DATA SUPPLEMENT

Randomized Trial of Azithromycin to Eradicate *Ureaplasma* in Preterm Infants

Rose M. Viscardi[a#]; Michael L. Terrin[b]; Laurence S. Magder[b]; Natalie L. Davis[a]; Susan J. Dulkerian[a]; Ken B. Waites[d]; Namasivayam Ambalavanan[d]; David A. Kaufman[e]; Pamela Donohue[f], Deborah J. Tuttle[g]; Jörn-Hendrik Weitkamp[h]; Hazem E. Hassan[c,i] and Natalie D. Eddington[c]

[a]Departments of Pediatrics, and [b]Epidemiology and Preventive Medicine, University of Maryland, Baltimore School of Medicine, Baltimore, MD;

[c]University of Maryland, Baltimore School of Pharmacy, Baltimore, MD;

[d]Departments of Pathology and Pediatrics, University of Alabama at Birmingham School of Medicine, Birmingham, AL;

[e]Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, VA;

[f]Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD; and

[g]Department of Pediatrics, Christiana Care Health System, Newark, DE;

[h]Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN.

#Corresponding Author: Rose M. Viscardi (email:rviscard@som.umaryland.edu), telephone: 410-328-6003), Professor of Pediatrics and Medicine, University of Maryland School of Medicine, 110 Paca Street, 8th floor, Baltimore, MD 21201, United States

LIST OF ELEMENTS IN THIS DATA SUPPLEMENT

1. SUPPLEMENTARY TABLE 1S

Primary and Secondary Outcomes, Stratified by Race.

	***White* (N=51)**			***Non-White* (70)**		
Outcome	Azithromycin (N=36)	Placebo (N=15)	P-value[e]	Azithromycin (N=24)	Placebo (N=46)	P-value[e]
Ureaplasma-free survival	32 (89%)	9 (60%)	0.04	23 (96%)	28 (61%)	<.001
Survival	32 (89%)	13 (87%)	>.99	23 (96%)	42 (91%)	.65
Ureaplasma clearance post-treatment	13/13 (100%)	0/5 (0%)	<0.001	6/6 (100%)	4/20 (20%)	<0.001
Discharge to home	24 (67%)	7 (47%)	.16	15 (63%)	23 (50%)	.47
Survival free of Physiologic BPD[a]	14/35 (40%)	8 (53%)	.44	17 (71%)	28/44 (64%)	.49
Physiologic BPD[a,b]	19/33 (58%)	5/13 (38%)	.27	6/23 (26%)	13/41 (32%)	.57
Modified Shennan BPD[b]	20/34 (59%)	7/13 (54%)	.84	8/23 (35%)	16/43 (37%)	.78
Moderate-Severe BPD[b]	23/34 (68%)	7/13 (54%)	.46	8/23 (35%)	16/43 (37%)	.78
Postnatal Steroids	13 (36%)	3 (20%)	.26	2 (8%)	11 (24%)	.08
Passed Hearing Screen[c]	27/31 (87%)	13/13 (100%)	.30	23/23 (100%)	39/41 (95%)	.53
Total Duration IMV, median (IQR), d[d]	19.0 (7.5, 42.0)	10 (2, 59)	.46	8 (2, 12.5)	2.5 (1, 44)	.95
Total Duration Supplemental Oxygen, Median (IQR), d[d]	87.5 (52.0, 129.5)	69 (58, 118)	.51	56.5 (31, 83.5)	62.5 (27, 125)	.20
Duration hospitalization, median (IQR), d[d]	105 (77.5, 150.0)	98 (73, 120)	.31	72.5 (54.5, 85.0)	87.0 (66.0, 111.0)	.02

Abbreviations: IMV, intermittent mandatory ventilation; IQR, interquartile range

[a]Three participants could not be classified with respect to physiologic BPD and are excluded from these percentages.

[b]Excludes 8 participants (3 azithromycin and 5 placebo) who did not live long enough to be assessed for BPD

[c] Based on those who survived until discharge but excludes 2 survivors who did not have a hearing screen.

[d]In computing the median and interquartile range (IQR), those who died are included as having the worst outcomes

[e]P-values for binary outcomes are based on a score test from Generalized Estimating Equations to account for correlations between twins, or Fisher's Exact Test when one of the cell sizes has an expectation of less than 5. P-values for quantitative outcomes are based on non-parametric tests using multiple outputation to account for correlations between twins

2. SUPPLEMENTARY TABLE 2S

Morbidities of Prematurity Stratified by Race.

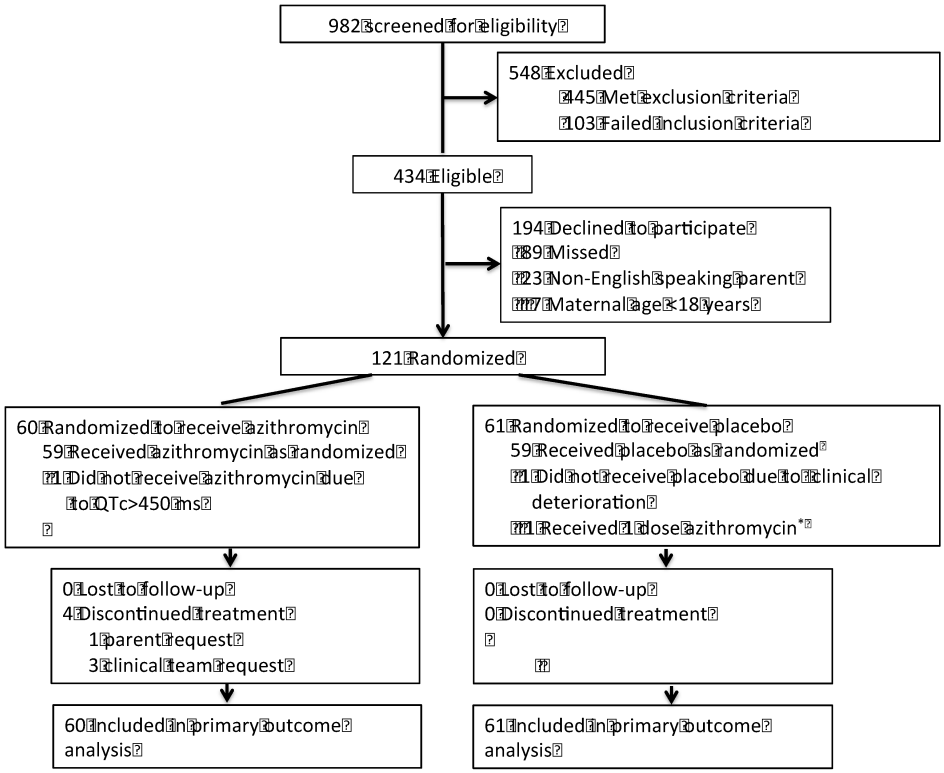
	White (N=51)			Non-White (N=70)		
Morbidity	Azithromycin (N=36)	Placebo (N=15)	P-value[a]	Azithromycin (N=24)	Placebo (N=46)	P-value[a]
Pneumothorax	6/32 (19%)	0/13 (0%)	.16	1/23 (4%)	4/44 (9%)	.65
PDA	18/35 (51%)	5/12 (42%)	.34	7/20 (35%)	16/44 (36%)	.93
Feeding Intolerance	16/32 (50%)	8 (53%)	.37	4/19 (21%)	26/43 (60%)	.008
Gastro-esophageal Reflux	8/36 (22%)	1 (7%)	.25	6 (25%)	10 (22%)	.76
Intestinal Perforation	1 (3%)	2 (13%)	.20	1 (4%)	2 (4%)	>.99
NEC ≥Stage 2	3 (8%)	1 (7%)	>.99	1 (4%)	4 (9%)	.65
Culture-confirmed Sepsis	6 (17%)	2 (13%)	>.99	2 (8%)	12 (26%)	.12
IVH>Grade 2	4/33 (12%)	1/12 (8%)	>.99	3/20 (15%)	1/42 (2%)	.095
PHH requiring shunt	4 (11%)	0 (0%)	.31	2 (8%)	0 (0%)	.11
PVL	3 (8%)	2 (13%)	.62	1 (4%)	3 (7%)	>.99
ROP> Stage 2	10/33 (30%)	3/13 (23%)	0.73	1/23 (4%)	1/43 (2%)	>.99

Abbreviations: PDA, patent ductus arteriosus; NEC, necrotizing enterocolitis; IVH, intraventricular hemorrhage; PHH, post-hemorrhagic hydrocephalus; PVL, periventricular leukomalacia; ROP, retinopathy of prematurity

[a]P-values are based on a score test from Generalized Estimating Equations to account for correlations between twins, or Fisher's Exact Test when one of the cell sizes has an expectation of less than 5.

3. SUPPLEMENTARY FIGURE S1

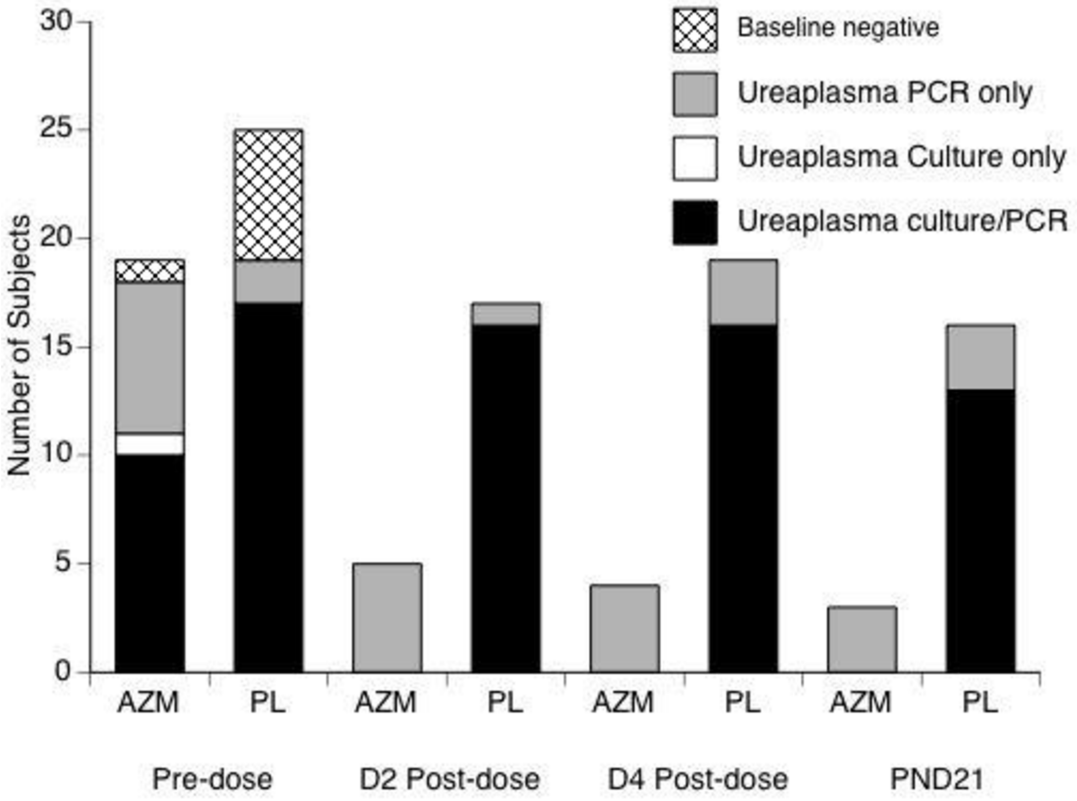
Consolidated Standards of Reporting Trials Diagram of the Azithromycin in Preterms Trial



Consolidated Standards of Reporting Trials Diagram of the Azithromycin in Preterms Trial. One participant in each treatment arm did not receive assigned treatment. *One participant assigned to the placebo group, received one dose of azithromycin due to investigational pharmacy error, but received placebo for other 2 doses. All participants were included in intent-to-treat analysis.

4. SUPPLEMENTARY FIGURE S2

The number of participants positive by culture or PCR at each time point by treatment assignment among those who were *Ureaplasma*-positive by culture or PCR at any time during the study.



Ureaplasma spp. clearance from the respiratory tract in neonates assigned to azithromycin (AZM) and placebo (PL). The number of participants in the azithromycin (AZM) and placebo (PL) groups with *Ureaplasma* detected by both culture and PCR (black), culture alone (white), and PCR alone (gray) at baseline pre-dose, 2 and 4 d post-third dose, and postnatal day 21 (PND21). The number of positive subjects who were negative at baseline, but positive at 1 or later timepoint are indicated by hatched bar. All follow-up cultures were negative in the azithromycin-treated group, but 21/25 (84%) of placebo-treated subjects were culture-positive at one or more follow-up time point.

5. SUPPLEMENTARY METHODS

Statistical Methods

The statistical analyses are influenced by twins who cannot be assumed to be independent, and the small number of some outcome events. To address these challenges, we used generalized estimating equations [1], multiple outputation[2], bootstrap, nonparametric tests, and exact tests as described below.

Analysis of Binary Outcomes

Treatment groups were compared with respect to the proportion of infants who survived *Ureaplasma*-free, and other secondary binary outcomes such as survival, physiologic BPD, etc. To take into consideration correlation of outcomes in twins, the statistical significance of observed differences was assessed based on generalized estimating equations, using an identity link, and assuming an exchangeable correlation between outcomes from twins. Since in simulations, these models did not perform well when the number of outcomes was very small, when any expected cell size was 5 or less in the implicit 2x2 table, we used Fisher's Exact Test by inverting two separate one-sided tests as implemented in SAS 9.4[3].

Analysis of Quantitative Outcomes

Treatment groups were compared with respect to the median value of several quantitative outcomes including duration of mechanical ventilation, duration of supplemental oxygen, and duration of hospital stay. One challenge in these analyses is how to include children who died during hospitalization. To include these children in the analysis appropriately, we used a rank-based (Wilcoxon test) analysis and gave these children the worst ranks. To address the challenge of the correlation between twins, we used multiple outputation [2]. This technique involves repeated analyses after randomly removing one child in each twin set, and combining the results.

REFERENCES

1. Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. *Biometrics*. 1988;44:1049-60.
2. Follmann D, Proschan M, Leifer E. Multiple outputation: inference for complex clustered data by averaging analyses from independent data. *Biometrics*. 2003;59:420-9.
3. Santner TJ, Snell MK. Small sample confidence intervals for p1-p2 and p1/p2 in 2x2 contingency tables. *J American Statistical Association*. 1980;75:386-94.