg/kg GSK2140944. BAL fluid was performed by inserting a catheter in the trachea and instilling four aliquots of 0.4 ml normal saline followed by immediate removal of this fluid as previously described (13). BAL fluid samples were centrifuged to remove blood and cellular debris. Supernatants were stored at −80°C.

(iii) GSK2140944 concentration determination. Plasma and BAL fluid concentrations of GSK2140944 were analyzed by GlaxoSmithKline (Collegeville, PA, USA) using a validated liquid chromatography-tandem mass spectrometry (LC/MS/MS) assay. A linear regression weighted by 1/concentration gave the best fit for the calibration curve over the concentration ranges of 10 to 100,000 ng/ml for plasma (coefficient of determination [r^2] = 0.997) and 1 to 5,000 ng/ml for BAL fluid (r^2 = 0.997). The accuracy of check samples (10 to 50,000 ng/ml for plasma and 5 ng/ml for BAL fluid) was within 8%.

(iv) Urea concentration analysis. Aliquots of plasma and BAL fluid were taken for the determination of urea to derive drug levels in epithelial lining fluid (ELF) (14). Urea concentrations were analyzed using a colorimetric enzymatic assay (Teco Diagnostics, Anaheim, CA, USA) via a spectrophotometric detection method (Cary 50 Series; Varian, Walnut Creek, CA, USA). The assay was linear with an r^2 of ≥0.999 for both BAL fluid and plasma urea concentrations over the range of 0.1 to 2.0 mg/dl. Quality control samples of 0.15 and 1.5 mg/dl had intraday and interday coefficients of variation of 1.68 and 0.81% and 7.3 and 3.7%, respectively.

***In vivo* efficacy.** A total of six MRSA isolates were tested in *in vivo* efficacy studies (Table 1). Treatment and control regimens were initiated 3 h postinoculation (0 h) with the test strain. GSK2140944 doses of 1.56 to 400 mg/kg were administered as 0.2-ml injections every 6 h (q6h) for 24 h. Two comparators were employed throughout the studies, levofloxacin and linezolid. A previously described levofloxacin dose of 10.6 mg/kg every 8 h (32 mg/kg/day) was utilized that simulated the area under the concentration-time curve for the free, unbound fraction of a drug (*f*AUC) profile of 500 mg once daily given in healthy volunteers (15, 16); the target *f*AUC of 42 to 53 mg · h/liter observed in healthy volunteers (15) was achieved in a mouse lung infection model with a resultant *f*AUC of 44 mg · h/liter (14) from a 32-mg/kg dose. A 5-mg/kg dose of uranyl nitrate was administered to levofloxacin-treated mice intraperitoneally 3 days prior to inoculation to predictably impair renal function of mice so that the elimination of levofloxacin in mice mimicked the human profile (15). Linezolid was administered to simulate a *f*AUC in ELF of 960 mg · h/liter, which was observed in the ELF after a 600-mg q12h dose (12, 17, 18) in healthy volunteers. A regimen of 240 mg/kg q12h was given to mimic this exposure (12, 18). Control animals received 0.2 ml of normal saline s.c. every 6 h for 24 h.

At 0 h, a group of untreated control mice ($n = 6$) was sacrificed to establish the initial bacterial burden. At 24 h postinitiation of dosing (24 h), all groups of control and treatment mice ($n = 6$ per each group) were euthanized via CO_2 inhalation, followed by cervical dislocation. The lungs were then removed and homogenized for bacterial enumeration. Dilutions in normal saline of each lung homogenate were plated on Columbia CNA agar with 5% sheep blood (BD Biosciences, Sparks, MD) using spiral plating techniques (Spiral Biotech Auto plate 4000, model AP4000) and incubated overnight at 37°C.

Data analysis. (i) Pharmacokinetics. Pharmacokinetic parameters for single doses of GSK2140944 were calculated using first-order input and elimination, by nonlinear least-squares techniques (Phoenix 32 WinNonlin version 6.3; Pharsight, Mountain View, CA). Compartment model selection was based on visual inspection of the profile and use of the correlation between the observed and calculated concentrations. The mean pharmacokinetic parameters derived from single-dose studies were used to construct concentration-time profiles for all multidose regimens

TABLE 2 Pharmacokinetics of GSK2140944 after a single subcutaneous dose in a murine (BALB/c) *S. aureus* lung infection model[a]

Model	Dose (mg/kg)	V (liter/kg)	K_{01} (h^{-1})	K_{10} (h^{-1})	$AUC_{0-\infty}$ (mg · h/liter)	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (mg/liter)
I−	6.25	2.055	10.000	1.091	2.788	0.636	0.249	2.319
	50	2.130	11.338	0.940	24.965	0.737	0.239	18.739
	200	3.959	11.953	0.453	111.417	1.529	0.285	44.406
I+	0.78	3.172	10.156	0.515	0.478	1.345	0.309	0.210
	6.25	2.262	4.500	0.688	4.018	1.008	0.493	1.969

[a] I−, neutropenic; I+, immunocompetent; V, volume of distribution; K_{01}, transfer rate constant into the central compartment; K_{10}, transfer rate constant out of the central compartment; $AUC_{0-\infty}$, AUC from 0 h to infinity; $t_{1/2}$, elimination half-life; T_{max}, time to maximal concentration; C_{max}, maximal concentration.

evaluated in the *in vivo* efficacy. The AUC was calculated using the linear trapezoidal rule formula. Free, unbound drug concentrations in plasma were calculated using a murine protein binding value of 22.46% (data on file at GlaxoSmithKline).

(ii) Pulmonary distribution. The concentration of GSK2140944 in ELF was determined using the urea dilution method:

$$GSK2140944_{ELF} = GSK2140944_{BAL} \times (Urea_{Plasma}/Urea_{BAL})$$

where GSK2140944_{BAL} is the concentration of GSK2140944 in the BAL fluid sample and Urea_{BAL} and Urea_{Plasma} are the concentrations of urea in the paired BAL fluid and plasma samples, respectively. The penetration of GSK2140944 into the ELF was estimated by the ratio of the AUC in ELF and *f*AUC in plasma calculated by the linear trapezoidal formula from observed data. For doses of 0.78, 6.25, and 50 mg/kg, area under the concentration-time curve from 0 to 3 h (AUC_{0-3}) in both plasma and ELF was used, while AUC_{0-4} was used for 200 mg/kg as directed by the different sampling times for each regimen.

(iii) In vivo efficacy. Antibacterial efficacy was calculated as the mean change in lung bacterial density in treated or control animals at 24 h compared with the average bacterial density at the initiation of dosing (0 h). The lung bacterial density was expressed as $\log_{10}$ CFU and reported as the mean for each treatment group ± standard deviation (SD).

(iv) Pharmacodynamic assessment. *f*AUC/MIC was previously determined to be the pharmacodynamic parameter most closely associated with GSK2140944 activity (19); therefore, data analyses were performed only for this parameter. The relationship between *f*AUC/MIC and efficacy was determined using the sigmoidal E_{max} inhibitory model (Phoenix 32 WinNonlin version 6.3; Pharsight, Mountain View, CA, USA) for each isolate utilized in efficacy studies, in addition to a composite data set of all six strains evaluated. The exposure indices required for stasis, 1-$\log_{10}$-unit decrease, and 2-$\log_{10}$-unit decrease were calculated from each of these models.

RESULTS

Bacterial isolates. The MICs of GSK2140944 against the six isolates studied ranged from 0.125 to 0.5 mg/liter (Table 1). *S. aureus* 456, F40-14, 146, and 156 were clinical isolates, while it is not known whether *S. aureus* 508 and 509 were clinical isolates. Four out of six strains were resistant to levofloxacin, whereas all six strains were susceptible to linezolid.

Pharmacokinetics. The pharmacokinetic profile of GSK2140944 was best described using a one-compartment model with first-order input and elimination. The pharmacokinetic parameters are shown in Table 2. GSK2140944 showed relative linearity across the dose range, allowing for extrapolation of all doses using mean pharmacokinetic parameters (Fig. 1).

Pulmonary distribution. GSK2140944 distributed well into the ELF of infected animals. The mean penetration of GSK2140944 into ELF was 1.27 ± 0.09 with the range of 1.14 to 1.37.

In vivo efficacy/pharmacodynamics. Initial lung bacterial density at 0 h averaged between 5.6 and 6.3 $\log_{10}$ CFU, which increased by 0.3 to 3.4 $\log_{10}$ CFU in untreated mice after 24 h. The *f*AUC/MIC values required to achieve various efficacy endpoints against six isolates are presented in Table 3. When calculated as a composite, *f*AUC/MIC required for stasis and 1 $\log_{10}$ CFU were 49 and 130 ($r^2 = 0.79$), which are graphically displayed in Fig. 2. The activities of the comparators, levofloxacin and linezolid, were predictive given their phenotypic profiles; linezolid showed 0.8 to 2.6 $\log_{10}$ CFU decrease against all six isolates (MIC, 1 to 4 mg/liter), whereas levofloxacin showed 1.6 to 2.8 $\log_{10}$ CFU increase for the four isolates with a MIC of ≥8 mg/liter and 1.5 to 2.6 $\log_{10}$ CFU decrease for the two isolates with a MIC of ≤0.25 mg/liter.

DISCUSSION

GSK2140944 is a novel bacterial type II topoisomerase inhibitor under development in both oral and intravenous formulations with *in vitro* activity against key causative pathogens of respiratory infections, including MRSA. We evaluated the pharmacokinetic and pharmacodynamic properties of GSK2140944 against diverse MRSA isolates using the neutropenic murine lung infection model. GSK2140944 was found to possess *in vitro* activity against a phenotypically and genotypically diverse group of MRSA isolates that translated into efficacy *in vivo*.

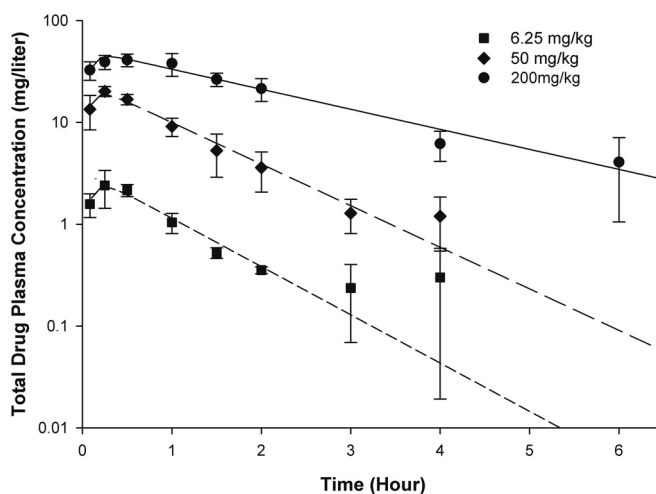When selecting isolates for the purposes of early pharmacody-

FIG 1 Total drug plasma concentrations of GSK2140944 following single subcutaneous doses in a murine neutropenic *S. aureus*-infected lung model. Values are means ± standard deviations (error bars).

TABLE 3 Pharmacodynamic profile for GSK2140944 in a neutropenic murine *S. aureus*-infected lung model[a]

| Isolate or measure | MIC (mg/liter) | *f*AUC/MIC | | | Max Δ log CFU |
		r^2	Stasis	1-Log-unit decrease	
S. aureus isolates					
456	0.125	0.95	24	107	−2.87
508	0.25	0.98	99	162	−3.14
F40-14	0.25	0.92	31	104	−1.37
146	0.5	0.89	45	87	−2.48
156	0.5	1.00	53	121	−1.82
509	0.5	0.98	105	310	−1.60
Statistical measures					
Mean			59	148	
SD			35	83	
Median			49	114	

[a] r^2, coefficient of determination; Max Δ log CFU, maximum change in the log CFU.

namic analyses, it is important to target organisms that are relevant to the likely distribution anticipated in clinical practice and in the range of expected *in vitro* activity for the compound under study. In a recent study of 201 clinical *S. aureus* isolates, GSK2140944 exhibited a rather narrow MIC distribution where 98% of organisms had MICs of 0.125 to 0.5 mg/liter with MIC_{50} and MIC_{90} values of 0.25 and 0.5 mg/liter, respectively (10). Moreover, another surveillance study of 1,008 clinical isolates of *S. aureus* collected in 2011 through 2012 reported the same MIC_{50} and MIC_{90} values (20) with 97% of isolates with MICs of ≤0.5 mg/liter. GSK2140944 was similarly active *in vitro* against the six strains studied herein, for which MIC values were between 0.125 and 0.5 mg/liter.

In dose fractionation studies using 2 to 512 mg/kg/day with one MRSA isolate and one *Streptococcus pneumoniae* isolate, Bulik et al. demonstrated that *f*AUC/MIC was closely correlated with the efficacy of GSK2140944 using a neutropenic murine thigh infection model (19). Moreover, while GSK2140944 is a novel bacterial topoisomerase inhibitor whose mode of action is distinguished from available fluoroquinolones, it shares the same target enzymes (i.e., DNA gyrase and topoisomerase IV) (7) and could reasonably be expected to follow traditional fluoroquinolone pharmacodynamics.

In our study, the *f*AUC/MIC target required for stasis and a 1 log_{10} CFU decrease varied across the six isolates tested; this variability is not uncommon in this model and emphasizes the reasoning for testing multiple strains. Similar degrees of variability were also noted in the aforementioned study by Bulik et al. which evaluated the pharmacodynamics of GSK2140944 against 2 methicillin-susceptible *S. aureus* (MSSA) isolates and 3 MRSA isolates (MIC = 0.5 to 2 mg/liter) with the doses ranging from 2 to 2,048 mg/kg/day in a neutropenic murine thigh infection model (19). In their study, *f*AUC/MIC targets for stasis and 1 log_{10} CFU decrease ranged from 3.97 to 35.5 and 12.6 to 103.2, respectively. When considering this in the context of identified median *f*AUC/MIC required for a 1-log-unit decrease, the median identified in our lung infection model was similar to the upper end of the range identified in the thigh model (i.e., 114 versus 103), but nearly double that of the median thigh value, 59. While the reason for this difference is unclear, certainly these data sets were derived from

two distinctive groups of isolates using varied models of infection, and factors such as drug distribution to the site of infection and strain variability could be contributing. Of note, the Bulik et al. study also reported *f*AUC/MIC targets for a 2 log_{10} CFU decrease. Similar targets in our model were not calculated, as only three of six isolates achieved such a response. The inability to reach a 2 log_{10} CFU reduction has been encountered among other currently available bactericidal and bacteriostatic anti-MRSA antimicrobials in the neutropenic and immunocompetent *in vivo* lung infection model (18, 21–24).

It is clearly of importance to understand our identified targets in the context of clinical pharmacokinetic data. Tiffany et al. performed a pharmacokinetic study in 48 healthy volunteers with repeated doses of GSK2140944 from 400 to 1,500 mg infused over 2 or 3 h two or three times a day for 7 to 10 days (25). Repeated intravenous doses of 1,000 mg twice daily achieved an average steady-state AUC_{0-12} of 26.1 mg · h/liter with 24.0% coefficient of variation (CV). If not considering the variability among patients versus healthy volunteers studied, this value would translate to an average *f*AUC_{0-24} of 35.0 mg · h/liter after applying 33% protein binding of GSK2140944 in humans. Therefore, it could be inferred that at the MIC_{90} of GSK2140944 against *S. aureus* (i.e., 0.5 mg/liter), this dose would achieve a *f*AUC/MIC of 70.0, which falls in between our stasis and 1 log_{10} CFU targets from our neutropenic model.

Another important consideration is the relative pulmonary distribution of GSK2140944 in humans and mice. A study by Hossain et al. generated a population pharmacokinetic model using plasma and ELF concentrations derived from 22 healthy volunteers after giving a single dose of GSK2140944 (1,000 mg) intravenously over 2 h (26). Plasma samples were drawn at 0.5 h, 1 h, 2 h (at the end of infusion), 3 h, 4 h, 6 h, 8 h, and 12 h, while BAL fluid samples were drawn at 2 h, 4 h, 8 h, and 12 h. Their mean ELF penetration estimate was 1.88 (%CV, 17.9; 95% confidence interval, 1.22 to 2.54) compared with 1.27 identified in mice. While the presence of lung infection (12, 27–30) could alter pulmonary distribution, it is possible that the *f*AUC/MIC blood targets of GSK2140944 in patients may be
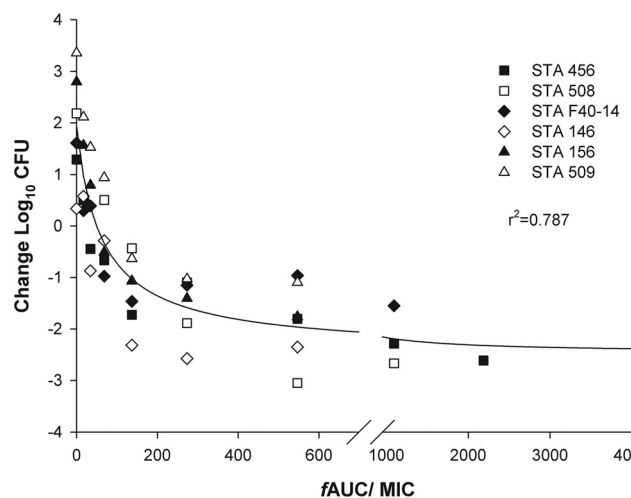

FIG 2 Composite free drug pharmacodynamic profile (*f*AUC/MIC) of GSK2140944 versus six *S. aureus* (STA) isolates in the murine neutropenic lung infection model.

lower than the targets identified from mice in our study. Namely, secondary to enhanced pulmonary distribution in humans, a similar fAUC in blood between humans and mouse could result in a greater ELF AUC in humans.

In conclusion, GSK2140944 demonstrated *in vitro* activity and *in vivo* efficacy against all six MRSA isolates tested, and these data provide a pharmacodynamic understanding of GSK2140944 that should help guide further development as a potential treatment option for pulmonary infections, including those caused by MRSA.

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