Intrapulmonary pharmacokinetics of GSK2251052 in Healthy Volunteers

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Running Title: Pulmonary and Plasma PK of GSK2251052

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The plasma and intrapulmonary pharmacokinetics (PK) of intravenous (IV) GSK2251052, a novel, boron-containing antimicrobial, were evaluated in healthy adult subjects. Thirty subjects underwent bronchoscopy and timed bronchoalveolar lavage (BAL) following either a single dose (Cohort 1) or after 5 twice daily doses (Cohort 2) of 1500mg IV GSK2251052. Serial PK and safety assessments were obtained throughout the study. Bronchoscopy was performed on a single occasion in each subject at 2, 6, or 12 hours after start of infusion. Non-compartmental analysis was performed to calculate PK parameters. Thirty subjects completed the study. The mean CL, Vss, and t1/2 values were 22 L/h, 231L, and 10.7 h, respectively. Approximately 30% of the dose was excreted unchanged in urine.

GSK2251052 concentrations in epithelial lining fluid (ELF) and alveolar macrophages (AM) were approximately 50%, and 500 to 600%, respectively, compared to plasma. GSK2251052 exposures in ELF and AM were comparable following single and repeat dose administration. The most frequently reported drug-related adverse event (AE) was mild to moderate infusion site reactions (7 subjects) occurring primarily in the repeat dose cohort. No serious drug-related AEs or clinically significant trends in laboratory values, vital signs, or electrocardiograms were observed. GSK2251052 given as a 1500mg infusion was generally tolerated following single or repeat dose administration. GSK2251052 distributes into both the ELF and AM of healthy volunteers, which supports further study in patients with pneumonia.
INTRODUCTION

GSK2251052 is a novel, boron-containing inhibitor of bacterial enzyme leucyl-tRNA synthetase (LeuRS) with in vitro activity against Gram-negative pathogens, including multidrug resistant strains of Enterobacteriaceae, and was previously in development for the treatment of serious Gram-negative bacterial infections. LeuRS is an essential enzyme that catalyzes the coupling of the amino acid leucine onto its corresponding tRNA^Leu which are used by the ribosome for protein synthesis. Inhibition of LeuRS prevents protein synthesis and stops growth of the bacteria. Through the unique chemical binding properties of the boron atom, GSK2251052 binds to the editing active site of bacterial LeuRS and forms a boron adduct with the 3’ terminus of tRNA^Leu which locks the tRNA^Leu to LeuRS in an unproductive state (1).

GSK2251052 has in vitro activity against common Gram-negative pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterobacter* species. GSK2251052 has an MIC$_{90}$ value of 1 μg/mL against recent clinical isolates, including resistant strains of *E. coli*, *Klebsiella spp.*, *Enterobacter spp.*, *Citrobacter spp.*, *Proteus spp.*, *Morganella morganii*, and *Serratia marcescens* (2). The microbiological activity of GSK2251052 was not affected by clinically relevant resistance mechanisms, including efflux pumps (3), ESBL, KPC and AmpC (1,2).

Following single and repeat intravenous dosing, GSK2251052 exhibited dose proportional increases in area under the plasma concentration-time curve (AUC) and maximum plasma concentrations ($C_{\text{max}}$). On average, the systemic clearance was approximately 18 L/h, while the elimination half-life was approximately 11 hours (4). Intravenous regimens of 750mg or 1500mg are expected to achieve target plasma exposures associated with efficacy in animal models (5-7).

Data from preclinical lung infection models indicate that GSK2251052 achieves sufficient pulmonary concentrations to demonstrate efficacy against *K. pneumoniae* and *P. aeruginosa*, major
pathogens implicated in hospitalized and ventilator associated pneumonia (6-8). The purpose of this study was to evaluate plasma and intrapulmonary pharmacokinetics of GSK2251052 in healthy volunteers to assess its potential utility in patients with pneumonia.

**MATERIALS AND METHODS**

**Study design and subjects:** This was a prospective, open-label, non-randomized, two-part study that evaluated plasma and intrapulmonary concentrations of intravenous GSK2251052 administered after single and repeat doses in healthy adults. Key eligibility criteria included healthy male or female subjects aged 18-55 years, BMI inclusive of 18.5-30 kg/m², and no clinically significant abnormalities in vital signs, electrocardiographs (ECGs), chemistry, hematology or liver function tests. Key exclusion criteria included women of child-bearing potential, pregnant or lactating females, presence of contraindications to bronchoalveolar lavage including hypercapnia >50 mm Hg, refractory hypoxemia, reactive airway disease or asthma, unstable angina or acute myocardial infarction in the previous 6 months, heart failure, and severe hemostatic alterations, use of medications that could not be interrupted during study, positive HIV, hepatitis B or C status, and recent alcohol or smoking history. Adverse events were graded based on the FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (9). All subjects gave written informed consent. The protocol was approved by Western Institutional Review Board.

Fifteen subjects received a single 1500 mg dose on Day 1 and assigned to 1 of 3 sub-groups (5 per group) for bronchoscopy at either 2, 6, or 12 hours after the start of infusion. In the repeat dose cohort, another 15 subjects received GSK2251052 1500 mg q12h for 5 doses over 3 days and similarly assigned to 1 of the 3 bronchoscopy sub-groups (5 per group). GSK2251052 was administered as a 1 hour intravenous infusion in all subjects. Each subject participated in the study for approximately 6 or 7 weeks which included a 30-day screening period, 1 or 3 day treatment period, and a 2 week follow-up.

The study was conducted at Davita Clinical Research, Minneapolis, MN.
**Bronchoscopy/Bronchoalveolar Lavage.** One standardized bronchoscopy with bronchoalveolar lavage (BAL) was conducted by an experienced pulmonologist in each subject at one of three protocol-specified time-points: 2, 6, or 12 hours post start of infusion. Prior to bronchoscopy, subjects were pre-medicated with the following: nebulized 4% lidocaine with atropine, 2% topical lidocaine, 1% lidocaine (if needed), codeine, midazolam, and fentanyl. A fiber-optic bronchoscope (Olympus P-20 and P-20D, Olympus America Inc., Melville, NY, USA) was inserted into a subsegment of the right middle lobe. Four 50 mL aliquots of sterile 0.9% normal saline solution were instilled into the middle lobe of the right lung, and each specimen was immediately aspirated. The volume of the first aspirated sample (BAL1) was collected separately, measured, recorded and placed on ice. The volumes of samples 2, 3, and 4 were combined (BAL2), measured and recorded and immediately placed on ice. A minimum 4 mL aliquot was removed from both BAL1 and BAL2, refrigerated, and sent for cell count and differential. The remaining volume of BAL1 and BAL2 was immediately centrifuged at 400g for 5 minutes, to separate alveolar macrophages (pellet) and epithelial lining fluid (supernatant). A minimum 2 mL aliquot of supernatant was removed for determining urea concentrations in BAL fluid for both BAL1 and BAL2, and refrigerated until shipped to MEDTOX Laboratories (St. Paul, MN) for urea analysis. Remaining BAL1 and BAL2 supernatant and associated cell pellets (4 samples total) were transferred into storage vials and frozen at -70°C until analyzed for GSK2251052 concentrations in alveolar macrophages (pellet) and epithelial lining fluid (supernatant). Plasma urea concentrations were also determined at the same time as bronchoscopy to provide a dilution ratio for the BAL samples.

**Pharmacokinetic Samples.** Blood samples, for plasma, were obtained for GSK2251052 concentration analysis at the following sampling times: pre-dose (within 30 minutes of dosing), during the infusion at exactly 0.5 and 1 h (end of infusion), and 1.25, 1.5, 2, 4, 5, 6, 8, 10, 12, 24, 36 and 48 h after the start of the infusion on day 1 (single dose cohort) or on day 3 after the 5th dose (repeat dose cohort). Blood draws at 2, 6, and 12 h coincided with the BAL procedure, or if necessary, within 15 min of the BAL. Blood samples (approximately 2 mL) were collected into anticoagulant K2 EDTA tubes and
immediately chilled on crushed ice. Plasma was separated by centrifugation, at approximately 1,700g and 4°C for approximately 10 minutes and transferred to polypropylene specimen containers. Plasma samples were frozen and stored at approximately -80°C prior to analysis. Pooled urine was collected at the following times: 0-6h, 6-12h, 12-18h, 18-24h, 24-36h, 36-48h after the start of the infusion. Urine samples were collected into amber polypropylene containers cooled with crushed ice or refrigeration. At the end of each collection period, following recording volume and/or weight, an aliquot (approximately 7.5 mL) of the urine sample was transferred to a plastic container and stored at -80°C prior to analysis.

Bioanalytical procedures for determination of plasma, urine and pulmonary GSK2251052 concentrations. Bioanalytical methods were developed, validated and used for the determination of plasma, urine, and pulmonary GSK2251052 concentrations by CPR Pharma Services (Thebarton, Australia). The plasma concentrations of GSK2251052 were measured using a method based on protein precipitation followed by LC/MS/MS analysis. Briefly, a 100 μL aliquot of plasma was added to 300 μL methanol containing trifluoroacetic acid (0.1% v/v) and [2H2 13C]-GSK2251052 in polypropylene tubes and mixed. The samples were centrifuged for 5 minutes and the supernatants then carefully transferred to clean glass tubes and dried under warm nitrogen. The samples were reconstituted in 200 μL methanol : water (5:95, v:v) containing 0.1% trifluoroacetic acid before transfer to polypropylene (0.3 mL) vials or 96 well plates prior to analysis. Next, 20μl sample aliquots were injected onto an LC/MS/MS system consisting of a Shimadzu HPLC system with a Waters X-bridge column (3.5 μm) and an MDS Sciex 4000 API-4000 mass spectrometer (Applied Biosystems /MDS Sciex, Canada). Mobile phase A consisted of methanol : water (5:95, v:v) containing 0.1% trifluoroacetic acid and mobile phase B consisted of methanol : water (95:5, v:v) containing 0.1% trifluoroacetic acid. The column was eluted at a flow rate of 0.3 mL/min with a gradient starting at 0% B and increased linearly to 30% B from 0 to 3 min. MS/MS analyses were performed using a TurboIonSpray™ interface operated in the positive mode and a probe temperature of 400°C. The analyte and its internal standard were measured by multiple reaction monitoring of the following [M+H]+ transitions: GSK2251052, m/z 238 → 202 and...
Data collection and integration were performed using Analyst software (v1.5, Applied Biosystems/MDS Sciex, Canada). Quantification was based on analyte/internal standard peak area ratios and calculated using a weighted 1/x² linear regression model. The operating range of the assay was 1 to 2000 ng/mL.

The LC/MS/MS method used to determine concentrations of GSK2251052 in urine was very similar to that described for plasma. The method used a dilution procedure where aliquots of urine (30 μL) were added to an internal standard solution of [2H213C]-GSK2251052 in 1000 μL methanol containing trifluoroacetic acid (0.1% v/v). The samples were analyzed following injection of a 2 μL aliquot onto the LC/MS/MS system described previously for the plasma method. The operating range of the urine assay was 100 to 200000 ng/mL. Plasma and urine samples were analyzed in a total of 7 and 5 analytical runs respectively. The mean inter-run precision and accuracy were within 3 and 4%, respectively, for plasma and 3% for urine.

Determination of GSK2251052 concentrations in human epithelial lining fluid (ELF) utilized a dilution procedure followed by LC/MS/MS analysis and detection. Interference free ‘blank’ ELF was prepared from control human bronchoalveolar lavage (BAL) fluid by centrifugation for 5 minutes at 300 g (Eppendorf 5810). The supernatant, or ELF, was transferred to a polypropylene container and stored at -80°C until use. The residual pellet, containing alveolar macrophages (AM), was re-suspended in phosphate buffered saline (PBS) and stored at -80°C until use. The analytical methodology used was similar to that previously described for urine and plasma. Aliquots of ELF samples (50 μL) were added to an internal standard solution (50 μL) in water and injections (up to 10 μL) were made onto the HPLC system. The column was eluted at a flow rate of 0.3 mL/min with a gradient starting at 0% B and increased linearly to 25% B from 0 to 9 min. The operating range of the assay was 1 to 2000 ng/mL. ELF and AM samples were each analyzed in 1 analytical run.
The AM concentrations of GSK2251052 were measured by using a validated method based on protein precipitation followed by LC/MS/MS analysis. Interference free ‘blank’ alveolar macrophage fluid was prepared from control human BAL fluid as described above. The PBS suspended AM sample (30 μL) was placed into a 1.5 mL microfuge tube, followed by 20 μL of methanol containing 0.1% TFA and the internal standard (50 μL in water). The sample was mixed to a vortex and centrifuged at 13000 rpm for 10 minutes in an Eppendorf 5244 centrifuge and 10 μL of the supernatant was injected onto the LC/MS/MS system. HPLC methods were as previously described for ELF analysis. The operating range of the assay was 1 to 2000 ng/mL. The mean inter-run precision and accuracy were within 2 and 3%, for ELF and 5% and 3% for AM, respectively.

**Urea in serum and ELF.** The analysis of urea nitrogen in serum and bronchioalveolar lavage (BAL) fluid was performed via spectrophotometric assay on a Beckman Coulter AU480® Chemistry Analyzer using a Beckman Coulter Urea nitrogen reagent (Kit #OSR6134) by MedTox Laboratories. The standard curve for serum was prepared as recommended by the manufacturer and ranged from 1.00 to 100 mg/dL. For BAL fluid samples, a modification in the manufacturer’s procedure was made, and standard curves were prepared in normal saline over a range of urea nitrogen concentrations from 0.050 to 8.000 mg/dL. For both assays, standard curves were linear and the relative accuracy ranged from 96.2 to 101.6% in serum and from 102 to 103.8% in BAL. Calibrators were prepared in physiological saline solution and quality control samples were prepared in human BAL. The analyzer measured urea nitrogen based on the urea hydrolysis. Briefly, urea is hydrolyzed enzymatically by urease to yield ammonia and carbon dioxide. The ammonia and α-oxoglutarate are converted to glutamate in a reaction catalyzed by L-glutamate dehydrogenase (GLDH). Simultaneously, a molar equivalent of reduced NADH is oxidized. Two molecules of NADH are oxidized for each molecule of urea hydrolyzed. The rate of change in absorbance at 340 nm, due to the disappearance of NADH, is directly proportional to the urea nitrogen concentration in the sample. This assay measured urea nitrogen concentrations. The urea nitrogen measurements were converted into urea by multiplying the results by a factor of 2.14.
Calculation of recovered ELF volume and GSK2251052 concentrations in ELF and AM.

The calculation of ELF volume and GSK2251052 concentrations in ELF and AM were performed with BAL2 fluid (10,11). The relative amount of ELF recovered within the BAL fluid was calculated according to the urea dilution method by (12). The concentration of GSK2251052 in ELF was estimated from the concentration of GSK2251052 in BAL aspirate supernatant and the ratio of the urea concentration in BAL aspirate supernatant to that in serum. Thus, the concentration of GSK2251052 in ELF was determined as:

$$052_{\text{ELF}} = 052_{\text{BAL}} \times \left( \frac{\text{UREA}_{\text{serum}}}{\text{UREA}_{\text{BAL}}} \right)$$

where $052_{\text{BAL}}$ is the concentration of GSK2251052 measured in the BAL aspirate supernatant, $\text{UREA}_{\text{serum}}$ is the concentration of urea in serum, and $\text{UREA}_{\text{BAL}}$ is the concentration of urea in the BAL aspirate supernatant.

The concentration of GSK2251052 in AM was determined as:

$$052_{\text{AM}} = \frac{(052_{\text{BAL-P}})}{(WBC_{\text{BAL}} \times V_{\text{BAL-S}} \times \text{average cell volume} \times \% \text{ cell count})}$$

where $052_{\text{BAL-P}}$ is the amount of GSK2251052 in BAL pellet in the 5mL cell suspension, $V_{\text{BAL-S}}$ is the volume of BAL fluid recovered from the BAL procedure, $WBC_{\text{BAL}}$ is the white blood cell concentration in BAL fluid prior to centrifugation (cells/mm$^3$), and average cell volume is based on a mean value of 2.48µL/10$^6$ cells (12). A differential cell count of the BAL fluid was performed to determine percent cell count (% cell count) included both macrophages and monocytes.

Pharmacokinetics and Statistical Analyses. Plasma pharmacokinetic parameters for each subject were estimated by noncompartmental analysis using WinNonlin version 5.2 (Pharsight Corporation, Cary, NC). The maximal drug concentration in plasma ($C_{\text{max}}$), time needed to reach the maximum concentration ($T_{\text{max}}$), area under the concentration-time curve extrapolated to infinity [$\text{AUC}_{0-\infty}$], area under the concentration-time curve over the dosing interval [$\text{AUC}_{0-12}$], elimination rate constant ($\lambda_z$), elimination half-life ($t_{1/2}$), systemic clearance (CL), renal CL (CL$_{R}$), urinary recovery of GSK2251052 ($\text{Ae}$), percentage of GSK2251052 excreted in urine ($\text{fe}$), and the volume of distribution at steady-state (V$_{\text{SS}}$) were calculated.
(Vss) were determined. GSK2251052 pharmacokinetic parameters were summarized as geometric mean values with between-subject variability estimates (percent coefficient of variation [%CV]) except for $T_{\text{max}}$ which was summarized as the median and range.

$C_{\text{max}}$ and AUC$_{0-12}$ for ELF and AM were determined from the composite profile using the mean values at 2, 6, and 12 hours post dose on Days 1 and 3. AM/plasma and ELF/plasma ratios were determined based on the AUC$_{0-12}$ and $C_{\text{max}}$ values and at each sampling time by dividing concentrations of GSK2251052 in the AM or ELF by those in the plasma, respectively, at each sampling time. GSK2251502 is only approximately 10% protein bound (unpublished data), therefore total plasma concentrations were used in all calculations.

RESULTS

Subjects. Thirty (30) subjects enrolled (15 in single dose and another 15 in repeat dose cohorts) and completed the study. The mean age ($\pm$SD) of the subjects was 30.8$\pm$9.3 years and 34.6$\pm$10.9 years and mean BMI ($\pm$SD) was 26.6$\pm$2.2 kg/m$^2$ and 24.2$\pm$2.9 kg/m$^2$ in Cohort 1 (single dose) and Cohort 2 (repeat dose), respectively. The majority of subjects were male (n=28, 93%) and of Caucasian heritage (n=25, 83%).

GSK2251052 was generally tolerated with no serious adverse events (AE) or withdrawals. In the single dose cohort, diarrhea, infusion site pain, and hypotension were reported by 1 subject each. All events were considered mild in severity except for hypotension occurring post BAL procedure, which was moderate and not considered study-drug related. In the repeat dose cohort, 10 subjects reported AEs of which 5 subjects had multiple AEs. All AEs were reported to be mild and consisted of the following: infusion site reactions, influenza-like illness, chest pain, dizziness, headache, hiccups, oropharyngeal pain, rhinitis, contact dermatitis, and orthostatic hypotension. The most common drug-related AE was infusion site reactions in 6 subjects followed by chest pain, dizziness, orthostatic hypotension in 2 subjects or less. The reason for one subject’s chest pain appeared due to musculoskeletal pain, however,
given no previous reports of chest pain with GSK2251052, the investigator could not rule out relation to
study drug. Infusion site reactions occurred in 7/30 subjects (23%) (1 in single dose, 6 in repeat dose)
and consisted of phlebitis, irritation, pain, or infiltration of Grade 1 or 2 in severity. All subjects
completed treatment and no subject prematurely withdrew from the investigational product or study.
Subjects reported pain, burning, tingling and warmth within 5 to 15 minutes from the start of infusion,
usually with the second or third dose, and symptoms were progressive in nature. Symptoms resolved
soon after dose completion without intervention in the majority of subjects. In two subjects, peripheral
vein infiltration occurred in the forearm requiring placement of an alternate line. Additionally, one
subject experienced Grade 1 erythema in a 4 to 8 cm area surrounding the vein where there appeared to be
infiltration with possible extravasation of drug substance. Another subject developed red streaking along
the vein of Grade 1 severity. No other subjects in the study reported injection site reactions.

Plasma Pharmacokinetics. Following single dose administration of 1500 mg GSK2251052 via
intravenous infusion over 1 hour, GSK2251052 was eliminated slowly with a median t1/2 value of 10.7
hours (Table 1). On average, CL was low, 23.1 L/h on Day 1 and 20.6 L/h on Day 3. Average CLR was
6.62 L/h and the fe GSK2251052 recovered in urine after 48 hours was approximately 27.8% of the total
administered dose. The mean Vss (231 L) exceeded the total body water. Following single dose
administration, geometric mean (CV%) GSK2251052 AUC0-∞ and Cmax were 65.0 ug•h/mL (13.6%) and
21.6 ug/mL (20.4%), respectively (Table 1). Following repeat dose administration, AUC0-12 was
72.9 ug•h/mL (15.4%) and Cmax was 24.0 ug/mL (15.4%) (Table 1).

ELF and AM. The numbers (mean ± SD) of cells recovered in pooled BAL (BAL2) from the
single dose cohort and repeat dose cohort were 163 x 10^6 ± 66.1 cells/L and 182 x 10^6 ± 208 cells/L,
respectively. The mean (± SD) percentages of cells that were classified as monocytes and macrophages
were 90.1 ± 11.1% and 88.4 ± 9.87%, for the single and repeat dose cohorts, respectively.
The concentrations of GSK2251052 in AM, ELF, and plasma and AM:plasma and ELF:plasma
ratios are displayed in Table 2 (composite PK parameters) and Table 3 (point-to-point comparison) and
graphically in Figure 1. Following a single dose, GSK2251052 concentrations in ELF were 53.8% based on composite AUC and, on average (CV%), 56.5% (32.7%), 54.7% (41.0%), and 47.4% (14.9%) at the 2, 6, and 12 hour BAL sampling points, respectively, relative to plasma. ELF concentrations of GSK2251052 after multiple, twice daily doses were 53.4% based on composite AUC and, on average (CV%), 60.2% (24.6%), 53.4% (57.3%), and 43.6% (16.8%) at 2, 6, and 12 hours, respectively, compared to plasma.

In contrast, GSK2251052 concentrations in AM relative to plasma were 621% based on composite AUC and, on average (CV%), 573% (29.5%), 726% (44.6%), and 544% (30.5%), at the 2, 6, and 12 hour BAL sampling points, respectively, relative to plasma following single dose and 498% based on composite AUC and, on average (CV%), 549% (16.7%), 405% (35.3%), and 566% (53.4%), at the 2, 6, and 12 hour BAL sampling points, respectively, relative to plasma following multiple dosing.

**DISCUSSION**

This is the first study to document the distribution of GSK2251052 in AM and ELF over the dosing interval in healthy volunteers. All subjects completed the study and all had acceptable cell recovery from pulmonary fluids for determination of drug concentrations. Plasma pharmacokinetic data from this study were consistent with those observed in the first in human study further supporting these study findings (4).

It is widely believed that antibiotic concentrations in the pulmonary compartment correlate with efficacy for the treatment of pneumonia. Agents that distribute adequately into ELF are expected to be active against extracellular pathogens, while those that concentrate in AM are anticipated to be effective against intracellular pathogens. On average, GSK2251052 concentrations in ELF were approximately half of those observed in plasma while AM concentrations were approximately five-fold greater than plasma. There was no apparent delay in reaching the ELF and AM sites indicating rapid pulmonary penetration after the first dose of GSK2251052. This is especially important in critically ill patients, in
whom it is vital to rapidly achieve sufficient antibiotic concentrations at the site of infection. As expected based on the elimination $t_{1/2}$ of GSK2251052, there was some accumulation observed in plasma, ELF, and AM with steady-state dosing. After both single and repeat dose administration, drug concentrations declined in parallel in the ELF, AM, and plasma sites as the ELF:plasma and AM:plasma ratios were maintained over the sampling interval.

The degree of ELF penetration by GSK2251052 compared to plasma observed in humans (approximately 50%) is slightly higher than observed in mice (approximately 34%) (6). Unbound drug concentrations were used in mice for determination of ELF/fAUC while total drug concentrations were used in the present study for ELF/AUC determination. In the neutropenic murine pneumonia infection model, the PK/PD index most associated with efficacy was AUC/MIC, with free drug ratios of 7 to 10 in ELF and 10 to 30 in plasma demonstrating efficacy (6). Based on the composite AUC for ELF observed in this study [AUC(0-12) of 29.4 μg•h/mL], achieving these target values (with BID dosing) should be possible for pathogens with MICs up to and including 4 μg/mL. GSK2251052 has an MIC90 value of 1 μg/mL against recent clinical isolates, including resistant strains of Escherichia coli, Klebsiella pneumoniae, Enterobacter spp., Citrobacter spp., Proteus spp., Morganella morganii, and Serratia marcescens (2). A population pharmacokinetic plasma/lung model has been developed and was planned to be used to conduct Monte Carlo simulations to help select the dose regimen(s) that would be considered adequate to treat patients with pneumonia (13).

The overall safety profile of GSK2251052 administered IV in this study was similar to that seen in prior IV studies. Also seen previously, mild decreases in reticulocytes, red cell counts, and hemoglobin were observed following multiple dosing; however, these changes were reversible upon drug discontinuation. The clinical relevance of these findings is unknown. Contrary to the first in human (FIH) study, repeat dosing in 7/15 subjects (and 1 single dose subject) was associated with mild infusion site reactions during the infusion. Most subjects completed dosing in the original infusion site despite the reaction, while a few subjects resumed therapy after a new line was placed in the alternate arm. No
subject withdrew from the study due to this adverse event. Since the dose and duration of infusion in the present study were not changed from the FIH study, these reactions are thought to be related to a higher final drug concentration of 15 mg/mL in 100 mL normal saline employed in this study. In future studies, GSK2251052 will be diluted in a higher infusion volume to mitigate risk of infusion related reactions.

In the present study, GSK2251052 penetration was approximately 50% in ELF and 500-600% in AM relative to plasma in healthy volunteers. GSK2251052 was generally tolerated following a 1500mg infusion, except for injection site reactions reported in the repeat dose cohort that appeared to be concentration-dependent. Recently, the development of GSK2251052 was discontinued by GlaxoSmithKline due to the identification of microbiological findings of resistance in a small number of patients in a Phase 2b trial for the treatment of complicated urinary tract infections (cUTI). Although therapeutic response (resolution of clinical signs and symptoms and clearance of baseline pathogen from the urine) was observed in some patients, a sub-population of patients had rapid emergence of GSK2251052 resistance in bacterial isolates during therapy, prompting discontinuation of the cUTI trial and subsequent termination of a concomitant complicated intra-abdominal infection phase 2 trial. Details of this finding will be the subject of a future manuscript.

Given the favorable pharmacodynamics of this boron-containing class of antimicrobials, the potential to successfully address the unmet need for treatment of multi-drug resistant Gram-negative infections worldwide remains hopeful (14).


FIG. 1  GSK2251052 concentrations in plasma, epithelial lining fluid (ELF), and alveolar macrophages (AM) on Day 1 and 3
TABLE 1 Plasma pharmacokinetic parameters of GSK2251052

<table>
<thead>
<tr>
<th>Treatment Cohort</th>
<th>N</th>
<th>AUC$_{0-\infty}$ (ug•h/mL)</th>
<th>C$_{max}$ (ug/mL)</th>
<th>CL (L/h)</th>
<th>V$_ss$ (L)</th>
<th>t$_{1/2}$ (h)</th>
<th>T$_{max}$ (h)</th>
<th>CLR (L/h)</th>
<th>fe (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single dose, Day 1</td>
<td>15</td>
<td>65.0 (13.6)</td>
<td>21.6 (20.4)</td>
<td>23.1 (13.6)</td>
<td>231 (20.7)</td>
<td>10.7 (8.62 – 12.3)</td>
<td>1.00 (0.50 – 1.05)</td>
<td>6.62 (16.0)</td>
<td>26.8</td>
</tr>
<tr>
<td>Repeat dose, Day 3</td>
<td>15</td>
<td>72.9 (12.5)</td>
<td>24.0 (15.4)</td>
<td>20.6 (12.5)</td>
<td>1.00</td>
<td>(0.53 – 1.00)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Data presented as geometric mean (CV%)
2. Data presented as median (range)
**TABLE 2** Area under the concentration-time curve (AUC\textsubscript{0-12}) and maximum concentration (C\textsubscript{max}) for GSK2251052 in plasma, alveolar macrophages (AM), and epithelial lining fluid (ELF)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Visit</th>
<th>N</th>
<th>AUC\textsubscript{0-12} (\text{ug·h/mL})\textsuperscript{a}</th>
<th>C\textsubscript{max} (\text{ug/mL})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Composite</td>
<td>Plasma Ratio</td>
</tr>
<tr>
<td>Plasma</td>
<td>Day 1</td>
<td>5</td>
<td>30.0</td>
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<td></td>
<td>Day 3</td>
<td>5</td>
<td>55.1</td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>Day 1</td>
<td>5</td>
<td>186</td>
<td>6.21</td>
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<tr>
<td></td>
<td>Day 3</td>
<td>5</td>
<td>275</td>
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<tr>
<td>ELF</td>
<td>Day 1</td>
<td>5</td>
<td>16.1</td>
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<tr>
<td></td>
<td>Day 3</td>
<td>5</td>
<td>29.4</td>
<td>0.534</td>
</tr>
</tbody>
</table>

\textsuperscript{a.} AUC(0-12) based on concentrations at 2, 6 and 12 hour sampling times.
TABLE 3  Concentrations of GSK2251052 in alveolar macrophages (AM) and epithelial lining fluid (ELF) and corresponding plasma ratios for each study day and sampling time

<table>
<thead>
<tr>
<th>Treatment Cohort</th>
<th>BAL Sampling Time1</th>
<th>N</th>
<th>GSK2251052 Concentration in AM (ug/mL)</th>
<th>AM:Plasma Ratio</th>
<th>GSK2251052 Concentration in ELF (ug/mL)</th>
<th>ELF:Plasma Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single dose, Day 1 N=15</td>
<td>2-hour</td>
<td>5</td>
<td>33.3 (29.8)</td>
<td>5.73 (29.5)</td>
<td>3.30 (31.5)</td>
<td>0.565 (32.7)</td>
</tr>
<tr>
<td></td>
<td>6-hour</td>
<td>5</td>
<td>147 (5.14)</td>
<td>7.26 (44.6)</td>
<td>1.05 (40.2)</td>
<td>0.547 (41.0)</td>
</tr>
<tr>
<td></td>
<td>12-hour</td>
<td>5</td>
<td>7.340 (33.9)</td>
<td>5.44 (30.5)</td>
<td>0.635 (12.9)</td>
<td>0.474 (14.9)</td>
</tr>
<tr>
<td>Repeat dose, Day 3 N=15</td>
<td>2-hour</td>
<td>5</td>
<td>45.9 (24.6)</td>
<td>5.49 (16.7)</td>
<td>5.01 (25.1)</td>
<td>0.602 (24.6)</td>
</tr>
<tr>
<td></td>
<td>6-hour</td>
<td>5</td>
<td>16.5 (34.5)</td>
<td>4.05 (35.3)</td>
<td>2.17 (55.8)</td>
<td>0.534 (57.3)</td>
</tr>
<tr>
<td></td>
<td>12-hour</td>
<td>5</td>
<td>16.0 (52.8)</td>
<td>5.66 (53.4)</td>
<td>1.16 (16.5)</td>
<td>0.436 (16.8)</td>
</tr>
</tbody>
</table>

Data presented as mean (CV%)

1. In each treatment cohort, subjects were divided into 3 subgroups based on time of bronchoscopy (2, 6, or 12 hours post dose).