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Pharmacokinetics/Pharmacodynamics of Peptide Deformylase Inhibitor GSK1322322 against *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus* in Rodent Models of Infection

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GSK1322322 is a novel inhibitor of peptide deformylase (PDF) with good *in vitro* activity against bacteria associated with community-acquired pneumonia and skin infections. We have characterized the *in vivo* pharmacodynamics (PD) of GSK1322322 in immunocompetent animal models of infection with *Streptococcus pneumoniae* and *Haemophilus influenzae* (mouse lung model) and with *Staphylococcus aureus* (rat abscess model) and determined the pharmacokinetic (PK)/PD index that best correlates with efficacy and its magnitude. Oral PK studies with both models showed slightly higher-than-dose-proportional exposure, with 3-fold increases in area under the concentration-time curve (AUC) with doubling doses. GSK1322322 exhibited dose-dependent *in vivo* efficacy against multiple isolates of *S. pneumoniae*, *H. influenzae*, and *S. aureus*. Dose fractionation studies with two *S. pneumoniae* and *S. aureus* isolates showed that therapeutic outcome correlated best with the free AUC/MIC (*f*AUC/MIC) index in *S. pneumoniae* (R^2, 0.83), whereas *f*AUC/MIC and free maximum drug concentration (fC_{max})/MIC were the best efficacy predictors for *S. aureus* (R^2, 0.9 and 0.91, respectively). Median daily *f*AUC/MIC values required for stasis and for a 1-$\log_{10}$ reduction in bacterial burden were 8.1 and 14.4 for 11 *S. pneumoniae* isolates (R^2, 0.62) and 7.2 and 13.0 for five *H. influenzae* isolates (R^2, 0.93). The data showed that for eight *S. aureus* isolates, *f*AUC correlated better with efficacy than *f*AUC/MIC (R^2, 0.91 and 0.76, respectively), as efficacious AUCs were similar for all isolates, independent of their GSK1322322 MIC (range, 0.5 to 4 μg/ml). Median *f*AUCs of 2.1 and 6.3 μg · h/ml were associated with stasis and 1-$\log_{10}$ reductions, respectively, for *S. aureus*.

The steady appearance and spread of resistance to marketed antibiotics in bacterial pathogens causing major human diseases have constituted a public health concern for many years (1–3). Moreover, hospitalizations associated with drug-resistant infections have substantial implications for the health care system, such as increased risk of patient mortality, longer stays, and higher hospital costs (4, 5). Although a review of the antibacterial pipeline shows an increase in the number of antibiotic candidates in clinical development (6), with five new FDA approvals since the beginning of 2014 (http://www.pewtrusts.org/antibiotics), there is still a need for novel-acting antimicrobial agents (3, 7). GSK1322322 is a novel inhibitor of peptide deformylase (PDF), an essential metalloprotease that removes the *N*-formyl group from all nascent polypeptides (8–10) and, so far, a clinically unexploited antibacterial target. GSK1322322 shows good *in vitro* antibacterial activity against organisms associated with community-acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infections (ABSSSI), including strains carrying resistance determinants for commonly used antibacterial agents, with MIC_{90}s of 2 μg/ml against *Streptococcus pneumoniae* and 4 μg/ml against *Haemophilus influenzae* and *Staphylococcus aureus* (11). This compound has also demonstrated good safety, tolerability, and pharmacokinetic (PK) properties in phase I clinical trials (12–14), as well as efficacy in human proof-of-concept clinical studies (15). A considerable number of PDF inhibitors have been discovered over the last decade of research (16), and two, BB-83698 (17) and LBM415 (18), have progressed to phase I clinical trials but have not been further developed. The reasons for the discontinuation of BB-83698 have not been reported, but in the case of LBM415, reversible methemoglobinemia was detected at the highest dose tested in human volunteers (19). These findings were structure based and not related to the compound's mechanism of action (19).

Pharmacokinetic/pharmacodynamic (PK/PD) studies with BB-83698 and LBM415 determined that the area under the concentration-time curve (AUC)/MIC ratio was the parameter which best correlated with efficacy (W. A. Craig and D. R. Andes, presented at the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy [ICAAC], Chicago, IL, 2001; W. A. Craig and D. R. Andes, presented at the14th European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], Prague, Czech Republic, 2004). Here we report the characterization of the *in vivo* PD of GSK1322322 in immunocompetent animal models of infection with *S. aureus*, *S. pneumoniae*, and *H. influenzae* in order to determine the PK/PD parameter and

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magnitude that best correlates with efficacy in all three organisms.

(The clinical development of GSK1322322 has been terminated at GlaxoSmithKline [GSK] [https://clinicaltrials.gov/ct2/show/NCT01953809].)

MATERIALS AND METHODS

Bacterial strains and growth conditions. All strains used in this study were either clinical isolates obtained from the GSK Microbiology Department Culture Collection or reference strains obtained from the American type Culture Collection (ATCC). *S. pneumoniae* and *S. aureus* isolates, including methicillin-, macrolide-, and quinolone-resistant strains, were cultured at 37°C on Trypticase soy agar (TSA) with 5% sheep blood or in cation-adjusted Mueller-Hinton (MH) broth. *H. influenzae* strains were cultured at 37°C on chocolate agar II plates or in *Haemophilus* test medium (HTM) broth.

***In vitro* susceptibility testing of GSK1322322.** PDF inhibitor GSK1322322 was obtained from GlaxoSmithKline Pharmaceuticals (Collegeville, PA) and dissolved in dimethyl sulfoxide (DMSO). MIC endpoints were determined in triplicate by broth microdilution methodology according to Clinical and Laboratory Standards Institute (CLSI) guidelines (20). The MIC was defined as the lowest concentration of compound that inhibited visible growth of the organism.

Animals. Specific-pathogen-free (SPF) CD1 mice (Charles River, Raleigh, NC) weighing approximately 20 g were used in the respiratory tract infection models. SPF male Cr1:Sprague-Dawley (CD) rats (Charles River) weighing 95 to 105 g were used in the abscess infection models. Animals were allowed access to food and water *ad libitum*.

All studies, conducted in accordance with the GlaxoSmithKline policy on the care, welfare and treatment of laboratory animals, were reviewed by the Institutional Animal Care and Use Committee at GSK and met or exceeded the standards of the American Association for the Accreditation of Laboratory Animal Care, the U.S. Department of Health and Human Services, and all local and federal animal welfare laws.

Mouse respiratory tract infection (RTI) model. All *S. pneumoniae* and *H. influenzae* isolates were subcultured, respectively, onto TSA plates supplemented with 5% sheep blood, or onto chocolate agar plates, and incubated overnight at 37°C. Colonies were harvested from the agar plates and suspended in phosphate-buffered saline (PBS). Immediately prior to infection, a 5- to 10-fold dilution of the bacterial suspensions was prepared in cooled (approximately 42°C) molten nutrient agar. Mice were anesthetized with 5% isoflurane in 1.5 liter/min of oxygen and infected by intrabronchial instillation with a 20-μl aliquot of the agar suspensions via nonsurgical intubation. The final inocula ranged from 2.3×10^5 to 1.9×10^6 CFU/mouse for *S. pneumoniae* isolates and from 1.5×10^6 to 2.4×10^6 CFU/mouse for *H. influenzae* isolates. No correlation was observed between starting inocula and the growth of the strains in the lungs or the efficacy of GSK1322322. Mice used for the PK evaluation of GSK1322322 in this animal model were infected with *S. pneumoniae*.

Rat abscess infection model. All *S. aureus* isolates were subcultured into brain heart infusion broth (BHI) and incubated overnight at 37°C without shaking. Inocula were obtained from the overnight cultures by diluting twice 1:2, and once 1:10, into sterile saline. A final 10-fold dilution was done into 0.6% (wt/vol) semisolid nutrient agar immediately prior to infection. Rats were inoculated with 1 ml of this suspension (1.3×10^6 to 3.2×10^6 CFU/rat) by subcutaneous injection in the groin area. Rats used for the PK evaluation of GSK1322322 in this animal model were infected with *S. aureus*.

GSK1322322 administration. Dosing solutions of GSK1322322 were prepared in 20% polyethylene glycol (PEG) immediately prior to each dose and were administered by oral gavage in a volume of 0.4 ml/mouse (RTI model) or 2 ml/rat (abscess model), starting 1 h postinfection. In all studies, additional groups of infected animals were either left untreated (1-h baseline controls) or given a vehicle only and served as 24-h (dose fractionation studies) or 48-h (dose ranging studies) nontreated controls (NTC).

PK/PD index determination. Dose fractionation studies were performed with two *S. pneumoniae* isolates (Ery-2 and 1302005S) in the mouse RTI model and two *S. aureus* isolates (A-24 and 1307005A) in the rat abscess model. Groups of 3 to 5 *S. pneumoniae*-infected mice were treated with 1, 2, 4, or 8 doses of GSK1322322 over a 24-h period, for a total of 20 dosing regimens, with doses ranging from 20 to 600 mg/kg (of body weight)/day. Groups of 5 *S. aureus*-infected rats were treated with 1, 2, or 4 doses of GSK1322322 over a 24-h period, for a total of 18 dosing regimens, with doses ranging from 37.5 to 1,200 mg/kg/day.

PK/PD magnitude determination. Eleven *S. pneumoniae* isolates and five *H. influenzae* isolates were used to evaluate the efficacy of GSK1322322 in dose ranging studies in the mouse RTI model (4 or 5 mice/group). The compound was given every 8 h for 2 days at 4 different doses ranging from 18.75 to 300 mg/kg in *S. pneumoniae* infections and at 4 different doses ranging from 37.5 to 300 mg/kg in *H. influenzae* infections. Eight *S. aureus* isolates were used in dose ranging studies in the rat abscess model (5 rats/group), with GSK1322322 administered every 12 h for 2 days at 4 or 5 different doses ranging from 4.7 to 150 mg/kg, depending on the strain.

Animals were euthanized at 1 h (untreated baseline controls), 24 h (dose fractionation studies), or 48 h (dose ranging studies) postinfection. Lungs or abscesses were removed using aseptic technique, placed in bags, and homogenized in 1 ml of PBS (lungs) or sterile saline (abscesses) for 2 min in a Stomacher 80 Biomaster (Seward, Ltd., Worthing, United Kingdom). For enumeration of viable bacteria, 20 μl of 10-fold serial dilutions in PBS (lungs) or sterile saline (abscesses) were plated in triplicate on TSA supplemented with 5% sheep blood (*S. aureus* and *S. pneumoniae* infections) or chocolate agar plates (*H. influenzae* infections) by a modified Miles-Misra technique using the Hamilton Microlab AT-Plus2 liquid handling system. The colonies were counted following overnight incubation at 37°C. The lower limits of quantification were 1.7 $\log_{10}$ CFU/lung and 1.2 $\log_{10}$ CFU/abscess.

Drug PK studies. Single-dose PK studies were performed in *S. pneumoniae* Ery-2-infected mice (3 mice/group). Animals were administered oral doses (0.4 ml/mouse) of GSK1322322 at 10, 37.5, 75, 150, 300, or 600 mg/kg at 1 h postinfection. PK parameters could not be obtained from the 10-mg/kg dose, as most of the values were below the limit of quantification (0.05 μg/ml). The PK of GSK1322322 was also evaluated in *S. aureus* A24-infected rats (3 rats/group). GSK1322322 was administered at 37.5, 75, 150, or 300 mg/kg 1 h postinfection by oral gavage in a volume of 2 ml/rat.

Approximately 30 μl (mice) or 40 μl (rats) of whole blood was collected serially from the lateral tail vein at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 h postdose. The lateral tail vein was punctured using a microlancet (mice) or a 23-gauge butterfly needle (rats), and blood was collected into heparin-coated capillary tubes (mice) or into a heparinized Eppendorf tube (rats). The blood was transferred into a microcentrifuge tube, and a 10-μl (mice) or a 25-μl (rats) aliquot was mixed with an equal volume of cold high-performance liquid chromatography (HPLC)-grade water. All samples were frozen immediately on dry ice and maintained at −80°C. Sample analysis using high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) with electrospray ionization, working in multiple-reaction monitoring mode, was performed at GSK (Waters Acquity ultra-HPLC [UHPLC] connected to an API Sciex 4000 tandem quadrupole mass spectrometer). The lower limit of quantification was 0.05 μg/ml.

Data handling and analysis. Exposure data are presented as the mean (±standard deviation) total concentration in blood (in micrograms per milliliter) from three animals per dose group. PK statistics were calculated nonparametrically. AUC was calculated using the trapezoid rule. The peak concentration (C_{max}) was the highest observed concentration of drug in blood. Time above the MIC (T>MIC), the time over which blood concentrations remain above a specific MIC expressed either as hours or as a

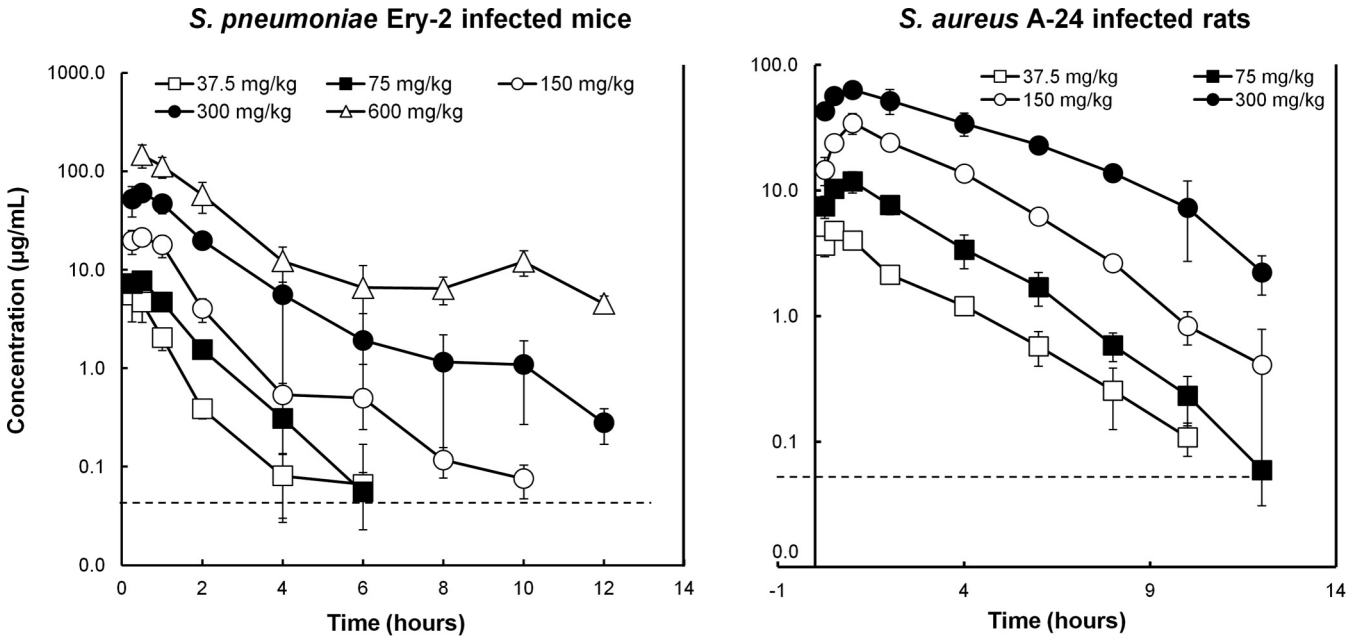


FIG 1 Total concentrations of GSK1322322 in blood after single-dose oral administration to mice with a lung infection caused by *S. pneumoniae* Ery-2 or to rats with an abscess infection caused by *S. aureus* A-24. Groups of three animals were used for all data points. The dashed lines represent the lower limits of quantification. The error bars represent standard deviations.

percentage over a 24-h period (%T>MIC), was calculated by fitting a linear interpolation model between observations and computing the percentage of the line that lies above the MIC. All parameters were calculated based on free (*f*) drug exposure profiles (*f*AUC, *f*AUC/MIC, fC_{max}/MIC, and %*f*T>MIC). Protein binding of GSK1322322 was determined in rat and mouse plasma by ultrafiltration at 5 μg/ml.

For repeat dosing, it was assumed that negligible residual concentrations remained at the time the next dose was administered. The outcome measure for comparison of treatments was the number of bacteria isolated from abscesses ($\log_{10}$ CFU/abscess) or lungs ($\log_{10}$ CFU/lungs). Mean $\log_{10}$ CFU from each group were correlated with predicted mean PK parameters using the following E_{max} model, where *c* was the PK parameter of interest (dose fractionation studies) or the dose (dose ranging studies), and *E*min, *E*max, *K*, and *m* were coefficients fitted from the data: log drop = $E_{min} + \{(E_{max} - E_{min})/[1 + (c/K)^m]\}$ + error.

Data from dose fractionation studies with both isolates of each genus were analyzed separately and pooled. A combination of the E_{max} model and an intermediary model linking PK statistics with dose was used to correlate efficacy with free blood levels for AUC/MIC, C_{max}/MIC, and %T>MIC. The coefficient of determination (R^2) was used to estimate the variance that could be due to regression with each of the PK/PD indices. The static dose was defined as the dose required to prevent growth over 24 or 48 h; i.e., mean bacterial counts were equivalent to the 1-h NTC. Doses for 1-$\log_{10}$ reductions were defined as those producing mean bacterial counts equivalent to 1 $\log_{10}$ CFU less than the 1-h NTC.

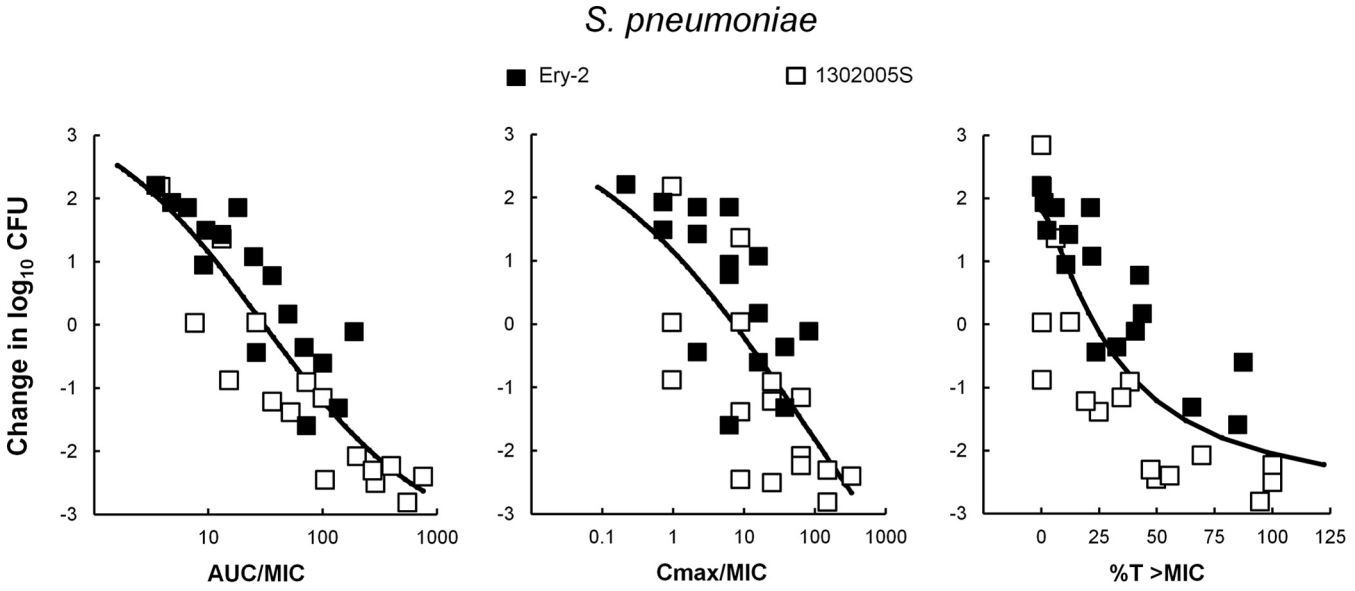
RESULTS

Pharmacokinetics of GSK1322322 in mice and rats. The PK characteristics of GSK1322322 were determined following single oral dose administration of 37.5, 75, 150, 300, or 600 mg/kg to mice infected with *S. pneumoniae* Ery-2 (Fig. 1). Time to maximum concentration (T_{max}) was observed by 30 min with all doses, with an average free C_{max} ranging from 1.8 ± 0.8 to 46.1 ± 12.2 μg/ml (Table 1). Higher-than-dose-proportional increases were observed in the values for free drug AUC from 0 to 12 h (AUC_{0-12}),

TABLE 1 Blood pharmacokinetic parameters of GSK1322322 following single oral administration to mice ($n = 3$) with a lung infection caused by *S. pneumoniae* Ery-2 or to rats ($n = 3$) with an abscess infection caused by *S. aureus* A-24

Infection (organism)[a]	Dose (mg/kg)	Free C_{max}[b] (μg/ml)	T_{max} (h)	Free AUC_{0-12}[b] (μg · h/ml)	$t_{1/2}$[b] (h)
Mouse lung (*S. pneumoniae* Ery-2)	37.5	1.8 ± 0.8	0.25	1.8 ± 0.4	1.8 ± 1.9
	75	2.5 ± 0.3	0.5	3.6 ± 0.1	1.6 ± 0.2
	150	6.8 ± 1.1	0.5	10.9 ± 2.6	1.5 ± 0.3
	300	19.3 ± 2.7	0.5	36.8 ± 5.8	1.9 ± 0.3
	600	46.1 ± 12.2	0.5	101.4 ± 28.6	4 ± 0.9
Rat abscess (*S. aureus* A-24)	37.5	2.3 ± 0.2	0.5	6.3 ± 0.3	1.8 ± 0.3
	75	5.7 ± 1.0	1	18.1 ± 1.1	1.5 ± 0.1
	150	16.4 ± 3.0	1	58 ± 5.6	1.6 ± 0.3
	300	30.3 ± 4.2	1	150.5 ± 22.5	2.3 ± 0.4

[a] The levels of protein binding were 68.8% with infection of mice with *S. pneumoniae* Ery-2 and 52.6% with infection of rats with *S. aureus* A-24.
[b] Mean values ± standard deviations.



	R^2		
Strain	AUC/MIC	Cmax/MIC	%T > MIC
Ery-2	0.84	0.65	0.78
1302005S	0.89	0.66	0.75
Overall	0.83	0.64	0.63

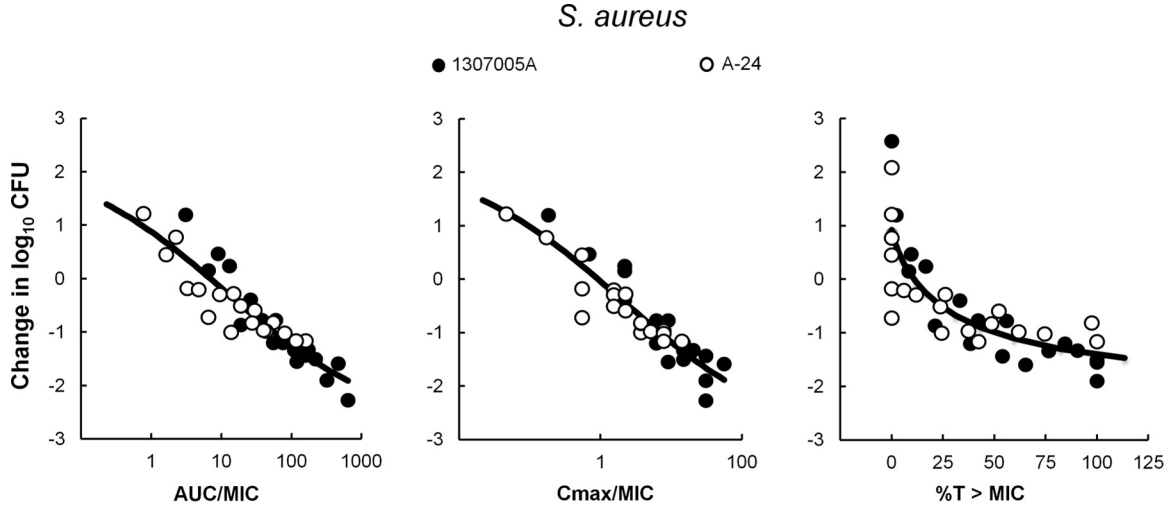
FIG 2 Relationship between GSK1322322 PK/PD indices and efficacy over 24 h against *S. pneumoniae* 1302005S and *S. pneumoniae* Ery-2 in a mouse lung model of infection. Free drug concentrations were used for index calculations. Efficacy is expressed as change in CFU/lung over time compared to that at the start of therapy. Each symbol represents the mean CFU/lung from 3 to 5 mice. The sigmoid line represents the best fit using a combination of the E_{max} model and an intermediary model linking PK statistics with dose. R^2 is the coefficient of determination.

which ranged from 1.8 ± 0.4 to 101.4 ± 28.6 μg · h/ml; i.e., a 16-fold dose increase resulted in a 56-fold increase in AUC (Table 1). A similar elimination half-life ($t_{1/2}$) was observed with doses from 37.5 to 300 mg/kg (1.5 to 1.9 h), although it increased to 4 h at the highest dose tested. These PK parameters were also determined in rats infected with *S. aureus* A-24 after oral administration of single GSK1322322 doses of 37.5, 75, 150, or 300 mg/kg (Fig. 1). Blood concentrations of GSK1322322 increased in a dose-dependent manner across this dose range. The average free C_{max} increased with the dose and ranged between 2.3 ± 0.2 and 30.3 ± 4.2 μg/ml, while the T_{max} (0.5 to 1 h) was not affected by dose (Table 1). Slightly higher-than-dose-proportional increases were observed in the free drug AUC_{0-12} values, which ranged from 6.3 ± 0.3 to 150.5 ± 22.5 μg · h/ml. The $t_{1/2}$ increased slightly with dose, oscillating between 1.5 and 2.3 h. Levels of protein binding of GSK1322322 were 52.6% and 68.8% in rat and mouse plasma, respectively.

PK/PD index determinations. The relationship between the antibacterial effect of 20 different dosing regimens of GSK1322322 after 24 h of treatment with each of the PD indices, *f*AUC/MIC, fC_{max}/MIC, and %*f*T > MIC, was evaluated with two isolates of *S. pneumoniae*, Ery-2 and 1302005S, in a mouse RTI model. In this model, the organisms grew means of 4.28 and 2.84 $\log_{10}$ CFU/lung, respectively. Data for the two isolates were analyzed separately and combined (Fig. 2). Independently of how the analysis was performed, therapeutic outcome correlated best with the *f*AUC/MIC index (R^2 = 0.84 and 0.89 for *S. pneumoniae* Ery-2 and 1302005S, respectively; R^2 = 0.83 for the two isolates combined). Correlation of *in vivo* efficacy with the other two parameters was less strong (fC_{max}/MIC, R^2 = 0.65 and 0.66 for *S. pneumoniae* Ery-2 and 1302005S, respectively, and 0.64 for both isolates combined; %*f*T>MIC, R^2 = 0.78 and 0.75 for *S. pneumoniae* Ery-2 and 1302005S, respectively, and 0.63 for the two isolates combined). Interestingly, %*f*T>MIC correlated better with efficacy when each *S. pneumoniae* isolate was analyzed independently than with the pooled data.

The relationship between the decrease in abscess bacterial counts at the end of 24 h of therapy with 18 different GSK1322322 dosing regimens, and each of the PD indices was also evaluated with two isolates of *S. aureus*, 1307005A and A-24, in a rat groin abscess model. The organisms grew means of 2.58 and 2.09 $\log_{10}$ CFU/abscess in this model, respectively. Data for the two isolates studied are presented in Fig. 3 and were analyzed both separately and combined. Free AUC/MIC and fC_{max}/MIC were shown to best correlate with efficacy when the two isolates were analyzed together, with R^2 values of 0.9 and 0.91, respectively, versus a coefficient of determination of 0.71 for %*f*T>MIC (Fig. 3), although correlations for all three parameters were very similar when the two isolates were analyzed separately. This indicates that increasing the number of isolates used in the determination of the PK/PD index could help better discern between them.

Dose-response studies with *S. pneumoniae* isolates. To determine the magnitude of the PK/PD parameter necessary to achieve efficacy against *S. pneumoniae*, dose ranging studies were performed against 11 *S. pneumoniae* isolates, with GSK1322322 MICs of 0.25 to 2 μg/ml (Table 2), in the mouse RTI model. The



	R^2		
Strain	AUC/MIC	Cmax/MIC	%T > MIC
1307005A	0.96	0.94	0.94
A-24	0.93	0.92	0.84
Overall	0.90	0.91	0.71

FIG 3 Relationship between GSK1322322 PK/PD indices and efficacy over 24 h against *S. aureus* 1307005A and *S. aureus* A-24 in a rat abscess model of infection. Free drug concentrations were used for index calculations. Efficacy is expressed as change in CFU/abscess over time compared to that at the start of therapy. Each symbol represents the mean CFU/abscess from five rats. The sigmoid line represents the best fit using a combination of the E_{max} model and an intermediary model linking PK statistics with dose. R^2 is the coefficient of determination.

compound was given every 8 h for 2 days at 4 different doses ranging from 18.75 to 300 mg/kg. The strains grew to a mean of 5.6 to 8.9 $\log_{10}$ CFU/lung in the vehicle-treated controls after 48 h. The mean maximal reduction in bacterial counts after treatment with GSK1322322 was 1.2 to 2.8 $\log_{10}$ CFU/lung (Table 2). The *f*AUC and *f*AUC/MIC ratio necessary to achieve a static effect or a 1-$\log_{10}$ kill were determined for each isolate and are summarized in Table 2. For individual line fits, the R^2 values ranged from 86% to 100%. Free daily AUC and AUC/MIC values associated with stasis oscillated between 2.0 and 26.5 (mean, 11 ± 7.5; median, 8.2) and 1.5 to 26 (mean, 11 ± 7.9; median, 8.1), respectively (Table 2). For a 1-$\log_{10}$ reduction in bacterial counts, values ranged from 3.6 to 31 (mean, 17 ± 8.2; median, 19) and 3.4 to 60.3 (mean, 19.5 ± 17.8; median, 14.4), respectively (Table 2).

Dose-response studies were also performed against five *H. influenzae* isolates, with GSK1322322 MICs of 1 to 4 μg/ml, in a mouse RTI model with administration of the compound every 8 h for 2 days at 4 different doses ranging from 37.5 to 300 mg/kg. In the vehicle-treated control groups, organisms grew to a mean of 5.4 to 6.6 $\log_{10}$ CFU/lung after 48 h. GSK1322322 was efficacious against these isolates, with mean maximal reduction in bacterial counts ranging from 2.7 to 3.0 $\log_{10}$ CFU/lung (Table 2). Free daily AUC and AUC/MIC values associated with stasis and 1-$\log_{10}$ kill for the individual isolates are summarized in Table 2. The R^2 values for individual line fits ranged from 91.9% to 99.9%. The *f*AUC necessary to achieve a static effect and a 1-$\log_{10}$ reduction in bacterial counts ranged from 7.1 to 15.3 (mean, 11.8 ± 4.2; median, 14.5) and 13.4 to 37.3 (mean, 21.5 ± 10.2; median, 16.7), respectively, whereas the *f*AUC/MIC ratios required to reach those endpoints were 3.7 to 7.6 (mean, 6.6 ± 1.6; median, 7.2) and 8.3 to 13.9 (mean, 11.6 ± 2.6; median, 13), respectively (Table 2).

The relationships between the daily free AUC or AUC/MIC and efficacy against both sets of isolates are shown graphically in Fig. 4. Although the exposure-response relationship was very good for individual *S. pneumoniae* isolates, the correlation was weaker when all data were considered together, irrespectively of relating efficacy to *f*AUC or to *f*AUC/MIC, with R^2 of 0.65 and 0.62, respectively (Fig. 4). This was not the case for the combined set of *H. influenzae* isolates, where the correlation between efficacy and *f*AUC/MIC (R^2 of 0.93) was stronger than that observed with *f*AUC (R^2 of 0.89), and both relationships were greater than in the case of *S. pneumoniae* (Fig. 4).

Dose-response studies with *S. aureus* strains. While both *f*AUC/MIC and fC_{max}/MIC correlated well with efficacy against *S. aureus*, AUC (with or without consideration of MIC) was chosen as the primary PK/PD driver because it demonstrated the most consistent correlation across the organisms tested. Dose ranging studies were performed in the rat groin abscess model of infection against eight *S. aureus* isolates with GSK1322322 MICs of 0.5 to 4 μg/ml (Table 2). All *S. aureus* isolates grew well in this nonneutropenic model, to a mean of 6.4 to 8.1 $\log_{10}$ CFU/abscess at 48 h in the vehicle-treated controls. GSK1322322 was administered twice daily for 2 days at 4 or 5 different doses ranging from 4.7 to 150 mg/kg, depending on the isolate. GSK1322322 demonstrated good efficacy against all isolates, with mean maximal reductions in bacterial counts of 1.8 to 3.4 $\log_{10}$ CFU/abscess (Table 2). The free daily AUC and AUC/MIC ratio determined for each isolate are summarized in Table 2. The R^2 values for the individual line fits ranged from 96.6% to 99.9%. AUC values associated with stasis and a 1-$\log_{10}$ reduction in bacterial counts oscillated from 0.2 to 3.9 (mean, 2.3 ± 1.2; median, 2.1) and 2.3 to 14.2 (mean, 6.7 ± 3.7; median, 6.3), respectively. Interestingly, the *f*AUC required to

TABLE 2 *In vitro* and *in vivo* activities of GSK1322322 against *S. pneumoniae*, *H. influenzae*, and *S. aureus* isolates

Organism	MIC (μg/ml)	R^2 for line fit (%)	Stasis		1-$\log_{10}$ reduction		Max killing ($\log_{10}$ reduction)
			*f*AUC	*f*AUC/MIC	*f*AUC	*f*AUC/MIC	
S. pneumoniae							
10127	0.25	99.9	2.0	8.1	3.6	14.4	2.8
1316009S	0.25	96.1	6.5	26.0	15.1	60.3	1.8
ATCC 10813	0.5	100	8.4	16.9	22.2	44.5	1.2
1307007S	1	95.8	16.1	16.1	20.1	20.1	2.7
ATCC 6303	1	99.8	19.8	19.8	24.8	24.8	2.8
1629	2	100	15.8	7.9	21.2	10.6	2.4
298443	2	99.9	26.5	13.2	31.0	15.5	2.3
336808	2	86	8.2	4.2	19.0	9.5	2.6
338860	2	99.5	2.9	1.5	6.7	3.4	2.8
340449	2	94.6	8.0	4.0	14.9	7.4	2.1
L11259	2	100	7.1	3.5	9.0	4.5	2.0
Mean ± SD	NA[d]	NA	11.0 ± 7.5	11.0 ± 7.9	17.0 ± 8.2	19.5 ± 17.8	2.3 ± 0.5
Median	NA	NA	8.2	8.1	19.0	14.4	2.4
H. influenzae							
1998-100-126H	1	99.7	7.3	7.3	13.4	13.4	2.9
503-008H	1	99.8	7.1	7.1	13.9	13.9	2.7
08003H	2	99.9	14.5	7.2	16.7	8.3	2.7
H128	2	96.6	15.3	7.6	26.0	13.0	2.7
19001H	4	91.9	14.8	3.7	37.3	9.3	3.0
Mean ± SD	NA	NA	11.8 ± 4.2	6.6 ± 1.6	21.5 ± 10.2	11.6 ± 2.6	2.8 ± 0.1
Median	NA	NA	14.5	7.2	16.7	13.0	2.7
S. aureus							
1312007A	0.5	96.6	3.9	7.8	9.0	17.9	1.8
1307005A[a]	0.5	98.9	3.8	7.6	7.7	15.5	2.0
X32601[a,b,c]	1	99.8	1.6	1.6	4.3	4.3	3.2
1309006[a]	1	99.7	0.2	0.2	2.3	2.3	2.9
PVL-2[a,b]	2	99.2	1.9	0.9	5.5	2.8	3.4
PK-2	2	97	2.3	1.1	14.2	7.1	2.5
A-24	4	99.9	2.7	0.7	3.8	1.0	3.1
T63256[a,b,c]	4	99.9	2.0	0.5	7.1	1.8	3.4
Mean ± SD	NA	NA	2.3 ± 1.2	2.6 ± 3.2	6.7 ± 3.7	6.6 ± 6.6	2.8 ± 0.6
Median	NA	NA	2.1	1.0	6.3	3.5	3.0

[a] Macrolide resistant.
[b] Methicillin resistant.
[c] Quinolone resistant.
[d] NA, not applicable.

achieve efficacy did not increase with increasing MIC. Consequently, more variability was observed among the *f*AUC/MIC values, which ranged from 0.2 to 7.8 (mean, 2.6 ± 3.2; median, 1) for a static effect and from 1 to 17.9 (mean, 6.6 ± 6.6; median, 3.5) for a 1-$\log_{10}$ kill (Table 2).

A comparison of the relationships between the daily *f*AUC or *f*AUC/MIC and efficacy against the eight *S. aureus* isolates showed that AUC correlates better with efficacy than AUC/MIC, with R^2 of 0.91 and 0.76, respectively (Fig. 5). Recently, we have reported that GSK1322322 could prevent the *in vitro* growth of *S. aureus* strains for up to 6 h at concentrations 8- to 32-fold below the MICs and that this sub-MIC effect appeared more substantial on those strains at the higher end of the MIC spectrum (21). Therefore, we investigated the activity of GSK1322322 at concentrations below its MIC against the eight isolates used in this study. As shown in Table 3, a very strong growth inhibition could be observed against all isolates with GSK1322322 MICs of 2 and 4 μg/ml at concentrations of 1/16 its MIC. This could perhaps explain why increases in MIC did not seem to have an effect on the exposure necessary for efficacy against this organism.

DISCUSSION

In an era characterized by steady increases in bacterial resistance to most commonly used antibacterial agents combined with a scarcity of new molecules reaching phase III clinical trials, the development of new inhibitors of essential bacterial pathways with acceptable safety, tolerability, and efficacy properties for human use has become a pressing need. Antimicrobial PK/PD studies can help determine the therapeutic potential of a drug by integrating its PK properties, *in vitro* potency, and *in vivo* efficacy and can be used to design dosing regimens in humans that balance efficacy and safety, to avoid over- or underdosing and to minimize resistance development (22–27). GSK1322322 is a potent PDF inhibitor that has progressed to phase IIa clinical trials (14). These studies were done to characterize the PK/PD relationship of GSK1322322 against major respiratory and skin pathogens in or-

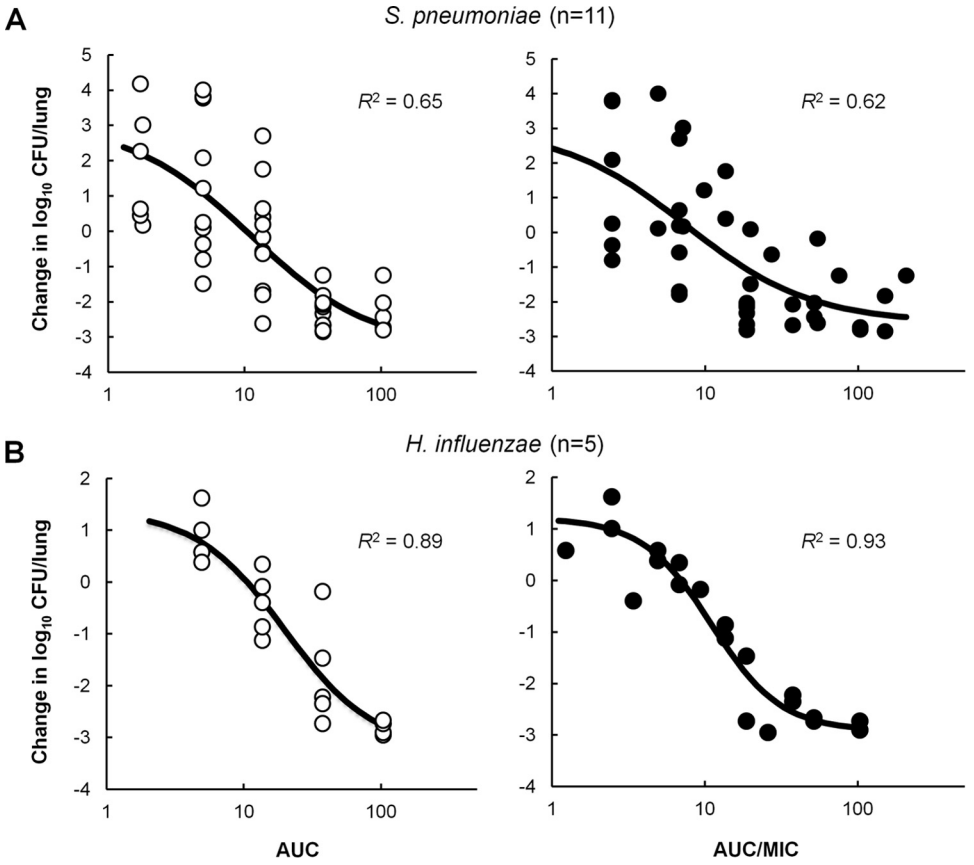


FIG 4 Relationship between GSK1322322 free drug 24-h AUC (open circles) or AUC/MIC (filled circles) and efficacy against 11 *S. pneumoniae* isolates (A) and 5 *H. influenzae* isolates (B). Each symbol represents the mean CFU/lung from 4 or 5 mice. The sigmoid line represents the best-fit curve using a combination of the E_{max} model and an intermediary model linking PK statistics with dose. R^2 is the coefficient of determination.

der to guide the progression of this compound through clinical studies and to inform the development of susceptibility breakpoints.

Oral PK studies over a broad range of doses in two different rodent animal models showed slightly higher-than-dose-proportional pharmacokinetics, with doubling doses resulting in 3-fold increases in AUC. In rodents, the half-life was short (approximately 2 h) and protein binding moderate (50 to 70% bound). GSK1322322 is 66% protein bound in human plasma and has been shown to have rapid absorption (T_{max} of 0.5 to 1 h) and

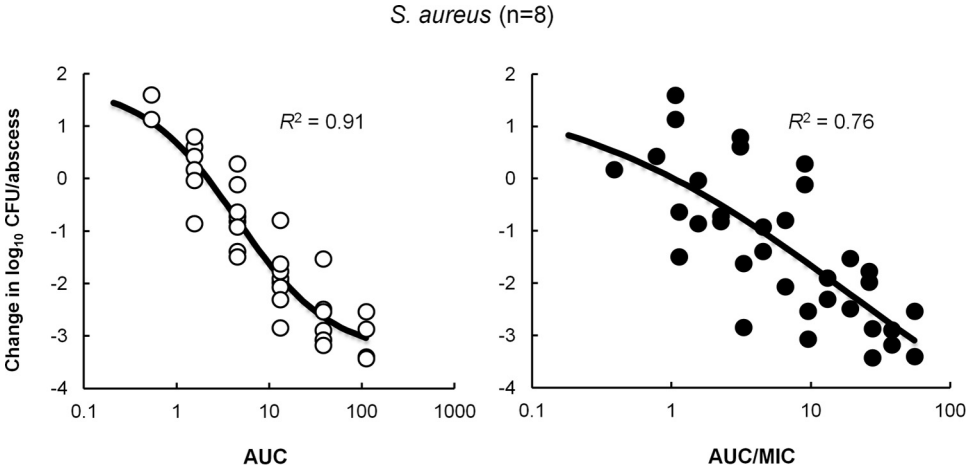


FIG 5 Relationship between GSK1322322 free drug 24-h AUC (open circles) or AUC/MIC (filled circles) and efficacy against eight *S. aureus* isolates. Each symbol represents the mean CFU/abscess from five rats. The sigmoid line represents the best-fit curve using a combination of the E_{max} model and an intermediary model linking PK statistics with dose. R^2 is the coefficient of determination.

TABLE 3 *In vitro* and *in vivo* characteristics of GSK1322322 against eight *S. aureus* isolates

S. aureus isolate	MIC (μg/ml)	Fraction of MIC that inhibits 95% growth for 6 h	*f*AUC (stasis)
1312007A	0.5	1/2	3.9
1307005A	0.5	1/4	3.8
X32601	1	1/8	1.6
1309006	1	1/8	0.2
PVL-2	2	1/16	1.9
PK-2	2	1/16	2.3
A-24	4	1/32	2.7
T63256	4	1/16	2.0

nonlinear PK in single-dose oral first-time-in-human (FTIH) studies (12).

Analysis of the fractionated GSK1322322 dosing regimens evaluated in *S. pneumoniae*- and *S. aureus*-infected animals suggested that efficacy is dose dependent and independent of the dosing interval. Results with two *S. pneumoniae* isolates indicated that the daily *f*AUC/MIC is the most important index for efficacy, irrespective of analyzing the data separately or with both isolates combined. On the other hand, although regression analysis based on the coefficient of determination (R^2) suggested a reasonable correlation between all three parameters and *in vivo* efficacy for each of the *S. aureus* isolates independently, when data from the two isolates were combined, daily *f*AUC/MIC and fC_{max}/MIC became the best indices for predicting efficacy of GSK1322322 against *S. aureus*. Clearly, increasing the number of isolates used in these dose fractionation studies can help to better differentiate among the indices, which are logically interrelated (28). As the *f*AUC/MIC ratio demonstrated the best correlation for *S. pneumoniae* and was equally as predictive as fC_{max}/MIC for *S. aureus*, *f*AUC/MIC was selected from the dose fractionation studies as the key PK/PD parameter for GSK1322322.

Dose ranging studies were undertaken to determine the magnitude of the *f*AUC/MIC necessary for efficacy of GSK1322322 against 11 *S. pneumoniae*, 5 *H. influenzae*, and 8 *S. aureus* isolates. A comparable analysis using *f*AUC as the PK/PD parameter was also performed with all three species. Strains with a wide range of GSK1322322 MICs (4- to 8-fold different) and several antibiotic resistance phenotypes (*S. aureus* isolates) were used for these studies. As expected, given the novel mechanism of action of this compound, GSK1322322 was efficacious against all isolates tested, irrespective of their resistance phenotypes. Moderate variability was observed for the combined dose-response curves of the different *S. pneumoniae* isolates (R^2 of 0.62), and median daily *f*AUC/MIC values associated with stasis and a 1-$\log_{10}$ kill in this organism were 8.1 and 14.4, respectively. Similar *f*AUC/MIC values were obtained for *H. influenzae*, 7.2 and 13, respectively, although in this case the correlation between the magnitude of the PK/PD parameter and efficacy remained strong even when all isolates were combined (R^2 of 0.93). No statistical differences were observed in the correlation of *f*AUC/MIC or *f*AUC and *in vivo* antibacterial effect of GSK1322322 in either of these two organisms, with similar mean/median magnitudes and coefficients of determination. PK/PD studies performed with other PDF inhibitors have also shown AUC/MIC to be the parameter that best correlated with efficacy (Craig and Andes, presented at the 41st ICAAC; Craig and Andes, presented at the 14th ECCMID). In fact, a median value of 31.4 was predicted to achieve a static effect with LBM415 against *S. pneumoniae* isolates in studies performed using a neutropenic mouse thigh infection model (Craig and Andes, presented at the 14th ECCMID). As 4-fold-lower magnitudes were necessary in immunocompetent animals (Craig and Andes, presented at the 14th ECCMID), the *f*AUC/MIC value would be similar to that obtained with GSK1322322 in the present studies. The results obtained with *S. pneumoniae* and *H. influenzae* support the concept, previously demonstrated with other antimicrobial agents, that the magnitude of the PK/PD index required for efficacy is generally similar for different organisms and among drugs within the same antimicrobial class (reviewed in reference 29).

Interestingly, the results obtained in the studies performed with *S. aureus* isolates were strikingly different in two major aspects. First, the *f*AUC/MIC necessary to achieve efficacy was much lower, with median values of 1 and 3.5 required for stasis and a 1-$\log_{10}$ kill, respectively (R^2 of 0.76). Second, the data suggested that *f*AUC, rather than *f*AUC/MIC, was a better predictor of efficacy for this novel class agent against the wild-type *S. aureus* population, as a stronger correlation was obtained with this parameter (R^2 of 0.91). Efficacious AUCs were similar against all isolates, independent of their GSK1322322 MIC, and median *f*AUCs of 2.1 and 6.3 μg · h/ml were required to achieve stasis and a 1-$\log_{10}$ reduction in bacterial counts, respectively. *In vivo* studies had already shown better-than-anticipated efficacy of PDF inhibitors against *S. aureus*, and further *in vitro* investigation unveiled a pronounced inhibitory effect of PDF inhibitors on the first 6 to 8 h of *S. aureus* growth at concentrations 8- to 32-fold below the MIC, a property that did not extend to *S. pneumoniae* or *H. influenzae* isolates (21). In fact, sub-MICs of GSK1322322 could inhibit ≥95% growth of the eight *S. aureus* strains used in this study, with the lowest fractions of MIC inhibiting growth of those strains with the highest MICs. This could explain the lack of a linear relationship between the drug exposure required for efficacy and the MIC of the isolate causing the infection, particularly as repeated *in vivo* administration of the compound (as done in these studies) would result in exertion of this effect at regular intervals. It has already been reported for *S. aureus* that subinhibitory concentrations of certain antimicrobials can suppress virulence factor production (30), including production of alpha-toxin (31, 32), increase susceptibility to phagocytosis (31), and modulate adherence to fibronectin (33, 34). Given that antibacterial agents are often present at subinhibitory concentrations during the normal course of antibiotic therapy, these types of effects should perhaps be taken into account when evaluating the magnitude of the PK/PD indices against certain organisms.

Much higher *f*AUC/MIC ratios (median of 57.8) were necessary to achieve efficacy with LBM415 against five *S. aureus* isolates (Craig and Andes, presented at the 14th ECCMID), although the use of a neutropenic model and higher inocula may have contributed to this discrepancy. We have observed that the presence of neutrophils can reduce the free GSK1322322 AUC necessary to achieve efficacy against *S. aureus* 6- to 9-fold (data not shown). A very strong impact has also been reported with tedizolid (35, 36), for which it has been speculated that the majority of the bacterial killing in normal animals is due to the effect of the drug mediated through neutrophils (35). In addition, increases in the magnitude of the daily AUC/MIC values required for stasis have been observed with *S. aureus* for daptomycin and linezolid (4-fold) and for vancomycin (10-fold) when the starting inocula increased

from 10^5 to 10^7 CFU/thigh in the neutropenic model (37). No differences could be seen between *f*AUC/MIC and *f*AUC in the LBM415 study, but four of the five isolates used had LBM415 MICs of 1 μg/ml (Craig and Andes, presented at the 14th ECCMID). This stresses the importance of performing PK/PD studies with strains covering a wide range of MICs, as this phenomenon was noticeable only because strains with 8-fold differences in their GSK1322322 MICs were used.

In conclusion, these studies show that GSK1322322 has dose-dependent antimicrobial activity against several isolates of *S. pneumoniae*, *H. influenzae*, and *S. aureus*, including some resistant to other antibacterial agents. Free AUC/MIC is the PK/PD index that best predicts efficacy against *H. influenzae* and *S. pneumoniae*, with median values of ~8 and 14 required to achieve stasis and 1-$\log_{10}$ killing, respectively. Initial phase I PK studies with repeat oral dosing of GSK1322322 suggest that these values would be achievable in the clinic (14). Of interest, *f*AUC appears to be a better parameter than *f*AUC/MIC for predicting efficacy against *S. aureus*, with magnitudes at least 4-fold lower than those necessary for the other two pathogens. This could be due to the potent effect that subinhibitory concentrations of this compound have on the early growth of *S. aureus* isolates, a phenomenon that seems more pronounced against those isolates with higher MICs. These findings highlight the importance of performing PK/PD studies with strains that encompass a wide range of MICs and suggest that PK/PD indices and magnitudes could potentially be different among bacterial species if the compound affects growth, and perhaps virulence, of a particular pathogen at sub-MIC levels.

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