

1 **Study Protocol:**

2 **Design**

3 The study was a double-masked, placebo-controlled phase 2 trial with 3 treatment groups  
4 (ClinicalTrials.gov NCT02707523). All subjects, their families, medical providers, and study  
5 staff were blinded to study medication allocation, with the exception of the biostatistician and the  
6 pharmacist.

7  
8 **Recruitment and Enrollment**

9 *Subject Recruitment:*

10 16 patients per group [placebo (saline), 10mg/kg azithromycin and 20mg/kg azithromycin group;  
11 total=48] were recruited from the Pediatric Intensive Care Unit (PICU) at Children’s of  
12 Alabama. All children with RSV infection and required positive pressure ventilation (invasive  
13 and non-invasive) were screened for eligibility.

14 *Patient Compensation:*

15 None

16 *Race and ethnicity:*

17 The race and ethnicity were classified based on either patient self-identification or by hospital  
18 staff during admission. Race and ethnicity were assessed in the study to provide a baseline  
19 characterization of patients cared for in this region.

20

21 **Eligibility/Inclusion Criteria:**

22 **Inclusion Criteria:**

23 1. Admission to the PICU at Children's of Alabama with a diagnosis of RSV infection and  
24 requiring positive pressure ventilation (invasive and non-invasive). Non-invasive positive  
25 pressure ventilation is defined as need for BiPAP (bilevel positive airway pressure) or high flow  
26 oxygen (namely, >1 L/kg/min of flow, with a minimum of 5 L/min flow for children <5 kg).

27 2. Enrollment into the study within 48 hours of ICU admission and placement on positive  
28 pressure ventilation

29 3. Age 0-16 years

30 Exclusion Criteria:

31 1. AZM use within 7 days of PICU admission

32 2. Contraindication to AZM use

33 - Patients with electrocardiogram QT interval corrected for heart rate (Qtc)  $\geq$  450 ms

34 - Patients with significant hepatic impairment (direct bilirubin >1.5 mg/dL)

35 - Known hypersensitivity to azithromycin, erythromycin, any macrolide, or ketolide drug

36 - Cardiac arrhythmia

37 3. History of pyloric stenosis

38 4. Patients requiring chemotherapy or on chronic steroids for malignancy or post bone marrow  
39 transplantation

40 5. Positive pressure ventilation greater than 48 hours prior to PICU admission

41

42 Informed Consent:

43 Consent was obtained in a quiet setting prior to the initiation of the study. The legal guardian was  
44 given opportunity to delay consent until he/she has taken adequate time to read and understand  
45 the written consent and discuss the study with others. Subjects were not consented in writing

46 until they were able to demonstrate adequate understanding of all aspects of the study and  
47 consent process. A copy of the consent form was given to the patient/legal guardian. The signed  
48 consent form remained in the patient's study files at the clinical center.

49

50 Randomization:

51 All enrolled subjects were randomized according to a permuted-block design to receive either  
52 placebo (saline) or AZM (Fresenius Kabi, USA) at 10mg/kg/day (referred to as "standard dose")  
53 or 20mg/kg/day (referred to as 'high dose") intravenously (IV) every 24 hours for 3 days. The  
54 randomization schedule was created by the study biostatistician and provided to the study  
55 pharmacist for implementation. The FDA required the study enrollment to be done in two stages,  
56 Stage 1: First 9 subjects (older than 6 months), and Stage 2: Remaining 39 subjects (of all ages)  
57 if no safety issues were found. The first stage enrolled 9 subjects equally to the 3 treatment  
58 groups. For this, the random assignment of treatment was determined by random permutation.  
59 For the second stage, we randomly assigned the 3 treatments equally to the 39 subjects using  
60 block randomization of size 3 or 6. In addition, to maintain blinding to the treatment assignment,  
61 placebo was randomly assigned to either 10 mg/kg or 20 mg/kg dose. Dr. Aban generated the  
62 randomization schedule as described above. Dr. Aban provided the randomization schedule (with  
63 randomization IDs) in an excel file to the study pharmacist in charge of dispensing the  
64 appropriate treatment.

65

66 Duration of Study:

67 The trial began in February of 2016, and due to the age restriction placed by the FDA, the first 9  
68 subjects enrolled were older than 6 months of age. Once this number was attained, and no safety

69 concerns were evident as determined by the DSMB, infants younger than 6 months of age were  
70 eligible for enrollment in the study. The study spanned three and a half RSV seasons, with the  
71 last patient enrolled in February of 2019. The trial ended once 48 subjects were enrolled into the  
72 study. All subjects completed the study. No long term follow up was provided.

73

#### 74 Primary and Secondary Outcomes

75 The primary outcomes of this trial were to ascertain 1. The safety and AZM  
76 pharmacokinetic and 2. If treatment with AZM resulted in decreased nasal MMP-9 levels.  
77 Notably, when the study protocol was expanded to include non-ventilated patients, the primary  
78 outcome also changed from endotracheal MMP-9 to nasal MMP-9 levels. The secondary  
79 outcomes were to determine the *in vivo* efficacy of AZM administration on improving clinical  
80 outcome measures, specifically duration of ventilatory support and oxygenation, as well as PICU  
81 and hospital length of stay. The CONSORT guideline was used for the reporting of this trial.

82

#### 83 Visit Schedule and Study Procedures

84 Overview:

85 If subjects met the inclusion criteria and have no contraindication to study participation, they  
86 were be enrolled into the study within 48 hours of positive pressure ventilation. Subjects were  
87 randomized via a permuted block design to receive either placebo (normal saline), 10mg/kg of  
88 IV azithromycin or 20 mg/kg of IV azithromycin, daily for 3 days. Prior to drug administration,  
89 assessment was performed to ensure that subjects do not have contraindications of AZM  
90 administration or development of any adverse events. Nasal and endotracheal aspirate (if

91 intubated) were obtained daily for the first 3 days (first sample obtained before drug  
92 administration).  
93  
94 Prior to drug administration, baseline labs (CBC with differential, liver and renal function,  
95 electrolytes) was reviewed. If the patient had evidence of hypokalemia, hypocalcemia or  
96 hypomagnesemia, the electrolyte abnormalities were corrected prior to drug administration. EKG  
97 was also reviewed, and if there was evidence of prolonged QT, the subject was excluded from  
98 the study. Vital signs (heart rate, blood pressure, temperature, saturations), ventilator or BiPAP  
99 or HFNC settings, presence of multi-organ failure were recorded. All these parameters were  
100 assessed prior to drug administration. Vital signs (heart rate, blood pressure, temperature,  
101 saturations) were also assessed immediately before the drug infusion, during, and 2 and 4 hours  
102 post completion of infusion.

103

104 During the hospitalization, all patients were treated according to the American Academy of  
105 Pediatrics guidelines for the management of bronchiolitis, namely supportive care. All decisions  
106 regarding medical treatment during hospitalization were made by the patient's primary care  
107 team.

108

109 Data Collection:

110 Nasal, endotracheal aspirates (if intubated) and serum samples were obtained once daily for the  
111 first 3 days. The collection window had at least 24 hours between each sample collection. If  
112 patients were extubated within the first 3 days of study window, only nasal samples will be  
113 collected. Clinical data was collected each day until the subject was discharged from the ICU.

114 An overview of clinical and biologic data is outlined in this table.

	Day 1	Day 2	Day 3	Day of PICU discharge	Day of hospital discharge
AZM level (serum, nasal, lung)	X	X	X		
RSV titer (nasal and lung)	X	X	X		
Active MMP-9, Total MMP-9, TIMP (nasal and lung)	X	X	X		
Other RSV cytokine activity (nasal and lung)	X	X	X		
Continuous vital signs monitoring <sup>1</sup>	X	X	X		
Baseline and continuous lab review <sup>2</sup>	X	X	X		
Electrocardiogram	X	X	X		
Study drug administration <sup>3</sup>	X	X	X		
Duration of positive pressure ventilation (MV, BiPAP, HFNC)	X	X	X	X	X
Duration of oxygenation	X	X	X	X	X
Duration of PICU stay				X	
Duration of hospital stay					X

115 <sup>1</sup>All assessments were done prior to dosing, during dosing until completion of infusion,  
116 immediately at the end of infusion, 2 and 4 hours post completion of infusion.

117 <sup>2</sup>Laboratories that were collected: CBC with differential, electrolytes, liver and renal function;  
118 record if done for clinical care.

119 <sup>3</sup>Study drug administration took place on 3 consecutive days (1 dose per day) after consenting.  
120 Study drug at 2 mg/ml was administered over 60 minutes.

121

#### 122 Withdrawal of Participant Consent and Discontinuation of Study Drug:

123 Study participation may be discontinued for any of the following reasons:

- 124 a. Subject decision to withdraw consent for study
- 125 b. Evidence of allergy to administered products
- 126 c. Intolerable adverse event as judged by study investigator

127

#### 128 **Safety Data**

##### 129 Adverse Events:

130 a. Adverse event: Any untoward medical occurrence in a patient or clinical investigation in  
131 which a subject is given a pharmaceutical product; does not necessarily have a causal  
132 relationship with such treatment, or

133 b. Any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or  
134 disease temporally associated with the use of a medicinal (investigational) product; not  
135 necessarily related to the product.

136 c. Change-from-baseline

137 AEs can take 2 forms:

- 138 i. Appearance of a new symptom or sign, or  
139 ii. Increased severity or frequency of an existing symptom or sign
- 140 d. Severity assessment: Severity refers to the intensity of the event and is generally indicated as  
141 mild (Grade I), moderate (Grade II), severe (Grade III), or life-threatening (Grade IV). Grading  
142 definitions are:
- 143 i. Mild (Grade I): mild transient symptoms, only requiring monitoring or symptomatic treatment,  
144 and clinically significant
- 145 ii. Moderate (Grade II): Moderate illness or condition which requires new or significantly altered  
146 specific therapy
- 147 iii. Severe (Grade III): Severe illness or condition: unresponsive to medical therapy.
- 148 iv. Life-threatening: Life-threatening illness or condition. Complicated by acute, life-threatening  
149 metabolic or cardiovascular complications (such as circulatory failure, hemorrhage, sepsis); life-  
150 threatening physiological consequences; or need for intensive care or emergent invasive  
151 procedure (e.g. requires major surgery).
- 152 e. Causality refers to the likelihood and extent that the investigational agent being studied  
153 contributed to the development of an AE. In making determination, consider temporal  
154 relationship to drug exposure, other possible exposures as etiology, known effects of the  
155 investigational agent, anticipated (expected) side effects derived from preclinical studies, and  
156 what is known about similar drugs (See drug insert).

157 *Relatedness of AEs to an Intervention (Agent)*

158 Definite (must have all 4)

- 159 • Has a reasonable temporal relationship to the intervention

- 160 • Could not have readily been produced by the subject's clinical state or have been due to  
161 environmental or other interventions
- 162 • Follows a known pattern of response to intervention
- 163 • Disappears or decreases with reduction in dose or cessation of intervention and recurs with re-  
164 exposure
- 165 Probable (must have 3)
- 166 • Has a reasonable temporal relationship to the intervention
- 167 • Could not have readily been produced by the subject's clinical state or have been due to  
168 environmental or other interventions
- 169 • Follows a known pattern of response to intervention
- 170 • Disappears or decreases with reduction in dose or cessation of intervention
- 171 Possible (must have 2)
- 172 • Has a reasonable temporal relationship to the intervention
- 173 • Could not have readily been produced by the subject's clinical state
- 174 • Could not readily have been due to environmental or other interventions
- 175 • Follows a known pattern of response to intervention
- 176 Unlikely (must have 2)
- 177 • Does not have a temporal relationship to the intervention
- 178 • Could readily have been produced by the subject's clinical state
- 179 • Could have been due to environmental or other interventions
- 180 • Does not follow a known pattern of response to intervention
- 181 • Does not reappear or worsen with reintroduction of intervention

182 f. Duration of event: Onset of signs and symptoms as well as resolution of the event should be  
183 recorded, typically defined as the point when all signs and symptoms have subsided. Some  
184 events continue or change in severity over time. Capturing this information is also important.  
185 Changes in severity such as when an event improves from severe to mild should be recorded as  
186 separate events, with separate intensities and durations collected.

187 g. Adverse reaction: Defined as an adverse event caused by a drug. Therefore, adverse reactions  
188 are a subset of all suspected adverse reactions for which there are reasons to conclude that the  
189 drug caused the event.

190 h. Suspected adverse reaction (21 CFR 312.32(a)): Suspected adverse reaction means any  
191 adverse event for which there is a reasonable possibility that the drug caused the event.  
192 Reasonable possibility means there is evidence to suggest a causal relationship between the drug  
193 and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about  
194 causality than adverse reaction, which means any adverse event caused by a drug.

195 i. Unexpected (21 CFR 312.32(a)): An adverse event or suspected adverse reaction is considered  
196 “unexpected” if it is not listed in the investigator brochure (or drug insert) or is not listed at the  
197 specificity or severity that has been observed; or, is not consistent with the risk information  
198 described in the general investigational plan or elsewhere in the protocol. “Unexpected” also  
199 refers to adverse events or suspected adverse reactions that are mentioned in the investigator  
200 brochure as occurring with a class of drugs or as anticipated from the pharmacologic properties  
201 of the drug, but are not specifically mentioned as occurring with the particular drug under  
202 investigation (e.g. adverse events associated with other macrolides, but not specifically AZM  
203 such as pyloric stenosis). In addition, adverse events that would be anticipated to occur as part of

204 the disease process are considered ‘unexpected’ for the purposes of reporting because they would  
205 not be listed in the investigator brochure.

206 j. Serious (21 CFR 312.32(a)): An adverse event or suspected adverse reaction is considered  
207 ‘serious’ if, in the view of either the investigator or sponsor, it results in any of the following  
208 outcomes: death, a life-threatening adverse event, inpatient hospitalization, or prolongation of  
209 existing hospitalization, a persistent or significant incapacity, or important medical event that  
210 may, based on appropriate medical judgment, jeopardize the patient or subject and may require  
211 medical or surgical intervention to prevent one of the other outcomes listed in the definition.

212 k. Life-threatening (21 CFR 312.32(a)): An AE or suspected adverse reaction is considered ‘life-  
213 threatening if, in the view of the investigator or sponsor, its occurrence places the patient or  
214 subject at immediate risk of death.

215 SAEs

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217 ‘serious’ if, in the view of either the investigator or sponsor, it results in any of the following  
218 outcomes: death, a life-threatening adverse event, inpatient hospitalization, or prolongation of  
219 existing hospitalization, a persistent or significant incapacity, or important medical event that  
220 may, based on appropriate medical judgment, jeopardize the patient or subject and may require  
221 medical or surgical intervention to prevent one of the other outcomes listed in the definition.

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223 threatening if, in the view of the investigator or sponsor, its occurrence places the patient or  
224 subject at immediate risk of death.

225

226 **Safety Monitoring:** An independent Data and Safety Monitoring Board (DSMB) was appointed.  
227 Members included two pediatric intensivists, a biostatistician, a clinical trials epidemiologist, and  
228 an infectious disease specialist. The DSMB 1) reviewed and analyzed the progress of the study,  
229 2) approved amendments to the trial protocol, 3) monitored the safety of the study treatments and  
230 4) reviewed data quality. Monitoring for serious or unexpected adverse events (SAEs) was  
231 performed throughout the ICU period. The DSMB met before subject recruitment began and  
232 subsequently meet every 4-6 months in person or by teleconference to review the study progress  
233 and AE and SAE reports.

234

#### 235 Data Processing and Management

236 All participant data were obtained and recorded in individual files that was stored in a secured  
237 file cabinet within a locked office. Upon study entry, all participants were given a unique  
238 identifier to be used through the entirety of the study and this too was maintained in a locked file  
239 cabinet in secured office. Following each study visit, the data obtained was entered into a  
240 database using the study participants' unique identifiers. All other personal data were omitted  
241 from electronic records. At the close of the study, any missing data was verified with chart  
242 review. Only authorized study personnel have access to patient data.

243

#### 244 Data Analysis

245 The primary outcome measured was MMP-9 activity. Based on our preliminary data, we expect  
246 that n=16 per group would be sufficient to detect a 20% and 25% decrease in MMP-9 activity in  
247 the treated group (10mg/kg and 20mg/kg; respectively) relative to placebo with 80% power.

248 Inmaculada Aban, Ph.D (Professor, UAB School of Public Health) is the biostatistician for this  
249 clinical trial.

250

### 251 **Nasal and Endotracheal Aspirate Collection and Processing**

252 Nasal samples were collected using a nasal swab inserted through the nares parallel to the palate  
253 (not upwards) until resistance was encountered or the distance inserted was equivalent to that  
254 from the nostril to the ear of the patient indicating contact with the nasopharynx. After gentle  
255 rubbing and rolling of the swab, it was left in place for several seconds to absorb secretions prior  
256 to removal. The swab was then immediately placed into a sterile vial containing 3 ml of viral  
257 transport media (Quidel, San Diego, CA), and kept at 4°C for further analysis.

258

259 Endotracheal aspirates were collected via endotracheal tube suctioning using an 8 Fr suction  
260 cannula (CareFusion, San Diego, CA, USA) and centrifuged at 500xg for 10 min to separate  
261 supernatant from cells and mucus (pellet) . Separate aliquots of supernatant were subsequently  
262 saved at 4°C for further analysis.

263

### 264 **Assay of AZM, Urea and Pharmacokinetic Analysis**

265 AZM and urea levels in tracheal aspirates and plasma samples were measured using liquid  
266 chromatography-tandem mass spectrometric assays. For quantitation of AZM, all samples were  
267 treated with 200 µL of acetonitrile containing terfenadine (5 ng/mL) as an internal standard.  
268 Samples were then vortexed briefly and centrifuged at 20,000xg for 5 minutes. The supernatant  
269 was transferred to autosampler vials for analysis. Detection was performed using an Applied  
270 BioSystems 4000 QTRAP (Applied Biosystems, Foster City, CA) triple quadrupole mass

271 spectrometer operated in the positive ion mode. Separation of AZM and the internal standard  
272 from the biological matrix was achieved using a Phenomenex Luna C18, 100 X 2 mm 5 µm  
273 particle column. The mobile phase was delivered at a flow rate of 400 µL/min using a gradient  
274 elution profile consisting of DI water with 0.1% formic acid (A) and acetonitrile with 0.1%  
275 formic acid (B). The AZM and internal standard were detected using multiple reaction  
276 monitoring (MRM) for the following transitions: AZM (m/z 749.6→591.5), and Terfenadine  
277 (m/z 472.4→436.3).

278  
279 Samples (20 µL) for urea analysis were prepared by diluting each sample matrix with 955 µL of  
280 90:10 acetonitrile:water followed by the addition of 25 µL of internal standard (10 µg/mL 4-  
281 aminobenzoic acid). The samples were vortexed, centrifuged and the resulting supernatant was  
282 transferred to autosampler vials and analyzed by LC/MS/MS. Separation of urea and the internal  
283 standard from the biological matrix was achieved using a Waters Spherisorb Silica, 150 X 3.2  
284 mm 5 µm particle column (Waters Corporation, Milford, MA). The mobile phase was delivered  
285 at a flow rate of 400 µL/min using a gradient elution profile consisting of DI water with 0.1%  
286 formic acid (A) and acetonitrile with 0.1% formic acid (B). The urea and internal standard were  
287 detected using multiple reaction monitoring (MRM) for the following the following transitions:  
288 Urea (m/z 61.1→44.1), and 4-aminobenzoic acid (m/z 138.1→120.1).

289  
290 Dilution estimations of recovered samples from endotracheal compartment were calculated using  
291 the urea dilution method. The following equation was used to calculate the corrected drug level  
292 in these samples:

293

$$AZM_{corrected} = \frac{Urea_{plasma}}{Urea_{matrix}} \times AZM_{measured}$$

294

295 Where  $Urea_{matrix}$  was the amount of urea in the endotracheal samples,  $Urea_{plasma}$  was the amount  
296 of urea in the plasma of the corresponding patient,  $AZM_{measured}$  was the measured amount of  
297 AZM in the matrix being assayed.

298

299 Peak plasma levels ( $C_{max}$ ) and time to  $C_{max}$  ( $T_{max}$ ) of AZM in all matrices were determined by  
300 visual evaluation of the concentration versus time profile. The AUC (area under the curve)  
301 values from 0 to 144 hours for each matrix was calculated using the trapezoidal rule. The half-  
302 life ( $t_{1/2}$ ) of AZM in plasma and endotracheal samples were calculated as  $\ln(2)/k_e$ , where  $k_e$  is the  
303 terminal elimination rate constant estimated by linear regression analysis of the terminal portion  
304 of the concentration-time profile.

305

306 Penetration ratios for AZN in samples from the endotracheal compartment were calculated as  
307  $AUC_{endotracheal}/AUC_{plasma}$ .

308

### 309 **Biologic Outcome Measurement**

310 Measurement of active MMP-9, total MMP-9 and Tissue Inhibitor of Metalloproteinase (TIMP)-  
311 1 was done using an established fluorometric assay (#F9M00 and #DTM100, respectively; R&D  
312 Systems, Calbiochem, Billerica, MA, USA). Interleukin (IL)-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-  
313 12, IL-13, tumor necrosis factor (TNF)- $\alpha$ , and interferon (IFN)- $\gamma$  were analyzed via  
314 electrochemiluminescence using multi-array plates on a Meso Scale Discovery (MSD) Sector  
315 Imager 2400 (MSD, Gaithersburg, MD) using the MSD V-PLEX Cytokine Panel 1 Human Kit

316 (No. K15049D-1, Rockville, Maryland, USA). All samples were processed as indicated by the  
317 manufacturer's protocol.

318

319 In brief, RSV loads were measured by reverse-transcription (RT) quantitative PCR using known  
320 concentrations of RSV to derive a standard curve. Standards and negative controls were included  
321 and tested with each PCR assay. RSV quantification was reported as log<sub>10</sub> copies/mL.

322

### 323 **Clinical Data Assessment**

324 The clinical data collected included duration of respiratory support, focusing on days of  
325 mechanical ventilation, non-invasive positive pressure ventilation via BiPAP or high flow nasal  
326 cannula, and total duration of supplemental oxygen. Other clinical data assessed included length  
327 of PICU and hospital stay. Co-morbidities such as history of prematurity, apnea, seizure  
328 disorder, chromosomal abnormalities, and the presence of multi-organ failure were identified.

329

### 330 **Sample Size Determination and Statistical Analysis**

331 This study was designed to have a total sample size of 48 participants divided into three  
332 groups, with 16 per group to detect a 25% and 37.5% decrease in lung MMP-9 activity in the  
333 treated groups (10mg/kg and 20mg/kg, respectively) relative to placebo with 80% power using  
334 analysis of variance. The assumptions were based on MMP-9 levels found in endotracheal  
335 aspirate of children with RSV-induced respiratory failure (14).

336

337 Means, standard deviations, medians, quartiles and range (minimum, maximum)  
338 described continuous variables and counts and percentages for categorical variables. To compare

339 baseline characteristics among the treatment groups, we used Kruskal-Wallis test for age, height  
340 and weight due to evidence of non-normality of the distribution of the values. We used either  
341 Chi-Square or Fisher's exact test for categorical variables depending on expected cell counts.

342 We analyzed inflammatory markers by first performing  $\log_{10}$  transformation to address  
343 the skewness of the distribution and extreme outliers. We then calculated the difference at each  
344 time point relative to baseline to obtain the change (i.e. pre and post treatment) outcome and  
345 fitted a generalized linear mixed model (GLMM) with random intercepts and assumed unequal  
346 variances across treatment groups with change from baseline as the outcome. The model  
347 included treatment, day and treatment by day interaction. Point and confidence interval estimates  
348 of the mean change outcome at each time point and treatment group combination were obtained.  
349 If a confidence interval for the mean change does not include 0, this is evidence of an effect  
350 associated with that particular treatment as it is either a significant increase (or decrease) in the  
351 inflammatory marker relative to baseline. The Bonferroni approach was used to adjust for  
352 multiple testing and simultaneous confidence intervals. Thus, significance was determined using  
353 a cut-off of 0.002 and a 99.8% level was used to construct confidence intervals for each for each  
354 treatment at each time point. Note that the sample size justification was based on the assumption  
355 that the MMP-9 levels are normally distributed with common standard deviation. However, in  
356 the data for this trial, we observed evidence of non-normality and non-constant variance across  
357 the treatment groups. Thus, GLMM was deemed more appropriate than ANOVA. Furthermore,  
358 fitting GLMM allows us to combine unbalanced data from different time points to help gain  
359 more power in evaluating treatment effect.

360

361 We used a generalized linear model assuming a negative binomial distribution for clinical

362 outcomes (measured in number of days), using log link to address possible overdispersion due to  
363 some extreme observations. Furthermore, we fitted a model that allowed the variance to differ by  
364 treatment group. Spearman Correlation was used to examine associations. For this set of  
365 analyses, the standard 5% level of significance and 95% confidence interval was used as these  
366 are all exploratory outcomes. All analyses were done using SAS version 9.4.

367

368 **IRB and IND**

369 The study protocol was approved by the University of Alabama at Birmingham Institutional  
370 Review Board. An Investigational New Drug (IND) was obtained from the U.S Food and Drug  
371 Administration (FDA) for the use of AZM in this trial (IND 127632). An age restriction was  
372 placed by the FDA at the onset of enrollment for the first 9 patients whereby only infants older  
373 than 6 months of age could be screened and potentially enrolled in the study.

