Azithromycin to Prevent Bronchopulmonary Dysplasia in *Ureaplasma*-infected Preterm Infants: Pharmacokinetics, Safety, Microbial Response, and Clinical Outcomes of 20 mg/kg Single Intravenous Dose

Running Title: Azithromycin 20 mg/kg single dose in preterm infants

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Abstract: *Ureaplasma* respiratory tract colonization is associated with bronchopulmonary dysplasia (BPD) in preterm infants. Previously, we demonstrated that single dose intravenous azithromycin 10 mg/kg is safe, but inadequate to eradicate *Ureaplasma* in preterm infants. We performed a non-randomized, single-arm open-label study of the pharmacokinetics and safety of intravenous 20 mg/kg single-dose azithromycin in 13 mechanically ventilated neonates between 24<sup>0</sup> to 28<sup>6</sup> weeks gestation. Pharmacokinetic data from 25 neonates (12 dosed with 10 mg/kg iv and 13 dosed with 20 mg/kg iv) were analyzed using a population modeling approach. Using a two compartment model with allometric scaling of parameters on body weight (WT), the population PK parameter estimates were: clearance, 0.21 L/h x WT(kg)<sup>0.75</sup>; intercompartmental clearance, 2.1 L/h x WT(kg)<sup>0.75</sup>; central volume of distribution (Vd), 1.97 L x WT(kg); and peripheral Vd, 17.9 L x WT(kg). There was no evidence of departure from dose-proportionality in azithromycin exposure over the tested dose range. The calculated AUC<sub>24</sub>/MIC<sub>90</sub> for the single azithromycin 20 mg/kg dose was 7.5 h. Simulations suggest that 20 mg/kg for 3 days will achieve AUC<sub>24</sub>/MIC<sub>90</sub> of 7 h and maintain azithromycin concentrations >MIC<sub>50</sub> of 1 µg/ml for this group of *Ureaplasma* isolates for ≥ 96 h post first dose. Azithromycin was well tolerated with no drug-related adverse events. One of seven (14%) *Ureaplasma*-positive subjects and 3/6 (50%) *Ureaplasma*-negative subjects developed physiologic BPD. *Ureaplasma* was eradicated in all treated *Ureaplasma*-positive subjects. Simulations suggest that a multiple-dose regimen may be efficacious for microbial clearance, but the effect on BPD remains to be determined.
Introduction

Bronchopulmonary dysplasia, a disease of multifactorial etiology, causes significant morbidity and mortality in infants born preterm. Respiratory tract colonization with the genital mycoplasma species *Ureaplasma parvum* and *U. urealyticum* is associated with risk for development of BPD in extremely low gestation infants (1, 2). It is unknown whether eradicating *Ureaplasma* spp. from the respiratory tract of preterm infants with appropriate antibiotic therapy will prevent *Ureaplasma* infection-mediated lung injury. Our first step to address this question has been to conduct studies in the at-risk population to determine the optimal dose, safety, and *in vivo* anti-infective efficacy of specific antibiotics in preparation for future Phase III randomized, placebo-controlled trials.

Azithromycin, an azalide antibiotic has anti-inflammatory properties (3, 4), as well as antimicrobial activity against *Ureaplasma* spp. *in vitro* (5-7) and *in vivo* experimental models (8). Azithromycin exhibits two-fold greater potency than erythromycin against *Ureaplasma* isolates *in vitro* (5, 9). Azithromycin therapy may enhance *Ureaplasma* clearance in infected infants and inhibit the pulmonary inflammatory response in both infected and non-infected infants, possibly contributing to a decreased risk for BPD (10).

Although the efficacy of azithromycin and related macrolide, clarithromycin, to prevent BPD has been assessed in single center studies of at-risk preterm infants (11, 12), the optimal dosing regimens for these antibiotics have not been determined in pharmacokinetic and pharmacodynamic studies. Recently, we characterized the pharmacokinetics (PK) of a single dose of intravenous (IV) azithromycin 10 mg/kg in
preterm infants (13). We demonstrated that a single 10 mg/kg azithromycin IV dose in mechanically ventilated infants 24 to 28 weeks gestation was safe, but inadequate to eradicate *Ureaplasma* from the respiratory tract (13). Since increasing the dose is the most effective strategy to increase AUC$_{24}$/MIC$_{90}$ (14), the pharmacodynamic parameter that predicts azithromycin efficacy for clearance of other organisms in preclinical models (15) and human studies (16, 17), the current study was designed to collect additional data on azithromycin PK in preterm infants at a higher dose level (20 mg/kg iv), to assess the safety of such a regimen, and to determine the microbiological and clinical outcomes of the higher single dose. We hypothesized that intravenous azithromycin therapy will prevent BPD in *Ureaplasma* colonized preterm infants by accelerating pathogen clearance and/or down-regulating the pulmonary inflammatory response.

**Materials and Methods**

**Eligibility Criteria**

We conducted a Phase IIa non-randomized, open-label, single dose 20 mg/kg PK and safety study of intravenous azithromycin in mechanically ventilated preterm neonates with gestational age 24 weeks 0 days (240) to 28 weeks 6 days (286). Study subjects were recruited from the University of Maryland Medical Center, Baltimore, MD (UMB) and the University of Virginia, Charlottesville, VA from June 2009 to June 2010. The Institutional Review Board of each institution approved the study and parental informed consent was obtained. Infants between 24$^{\circ}$ to 28$^{\circ}$ weeks admitted to the neonatal intensive care unit less than 72 h of age were screened for study eligibility. Inclusion criteria were size appropriate for gestational age, mechanical ventilation for
any duration during the first 48 h of life, and presence of indwelling intravenous and arterial lines. Exclusion criteria included major lethal congenital anomalies, non-viability or planned withdrawal of life support, hypotension for greater than 6 continuous hours, corrected QT interval (QTc) >0.45 seconds, significant renal impairment, significant hepatic impairment, exposure to any other macrolide antibiotic, delivery for maternal indications, maternal receipt of a macrolide within seven days prior to delivery and participation in other clinical trials.

Drug administration and blood sampling

After baseline studies were completed, each enrolled infant received 20 mg/kg of intravenous azithromycin in concentration 2 mg/ml over 1 h. Six blood samples (0.5 ml each) were collected from an existing indwelling arterial catheter inserted for clinical indications or heelstick at 0-1, 1-4, 6-8, 24-48, 48-96 and 96-144 h post-dose. The sampling windows were selected to allow precise characterization of the distribution and the elimination phases of azithromycin (13). The blood samples were centrifuged, the plasma aspirated and frozen at -80°C. Azithromycin plasma levels were measured using a validated high performance liquid chromatography-tandem mass spectroscopy (HPLC/MS/MS) detection method (18). The lower limit of detection was 10 ng/ml. The precision determined by replicate injections of quality control samples was below 15 % CV for all concentrations, but the lowest concentration had a 19.7 %CV.

Pharmacokinetic data analysis
Pharmacokinetic (PK) data for the 20 mg/kg iv dose from the present study were combined with published data for the 10 mg/kg iv dose (13) and analyzed simultaneously using a population modeling approach. Overall, data from 25 neonates (12 dosed with 10 mg/kg iv and 13 dosed with 20 mg/kg iv) were available for the PK analysis. The non-linear mixed-effects modeling software NONMEM (version 7) (ICON Development Solutions, Ellicott City, MD) was used for the analysis. The first-order conditional estimation method with interaction was used throughout the modeling procedure. The final model was a two compartment structural model (ADVAN3 TRANS4 NONMEM subroutine) with first order elimination. The model selection was based on evaluation of the objective function (OF) value, pharmacokinetic parameter estimates and their relative standard errors, physiologic plausibility of the parameter estimates, and inspection of goodness-of-fit plots. The Likelihood Ratio Test was used for comparing rival hierarchical models where a decrease in OF (-2 log likelihood) of 6.6 points was necessary to consider the improvement in model performance statistically significant at \( p = 0.01 \) and 1 degree of freedom (19). An exponential error model was used to describe the inter-individual variability in the pharmacokinetic parameters as follows:

\[
P_i = TVP \exp(\eta_i)
\]

where \( \eta_i \) is the proportional difference between the hypothetical true parameter estimate of the \( i \)th subject (\( P_i \)) and the typical population parameter value (TVP) and is assumed to be normally distributed with a mean of 0 and a variance of \( \omega^2 \). The residual error (which includes model misspecification, intra-subject variability as well as errors in
dosing, sampling times and sample analysis) was described using a proportional error model as follows:

\[ Y_{\text{obs}} = Y_{\text{pred}} \times (1 + \epsilon) \]

Where \( Y_{\text{obs}} \) is the observed plasma concentration, \( Y_{\text{pred}} \) is the model predicted plasma concentration and \( \epsilon \) is a normally distributed parameter with a mean of 0 and variance of \( \sigma^2 \). The pharmacokinetic parameters were allometrically scaled on body weight with fixed exponents of 0.75 for the clearance parameters and 1 for the volume parameters. Such scaling resulted in significant drop (~64 points) in the NONMEM objective function indicating significant improvement in the model fit to the data. There was no evidence of departure from dose-proportionality in azithromycin exposure as the dose increased from 10 mg/kg iv to 20 mg/kg iv (i.e. dose was not a significant covariate for azithromycin clearance).

To evaluate the performance of the final model, the model was used to simulate 10 mg/kg and 20 mg/kg iv single-dose administration (500 simulated studies each with 12 neonates/arm with similar weight distribution as observed in the analyzed studies). The median, 5th and 95th percentiles of plasma concentrations at each time point were calculated for each study replicate. Subsequently, the medians of the aforementioned parameters were calculated across the 500 replicates and compared graphically to the observed data. Additionally, an i.v. azithromycin dosage regimen of 20 mg/kg/day for 3 days was simulated to evaluate whether this regimen will maintain azithromycin concentrations above the MIC\(_{50}\). All simulations were conducted using Pharsight® Trial Simulator software (version 2.2.1, Pharsight Corporation, Mountain View, CA).
**Ureaplasma culture and antibiotic susceptibility testing**

Two tracheal aspirate samples at least 2 h apart and one nasopharyngeal sample were obtained pre-dose and subsequent samples were obtained at 2 and 4-5 d post-dose, and 21 d postnatal age (PNA) as previously described (13). Follow-up specimens at the later time points were obtained from the endotracheal tube if the infant remained intubated and nasopharynx if the infant was extubated. All culture specimens were immediately inoculated in 10B broth (20) and placed on ice until processed in the laboratory. One-half of each culture specimen was serially diluted with 10B broth at UMB and incubated as a fresh culture and the other half of the original specimen was frozen for shipment on dry ice to the University of Alabama at Birmingham (UAB) Diagnostic Mycoplasma Laboratory for confirmation (20). Local cultures were incubated at 37°C and observed for broth color change indicative of urea hydrolysis. All presumptive-positive broth cultures from UMB and all original specimens collected at UVA were shipped to UAB frozen on dry ice for culture by quantitative 10B broth and A8 agar culture, species-specific PCR, and azithromycin susceptibility testing by broth microdilution as described previously (13). Ureaplasma eradication was defined as 3 negative cultures post-dose confirmed by UAB Diagnostic Mycoplasma Laboratory.

**Ureaplasma spp. detection and speciation by real-time PCR assay**

In addition to culture, all clinical specimens were tested for *U urealyticum* and *U parvum* by a real-time PCR. Individual Ureaplasma isolates obtained by culture were also tested by PCR to determine their species designations. Genomic DNA from clinical
specimens and the Ureaplasma isolates was extracted by the proteinase K method as described previously (21). A multiplex real-time PCR assay was used to detect and differentiate the 2 Ureaplasma species simultaneously using the Roche LightCycler 2.0 (Roche Diagnostics, Indianapolis, Ind) as previously described (13, 22).

**Clinical Outcomes**

All infants were monitored for safety and tolerability of the drug during the study period as previously described for the 10 mg/kg cohort (13). For the BPD endpoint, the physiologic definition of BPD based on oxygen-saturation monitoring was used (23). For neonates at 36 ± 1 week post-menstrual age (PMA) or pre-discharge who were on positive pressure support or receiving > 30% supplemental oxygen with oxygen saturations between 90% and 96%, the diagnosis of BPD was assigned with no further testing. For neonates at 36 ± 1 week PMA or pre-discharge who were receiving oxygen < 30% with oxygen saturations between 90% and 96% or receiving oxygen ≥ 30% with oxygen saturations >96% underwent a timed step-wise reduction by 2% increments every 5 minutes to room air and a period of observation in room air for 30 minutes as described by Walsh et al. (23). For infants receiving supplemental oxygen by nasal cannula, the delivered fraction of inspired oxygen concentration or “effective FiO₂” (23) was calculated by the technique described by Benaron et al. (24) that was used in the STOP-ROP trial (25) which is based on weight, oxygen liter flow, and oxygen concentration. Failure of the room air challenge was defined as oxygen saturations 80-89% for 5 consecutive min or <80% for 15 s. No BPD was defined as treatment with room air with oxygen saturation ≥90% or passing the timed, oxygen-reduction test.
Study subjects who were transferred or discharged at ≤ 35 wk PMA on supplemental oxygen were considered to have BPD.

RESULTS

Study enrollment

Forty-four of 102 infants (43%) 24^0 to 28^6 week gestation less than 72 h age upon admission who were screened at both study sites were eligible for the study. Parents of 41 infants were approached for consent, 3 were missed, and 13 consented to the study. Reasons for non-eligibility were: death or considered non-viable, N=13; delivery for maternal indications, N=10; no arterial access, N=7; maternal receipt of macrolide, N=6; never intubated within the first 48 h, N=5; major anomalies, N=4; or other exclusions, N=13. All enrolled subjects received the single azithromycin 20 mg/kg intravenous dose.

Subject characteristics

Seven (54%) of the 13 enrolled subjects in the 20 mg/kg cohort were Ureaplasma-positive prior to receiving study drug. Four subjects were colonized with U. parvum alone (57%), 2 with U. urealyticum alone (29%) and 1 with both species (14).

As shown in Table 1, the gestational age, birthweight, and race distribution of the Ureaplasma-positive and negative groups were similar. The Ureaplasma-positive infants experienced longer duration of mechanical ventilation at the time of study entry, but there was no difference between the 2 subgroups for postnatal age at the time of azithromycin dosing.
Pharmacokinetic analysis

Plasma concentrations (N=149) from 25 neonates (12 dosed with 10 mg/kg iv and 13 dosed with 20 mg/kg iv) were available for the PK analysis. Diagnostic plots for the updated population PK model of azithromycin are presented in Figure 1. These plots indicate that the model described the data well with no systematic bias. The population PK parameters of azithromycin in preterm infants are presented in Table 2. These parameters were in agreement with the parameter estimates of the model built based on the 10 mg/kg iv single dose data only (13).

In this study, we defined the time-concentration profile of azithromycin 20 mg/kg. Following intravenous administration, azithromycin achieved a high serum concentration above MIC$_{90}$ and rapidly decreases to a level below MIC$_{50}$ (1 µg/ml) within a few hours (Figure 2). Based on the updated model, for the average observed weight of 0.87 kg neonate administered azithromycin 20 mg/kg iv dose, the estimated area under the plasma concentration-time curve from time zero to infinity (AUC$_{\text{inf}}$) is 105 µg.h/mL and the AUC from time zero to 24 h post dose is approximately 30 µg.h/mL. In adults, 30% of the azithromycin dose is bound to plasma proteins, but the percent binding in newborns is unknown. Without correction for possible plasma protein binding, the calculated AUC$_{24}$/MIC$_{90}$ for the single azithromycin 20mg/kg dose is 7.5 hours, using MIC$_{90}$ of 4 µg/mL (13).

The updated PK model was used to predict azithromycin AUC$_{24}$/MIC$_{90}$ and plasma concentrations versus time profiles with administration of 20 mg/kg for 3 days in preterm infants (Figure 3). Simulations suggest that 3 day dosing will provide an
AUC$_{24}$/MIC$_{90}$ of 7 h and maintain plasma concentrations above the MIC$_{50}$ for more than 96 hours post first dose on average.

**Ureaplasma culture, clearance and AZI susceptibility**

Pre-dose, 6 subjects were *Ureaplasma* culture and PCR positive and 1 subject was culture negative, but PCR positive (Figure 4). All subjects in the 20 mg/kg cohort were culture negative at all follow-up time points. Four of the seven original PCR-positive subjects remained PCR-positive 2 days post-dose and one remained PCR-positive 5 days post-dose. All samples at postnatal day 21 were PCR-negative. Therefore, there were no treatment failures. The MICs for *Ureaplasma* isolates for the combined 10 and 20 mg/kg single dose cohorts ranged from 0.5-8 µg/ml.

**Clinical Outcomes**

Four of 13 (31%) infants developed physiologic BPD. One of seven (14%) *Ureaplasma*-positive subject developed BPD compared to 3/6 (50%) *Ureaplasma*-negative infants (p=0.164). All study subjects survived to discharge. The single dose of azithromycin at 20 mg/kg was well tolerated and there were no reported azithromycin related side effects. Severe intraventricular hemorrhage (IVH; ≥Grade 3) was observed on cranial ultrasounds in 3 subjects (1 *Ureaplasma*-positive and 2 *Ureaplasma*-negative) pre-dose and one *Ureaplasma*-negative subject between study day 3 and 7. Periventricular leukomalacia was noted in one *Ureaplasma*-negative infant on cranial ultrasound obtained within 2 days post-dose. Three infants in the *Ureaplasma*-positive group failed the hearing screen in both ears at discharge. There
were no reported occurrences of necrotizing enterocolitis in the 20 mg/kg single dose group. Of the serious adverse events described above, none were attributed to the drug.

DISCUSSION

In the present population PK analysis of azithromycin in preterm infants, a two-compartment model with allometric scaling of all parameters on body weight (WT) provided the best fit to the data. Azithromycin fixed-effects pharmacokinetic parameter estimates from the current analysis, combining the 10 and 20 mg/kg i.v. single dose data, were comparable to results from our previous analysis of the 10 mg/kg data alone (13). In the present analysis, dose was tested as a covariate on azithromycin clearance and inclusion of this covariate did not lead to any appreciable improvement in the model fit. Therefore, with the limited available data, there is no evidence of departure from dose-proportionality in azithromycin exposure over the 10 to 20 mg/kg dose range in premature neonates. As such, the final PK model was a linear model that was able to reasonably predict the observed concentrations for both dose levels in simulations (Figure 2). With the increased number of neonates included in the current analysis, the importance of scaling all four PK parameters (elimination clearance, intercompartmental clearance as well as volumes of the central and peripheral compartments) was more evident.

The increased number of neonates included in the current analysis allowed for more reliable estimation of residual between-subject variability in azithromycin PK. This has resulted in improvement in the model fit at the individual subjects level compared to
the previous analysis of the 10 mg/kg data (13). The model replicated the between-
subject variability fairly well in simulation, indicating that estimation of the between-
subject variability on all PK parameters in the current analysis was not due to over
parameterization that would have caused variability to be over-inflated in simulations.
Overall, the good agreement between observed and simulated concentrations for single
azithromycin intravenous doses suggest that the model may be a useful tool for
simulating multiple-dose regimens for azithromycin in preterm neonates. Uncertainty
remains due to lack of information regarding time-linearity as well as impact of
azithromycin accumulation with repeated dosing in premature neonates. Our next multi-
dose study will address the sources of uncertainty.

In this study, we defined the time-concentration profile of azithromycin 20
mg/kg. We observed that following intravenous administration, azithromycin achieves a
high serum concentration above MIC\textsubscript{90} and rapidly decreases to a level below MIC\textsubscript{50}
within a few hours. This finding agrees with the predicted azithromycin plasma
concentration versus time profile based on the PK model derived from the 10 mg/kg
study (13). Previous pharmacodynamic studies indicate that the AUC\textsubscript{24}/MIC\textsubscript{90} best
predicts azithromycin efficacy. The target range of AUC\textsubscript{24}/MIC\textsubscript{90} required for bacterial
eradication range from >5 for common respiratory tract infections (17) and 25-35 for S.
\textit{pneumoniae}-associated community acquired pneumonia in adults (16), but the optimal
target for \textit{Ureaplasma} clearance from the preterm infant respiratory tract has not been
established. In the current study, a single intravenous azithromycin 20 mg/kg dose
achieved an AUC\textsubscript{24}/MIC\textsubscript{90} of 7.5 hours. Despite apparent clearance of ureaplasmas in
all subjects treated with 20 mg/kg compared to 43\% failure rate in the 10 mg/kg group
(13), simulations suggest that multiple azithromycin dose regimen of 20 mg/kg x 3 days will achieve comparable AUC$_{24}$/MIC$_{90}$ with prolonged time above MIC$_{50}$ that we speculate might contribute to more effective clearance, reduced pulmonary inflammation, and improved clinical outcomes. Efficacy of azithromycin for *Ureaplasma* clearance and impact on pulmonary inflammation will need to be evaluated in a larger randomized trial.

The importance of pharmacokinetic/pharmacodynamic studies is underscored by failure of a previous low dose regimen of azithromycin (11) and of the related macrolide erythromycin to eradicate *Ureaplasma* spp. from the newborn respiratory tract or prevent BPD (26-29). Clarithromycin (10 mg/kg twice per day for 10 days) treatment of *Ureaplasma* nasopharyngeal-colonized preterm infants 750 to 1250 gm birthweight in a large center in Ankara, Turkey resulted in a reduced BPD rate in the treatment group compared to placebo (2.9% vs 36.4%; p<0.001), but failed to eradicate *Ureaplasma* colonization in 31.5%. Follow-up cultures were not done in the placebo group to determine the rate of spontaneous clearance and hence the effective eradication rate of clarithromycin treatment. This study suggests that BPD may be reduced by macrolides without complete eradication of the organism, potentially due to immunomodulatory effects. However, neither systemic nor pulmonary inflammatory mediators were assessed in the clarithromycin study.

Both azithromycin and clarithromycin are pro-arrhythmic with prior reports of occurrences of QT-interval prolongation, and torsades de pointes (30). Recently, a retrospective study of a large Tennessee Medicaid cohort detected a small absolute increased risk of cardiovascular death (hazard ratio, 2.88; 95% CI, 1.79-4.63) in adults
who took a five day course of azithromycin compared to individuals who took no
antibiotics (30). There were 47 additional cardiovascular deaths per 1 million
azithromycin courses. The increased risk for cardiovascular death was highest among
patients with a high baseline risk for cardiovascular disease.

The implications of that study for azithromycin use in newborns and children are
unclear. There is a single case report in the literature of cardiac arrest with suspected
prolonged QT in a 9 month old who inadvertently received 50 mg/kg azithromycin
intravenously over 20 minutes (31). Prolonged QT interval is rare among newborns with
an incidence of the heritable long QT syndromes estimated between 1 per 3000 and 1
per 5000 births (32). Based on a retrospective study of prolonged QT interval in
neonates, Villain and co-workers (33) suggest that infants presenting with a QTc<0.5
second normalize their QTc over time while infants with a QTc>0.6 second are at risk
for severe arrhythmias and sudden cardiovascular death. Prolonged QTc was not
detected in any of the 171 preterm infants we screened for the 10 mg/kg and 20 mg/kg
single dose studies. However, careful screening of potential subjects for prolonged QT
interval in subsequent studies of multi-dose azithromycin is recommended.

In conclusion, we did not observe any large threats to safety with a 20 mg/kg
dose of azithromycin in the preterm population and developed a PK model that can be
used for future studies. Based on findings from our two studies, we are prepared to
conduct a Phase IIa multi-center trial with a larger sample size to study the PK and
efficacy of multi-dose azithromycin to eradicate Ureaplasma from the preterm infant
respiratory tract.
Acknowledgement: This study was supported by a grant NIH NICHD R21 HD056424.

Technical support for performance of mycoplasma cultures, antibiotic susceptibility testing, and PCR assays from Donna Crabb and Amy Ratliff is gratefully acknowledged.

Conflict of Interest: Dr. Ahmed A Othman is a former employee of University of Maryland and is currently an employee of Abbott Laboratories.

REFERENCES


FIGURE LEGENDS

Figure 1. Diagnostic scatter plots for the population PK model including data from the 10 and 20 mg/kg iv single doses of azithromycin (AZI). (A) Population-predicted versus observed AZI plasma concentrations; (B) Individual-predicted versus observed AZI plasma concentrations; (C) Conditional weighted residuals versus population predicted plasma concentrations; and (D) Conditional weighted residuals versus time. (The solid lines represent the lines of identity in A and B and zero residuals in C and D.)

Figure 2. Observed and model-simulated azithromycin plasma concentration versus time profiles following administration of 10 (A) and 20 (B) mg/kg single iv doses of azithromycin to preterm neonates relative to in vitro MIC$_{90}$ (4 µg/ml) and MIC$_{50}$ (1 µg/ml). Data for the 10 mg/kg dose were previously published (13).

Figure 3. Simulated azithromycin plasma concentration versus time profiles in preterm neonates with administration of a regimen of 20 mg/kg/day for 3 days based on updated PK model developed with the 10 and 20 mg single dose data. The MIC$_{90}$ and MIC$_{50}$ are depicted with dotted lines.

Figure 4. Microbial clearance following a single dose AZI 20 mg/kg. Seven subjects were Ureaplasma-positive prior to AZI dosing by culture confirmed by A8 agar colonial morphology and/or PCR. Two days post-dose, one subject remained culture and PCR-
positive and 5 were PCR-positive only. All respiratory samples were culture and PCR-
negative 5 days post-dose and 21 d postnatal age.
Table 1. Comparison of characteristics of the 20 mg/kg azithromycin single dose combined cohort and stratified by *Ureaplasma* respiratory tract colonization status

<table>
<thead>
<tr>
<th>Variable</th>
<th>All (N=13)</th>
<th>Ureaplasma positive N=7</th>
<th>Ureaplasma negative N=6</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wk)</td>
<td>25.6 ± 1.2</td>
<td>25.6 ± 1.3</td>
<td>25.7 ± 1.2</td>
<td>0.893</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>870 ±116</td>
<td>914 ± 129</td>
<td>818 ± 80</td>
<td>0.143</td>
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<tr>
<td>Sex (male)</td>
<td>10 (77)</td>
<td>4 (57)</td>
<td>6 (100)</td>
<td>0.067</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>6 (46)</td>
<td>3 (43)</td>
<td>3 (50)</td>
<td></td>
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<tr>
<td>African-American</td>
<td>6 (46)</td>
<td>4 (57)</td>
<td>2 (33)</td>
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<td>Asian</td>
<td>1 (8)</td>
<td>0</td>
<td>1 (17)</td>
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<td>Mixed</td>
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<td>0</td>
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<td>Ethnicity (Hispanic)</td>
<td>0</td>
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<td>Positive pressure support at study entry</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CPAP</td>
<td>4 (31)</td>
<td>3 (43)</td>
<td>1 (17)</td>
<td>0.308</td>
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<tr>
<td>IMV/HFOV</td>
<td>9 (69)</td>
<td>4 (57)</td>
<td>5 (83)</td>
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<td>IMV duration at study entry</td>
<td>27.7 ± 15.8</td>
<td>36.1 ± 16.2</td>
<td>17.8 ± 8.2</td>
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<tr>
<td>FiO2 at study entry</td>
<td>0.24 ± 0.01</td>
<td>0.22 ± 0.02</td>
<td>0.26 ± 0.04</td>
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<tr>
<td>Postnatal age @dose (h)</td>
<td>64.9 ± 30.3</td>
<td>60.2 ± 26.0</td>
<td>70.3 ± 36.5</td>
<td>0.573</td>
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</table>

* Data are presented as mean ± SD or N (percentages)
Table 2. Parameter estimates of the population Model

<table>
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<tr>
<th>Parameter</th>
<th>Estimate (%RSE)</th>
<th>% ISV (% RSE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl (L/hr)</td>
<td>0.21 x WT (kg)^0.75 (6)</td>
<td>31 (27)</td>
</tr>
<tr>
<td>V1 (L)</td>
<td>1.97 x WT (kg) (19)</td>
<td>86 (39)</td>
</tr>
<tr>
<td>Q (L/hr)</td>
<td>2.1 x WT (kg)^0.76 (9)</td>
<td>39 (39)</td>
</tr>
<tr>
<td>V2 (L)</td>
<td>17.9 x WT (kg) (9)</td>
<td>39 (31)</td>
</tr>
<tr>
<td>Residual Error (%)</td>
<td>24.5 (29)</td>
<td>---</td>
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</table>

ISV, Inter-subject variability, calculated as the square root of the estimated variance of inter-subject variability x100; RSE, Relative standard error; Wt (kg), body weight in kilograms.
Number of Subjects

Sampling Timepoints

- pre-dose
- 2d post-dose
- 4-5d post-dose
- 21d PNA

Number of Subjects:

- 0
- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10
- 11
- 12
- 13

Legend:
- PCR positive
- culture positive