FDA

Division of Anti-Infectives Drug Products

Advisory Committee

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Briefing Document for

Zithromax® Accelerated Dosing –

Treatment of Acute Otitis Media

AVAILABLE FOR PUBLIC DISCLOSURE

WITHOUT REDACTION

EXECUTIVE SUMMARY

This Briefing Document presents evidence to support the clinical efficacy and safety of a total 30 mg/kg dose of azithromycin given over three days or as a single dose for treatment of children with acute otitis media.

Acute otitis media is a major source of childhood morbidity, with a peak incidence in children < 2 years of age. Physicians frequently prescribe antibiotics to more rapidly improve the acute suppurative illness, reduce serious complications and reduce hearing loss associated with effusion.[1] However, most antibacterial medications currently available require dosing 2-3 times daily for 7-10 days, a dosing schedule which results in the possibility of missed doses and unfinished courses of therapy.[2] Noncompliance with treatment regimens that exceed the period of symptom resolution (typically 2-5 days) may be more likely to result in treatment failure and re-treatment; this, in turn, increases the use of antibiotics in the community.[3, 4] As a consequence, a short and convenient means of treating children with acute otitis media is desirable to improve compliance, improve patient and parental satisfaction, and minimize potential for development of resistant organisms.

Azithromycin is the first of a class of antibiotics designated chemically as "azalides". Its mechanism of action is inhibition of protein synthesis. Azithromycin has unique features that make it an excellent candidate for shortened course therapy of acute otitis media. Among these features are activity in *in vitro* and in *in vivo* against the pathogens commonly associated with acute otitis media, including *S. pneumoniae, M. catarrhalis,* and *H. influenzae* as well as high concentrations in phagocytes and tissues relative to serum, resulting in prolonged tissue and serum half-lives. Studies in mice and gerbils showed that phagocytes deliver azithromycin directly to the infection site, and in the gerbil model, azithromycin concentrations in bulla increased and persisted over the same time period that serum concentrations decreased.[5]

Pharmacokinetic data from clinical studies further show that an azithromycin regimen given over three days or as a single dose provides at least equivalent drug exposure to the currently approved 5-day regimen, and that azithromycin is present in middle ear fluid (MEF) at levels necessary for bacterial killing. Pharmacodynamic data also provide evidence that AUC/MIC (and perhaps *Cmax*) are the important pharmacokinetic parameters in the microbiologic efficacy of azithromycin.

In 1995, the FDA approved azithromycin oral suspension for treatment of children with acute otitis media based on a total 30 mg/kg dose administered over five days (10 mg/kg on day 1, 5 mg/kg/day on days 2-5). Based on pharmacokinetic data, a 3-day regimen providing the same total dose was approved in Europe and elsewhere in the world. Subsequently, studies were performed in the United States with the

3-day regimen and, in addition, a single-dose regimen, as outlined in the table below.

Study	Treatment	Total Daily Dose	Duration	Design
Pivotal Studies				
A0661014	Az 3-day Augmentin®	10 mg/kg 45 mg/kg (dosed b.i.d.)	3 days 10 days	Double-blind
A0661015	Az SD	30 mg/kg	1 day	Noncomparative
R-0581	Az SD Augmentin®	30 mg/kg 45 mg/kg (dosed b.i.d.)	1 day 10 days	Double-blind
Supportive Study				
AZM-NY-95-001	Az 3-day Az SD Ceftriaxone IM	10 mg/kg 30 mg/kg 50 mg/kg	3 days 1 day 1 day	Single-blind (between Az groups)

Az = Azithromycin; SD = Single Dose; IM = Intramuscular; b.i.d. = twice daily

The following table summarizes clinical efficacy results. Clinical cure at the Test of Cure (TOC) visit (day 28) was the primary endpoint for analysis, as recommended in the 1998 FDA Draft Guidance. The analysis of these data was performed on a modified intent-to-treat (MITT) basis.

Clinical Cure at Test of Cure (Day 28) Visit				
Study	Az 3-day	Az SD	Comparator	95% CI*
A0661014 Az- Augmentin®	74%	--	69%	 -5, 15%
AZM-NY-95-001 Az 3-day – Ceftriaxone Az SD - Ceftriaxone	77%	86%	77%	 -15, 17% -5, 25%
R-0581 Az SD - Augmentin®	--	74%	70%	 -7, 15%
A0661015	--	85%	--	--
Overall (all studies) Az 3-day – Ceftriaxone Az SD - Ceftriaxone	75%	82%	71%	 -3, 11% 5, 17%

Az=Azithromycin; SD = Single Dose;* Confidence Interval on difference between treatment groups.

These data demonstrate that azithromycin provides clinical efficacy equivalent to the comparative agents (Augmentin®, ceftriaxone IM) approved for this indication.

Two of these studies (A0661015, AZM-NY-95-001) provided for culture of baseline pathogens from middle ear fluid, allowing assessment of clinical cure by infecting pathogen.

Clinical Cure by Baseline Pathogen at Test-of-Cure (Day 28) Visit – Studies A0661015 and AZM-NY-95-001				
Baseline Pathogen	Az 3-day	Az SD	Ceftriaxone	95% CI*
S. pneumoniae Az 3-day – Ceftriaxone Az SD - Ceftriaxone	16/17 (94%)	84/96 (88%)	19/23 (83%)	 -13, 36% -15, 25%
H. influenzae	9/13 (69%)	35/52 (67%)	8/9 (89%)	-
M. catarrhalis	2/2	10/10	--	-

Az=Azithromycin; SD = Single Dose;* Confidence Interval on difference between treatment groups.

The results provide evidence for equivalent treatment effects in treatment of otitis media due to *S. pneumoniae*. The limited number of *H. influenzae* and *M. catarrhalis* isolates prohibited statistical comparisons, but the azithromycin efficacy rates are similar to those seen previously with azithromycin 5-day and to those presented in the labeling for other antibiotics approved for treatment of otitis media. Historically, otitis media caused by susceptible *H. influenzae* infection has been difficult to treat.

Safety data confirm that azithromycin is among the better tolerated antimicrobial agents available. As reflected in the incidence of treatment-related adverse events, the safety profile of azithromycin 30 mg/kg total dose was similar whether given over 3 days (9%) or 5 days (6%). When given as a single dose, the incidence of adverse events (14%) was slightly higher. The incidence of vomiting was 5%, 2%, 1% on the single, three and five day dosing regimens, respectively. Analysis of the clinical outcome of children who vomited showed they were no more likely to fail therapy than children who did not vomit. With all three regimens, no new side effects were observed and the severity distribution of the known side effects was similar.

Lastly, an analysis of compliance data from studies A0661014 and R-0581 comparing azithromycin to Augmentin® showed that patients were more likely to adhere to a regimen of azithromycin of three days or a single dose. While other variables, such as side effects and taste, may influence compliance, it is likely that a shorter treatment regimen plays a significant role.

In summary, a 30 mg/kg total dose of azithromycin is clinically effective and safe whether administered over 1, 3 or 5 days. Shorter course therapy offers benefits related to compliance and patient/parental satisfaction in a treatment regimen that is safe and effective.

Appendices

GLOSSARY*

Abbreviation	Definition
AOM	Acute Otitis Media
AUC	Area Under the Concentration vs. Time Curve
CFU	Colony Forming Units
CI	Confidence Interval
Cmax	Maximum Concentration
ED_{50}	Median Effective Dose
EOT	End of Therapy
FDA	Food and Drug Administration
Hib	*Haemophilus influenzae* type b
H. influenzae	*Haemophilus influenzae*
IL-1 beta	Interleukin-1 beta
IM	Intramuscular
iNOS	Inducible Nitric Oxide Synthase
lb	Pound
LOCF	Last Observation Carried Forward
MEF	Middle Ear Fluid
MIC	Minimal Inhibitory Concentration
MITT	Modified Intent-to-Treat
MNL	Mononuclear
M. catarrhalis	*Moraxella catarrhalis*
NCCLS	National Committee on Clinical Laboratory Standards
NDA	New Drug Application
PD50	Dose that protects 50% of animals
PP	Per Protocol
PCR	Polymerase Chain Reaction
PMNL	Polymorphonuclear
q12h	Every 12 hours
rRNA	Ribosomal RNA
sNDA	Supplemental NDA
S. pneumoniae	*Streptococcus pneumoniae*
S. pyogenes	*Streptococcus pyogenes*
TNF	Tumor Necrosis Factor
TM	Tympanic Membrane
Tmax	Time to Maximum Concentration
TOC	Test of Cure
U.S.	United States

* Glossary applies to main text and appendices.

1 INTRODUCTION

Azithromycin was approved in the United States in 1995 for the treatment of acute otitis media using a total dose of 30 mg/kg given over 5 days. This briefing document will provide an overview of the *in vitro*, pharmacokinetic, and clinical trial data supporting supporting the use of Zithromax given orally for the treatment of acute otitis media using a 1- or 3-day dosing regimen. These data demonstrate that the same 30 mg/kg total dose, given as a single dose or over three days, delivers clinical efficacy equivalent to presently available therapy while maintaining a favorable safety profile, improving patient compliance and easing the burden of medication delivery on the caregiver.

Approximately three-fourths of all outpatient antibiotics in the Untied States are prescribed for acute respiratory infections.[6] The majority of these antibiotics are given to children diagnosed with otitis media.[7] As reviewed by Pichichero and Cohen (1997)[8], poor compliance is the primary reason for treatment failure in these children. Poor compliance is related to the number of daily doses as well as the duration of therapy, among other factors.[9,10] Because parents tend to discontinue their child's antimicrobial therapy when the symptoms of otitis media have resolved (typically within 2-5 days), longer treatment courses are more likely to result in poor compliance and treatment failure. Therefore, an argument can be made that among equally effective antibiotic regimens, there are advantages to prescribing the shortest and most convenient therapy. Other possible benefits of short course therapy include fewer side effects, improved patient (and parental) satisfaction and, because of improved compliance, a potential attenuation of the selective pressure on the microbial flora by minimizing the need for a second course of antibiotics.

As Zithromax is already approved for the treatment of acute otitis media, the data in this submission is provided in order to justify an amendment to the label to allow a 30 mg/kg total dose of azithromycin to be administered over 1, 3, or 5 days.

2 BACKGROUND

An estimated 25 million cases of acute otitis media occur in U.S. children each year.[11] By age 3, more than half of all children have experienced one or more episodes of acute otitis media; by age 7, this percentage rises to 65-95%.[12, 13] *S. pneumoniae*, *M. catarrhalis*, and *H. influenzae* account for most cases of acute otitis media of bacterial etiology. Otitis media is a major source of childhood morbidity, with a peak incidence in < 2 year-olds. Antibiotic treatment is required to more rapidly improve the acute suppurative illness, reduce serious complications,

such as mastoiditis and reduce hearing loss associated with effusion.[1] While a 10-day course of amoxicillin is frequently recommended as the first-line course of antibiotic therapy, recent trends in the susceptibility profile of *H. influenzae* and *M. catarrhalis* indicate an increasing prevalence of beta-lactamase-production.[14]

Streptococcus pneumoniae is also becoming less sensitive to antibiotic treatment with beta-lactam antibiotics. Mean inhibitory concentrations to amoxicillin have increased in recent years, resulting in recommendations to double the amount of amoxicillin needed to treat otitis media.[15,16]

Other treatment options for otitis media include amoxicillin-clavulanate (Augmentin®), intramuscular ceftriaxone, and oral third generation cephalosporins (cefixime, others). These therapies provide coverage of the offending pathogens, but they do so with higher side effect rates, the requirement for injectable administration, or the need for multiple daily administrations over extended periods of time. These therapies all rely on a similar mechanism of action, namely attachment to penicillin binding proteins and disruption of the bacterial cell wall. The overlapping approach to bacterial killing inherent in these drugs means that the use of each contributes to the potential for resistance to the others.

Alternative antibiotic therapies that are easier to administer, that can be used in patients allergic to other classes, such as beta-lactams, and that rely on alternative mechanisms of bacterial killing that are stable in the presence of beta-lactamases, without compromising safety or efficacy, would be desirable.

Azithromycin is a well established antibacterial agent and is the first of a class of antibiotics designated chemically as “azalides", which are closely related to macrolides. Attributes of azithromycin include: a) prolonged serum and tissue half-lives, b) higher concentrations in phagocytes and tissues, and c) increased potency against Gram-negative organisms relative to other macrolides. It is therefore an excellent candidate for shortened course therapy for common outpatient infections.

Azithromycin is active *in vitro* against the pathogens responsible for otitis media, including *S. pneumoniae, H. influenzae* and *M. catarrhalis.* Animal model data were provided in support of the original NDA for five-day dosing.[17, 18,19,20, 21] Efficacy was predicted in clinical settings from *in vivo* experiments that correlated efficacy with total dose delivered.[18] Clinical studies were performed which led to its approval in the United States for a 5-day treatment regimen for otitis media using a total dose of 30 mg/kg: 10 mg/kg given on Day 1, followed by 5 mg/kg/day on Days 2-5. It has since been approved for the same total dose (e.g. 30 mg/kg) using a 3-day regimen, based on pharmacokinetic data, in Europe and elsewhere in the world. Clinical studies were recently performed with the same regimen in the United States.

The pharmacokinetic data for azithromycin suggest that it would be possible to deliver the entire treatment regimen in a single dose and still expect good clinical activity. Based on this assumption, a clinical program employing single dose therapy was also initiated.

3 GENERAL *IN VITRO* PRE-CLINICAL INFORMATION

3.1 Mechanism of Action

The mechanism of action of azithromycin is by inhibition of protein synthesis. Azithromycin binds primarily to residues A2058 and A2059 in the peptidyl transferase domain of 23S rRNA, thereby blocking nascent polypeptide synthesis in the channel as well as inhibiting the assembly of newly-forming 50S subunits.[22, 23, 24, 25] Nucleic acid synthesis is not affected.

3.2 Antimicrobial Spectrum of Activity

Azithromycin has been shown, *in vitro*, to be active against a wide variety of pathogens, including the main organisms causing acute otitis media: *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*, as seen in the table below.[26,27;28,29,30]

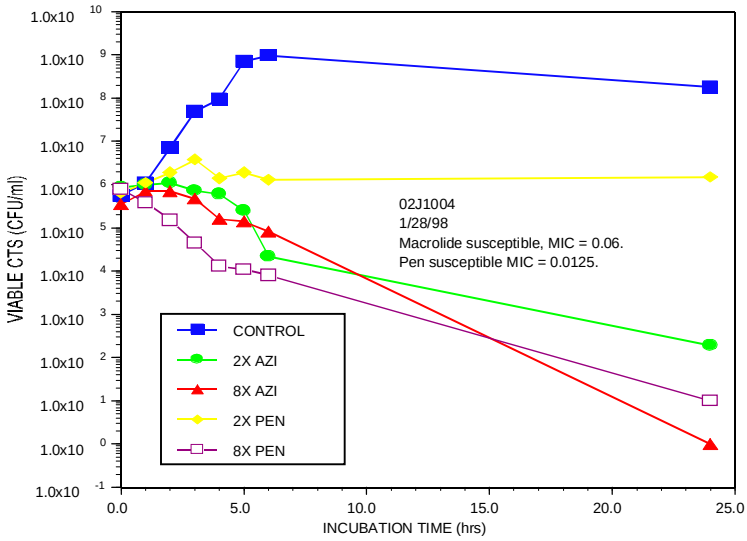
Azithromycin: *In Vitro* Activity

Organism	N	MIC_{50}	MIC_{90}
S. pneumoniae[a]	4193	≤0.12	2
mef(A)[b]	70	4	8
erm(B)[c]	65	>128	>128
pen S[d]	1212	≤0.25	≤0.25
pen I[d]	250 (15%)	≤0.25	16
pen R[d]	203 (12%)	1	>32
Haemophilus influenzae[a]	3116	1	2
Moraxella catarrhalis[a]	1411	≤0.12	≤0.12

A =Hoban, CID 2001;32 *Suppl 2): S81-S93
B= Shortridge, CID 1999;29:1186-1188
C= Montanari, J Clin Microbiol. 2001;39:1311-1315
D= Jones, Diagn Microbiol Infec Dis 2000;37: 93-98 (N=1665 total isolates)

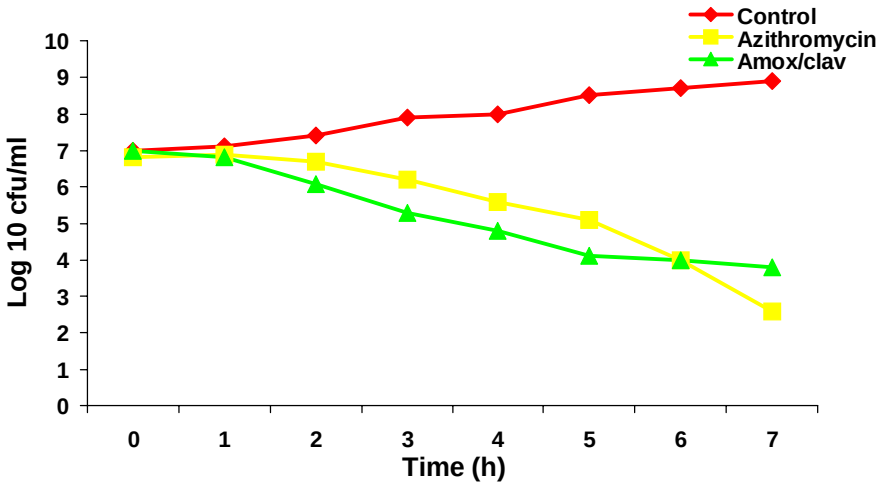
In addition, bactericidal activity has been demonstrated against *H. influenzae* and *S. pneumoniae*, as seen in the figures below.[31,32]

Azithromycin Time-kill Curve: Activity vs *S. pneumoniae*



Pfizer, data on file.

Bactericidal Activity of Azithromycin Against *H. influenzae*



- **MIC = 1.0 µg/ml for each organism**
- **antibiotic at 2x MIC**

Berry, V., AAC 1998; 42: 3193-3199.

3.3 Resistance: Mechanisms

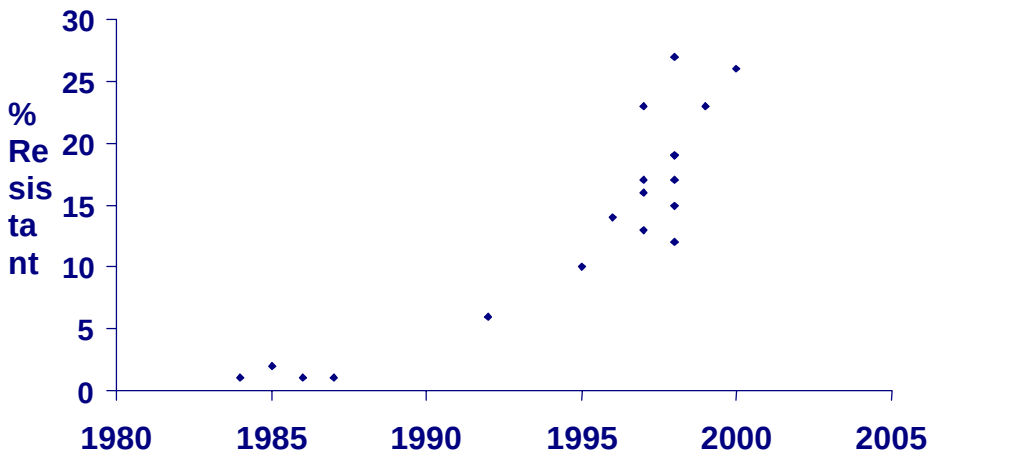
There are two widespread mechanisms of macrolide resistance in *S. pneumoniae* – *erm*(B) and *mef*(A).[23, 24, 25,33,34] *erm*(B) encodes a ribosomal methylase that adds two methyl groups to A2058 in 23S rRNA, thereby reducing the binding affinity for macrolides and two other structurally unrelated antibiotic classes, lincosamides and streptogramin B. *mef*(A) encodes an efflux pump specific for 14- and 15-membered macrolides. As the table above illustrates, the MIC_{90s} for *S. pneumoniae* are quite different with regard to the mechanism of macrolide resistance, and they tend to increase with the level of penicillin resistance.

3.4 Resistance: Epidemiology

3.4.1 *S. pneumoniae*

Surveillance data have demonstrated a rise in the prevalence of resistance of *S. pneumoniae* to macrolides in the United States (figure below).[26,35,36,37,38,39]

The current level of macrolide resistance in *S. pneumoniae* is approximately 20%-25% according to multiple surveillance studies.[40,41,42]. The *mef*(A) gene accounts for the majority of this resistance in the United States.[27,43]



A similar trend exists for the susceptibility of *S. pneumoniae* to beta-lactams. These surveillance data represent S. *pneumoniae* isolated from the respiratory tract as well as invasive sites and/or normally sterile sites. There are relatively few surveillance studies, however, that measure the susceptibility profile of *S. pneumoniae* isolated from the middle ear space. One surveillance study by Wald et al[44] of both invasive site isolates and middle ear isolates collected at eight children's hospitals in the U.S. demonstrated wide chronologic and geographic variation in the prevalence of resistance of *S. pneumoniae*; however, in every location, the middle ear isolates were less likely to be susceptible to beta-lactams (penicillin and ceftriaxone) than were systemic isolates, and the resistance rates were higher for younger children, those who received antibiotics in the previous month, those attending daycare, and those with a history of recurrent otitis media.

In short, *S. pneumoniae* resistance to macrolides, beta-lactams, and other antibiotics continues to be on the rise. Among the variables which are potentially driving this increase in resistance rates are the overuse of antimicrobials, social crowding and increased daycare attendance, international travel, and shifts in the demographic characteristics of the community, specifically its age distribution and level of vaccination.

4 PHARMACOKINETIC AND PHARMACODYNAMIC STUDIES

4.1 Animal Pharmacokinetic Studies

Pre-clinical investigations of the pharmacokinetics of azithromycin *in vitro* and in rats and dogs have demonstrated several unique features, including a very high affinity for tissues; a prolonged serum and tissue half-life after multiple dosing; and uptake and concentration in phagocytic cells without impairing their function.

Pharmacokinetic studies with oral azithromycin in animals showed serum concentrations to be proportional to dose. However, tissue concentrations up to several hundred times those in serum were achieved, with a half-life that varied from 26 to 160 hours after dosing from 1 to 10 days, respectively, in dogs. Additionally, initial radiolabelled studies in rats and dogs recovered only 64% and 57% of the drug after 7 days collection. These findings are markedly different from other antibiotics.[5,17]

Azithromycin has been shown *in vitro* and *in vivo* to concentrate in phagocytic cells, a phenomenon possibly related to its amphiphilic cationic properties. This uptake may be enhanced by as much as twofold when the temperature of the media is increased from 37°C to 40°C.[45] The uptake of azithromycin into macrophages, without impairing their function, is of potential benefit leading to increased delivery of the drug to infection sites and also possibly greater activity against pathogens absorbed into macrophages but not killed, such as *Staphylococcus aureus.*[46] For example, greatly increased concentrations of azithromycin in the peritoneal cavity of mice receiving intraperitoneal injections of sodium caseinate (to induce an inflammatory response) demonstrate the potential for azithromycin-laden phagocytes to deliver the drug to sites of infection. Also, in the gerbil model of otitis media, following a single azithromycin dose of 100mg/kg, azithromycin levels within bulla were found to increase and persist at 48 hours post dose during which time serum levels were decreasing. However, in the same study, bulla levels of comparative agents (amoxicillin, clarithromycin, erythromycin, roxithromycin) tended to decrease in parallel with serum elimination curves.[46]

The antimicrobial activity of azithromycin appears to correlate with the ratio of plasma AUC to MIC for certain key pathogens. In a recent series of studies comparing azithromycin with other macrolides, Craig, *et al*[47] reported on the ability of azithromycin to suppress pathogen growth at sub-minimal inhibitory concentrations (the post antibiotic effect), and related this effect to the pharmacokinetics of azithromycin *in vivo* using a mouse model. They showed that the bacteriostatic effect of azithromycin was independent of the dosing interval used within a 24-hour period, and that the high levels of azithromycin found in the white blood cell compartment contribute significantly to the efficacy of this drug. Finally, a strong, direct correlation was demonstrated in *S. pneumoniae* infection between azithromycin efficacy and the ratio of the area under the serum concentration-time

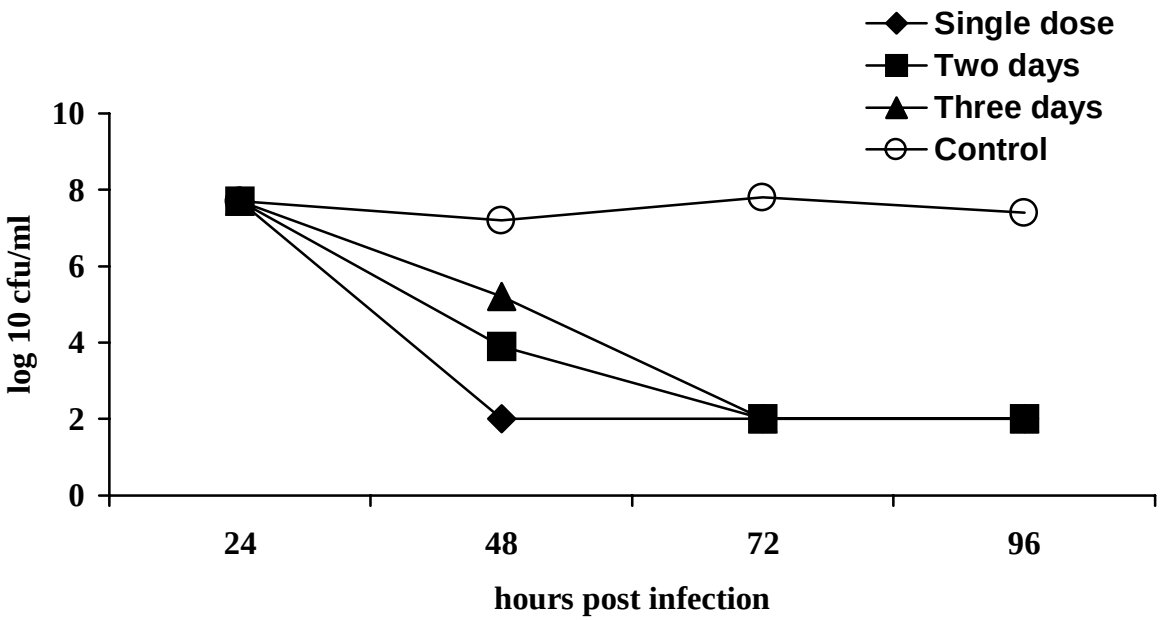
curve (AUC) to the minimal inhibitory concentration (MIC), the AUC/MIC ratio. Within a 100 fold range of AUC/MIC ratios tested (10 to 1,000), the greatest efficacy was observed at the highest AUC/MIC ratio tested. In contrast, efficacy with erythromycin and clarithromycin correlates best with the time that serum levels exceed the MIC.

Another animal model assessed the comparable tissue penetration of similar total doses of azithromycin administered by different dosing regimens.[18] In this study, rats had a paper disc impregnated with *Staphylococcus aureus* or a sterile control disc implanted in each thigh. Disc implantation marked day 1 of the study. The inflammatory tissue fluid from each thigh was analyzed for antimicrobial penetration after the administration of three different dosage regimens of azithromycin. These three regimens were (1) 200mg/kg of azithromycin on day 2; (2) 100mg/kg on days 2 and 3; and (3) 33 mg/kg bid on days 2, 3, and 4. Inflammatory fluid was analyzed at 5, 24, 48, 96, 120 and 144 hours post dosing. Azithromycin concentration in inflammatory fluid reached similar levels and sustained those levels for each dosing regimen.[18] These data demonstrate that the final concentration of azithromycin in inflammatory tissue was independent of the dosage regimen but dependent upon the total dose given. This was not seen with the other antibiotics tested.[18]

These animal models support the premise that the AUC/MIC ratio, and not time over $MIC_{90,}$ is the pharmacodynamic parameter that best correlates with azithromycin's antimicrobial activity in the models used. For other macrolides, such as clarithromycin and erythromycin, it was the time over the MIC_{90} that best correlated with efficacy.[47] This pharmacodynamic attribute of azithromycin was thought to be due to its post antibiotic effect and its pharmacokinetic profile, especially its high concentration and persistence within neutrophils.

C_{max} may also factor into the effectiveness of the regimen.[48] *In vivo* and *in vitro* models of *S. pneumoniae* infection demonstrate that single dose azithromycin therapy resulted in higher initial concentrations of antibiotic and significantly increased survival rates, and C_{max} was most predictive of clinical efficacy.[48] These findings have been extended to a gerbil model of middle ear infection challenged with H. influenzae in which a similar total dose of azithromycin was more effective in eradicating *H. influenzae* from the middle ear space, though more rapidly when administered as a single dose.

Figure 3. Eradication of *H. influenzae* after Therapy of 200 mg/kg Azithromycin administered over one, two or three Days



As seen above, the single dose treatment of otitis media appeared to result in faster reduction of organisms from the middle ear space, although all three regimens were active. This suggests that, in an *in vivo* setting, while the same total dose of azithromycin is effective for eliminating haemophilus from the middle ear, accelerating the dosing regimen–that is, giving the entire dose upfront--may reduce the burden of organisms more quickly. More information on this and other animal experiments is given in Appendix I.

In short, results from animal pharmacokinetic studies suggest that shorter treatment regimens using the same total dose deliver the same amount of azithromycin overall to infected sites, and that these shorter regimens are at least as effective as longer regimens in the treatment of various infections.

4.2 Human Pharmacokinetic Studies

The original submission for azithromycin provided pharmacokinetic and clinical trial data to support the use of a 5-day dosing regimen for the treatment of acute otitis media. To provide additional support to clinical trial data, there are available a series of pharmacokinetic studies which demonstrate that the total drug exposure conferred by a 5-day dosing regimen will be similarly demonstrated by a 3-day or a single-dose treatment regimen. In addition to offering supportive evidence for bioequivalence of these regimens, there are data to support sufficient exposure of

azithromycin in the middle ear at levels predicted by *in vitro* testing to kill pathogens associated with acute otitis media. The section that follows provides the following information:

a. 3-day and 5-day dosing of 1.5 grams in adults is bioequivalent
b. 1-day and 3-day dosing in adults provides similar PK exposure
c. 3-day and 5-day regimens in children provide similar drug exposure
d. middle ear fluid levels from 3- and 5- day dosing regimens reach or exceed MICs of offending pathogens

4.2.1 Bioequivalence of the 3- and 5-day regimens in Adults

Clinical study 066-087 was a comparative cross-over design study in 12 healthy adult volunteers. This study compared the pharmacokinetics of a total 1500-mg dose of azithromycin administered orally over 3 days (500 mg/day for 3 days) and over 5 days (500 mg on day 1 and 250 mg on days 2-5). Since leukocytes play an important role in delivering azithromycin to sites of infection in the body, this study also estimated azithromycin concentrations in leukocytes.

The ratio of serum $AUC_{0-\infty}$ (3-day)/$AUC_{0-\infty}$ (5-day) was 105% with 90% confidence intervals (CI) of 93% and 120% (p=0.49). Thus, the 3-day and 5-day regimens were bioequivalent with respect to serum concentrations of azithromycin. Two other studies (AZM-F-93-004; AZM-NY-90-011) confirmed the similarity of systemic exposure to azithromycin following the 3-day and 5-day dosing regimens in adults.

In mononuclear cells (MNL) the adjusted mean ratio of AUC_{0-288} (3day)/ AUC_{0-288} (5day) was 123% with 90% CI of 69% and 218% (p=0.54) (study 066-087). In polymorphonuclear cells (PMNL) the adjusted mean ratio of AUC_{0-288} (3day)/ AUC_{0-288} (5day) was 167% with 90% CI of 109% and 254% (p=0.054). Thus, the 3-day regimen produced concentrations in leukocytes at least as great as those produced by the 5-day regimen.

These data show high concentrations of azithromycin in leukocytes following both dose regimens, as well as relatively small differences in both serum exposure and leukocyte concentration and exposure following the 3-day and 5-day dosing regimens. These data support the observation that the pharmacodynamics of azithromycin is a function of total exposure to drug (AUC); consequently, these results suggest that these regimens should be equally effective in treating infections.

4.2.2 Bioequivalence of the single dose and 3-day regimens in Adults

A clinical study conducted by Amsden and Gray[49] provide data supporting the bioequivalence of the single-dose and 3-day regimens. This was an open label, randomized, two-way cross-over study in 12 healthy adults to evaluate the serum pharmacokinetics of 1.5 grams of azithromycin administered either as a single 1.5g

dose or as 500 mg doses given daily for 3 days. This study also estimated WBC exposure to azithromycin. The results are given in the table below.

Serum & WBC Azithromycin Pharmacokinetics 1.5 Gram Total Dose

Parameter (units)*	Single Dose	Three Day Dosing
Serum		
Cmax (mg/L)	1.46 (0.34-3.48)	0.54 (0.18-1.01)
AUC 0-infin (mg-h/L)	13.1 (3.02-20.6)	11.2 (2.98-24.5)
Granulocyte (PMN)		
Cmax (mg/L)	41.1 (27.8-63.3)	31.3 (14.8-53.3)
AUC 240 (mg-h/L)	6447 (4077-10 830)	5128 (2526-9317)
Monocyte/Lymphocyte		
Cmax (mg/L)	312.7 (32.9-709.0)	164.9 (38.9-563.0)
AUC 240 (mg-h/L)	20461 (4020-35 861)	15706 (4703-30 317)

*Mean (range)

Amsden & Gray, JAC, 2001.

The results of this study indicate that the single dose regimen and the 3-day dosing regimen of 1.5 g of azithromycin provide similar total serum exposures (13.1 vs. 11.2 μg.hr/ml) and similarly high and sustained leukocyte concentrations. The extensive uptake by leukocytes indicates that sufficient drug is present for at least 10 days following the start of therapy. Since the pharmacodynamics of azithromycin are a function of total exposure to drug (AUC), these results suggest that these regimens should be equally effective in treating infections.

4.2.3 Similarity of the 3- and 5-day regimens in Children

The dose in children for the treatment of AOM is 30 mg/kg, compared to 60 mg/kg for children with pharyngitis/tonsillitis. Clinical studies 066-683 and 066-095 were conducted in a total of 65 pediatric pharyngitis/tonsillitis patients. These were non-comparative, open label studies in children aged 3-16 years. Azithromycin pediatric oral suspension (POS) was administered following a standard low-fat breakfast. The objective of these studies was to assess the pharmacokinetics and safety of the two different dosing regimens of azithromycin POS in the fed state. Azithromycin was administered at 12 mg/kg/day for 5 days in Clinical Study 066-683 (31 patients), and at 20 mg/kg/day for 3 days in Clinical Study 066-095 (34 patients). In each study, azithromycin serum concentrations were measured over the 24-hour dosing interval following the last daily dose.

The area under the serum concentration versus time curve (AUC_{0-24}) was calculated from the azithromycin serum concentrations measured following the last daily dose administered (on day 3, for Study 066-095 and on day 5 for Study 066-683). The C_{24} is the azithromycin serum concentration 24 hours after the last daily dose (on day 4, for Study 066-095 and on day 6 for Study 066-683).

		Pharmacokinetic Parameter (Mean ± SD)			
	N	C_{max} (μg/ml)	T_{max} (hr)	AUC_{0-24} (μg·hr/ml)	C_{24} (μg/ml)
Study 066-095					
3-Day Dosing Regimen (20 mg/kg/day for 3 days)	11	1.05± 0.44 0.975 [a]	3 ± 2.0	7.92± 2.87 7.49 [a]	0.15 ± 0.04
Study 066-683					
5-Day Dosing Regimen (12 mg/kg/day for 5 days)	17	0.534± 0.361 0.428 [a]	2.2 ± 0.8	3.94± 1.90 3.51 [a]	0.09 ± 0.05

[a] geometric means.

Further analyses were conducted to compare overall azithromycin exposure over the entire dosing period following the 3-day and 5-day dosing regimens. Overall exposure for the 5-day (12 mg/kg/day for 5 days) dosing regimen was obtained by multiplying the AUC_{0-24} after the fifth daily dose by 5. Likewise, total exposure for the 3-day (20 mg/kg/day for 3 days) dosing regimen was estimated by multiplying the AUC_{0-24} after the third daily dose by 3. This was required because of the limited number of serum samples that could be collected from this pediatric population. These approximations may overestimate AUC values but provide a method for comparing the overall AUC exposure following the two regimens. The results are summarized below for all participating patients, as well as for the subset of patients who received the scheduled dose.

	N	AUC_{0-72} (μg·hr/ml)
3-Day Dosing Regimen 20 mg/kg/day for 3 days	11	7.92 X 3 = 23.8
5-Day Dosing Regimen 12 mg/kg/day for 5 days	17	3.94 X 5 = 19.7

The similarity of total exposure following the 3-and 5-day suggests that the two regimens will be equally effective in treating infections in pediatric patients.

4.2.4 Middle Ear Fluid Pharmacokinetic data

In the literature there are several reports of azithromycin middle ear fluid (MEF) levels obtained after administration of various dosing regimens. The table below summarizes these data.

Investigators	Dose	Assessment Time	Serum mg/L	MEF mg/L
Scaglione, 1999[50]	10 mg/kg (1 dose)	24 hours	--	1.05[a], 0.23[b]
Dagan, 2000[51]	10 mg/kg x 3 day	24-48 hours	0.07	3.51
Pukander, 1996[52]	10,5,5,5,5[c]	24 hours	0.043	8.61
Nahata, 1995[53]	10,5,5,5,5[c]	24 hours	0.047	--

a: Concentration in fluid containing cells b: Concentration in free cell fluid
c: 5-day dose regimen of 10 mg/kg on first day, followed by 5mg/kg on four days

These data demonstrate levels in the MEF ranging from 3.51 to 8.61 after delivery of a 30 mg/kg regimen--levels that far exceed serum levels, as is typically the case with azithromycin. These levels reach or exceed the MIC_{90s} for the common otitis media pathogens (*S. pneumoniae, M. catarrhalis,* and *H. influenzae*). Typically azithromycin is delivered to the infected space as a consequence of its accumulation in and release from macrophages and neutrophils.[54,55,56] Moreover, in a variety of studies, it has been shown that azithromycin-laden neutrophils release the drug as a consequence of contact with bacteria.[46]

4.2.5 Summary of Pharmacokinetic Studies

In summary, the pharmacokinetic data demonstrate that azithromycin is present in MEF at levels necessary for bacterial killing; that the 1-dose and 3-dose regimens of azithromycin provide at least equivalent exposure to the 5-dose regimen; and that AUC/MIC, and perhaps C_{max}, are the important pharmacokinetic parameters driving azithromycin's microbiologic efficacy.

In humans, a single dose regimen has been successfully used in the treatment of sexually transmitted diseases, including *Chlamydia trachomatis, Haemophilus ducreyi* and *Neisseria gonorrhoeae.* The improvement in compliance, with benefits for the patient and caregiver, as well as the possibility that higher peak levels would potentiate antimicrobial activity, justified the initiation of a clinical program in single dose treatment for otitis media.

5 AZITHROMYCIN CLINICAL PROGRAM

5.1 Summaries of Individual Studies in Support of Efficacy in Otitis Media

The efficacy of short-course azithromycin therapy in the treatment of pediatric subjects with otitis media was evaluated in three randomized, comparative trials (2 double-blind, 1 single-blind) and one open label noncomparative study.
The clinical efficacy of the 3-day dosing regimen was compared to that of Augmentin® in a double blind study (1014).[57] Similarly, the single-dose regimen was also compared to Augmentin® in a double blind study (R-0581).[58] Two other studies provided for culture of baseline pathogens from middle ear fluid. One of these was a single-blind study (AZM-NY-95-001)[59] comparing the single dose regimen to Augmentin®, as well as the azithromycin 3-day regimen. The fourth study looked at the microbiologic and clinical efficacy of single-dose azithromycin in an open label, noncomparative trial (1015).[60]

Each of these studies is summarized briefly below; more detailed summaries appear in Appendix II. In all four studies, "cure" rates are defined as complete resolution of specific signs/symptoms of acute otitis media. In three of the four studies (A0661014, A0661015, R-0581), the presence of middle ear fluid was not to be factored into the assessment of clinical response.

5.1.1 Protocol A0661014 (Comparative, 3-Day Regimen)

In this double-blind, comparative study, azithromycin oral suspension was administered as 10 mg/kg once daily (maximum of 500 mg/day) for 3 days. The comparative agent was an amoxicillin/clavulanate potassium suspension, administered as a 45 mg/kg daily dose (based on amoxicillin) given in divided doses every 12 hours for 10 days.

This study included 373 subjects (188 azithromycin, 185 amoxicillin/clavulanate), aged 6 months to 12 years, with signs and symptoms of acute otitis media. Overall, ear pain and/or fullness/bulging of the tympanic membrane were present in 98% of enrolled subjects at baseline. Other baseline characteristics were:

Demographic Parameter	Azithromycin	Amox/Clav
Mean age (years)	3.5	3.4
≤2 years old (%) > 2 years old (%)	61 (32%) 127 (68%)	53 (29%) 132 (71%)
% Male % Female	51% 49%	49% 51%
% White % Black %Hispanic % Other	83% 6% 8% 3%	80% 5% 9% 5%
Mean duration of symptoms (days)*	1.2 [range 0-9]	1.2 [range 0-8]

Amox/Clav = Amoxicillin/Clavulanate potassium; *Duration from onset of symptoms to study enrollment.

The following table shows clinical response outcomes.

Clinical Outcome—MITT Subjects

	Azithromycin	Amox/Clav		
	N (%)	N (%)	P-Value	95% CI
No. MITT Subjects	188	185		
Subjects Evaluable at EOT	185 (100%)	181 (100%)		
Success	153 (83%)	159 (88%)	0.186	-12.9%, 2.7%
Cure	100	114		
Improvement	53	45		
Failure	32 (17%)	22 (12%)		
Subjects Evaluable at TOC	182 (100%)	180 (100%)		
Success (Cure)	134 (74%)	124 (69%)	0.353	-5.2%, 14.6%
Failure	48 (26%)	56 (31%)		

Based on MITT subjects with observed values or classified as Failures due to additional antibiotics

Statistical analysis at EOT based on dichotomized categories Success and Failure

Amox/Clav = Amoxicillin/Clavulanate potassium; P-Value based on Fisher's exact test; 95% CI = 95% confidence interval based on normal approximation to the binomial; EOT = End-of-Therapy; TOC = Test of Cure; MITT = Modified Intent-to-Treat.

The once-daily administration of 10 mg/kg azithromycin for three days was as clinically effective as the administration of 45 mg/kg amoxicillin/clavulanate potassium in two divided doses q12h for 10 days for the treatment of acute otitis media.

Clinical Cure Rates by Age at TOC – MITT Subjects					
	Azithromycin		Amox/Clav		
Age Group	n/N	%	n/N	%	95% CI*
≤2 years old	35/58	60%	30/52	58%	-17.7%, 23.1%
>2 years old	99/124	80%	94/128	73%	-4.8%, 17.6%
Amox/Clav = Amoxicillin/Clavulanate potassium; CI = Confidence Interval on the difference between treatment groups (azithromycin-comparator)					

Cure rates at TOC were higher in the older subset of MITT subjects (>2 years old) than the younger subset of subjects (≤2 years old) in both the azithromycin 3-day group (80% and 60%, respectively) and the comparator group (73% and 58%, respectively). However, the number of younger subjects ≤2 years old was small (N ≤ 58/treatment group).

Overall, 99% of azithromycin versus 89% of amoxicillin/clavulanate subjects were compliant with their study regimen ($p<0.001$). This was based on subjects taking active drug in their study regimen.

The subjects in the azithromycin group were less likely to experience treatment-related adverse events, and had milder adverse events than the comparator group. The incidence of treatment-related adverse events in the azithromycin group (10.6%) was about half that of the amoxicillin/clavulanate potassium group (20.0%). The most commonly occurring treatment-related adverse events were gastrointestinal (diarrhea) and rash. Fewer azithromycin subjects than amoxicillin/clavulanate potassium subjects experienced treatment-related adverse events from these categories (diarrhea 5.9% versus 14.6%, respectively; rash: 0.0% versus 4.3%, respectively).

5.1.2 Protocol A0661015 (Noncomparative, Single-Dose Regimen)

In this noncomparative study, azithromycin oral suspension was administered as a single 30 mg/kg dose (maximum of 1500 mg).

This study included 248 subjects aged 6 months to 12 years. Overall, ear pain and/or fullness/bulging of the tympanic membrane were present in 98% of enrolled subjects at baseline. Other baseline characteristics were:

Demographic Parameter	Azithromycin
Mean age (years)	3.4
≤2 years old (%) > 2 years old (%)	86 (35%) 162 (65%)
% Male % Female	52% 48%
% White % Black % Hispanic % Other	46% 5% 44% 4%
Mean duration of diagnosis (days)*	2.5 [range 1-8]

* Duration from onset of symptoms to enrollment.

The following tables show clinical response outcomes overall and by pathogen.

Clinical Outcomes in Azithromycin Subjects – Observed Cases Analysis

	MITT Analysis*		
	N	(%)	95% CI***
Subjects Evaluable at EOT	240	(100%)	
Success	213	(89%)	84.5, 93.0
Cure	139	(58%)	--
Improvement	74	(31%)	--
Failure	27	(11%)	--
Subjects Evaluable at TOC**	242	(100%)	
Cure	206	(85%)	80.4, 89.8
Failure	36	(15%)	--

EOT=End of Therapy, TOC=Test of Cure, CI=Confidence Interval; * Total number of Clinical MITT subjects was 247; ** TOC visit had no option for "improvement";
*** Based on normal approximation to the binomial distribution

MITT Clinical Outcome by Baseline Pathogen in Subjects* Included in the Bacteriologic Analysis – Observed Cases – Based on Culture Data

EOT Assessment:						
	H. influenzae (N=42)		*M. catarrhalis* (N=10)		*S. pneumoniae* (N=76)	
Outcome	n (%)	95% CI***	n (%)	95% CI***	n (%)	95% CI***
Success	30 (71%)	56.6, 86.3	10 (100%)	95.0, 105	70 (92%)	85.4, 98.8
Cure	18 (43%)	--	5 (50%)	--	49 (64%)	--
Improvement	12 (29%)	--	5 (50%)	--	21 (28%)	--
Failure	12 (29%)	--	0 (0%)	--	6 (8%)	--
TOC Assessment:**						
	H. influenzae (N=44)		*M. catarrhalis* (N=10)		*S. pneumoniae* (N=76)	
Outcome	n (%)	95% CI***	n (%)	95% CI***	n (%)	95% CI***
Cure	28 (64%)	48.3, 79.0	10 (100%)	95.0, 105	67 (88%)	80.2, 96.1
Failure	16 (36%)	--	0 (0%)	--	9 (12%)	--

EOT=End of Therapy, TOC=Test of Cure, CI=Confidence Interval; * Some subjects are counted under more than one pathogen; ** TOC visit had no option for "improvement"; *** Based on normal approximation to the binomial distribution

Overall, clinical cure at the TOC visit for MITT evaluable subjects was 85%; by baseline pathogen, the corresponding rates were 64%, 100%, and 88% for *H. influenzae*, *M. catarrhalis*, or *S. pneumoniae*, respectively. Of note, 86% (6 of 7) subjects infected with *S. pneumoniae* with an azithromycin MIC of 8 µg/ml (presently considered resistant pathogens), were clinical cures. All of these organisms were documented to have had an efflux-pump based mechanism of resistance.

An assessment was performed of the clinical outcome by penicillin susceptibility of patients with *S. pneumoniae* isolated at baseline that treated with azithromycin. The results are provided in the table below:

Clinical Outcome of *S. pneumoniae* AOM by Penicillin Susceptibility

	Penicillin Susceptibility*	
	Resistant	Sensitivity
Cure	12 (75%)	43 (86%)
Failure	4	7

Penicillin Resistant Isolates by Macrolide Susceptibility			
	Susceptible	Resistant	
		8 μg/ml	>256 μg/ml
Cure	4	6 (86%)	2 (40%)
Failure	0	1	3

*based on oxacillin disc testing; a zone < 20 mm was considered resistant;10 isolates were not tested to both macrolides and penicillin

86% of patients with *S. pneumoniae* sensitive to penicillin were cured at the day 28 visit. 75% of 12 patients with isolates resistant to penicillin at baseline were cured.

Clinical Cure Rates by Age at TOC – MITT Subjects

Age Group	n/N	%	95% CI
≤2 years old	64/83	77%	67%, 87%
>2 years old	142/159	89%	84%, 94%

CI = Confidence Interval

As is typical in treatment of otitis media, the older subset of MITT subjects (>2 years old) responded more favorably than the younger subset of subjects (≤2 years old) at TOC (cure rates of 89% and 77%, respectively).

Overall, 12.1% subjects had treatment related adverse events, with the most common adverse events involving the gastrointestinal tract (vomiting 5.6%, diarrhea 3.2%, and abdominal pain 1.6%). All treatment-related adverse events were mild to moderate in severity and the majority resolved within a few days. One subject discontinued from the study due to a treatment-related adverse event (vomiting).

5.1.3 Protocol R-0581 (Comparative, Single-Dose Regimen)

In this double-blind, comparative study, azithromycin oral suspension was administered as a single 30 mg/kg dose. The comparative agent was an amoxicillin/clavulanate suspension, administered as a 45 mg/kg daily dose (based on amoxicillin) given in divided doses every 12 hours for 10 days.

This study included 346 treated subjects (173 azithromycin, 173 amoxicillin/clavulanate) who were outpatients between 6 months to 12 years of age with acute otitis media diagnosed based on clinical signs and symptoms of middle ear effusion and acute inflammation. Overall, ear pain and/or fullness/bulging of the tympanic membrane were present in 99% of enrolled subjects at baseline. Other demographic characteristics at baseline were:

Demographic Parameter	Azithromycin	Amox/Clav
Mean age	2.7	3.4
≤2 years old (%) > 2 years old (%)	75 (43%) 98 (57%)	63 (36%) 110 (64%)
% Male % Female	53% 47%	53% 47%
% White % Black %Hispanic % Other	50% 31% 16% 2%	52% 35% 13% 0%
Mean duration of symptoms (days)*	3.4 [1-18]	3.9 [1-17]

Amox/Clav = Amoxicillin/Clavulanate potassium; * Duration from onset of symptoms to enrollment

The following tables show clinical response outcomes overall.

Clinical Outcome at EOT and TOC—MITT Analysis			
	Azithromycin	Amox/Clav	
	N (%)	N (%)	95% CI
Total No. subjects treated	173	173	
Primary MITT analysis			
Subjects evaluable at EOT*	160	161	
Cure + Improvement	139 (87)	142 (88)	-9.2, 6.5
Cure	105 (66)	121 (75)	-20.1, 1.1
Improvement	34 (21)	21 (13)	
Failure	21 (13)	19 (12)	
Primary MITT analysis			
Subjects Evaluable at TOC*	151	154	
Cure + Improvement	114 (75)	116 (75)	-10.2, 10.5
Cure	112 (74)	108 (70)	-6.7, 14.8
Improvement	2 (1)	8 (5)	
Failure	37 (25)	38 (25)	
Failure	31 (21)	32 (21)	
Recurrence	6 (4)	6 (4)	

*Includes MITT subjects with observed values or classified as failures due to additional antibiotics
EOT = End-of-Therapy; TOC = Test of Cure; CI = Confidence interval

The administration of a single dose of azithromycin oral suspension (30 mg/kg) was as effective as the administration of 45 mg/kg amoxicillin/clavulanate in two divided doses q12h for 10 days for the treatment of acute otitis.

Clinical Cure Rates by Age at TOC – MITT Subjects					
	Azithromycin		Amox/Clav		
Age Group	n/N	%	n/N	%	95% CI*
≤2 years old	40/64	63%	27/53	51%	-8.3%, 31.4%
>2 years old	72/87	83%	81/101	80%	-9.7%, 14.8%
Amox/Clav = Amoxicillin/Clavulanate; CI = Confidence Interval on the difference between treatment groups (azithromycin-comparator).					

The older subset of MITT subjects (>2 years old) responded more favorably than the younger subset of subjects (≤2 years old) at TOC.

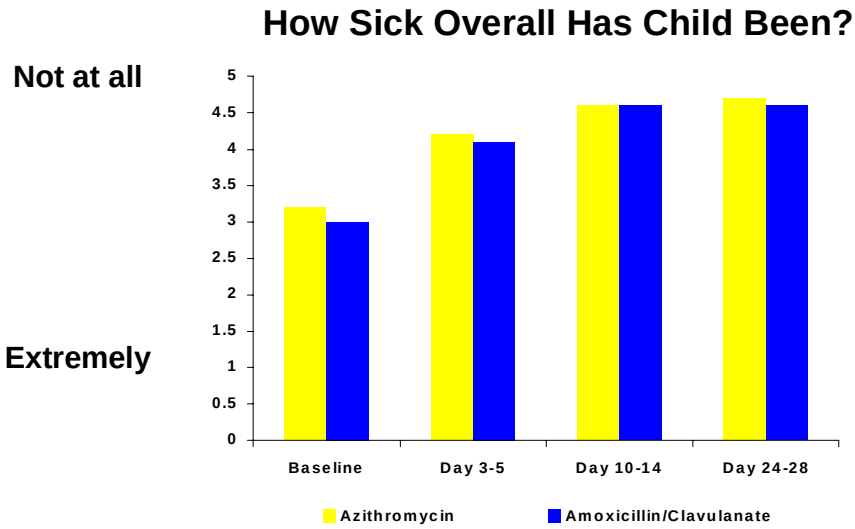
The subjects in the azithromycin group (16.8%) were less likely than subjects in the comparator group (22.5%) to experience treatment-related adverse events, and azithromycin subjects had milder adverse events than the comparator group. Diarrhea (12.7% vs. 6.4%) and rash (5.2% vs. 1.7%) were more common in the comparator than the azithromycin group, respectively.

Overall, 100% of azithromycin versus 84% of amoxicillin/clavulanate subjects were compliant with their study regimen ($p<0.001$). This was based on subjects taking active drug in their study regimens.

Indicators of Early Clinical Efficacy—Parental Questionnaires

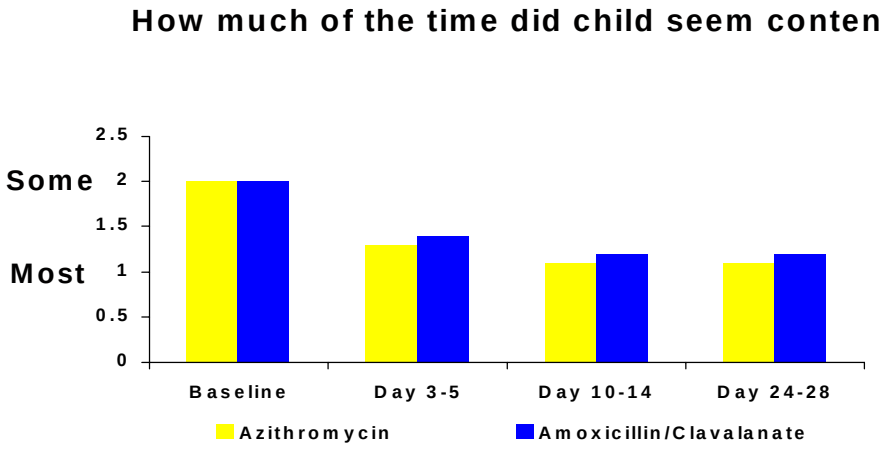
Although the studies in this submission were not designed to evaluate clinical response at the early (Day 3-6) study visit, data from parental surveys can be used to estimate the subject's clinical response to treatment at that time period. The results are given in the two figures below and in Appendix III.

R0581 Parental Questionnaire



Parental Questionnaire Confirms That Children Improved at Equal Rates

R0581 Parental Questionnaire



Parental Questionnaire Confirms That Children Improved at Equal Rates

As seen, there appears to be no difference in the clinical response between the two regimens at any of the study timepoints, as perceived by the parents. The importance of parental assessment cannot be understated, as it is the parents who make the decision to seek initial medical advice and whether to return for follow-up evaluations. Indeed, one study found that the parents were able to predict the presence of acute otitis media with a sensitivity and specificity of 71% and 80%, respectively, although symptoms of the disease are relatively nonspecific.[61]

5.1.4 Protocol AZM-NY-95-001 (Comparative, Single-Dose and 3-Day Regimens)

In this single-blind, comparative study, azithromycin oral suspension was administered as a single dose (30 mg/kg) or once daily for 3-days (10 mg/kg/day), and ceftriaxone was administered as a single IM dose (50 mg/kg). The two azithromycin regimens were blinded.

This study included 198 (66/treatment group) subjects up to 6.5 years. Overall, ear pain and/or fullness/bulging of the tympanic membrane were present in 100% of enrolled subjects at baseline.

Demographic Parameter	AZM Single	AZM 3-Day	Ceftriaxone
Mean age (years)	2.5	2.7	2.3
≤2 years old (%) > 2 years old (%)	26 (39%) 40 (61%)	27 (41%) 39 (59%)	35 (53%) 31 (47%)
% Male % Female	56% 44%	55% 45%	48% 52%
% White % Other	97% 3%	95% 5%	98% 2%
Mean duration of symptoms (days)*	1.5 [range 1-6]	2.4 [range 1-24]	1.7 [range 1-9]

AZM = Azithromycin; * Duration from onset of symptoms to enrollment

The following table shows the clinical outcome results.

Clinical Outcomes - MITT Analysis					
	AZM SD	AZM 3D	CX	95 % CI	
	N	N	N	AZM 3D-CX	AZM SD-CX
Observed Case MITT Analysis*					
Clinical Outcome at EOT					
Total Evaluable	64	63	62		
Cure + Improved	96.9%	95.2%	98.4%	-10.9%, 4.6%	-8.4%, 5.4%
Clinical Outcome at F/U					
Total Evaluable	65	66	64		
Cure + Improved	93.8%	92.4%	96.9%	-13.7%, 4.8%	-11.9%, 5.8%
Cure	86.2%	77.3%	76.6%	-15.4%, 16.9%	-5.4%, 24.6%
Improvement	7.7%	15.2%	20.3%		
Failure	6.2%	7.6%	3.1%		

*Includes MITT subjects with observed values or classified as failures due to additional antibiotics AZM SD = azithromycin single dose; AZM-3D = azithromycin 3-Day; CX = ceftriaxone.
EOT = End of Therapy (Days 14–15); F/U = Follow-Up (Days 28–30).
Missing values at Follow-up were computed using LOCF.

A single oral dose of azithromycin suspension (30 mg/kg), three day dosing of oral azithromycin suspension (10 mg/kg/day for 3 days), or a single dose of ceftriaxone (50 mg/kg) intramuscularly, had comparable safety and effectiveness in the treatment of acute otitis media.

Overall, for MITT evaluable subjects in the azithromycin 1-day group, clinical cure by baseline pathogen at the TOC (day 28) visit occurred in 7 of 8 (88%) subjects infected with *H. influenzae* and 17 of 20 (85%) infected with *S. pneumoniae*. The corresponding rates in the azithromycin 3-day group were 9 of 13 (69%) *H. influenzae* and 16 of 17 (94%) *S. pneumoniae*, and in the comparator group were 8 of 9 *H. influenzae* (89%) and 19 of 23 (83%) *S. pneumoniae*.

Clinical Cure Rates at TOC – MITT Subjects						
	AZM Single		AZM 3-Day		Ceftriaxone	
Age Group	n/N	%	n/N	%	n/N	%
≤2 years old (%)	19/25	76%	18/27	67%	23/33	70%
> 2 years old (%)	37/40	93%	33/39	85%	26/31	84%

	95% CI: ≤2 years old:	95% CI >2 years old:
AZM Single –AZM 3-Day	-19.4%, 38.1%	-8.8%, 24.6%
AZM Single – Comparator	-20.6%, 33.2%	-9.8%, 27.0%
AZM 3-Day – Comparator	-30.5%, 24.5%	-19.6%, 21.1%

AZM = Azithromycin

Cure rates at TOC were higher in the older subset of MITT subjects (>2 years old) than the younger subset of subjects (≤2 years old) in all three groups. However, the number of younger subjects ≤2 years old was small (N ≤ 33/treatment group).

Overall, all subjects (100%) completed their assigned treatment.

The incidence of treatment related adverse events was similar among the three treatment groups. Diarrhea, rash, and vomiting were the only treatment related adverse events that occurred in 2 or more subjects**.** Similar to what was observed in the all causality adverse events, diarrhea and rash occurred more frequently in the ceftriaxone group than in the two azithromycin groups, and vomiting was more frequent in the single dose azithromycin group than in the 3-day azithromycin or ceftriaxone group.

5.2 Overall Summary of Efficacy Studies

5.2.1 Clinical Efficacy

A summary of the pertinent clinical trials presented in the application is provided below. These studies have been analyzed for clinical cure at day 28 (Test of Cure visit). Signs/symptoms and acoustic reflectometry are discussed in Appendix IV.

Otitis Media Efficacy
Clinical Cure at Test of Cure Visit

Study	Azithromycin 10 mg/kg x 3d	Azithromycin 30 mg/kg x 1d	Comparator	95%CI
R-0581		112/151 (74%)	108/154 (70%)	
Az30-Augmentin®				-7, 15%
1015		206/242 (85%)		
1014	134/182 (74%)		124/180 (69%)	
Az10-Augmentin®				-5, 15%
95-001	51/66 (77%)	56/65 (86%)	49/64 (77%)	
Az30-Ceftriaxone				-5, 25%
Az10-Ceftriaxone				-15, 17%
Overall	185/248 (75%)	374/458 (82%)	281/398 (71%)	
Az30-Comparator				5, 17%
Az10-Comparator				-3, 11%

Source protocols: R-0581, A0661015, A0661014, AZM-NY-95-001.
Az30 = azithromycin 30 mg/kg/day, 1 day; Az10 = azithromycin 10 mg/kg/day for 3 days.

These data demonstrate consistently equivalent efficacy of azithromycin in relation to the comparative agents in the treatment of acute otitis media.

5.2.2 Clinical Cure by Baseline Pathogen

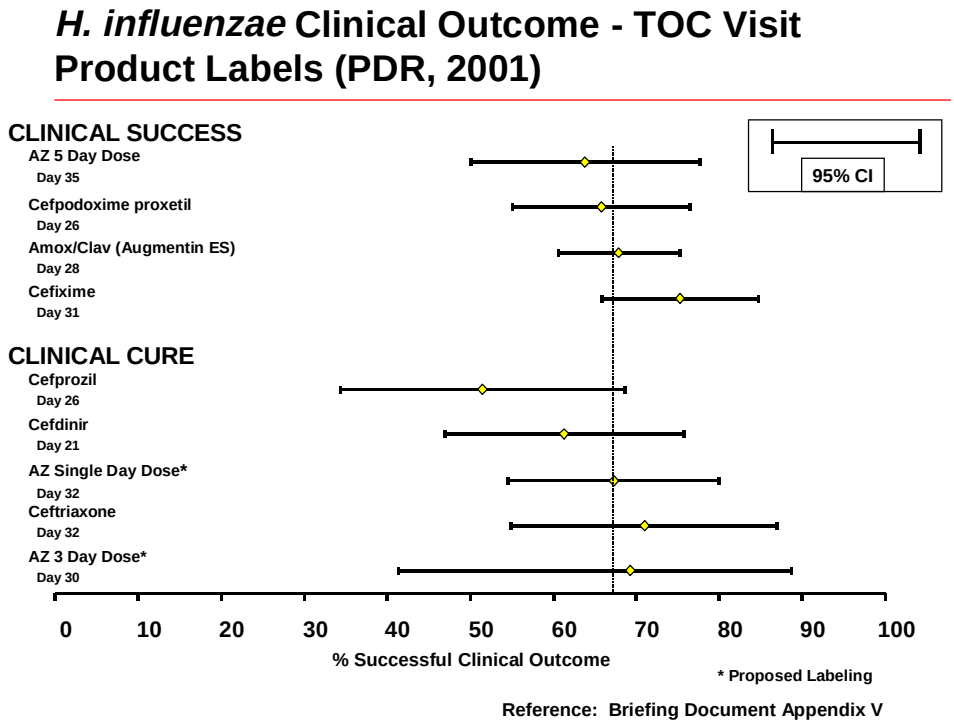
In addition, clinical cure data from studies 1015[60] and AZM-NY-95-001[59] were further analyzed by baseline pathogen. The results are presented below.

Otitis Media Efficacy
Clinical Cure by Baseline Pathogen at Test-of-Cure Visit

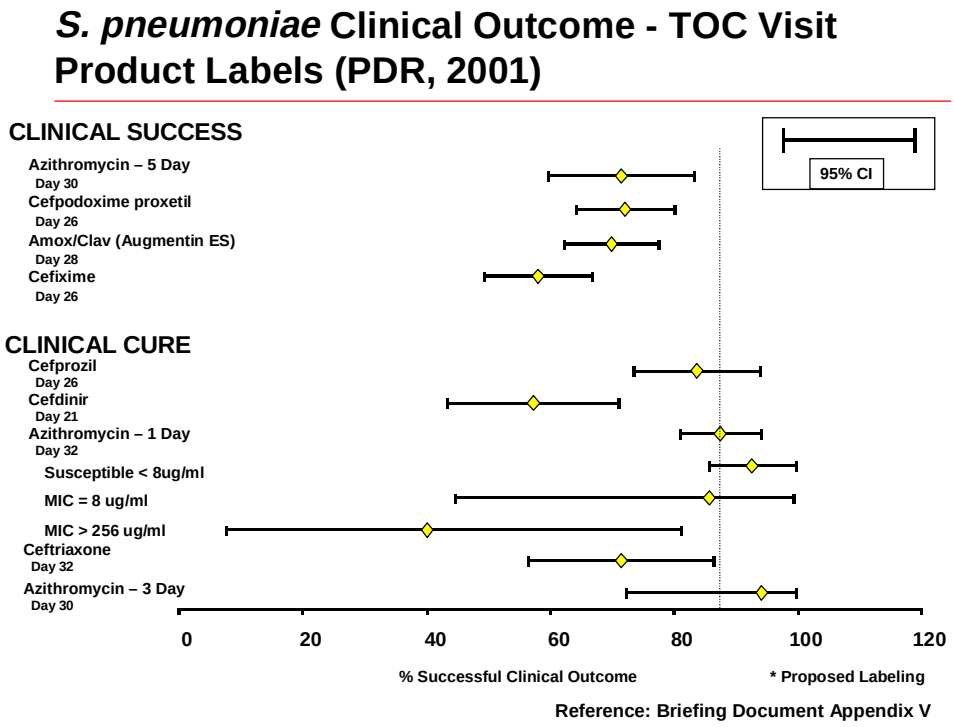
Pathogen	Azithromycin 10 mg/kg x 3d	Azithromycin 30 mg/kg x 1d	Ceftriaxone	95%CI
S. pneumoniae	16/17 (94%)	84/96 (88%)	19/23 (83%)	
Az30-Comparator				-15, 25%
Az10-Comparator				-13, 36%
H. influenzae	9/13 (69%)	35/52 (67%)	8/9 (89%)	*
M. catarrhalis	2/2	10/10		*

Source protocols: A0661015, AZM-NY-95-001; * small sample size

The *H. influenzae* clinical cure rates at the Test-of Cure Visit are consistent with data in the product labels for other antimicrobials commonly used for the treatment of acute otitis media, provided below.



Similarly, a comparison of the results at the day 28 visit for clinical success in treatment of acute otitis media due to *S. pneumoniae* for a variety of antimicrobials based on data presented in the package inserts, is given below.



These data demonstrate that the clinical response rates for patients with acute otitis media due to *S. pneumoniae* treated with azithromycin are likely to be at least as good as that of any other antibiotic used for treatment of otitis media.

5.3 Review of Compliance

As seen from the studies in this application, patients were more likely to adhere to a regimen of three days or less. Compliance rates of subjects taking active drug in their study regimen in study 1014, comparing azithromycin for three days with Augmentin® for 10 days, were 99% for azithromycin and 89% for Augmentin® ($p < 0.001$). Similarly, in study R-0581, comparing a single dose of azithromycin with 10 days of Augmentin®, 100% of subjects were compliant with single dose treatment compared to 84% on Augmentin® ($p < 0.001$). While improvements in adherence to therapy may be due to other variables (improved taste, less side effects, etc), it is likely that the number of daily doses and the shorter duration of treatment plays a significant role.

With beta-lactams, full compliance with prescribed therapy increases the likelihood of clinical success.[3,4] If patients do not complete their assigned therapy some

proportion of those who become clinical failures may receive additional antibiotic therapy and subsequently increase the overall use of antibiotics in the community.

5.4 Review of Safety

The side effect profiles of the three otitis media regimens, summarized from clinical studies, are given in the table below. Treatment-related adverse events are discussed here; all-causality adverse events follow similar trends and are presented in Appendix VI.

Adverse Events: Treatment Emergent, Treatment-Related
Azithromycin 30 mg/kg: 1-, 3- & 5-Day Regimens

	Dosing Duration		
	1-Day	**3-Day**	**5-Day**
Number of Subjects	487	1729	1888
Subjects with Treatment-Related Adverse Events	66 (14%)	148 (9%)	112 (6%)
Total number of Adverse Events	117	214	132
Subjects with common Adverse Events:			
Diarrhea	21 (4%)	45 (3%)	33 (2%)
Vomiting	24 (5%)	39 (2%)	21 (1%)
Abdominal pain	7 (1%)	30 (2%)	22 (1%)

Source:NDA Supplement 50-710, Overall Summary of Safety.

As shown, a 30 mg/kg dose of azithromycin given over 3 days has a similar safety profile to that previously described for the same total dose given over 5 days. When treatment-related adverse events are analyzed by day of onset, the two regimens remain similar. That is, treatment-related adverse events are reported with similar frequency on Day 1 of therapy: 53/1729 (3%) for the 3-day regimen versus 39/1888 (2%) for the 5-day regimen. The incidence of adverse events for the two regimens tapers off in a similar fashion over subsequent treatment days. In addition, no new side effects were observed and the severity distribution of these known side effects was similar.

Moreover, the side effect profile of 30 mg/kg given as a *single* dose was similar in scope and nature to that of the same total dose delivered over 3 or 5 days. The total side effect rate was slightly higher but this difference was not clinically significant. In addition, no new side effects were observed and the severity distribution of these known side effects was similar. The nominal rate of vomiting was similar with all

of the regimens. Understandably, the majority (40/66) of the side effects in the single dose regimen occurred on day 1.

It should be noted that children in the single dose studies that vomited within 30 minutes of receiving the dose were instructed to re-dose. A specific concern regarding the single dose treatment of otitis media involves the effect of vomiting on efficacy (i.e., In other words, if a child vomits the single dose of azithromycin, are they more likely to be a treatment failure?). The table below summarizes the outcome of children assigned to single dose therapy in any of the single dose studies who vomited.

Patients Who Vomited Single Dose Therapy

Patients Who Vomited:

Total	52/487 (10.7%)
Treatment related	24/487 (4.9%)
On the first day	36/487 (7.4%)
With outcome data	34

Clinical Outcome:

	Day 14	Day 28	Day 28
	Vomited	Vomited	Never Vomited
Cure	21	29 (85%)	345 (81%)
Improved	10		
Fail	3	5 (15%)	79 (19%)
Missing	2	2	

As presented above, children who vomited on the first day of dosing were not found to be any more likely of failing therapy than children who did not vomit. It is likely that children have absorbed most of their total dose prior to vomiting, given the absorption characteristics of azithromycin.

Analysis of safety laboratory data showed no consistent effect of azithromycin on hematologic indices, hepatic function, renal function, serum electrolyte, or other laboratory safety tests.

6 ADVANTAGES OF SINGLE DOSE AZITHROMYCIN THERAPY

Single dose treatment of otitis media with azithromycin offers the following advantages over presently available therapy:

1) Providing the maximally tolerated dose of azithromycin at the beginning of therapy takes advantage of the host response to infection by optimizing the loading of azithromycin into neutrophils and enabling its delivery to the site of infection.

The primary site of azithromycin accumulation is within phagocytes. Recruitment of neutrophils to the site of infection peaks when the bacterial burden is highest. Delivery of azithromycin to the infected site will therefore be best during periods of maximal neutrophil recruitment. Also, based on *in vitro* studies, the loading of azithromycin into cells is doubled when the temperature is increased from 37° to 40°C.[45] As a result, providing a large single dose of azithromycin at the inception of treatment, when bacterial burden and therefore the febrile response and neutrophil recruitment is highest, will optimize the delivery of azithromycin to the site of infection.

2) The delivery of the entire treatment in a single oral dose guarantees full course treatment and optimal compliance.

Inadequate courses of antibiotic therapy may result in treatment failure. Failure is less likely when full courses of appropriate antimicrobials are administered.

3) The only other single dose treatment option (ceftriaxone) must be delivered intramuscularly

Administration by injection is always painful to the recipient, carries with it the risk of needle-stick related transmission of infectious diseases, is relatively expensive, and is labor intensive. In clinics for sexually transmitted diseases, when given the choice between single oral therapy (azithromycin) and single IM injection (ceftriaxone), patients choose azithromycin. When possible, therapies delivered orally are always preferred.

4) Delivery of the entire dose in a single administration may potentiate the antimicrobial, and therefore, the clinical efficacy of azithromycin.

The concept of AUC_{24}/MIC has been suggested as a parameter that predicts the effectiveness of azithromycin. For that reason, delivering azithromycin as a single 30 mg/kg dose may offer a therapeutic advantage.

5) Single dose treatments decrease the burden of disease on the caregiver.

Therapy for pediatric patients is administered by the caregiver. The additional convenience of single dose treatment decreases the overall burden of disease, not only for the patient, but for the extended family as well.

6) The safety profile of single dose treatment with azithromycin remains favorable.

The side effect rate of azithromycin given as a single dose, when compared to the 3-day regimen, is marginally higher, but this difference is not clinically significant. The adverse event rate is not greater, and is nominally lower, than the rate of a commonly used treatment, Augmentin® (Study R0581). The scope and nature of the side effects is no different than that of the longer courses of therapy.

7 CONCLUSIONS

Data in this submission demonstrate that the 30 mg/kg regimen of azithromycin for treatment of acute otitis media, given as a single dose or over three days, delivers clinical efficacy equivalent to presently available therapy. Recent clinical studies confirm adequate antimicrobial activity against microorganisms commonly seen in practice today. The findings from the pivotal phase 3 studies submitted in this application, supplemented by additional safety data from the phase 4 experience, confirm that the drug is among the better tolerated antimicrobial agents available. In addition, the single dose treatment of otitis media makes use of the host response to infection in order to optimize the pharmacokinetic characteristics of azithromycin while offering advantages to the patient in terms of both compliance and convenience.

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APPENDIX I

ANIMAL MODEL EXPERIMENTS WITH AZITHROMYCIN

1. PHARMACODYNAMIC EXPERIMENTS IN MICE

1.1. Dose-fractionation evaluation of azithromycin versus an acute infection due to susceptible Streptococcus pneumoniae (08-16-99)

TO: Dr. P.F. Miller DATE: 08-16-99

FROM: D. Girard/S.M. Finegan

SUBJECT: *In vivo* dose-fractionation evaluation of CP-062,993 (azithromycin) Vs an acute infection produced by a susceptible *Streptococcus pneumoniae* (02J1016) in 14.9 gm CF-1 mixed sex mice.

DISCUSSION: This evaluation is one of at least three dose-fractionation experiments that will be conducted in our laboratory in order to characterize the PK/PD of azithromycin in-house using pre-clinical infection models. To date, the PK/PD dose-fractionation studies that have been conducted have been with one organism and in only one infection model. The data that was recently generated with linezolid in a single *S. aureus* trial addresses the limitations of working with too small a dataset and perhaps using mortality as an endpoint with a rapidly lethal *S. aureus* challenge.

The literature indicates that AUC/MIC is the key pharmacodynamic parameter for azithromycin. The purpose of this experiment was to characterize the PK/PD relationship for azithromycin using a dose-fractionation paradigm in a *S. pneumoniae* peritonitis model. If the key parameter were indeed AUC/MIC, we would expect the PD50s for all four dose regimens to be the same. In this experiment, since the PD50s are quite similar, this dataset would suggest that efficacy is AUC/MIC driven. However, a larger efficacy dataset will be required and the correlation between efficacy and PK parameters determined before stating that the key driver of efficacy for azithromycin in these pre-clinical infection models is AUC/MIC.

CP-062,993 was administered in 5% EtOH, 5% Tween 80 and 90% PBS q24h (once a day), q12h (twice a day), q6h (four times a day) or q3h (eight times a day) P.O. starting 0.5 h after an I.P. challenge with *Streptococcus pneumoniae* (02J1016). PD50 values are estimated using non-linear regression techniques from the survival data on day 4 following infection.

Compound	Dose (mg/kg/dose)	Mice Surviving/Total	PD50 (mg/kg/day)
CP-062,993	12.5	10/10	
Lot #17419-64-1F	3.12	8/10	17.8
MIC 0.03-0.25 µg/ml	0.78	1/10	(13.4-22.2)*
q3h	0.20	0/10	
CP-062,993	25.0	9/10	
Lot #17419-64-1F	6.25	8/10	17.6
MIC 0.03-0.25 µg/ml	1.56	2/10	(6.8-28.3)*
q6h	0.39	0/10	
CP-062,993	50.0	10/10	
Lot #17419-64-1F	12.5	6/10	22.7
MIC 0.03-0.25 µg/ml	3.12	1/10	(18.7-26.6)*
q12h	0.78	0/10	
CP-062,993	100.0	10/10	
Lot #17419-64-1F	25.0	8/10	18.0
MIC 0.03-0.25 µg/ml	6.25	0/10	(17.4-18.6)*
q24h	1.56	0/10	
Infected (non-treated) control			
10-5 dilution		0/10	
10-6 dilution (used in study)		0/10	
10-7 dilution		2/10	
BHI (non-infected) control		10/10	

*95% confidence limits Ref: 44414.076

Bacterial challenge : $5.4 * 10^3$ organisms/mouse

1.2. Dose-fractionation evaluation of azithromycin versus an acute infection due to susceptible Streptococcus pyogenes (09-20-99)

TO: Dr. P.F. Miller DATE: 09-20-99

FROM: D. Girard/S.M. Finegan

SUBJECT: *In vivo* dose-fractionation evaluation of CP-062,993 (azithromycin) vs an acute infection produced by a susceptible *Streptococcus pyogenes* (02C0203) in 13.7 gm CF-1 mixed sex mice.

DISCUSSION: This evaluation is the second of at least three dose-fractionation experiments that will be conducted in our laboratory in order to characterize the PK/PD of azithromycin in-house using pre-clinical infection models. The first study was conducted with azithromycin versus a susceptible *Streptococcus pneumoniae* (02J1016) in an acute peritonitis model where the data indicated that AUC/MIC was the key pharmacokinetic parameter that was driving efficacy.

Craig, *et al* has cited that AUC/MIC is the key pharmacodynamic parameter for azithromycin driving efficacy. The purpose of this experiment was to characterize the PK/PD relationship for azithromycin using a dose-fractionation paradigm in a *S. pyogenes* peritonitis model. If the key parameter were indeed AUC/MIC, we would expect the PD50s for all four dose regimens to be the same. In this experiment, since the PD50s are quite similar, this dataset would suggest that efficacy is AUC/MIC driven. However, a larger efficacy dataset will be required and the correlation between efficacy and PK parameters determined before stating that the key driver of efficacy for azithromycin in these pre-clinical infection models is AUC/MIC.

PROTOCOL: CP-062,993 was administered in 5% EtOH, 5% Tween 80 and 90% PBS q24h (once a day), q12h (twice a day), q6h (four times a day) or q3h (eight times a day) P.O. starting 0.5 h after an I.P. challenge with *Streptococcus pyogenes* (02C0203). PD50 values are estimated using non-linear regression techniques from the survival data on day 4 following infection and are expressed on a mg/kg/day basis.

Compound	Dose (mg/kg/dose)	Mice Surviving/Total	PD50 (mg/kg/day)
CP-062,993	3.12	10/10	
Lot #17419-64-1F	0.78	8/10	2.2
MIC 0.03-0.10 µg/ml	0.20	2/10	(1.0-3.4)*
q3h	0.05	0/10	
CP-062,993	6.25	10/10	
Lot #17419-64-1F	1.56	9/10	2.2
MIC 0.03-0.10 µg/ml	0.39	2/10	(1.7-2.7)*
q6h	0.10	0/10	
CP-062,993	12.5	10/10	
Lot #17419-64-1F	3.12	10/10	1.4
MIC 0.03-0.10 µg/ml	0.78	6/10	(1.4-1.4)*
q12h	0.20	0/10	
CP-062,993	25.0	10/10	
Lot #17419-64-1F	6.25	8/10	1.3
MIC 0.03-0.10 µg/ml	1.56	7/10	(0.8-1.8)*
q24h	0.39	0/10	
Infected (non-treated) control			
10-1 dilution		0/10	
10-2 dilution (used in study)		0/10	
10-3 dilution		0/10	
BHI (non-infected) control		10/10	

*95% confidence limits. Ref: 44414.111

Bacterial challenge : $3.4 * 10^6$ organisms/mouse

1.3. Pneumococcal lung infection maximum antimicrobial effect (Emax) dose-response model using a dose-fractionation paradigm with azithromycin (10-01-00)

TO: Infectious Diseases Project Team DATE: 10-01-00

FROM: D. Girard/H.W. Mathieu

SUBJECT: Results of a maximum antimicrobial effect (*Emax*) dose-response model using a dose-fractionation paradigm with azithromycin.

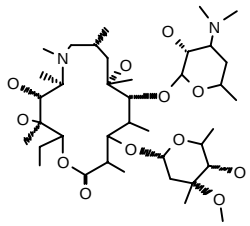
OBJECTIVE: To use a murine pneumococcal lung infection (02J1016) model in order to provide efficacy data for azithromycin, so that the pharmacodynamic parameter that best correlates with efficacy can be determined for azithromycin.

PROTOCOL: Immune competent CF-1 female mice were challenged with a log phase culture of *Streptococcus pneumoniae* 02J1016 (fully susceptible) via the intranasal route ($1.3 * 10^5$ CFU/mouse). Therapy was initiated 18 h following bacterial challenge using a q24h, q12h, q6h and q3h dosing interval that covered a 64-fold dose range of 50, 12.5, 3.12 and 0.78 mg/kg/day. *S. pneumoniae* clearance was determined by quantifying the bacterial population (CFU/mouse) at 18 and 42 h post-challenge following aseptic excision of infected lungs and standard plate counting methods using blood agar for cultivation. Subsequently, the change in $\log_{10}$CFU over 24 h (relative to the bacterial burden at the time of therapy) was determined for each dosing interval and the P50 value (the dose required to produce 50% of *Emax*) and static dose value (dose at which there was no net change in bacterial burden) were estimated from non-linear regression of these data. (We thank Steven Finegan and Caroline Cimochowski with their assistance with the aforementioned dosing paradigm.)

RESULTS/DISCUSSION: We utilized a dose-fractionation paradigm for azithromycin (varying the dosing interval while keeping the total daily dose for each dose level constant) in order to reduce the interdependence between pharmacokinetic parameters (total dose, AUC, peak level, time above MIC, etc.) in our pneumococcal pulmonary infection *Emax* model. The results of our experiments are presented below but without accompanying PK and PD estimates only un-substantiated observations can be made. These efficacy data for azithromycin vs. 02J1016 are consistent with a PD parameter of AUC/MIC driving efficacy, similar to the data that we have generated in our *S. pneumoniae* and *S. pyogenes* murine peritonitis models.

Compound	Dose (mg/kg/day)	Route	log_{10} CFU @42 h	P50 (mg/kg/day)	Static Dose (mg/kg/day)
CP-062,993	50	PO	<2.48	3.4	2.3
#15989-286-1F	12.5		<2.48	(2.8-4.0)*	
MIC 0.06 µg/ml	3.12		4.82		
Range (0.03-0.13)	0.78		6.13		
q24h regimen					
CP-062,993	50	PO	<2.48	8.9	6.4
#15989-286-1F	12.5		3.28	(7.9-10)*	
MIC 0.06 µg/ml	3.12		6.41		
Range (0.03-0.13)	0.78		6.36		
q12h regimen					
CP-062,993	50	PO	<2.48	2.6	1.8
#15989-286-1F	12.5		<2.48	(0.3-5.0)*	
MIC 0.06 µg/ml	3.12		3.87		
Range (0.03-0.13)	0.78		4.69		
q6h regimen					
CP-062,993	50	PO	<2.48	1.1	1.2
#15989-286-1F	12.5		<2.48	(1.1-1.2)*	
MIC 0.06 µg/ml	3.12		<2.48		
Range (0.03-0.13)	0.78		5.82		
q3h regimen					
Infected Control			6.52		

*95% confidence interval Ref: 43876-077



Azithromycin

1.4. Neutropenic thigh infection maximum antimicrobial effect (Emax) dose-response model using a dose-fractionation paradigm with azithromycin (10-20-99)

TO: Macrolide Project Team DATE: 10-20-99

FROM: D. Girard/S. M. Finegan

SUBJECT: Results of a maximum antimicrobial effect (*Emax*) dose-response model using a dose-fractionation paradigm with CP-062,993 (azithromycin).

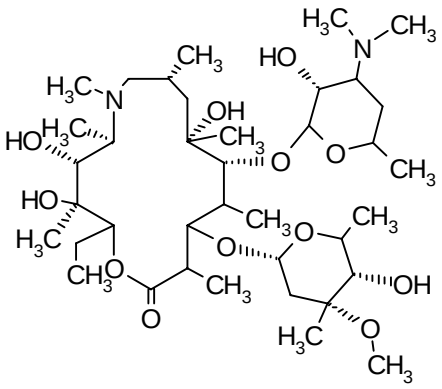
OBJECTIVE: To use a murine neutropenic thigh infection (02C0203) model in order to provide efficacy data for CP-062,993, so that the pharmacodynamic parameter that best correlates with efficacy can be determined for CP-062,993.

PROTOCOL: On day –3, CF-1 female mice were treated I.P with 150 mg/kg cyclophosphamide and on day –1 these same mice were treated I.P. with 100 mg/kg cyclophosphamide. On day 0, 22.5 gram CF-1 female mice were challenged with a 1:1000 dilution of an overnight culture of *Streptococcus pyogenes* 02C0203 (fully susceptible) I.M. ($4.0 * 10^5$ CFU/mouse). Therapy was initiated 1 h following bacterial challenge using a q24h, q12h, q6h and q3h dosing interval that covered a 64-fold dose range of 50, 12.5, 3.12 and 0.78 mg/kg/day. *S. pyogenes* clearance was determined by quantifying the bacterial population (CFU/mouse) at 24 h post-challenge following aseptic excision of infected thighs and standard plate counting methods using blood agar for cultivation. Subsequently, the change in $\log_{10}$CFU over 24 h (relative to the bacterial burden at the time of therapy) was determined for each dosing interval and the P50 value (the dose required to produce 50% of *Emax*) and static dose value (dose at which there was no net change in bacterial burden) were estimated from non-linear regression of these data. (We thank Heather Mathieu with her assistance with the aforementioned dosing paradigm.)

RESULTS/DISCUSSION: We utilized a dose-fractionation paradigm for CP-062,993 (varying the dosing interval while keeping the total daily dose for each dose level constant) in order to reduce the interdependence between pharmacokinetic parameters (total dose, AUC, peak level, time above MIC, etc.) in our murine neutropenic thigh infection *Emax* model. The results of our experiments are presented below but without accompanying PK and PD estimates only un-substantiated observations can be made. Although the q24h dosing interval for CP-062,993 resulted in the lowest P50 and static dose, the 95% confidence intervals overlap suggesting that there is no significant difference between the various dosing intervals for this one study. These data are consistent with a PD parameter of AUC/MIC.

Compound	Dose (mg/kg/day)	Route	log_{10} CFU @24 h	Norm P50 (mg/kg/day)	Static Dose (mg/kg/day)
CP-062,993	50	PO	<2.48	3.5	3.2
#17419-64-1F	12.5		<2.48	(3.5-3.5)*	
MIC 0.03-0.1 µg/ml	3.12		5.43		
q24h regimen	0.78		7.25		
CP-062,993	50	PO	<2.48	8.9	12.2
#17419-64-1F	12.5		3.52	(0-18.7)*	
MIC 0.03-0.1 µg/ml	3.12		5.40		
q12h regimen	0.78		6.76		
CP-062,993	50	PO	<2.48	11.7	7.7
#17419-64-1F	12.5		4.51	(6.8-16.5)*	
MIC 0.03-0.1 µg/ml	3.12		6.34		
q6h regimen	0.78		6.61		
CP-062,993	50	PO	<2.48	12.8	9.5
#17419-64-1F	12.5		5.02	(7.7-18.0)*	
MIC 0.03-0.1 µg/ml	3.12		6.35		
q3h regimen	0.78		6.61		
Infected Control			6.41		

*95% confidence interval Ref: 44414-143



CP-062,993 Azithromycin

1.5. Evaluation of azithromycin and clarithromycin given as the same total dose over 1, 2 and 3 days versus an intranasal lung infection due to susceptible Streptococcus pneumoniae (09-25-00)

TO: Mr. Dennis Girard DATE: 09-25-00

FROM: D. Girard/S.M. Finegan

SUBJECT: Accelerated dosing study. *In vivo* evaluation of CP-062,993 (azithromycin) and CP-097,593 (clarithromycin) giving the same total dose over 1, 2 and 3 days vs. an intranasal lung infection produced by a susceptible *Streptococcus pneumoniae* (02J1016) in 21.1 gm CF-1 female mice.

COMMENTS: Azithromycin and clarithromycin are both active against our susceptible *Streptococcus pneumoniae* 02J1016 that produces a more slowly developing disease relative to other pneumococcal strains in our *in vivo* panel. In our pulmonary infection model 100% mortality in no-drug controls is not observed until 7 days post-infection. Clarithromycin was chosen as a comparator to azithromycin since the pharmacokinetics for these two macrolides are quite different. While clarithromycin achieves relatively high blood levels quickly and is cleared fairly rapidly, azithromycin accumulates in tissues and leeches out slowly over time into the bloodstream yielding overall lower blood levels than clarithromycin but a much longer exposure time. Additionally, the research of Craig, *et. al* in preclinical species suggest that the outcomes observed with azithromycin best correlate with the PD parameter of AUC/MIC and time above MIC for clarithromycin. We tried to model the differences in PK/PD over a longer period and therefore in this experiment, we wanted to determine if the compounds work better against a lung infection with *Streptococcus pneumoniae* if the same total dose was given over a 1, 2, or 3-day period administered using a q.d. therapeutic regimen.

Azithromycin performs the best when given once rather than the total dose being spread out over 3 days. It is important to note that while the azithromycin 1 day dosing PD_{50} (20.4 mg/kg/regimen) is significantly different than the 3 day dosing PD_{50} (49.4 mg/kg/regimen), both are within the 95% confidence limits of the 2 day dosing regimen PD_{50} (27.6 mg/kg/regimen).

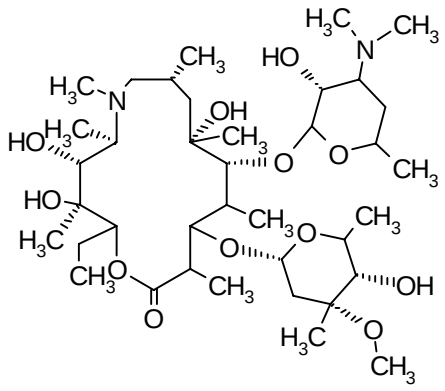
Clarithromycin fails (PD_{50} >200 mg/kg/regimen) using the q.d. regimen regardless as to the duration of therapy. This is puzzling since clarithromycin has a MIC equivalent to that of azithromycin against 02J1016 (0.01-0.06 ug/ml). However, since clarithromycin is cleared more rapidly from the animal and this infection takes longer to manifest itself and cause mortality, this is most likely an accurate result. All of our previous efficacy data with clarithromycin in this pneumococcal pneumonia model was generated following BID administration over two days of therapy where clarithromycin therapy is successful with PD_{50}s ranging from 5-25 mg/kg.

PROCEDURE: Azithromycin and clarithromycin were administered P.O. in Pfizer diluent q.d. for either 1, 2 or 3 days after an I.N. challenge with *Streptococcus pneumoniae* (02J1016).

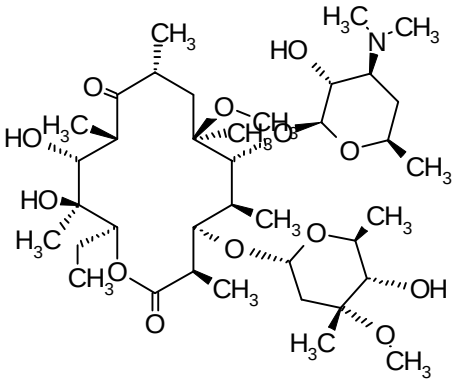
Compound	Dose (mg/kg/day)	Mice Surviving/Total	PD50 (mg/kg/regimen)
CP-062,993 P.O.	33.3	9/10	
Lot #17419-64-1F	8.3	1/10	49.4
MIC 0.02-0.13 µg/ml	2.1	0/10	(28.1-70.8)*
3 day regimen	0.52	0/10	
CP-062,993 P.O.	50.0	9/10	
Lot #17419-64-1F	12.5	4/10	27.6
MIC 0.02-0.13 µg/ml	3.12	0/10	(22.8-32.4)*
2 day regimen	0.78	0/10	
CP-062,993 P.O.	100.0	10/10	
Lot #17419-64-1F	25.0	7/10	20.4
MIC 0.02-0.13 µg/ml	6.25	1/10	(16.4-24.3)*
1 day regimen	1.56	0/10	
CP-097,593 P.O.	66.7	2/10	
Lot #34844-174-01	16.7	0/10	>200
MIC 0.01-0.06 µg/ml	4.2	0/10	
3 day regimen	1.04	0/10	
CP-097,593 P.O.	100.0	1/10	
Lot #34844-174-01	25.0	0/10	>200
MIC 0.01-0.06 µg/ml	6.25	0/10	
2 day regimen	1.56	0/10	
CP-097,593 P.O.	200.0	4/10	
Lot #34844-174-01	50.0	1/10	>200
MIC 0.01-0.06 µg/ml	12.5	1/10	
1 day regimen	3.12	0/10	
Infected (non-treated) control			
10-1 dilution		0/10	
10-2 dilution **(**used in study)		0/10	
10-3 dilution		0/10	
BHI (non-infected) control		10/10	

*95% confidence limits Ref: 47910.161

Bacterial challenge: $2.88 * 10^4$ organisms/mouse



CP-062,993



CP-097,593

1.6. Evaluation of azithromycin and clarithromycin given as the same total dose over 1, 2 and 3 days versus an acute infection due to susceptible Streptococcus pyogenes (10-30-00)

TO: Mr. Dennis Girard DATE: 10-30-00

FROM: D. Girard/S.M. Finegan

SUBJECT: Accelerated dosing study. *In vivo* evaluation of CP-062,993 (azithromycin) and CP-097,593 (clarithromycin) giving the same total dose over 1, 2 and 3 days vs. an acute infection produced by a susceptible *Streptococcus pyogenes* (02C0203) in 14.2 gm CF-1 mixed mice.

COMMENTS: Azithromycin and clarithromycin are both highly active against our susceptible *Streptococcus pyogenes* 02C0203. We chose to compare the outcomes for various lengths of therapy in this infection model, as the peritonitis caused by this strain lends itself to prolonged therapy since 100% mortality doesn't occur until day 2-3 post-challenge. Clarithromycin was chosen as a comparator to azithromycin since the pharmacokinetics for these two macrolides are quite different. While clarithromycin achieves relatively high blood levels quickly and is cleared fairly rapidly, azithromycin accumulates in tissues and leeches out slowly over time into the bloodstream yielding overall lower blood levels than clarithromycin but a much longer exposure time. Additionally, the research of Craig, *et. al* in preclinical species suggest that the outcomes observed with azithromycin best correlate with the PD parameter of AUC/MIC and time above MIC for clarithromycin. We tried to model the differences in PK/PD over a longer period and therefore in this experiment, we wanted to compare the outcomes for these compounds against a *Streptococcus pyogenes* acute infection giving the same total therapeutic dose over a 1, 2, or 3-day dosing period. We did this same type of experiment earlier with a susceptible *Streptococcus pneumoniae* (02J1016) and found that azithromycin performs best when administered in one dose rather than spreading the same total dose out over 3 days. All three regimens of clarithromycin failed (PD_{50}s > 200 mg/kg), so no useful information was gleaned from that portion of the experiment except to say that when therapy was administered using a b.i.d. regimen the PD_{50}s were in the range of 5-25 mg/kg.

Against 02C0203, azithromycin performs the best when given once rather than the total dose being spread out over 3 days. The azithromycin 1-day dosing PD_{50} (1.0 mg/kg/regimen) is significantly different than the 2 and 3 day dosing PD_{50}s (2.5 and 3.8 mg/kg/regimen, respectively). The 2 and 3-day dosing regimen PD_{50}s are also significantly different from each other.

Clarithromycin has the best activity when given more often (2-days and 3-days dosing versus 1-day dosing). The PD_{50}s for the 2 and 3-day dosing regimens (3.1 and 2.2 mg/kg/regimen) are equivalent, while the PD_{50} for the 1-day dosing regimen, 11.3 mg/kg/regimen, is clearly higher. However, when the 95% confidence limits for

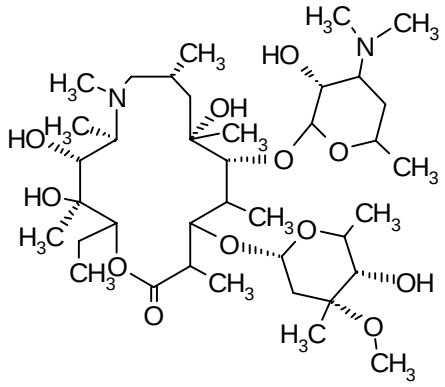
all three regimens are taken into account, it appears as though all three regimens are equivalent. The 2 and 3-day therapies are more consistent with historical data for clarithromycin administered b.i.d. for 1-day.

PROCEDURE: Azithromycin and clarithromycin were administered P.O. in Abbot diluent q.d. for either 1, 2 or 3 days after an I.P. challenge with *Streptococcus pyogenes* (02C0203) starting 1.0 hour after infection.

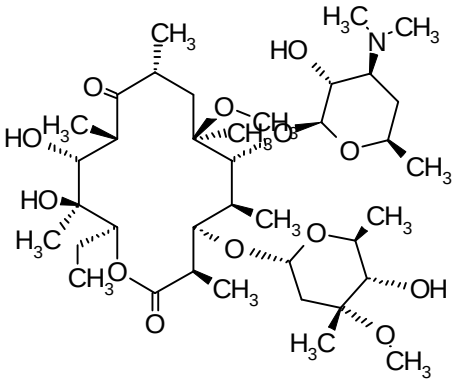
Compound	Dose (mg/kg/day)	Mice Surviving/Total	PD50 (mg/kg/regimen)
CP-062,993 P.O.	12.5	10/10	
Lot #17419-64-1F	3.12	10/10	3.8
MIC 0.01-0.10 µg/ml	0.78	3/10	(3.8-3.9)*
3 day regimen	0.20	0/10	
CP-062,993 P.O.	25.0	9/10	
Lot #17419-64-1F	6.25	10/10	2.5
MIC 0.01-0.10 µg/ml	1.56	7/10	(1.8-3.3)*
2 day regimen	0.39	1/10	
CP-062,993 P.O.	50.0	10/10	
Lot #17419-64-1F	12.5	10/10	1.0
MIC 0.01-0.10 µg/ml	3.12	8/10	(0.6-1.4)*
1 day regimen	0.78	3/10	
CP-097,593 P.O.	12.5	10/10	
Lot #34844-174-01	3.12	9/10	3.1
MIC 0.01-0.03 µg/ml	0.78	5/10	(2.6-3.7)*
3 day regimen	0.20	0/10	
CP-097,593 P.O.	25.0	8/10	
Lot #34844-174-01	6.25	9/10	2.2
MIC 0.01-0.03 µg/ml	1.56	8/10	(0.9-3.6)*
2 day regimen	0.39	0/10	
CP-097,593 P.O.	50.0	10/10	
Lot #34844-174-01	12.5	6/10	11.3
MIC 0.01-0.03 µg/ml	3.12	4/10	(3.1-19.4)*
1 day regimen	0.78	0/10	
Infected (non-treated) control			
10-1 dilution		0/10	
10-2 dilution (used in study)		0/10	
10-3 dilution		0/10	
BHI (non-infected) control		10/10	

*95% confidence limits Ref: 47910.189

Bacterial challenge: $1 * 10^6$ organisms/mouse



CP-062,993



CP-097,593

1.7. Evaluation of azithromycin and clarithromycin given as the same total dose over 1, 2 and 3 days versus an acute infection due to susceptible Enterococcus faecalis (12-11-00)

TO: Infectious Diseases Biology Date: 12-11-00

FROM: Caroline Cimochowski/D. Girard

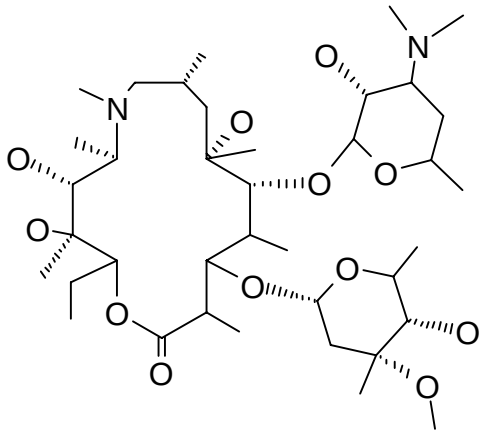
SUBJECT: Accelerated dosing paradigm. *In vivo* evaluation of azithromycin and clarithromycin vs. an acute infection of *Enterococcus faecalis* 03A1085 in 22 gram [F] DBA/2 mice comparing equivalent therapies (same total therapeutic dose) administered over 1, 2 and 3 days.

DISCUSSION: To date, we have demonstrated improved *in vivo* efficacy for azithromycin in preclinical infection models with *S. pneumoniae* and *S. pyogenes* when the total therapeutic dose is administered in an accelerated fashion (one day of therapy vs. two or three). Azithromycin and clarithromycin are both active against our susceptible *Enterococcus faecalis* 03A1085 that also produces a more slowly developing disease relative to more acutely lethal pathogens used in our *in vivo* models. Azithromycin (CP-062993) and clarithromycin (CP-097593) had previously been run in the mouse systemic disease (PD50) model utilizing our standard dosing paradigm (b.i.d. on day 1) and had PD50's of 20.1 mg/kg. s.c. and 1.7 mg/kg. s.c., respectively vs 03A1085. Since in this model we don't see 100% mortality until day 2-3 in our no drug controls, we were able to evaluate the influence of therapy duration on survival.

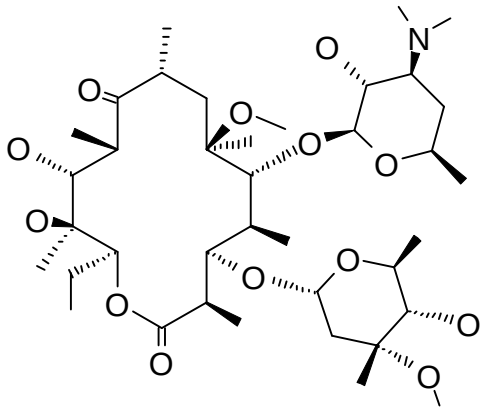
In this study, the same total therapeutic dose (i.e., 200 mg/kg/therapeutic regimen) was administered Q.D. on day one (i.e., 200 mg/kg on day 1), Q.D. on day one and two (i.e., 100 mg/kg on day 1 and 2) or Q.D. on day one, two and three (i.e., 67 mg/kg on day 1, 2 and 3). For azithromycin, efficacy was best with one dose (14.8 mg/kg s.c.) and results were statistically significant ($p < 0.05$). Interestingly, clarithromycin efficacy was also best with one dose (2.2 mg/kg s.c.), and as with azithromycin, the one dose confidence limits did not overlap with the 2 and 3 day regimens. However, the data for the 2 day and 3 day regimens were indistinguishable.

Compound	Dose (mg/kg/day)	Mice Surviving/Total	PD50 (mg/kg/total regimen)
CP-062993 s.c.	200	10/10	14.8
(17419-064-1F)	50	9/10	(10.2-19.5) [1]
Q.D. [1 day]	12.5	4/10	
MIC: 3-6 μg/ml	3.12	1/10	
w/serum: 6.25			
CP-062993	100	10/10	42.7
(17419-064-1F)	25	7/10	(42.2-43.2) [1]
Q.D. [2 days]	6.25	0/10	
	1.56	1/10	
CP-062993	66.6	9/10	59.3
(17419-064-1F)	16.6	4/10	(27.5-91.2) [1]
Q.D. [3 days]	4.15	2/10	
	1.04	3/10	
CP-097593	100	10/10	2.2
(34844-174-01)	25	10/10	(0.19-4.2) [1]
Q.D. [1 day]	6.25	7/10	
MIC: 3.12 μg/ml	1.56	3/10	
w/serum : 3.12			
CP-097593	50	10/10	23.7
(34844-174-01)	12.5	6/10	(5.4-42) [1]
Q.D. [2 days]	3.12	5/10	
	0.78	1/10	
CP-097593	33.3	9/10	18.8
(34844-174-01)	8.3	8/10	(7.2-30.3) [1]
Q.D. [3 days]	2.08	1/10	
	0.52	3/10	
Infected only	dilution 10^{-2}	0/10	
	dilution 10^{-3}	1/10 [2]	
	dilution 10^{-4}	0/10	
	3% yeast	10/10	
Bacterial challenge: 1.7×10^{7} per mouse			43907.208

1 95% confidence limits
2 PD50 adjusted



CP-062993 (azithromycin)



CP-097593 (clarithromycin)

1.8. Evaluation of azithromycin given as the same total dose (200 mg/kg) over 1, 2 and 3 days, and clarithromycin given on one day, versus an acute infection due to Haemophilus influenzae (non-type B) (01-09-01)

TO: Infectious Diseases Biology DATE: 01-09-01

FROM: Caroline Cimochowski/D. Girard

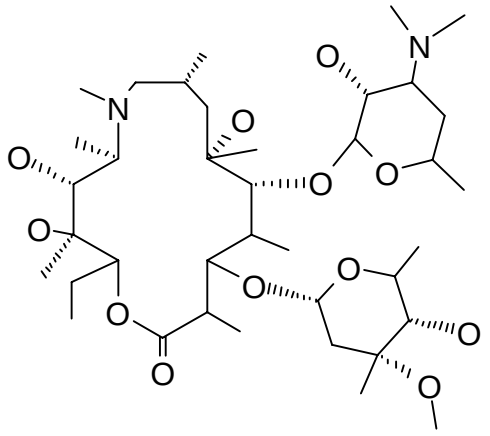
SUBJECT: Accelerated dosing paradigm. *In vivo* evaluation of azithromycin vs. an acute infection of *Haemophilus influenzae* 54A1100 (non-type B) in 20 gram [F] DBA/2 mice comparing equivalent therapies (same total therapeutic dose) administered over 1, 2 and 3 days. One set of clarithromycin-dosed mice was also run, with total dose administered in just one day.

DISCUSSION: To date, we have demonstrated improved *in vivo* efficacy for azithromycin in preclinical infection models with *S. pneumoniae, S. pyogenes, and Enterococcus faecalis* when the total therapeutic dose is administered in an accelerated fashion (one day of therapy vs. two or three). *Haemophilus influenzae* 54A1100 also produces a more slowly developing disease relative to more acutely lethal pathogens used in our *in vivo* models. Azithromycin (CP-062993) had previously been run in the mouse systemic disease (PD50) model utilizing our standard dosing paradigm (b.i.d. on day 1) and had a PD50 of 27 mg/kg. s.c. vs 54A1100. Since in this model we don't see 100% mortality until day 2 in our no drug controls, we were able to evaluate the influence of therapy duration on survival.

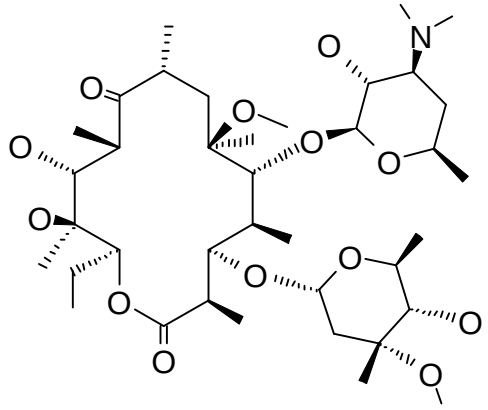
In this study, for azithromycin- the same total therapeutic dose (i.e., 200 mg/kg/therapeutic regimen) was administered Q.D. on day one (i.e., 200 mg/kg on day 1), Q.D. on day one and two (i.e., 100 mg/kg on day 1 and 2) or Q.D. on day one, two, and three (i.e., 67 mg/kg on day 1, 2 and 3). For azithromycin, efficacy was best with one therapeutic dose (25.3 mg/kg s.c.) and this accelerated dosing regimen was significantly better ($p < 0.05$) than the 2 and 3 day regimens. Furthermore, the 2 day regimen was also significantly better than the 3 day regimen ($p < 0.05$). Clarithromycin (with a one day dose regimen and an MIC of 8 µg/ml) failed (PD50: >200 mg/kg s.c.)

Compound	Dose (mg/kg/day)	Mice Surviving/Total	PD50 (mg/kg/total regimen)
CP-062993 s.c.	200	10/10	25.3
(17419-064-1F)	50	9/10	(14.3-36.2) [1]
Q.D. [1 day]	12.5	1/10	
MIC: 0.5-1 μg/ml	3.12	0/10	
Azithromycin			
CP-062993	100	10/10	49.9
(17419-064-1F)	25	5/10	(41.5-58.5) [1]
Q.D. [2 days]	6.25	1/10	
Azithromycin	1.56	0/10	
CP-062993	66.6	6/10	181.6
(17419-064-1F)	16.6	0/10	(180.1-183.1) [1]
Q.D. [3 days]	4.15	0/10	
Azithromycin	1.04	0/10	
CP-097593 s.c.	200	0/10	>200
(34844-174-01)	50	2/10	
Q.D. [1 day]	12.5	0/10	
MIC: 8 μg/ml	3.12	0/10	
Clarithromycin			
Infected only	dilution 10^{-2}	0/10	
	dilution 10^{-3}	0/10	
	dilution 10^{-4}	0/10	
	3% yeast	10/10	
Bacterial challenge: 1.3×10^{7} per mouse			43907.213

1 95% confidence limits



CP-062993 (azithromycin)



CP-097593 (clarithromycin)

2. GERBIL MODEL OF MIDDLE EAR INFECTION

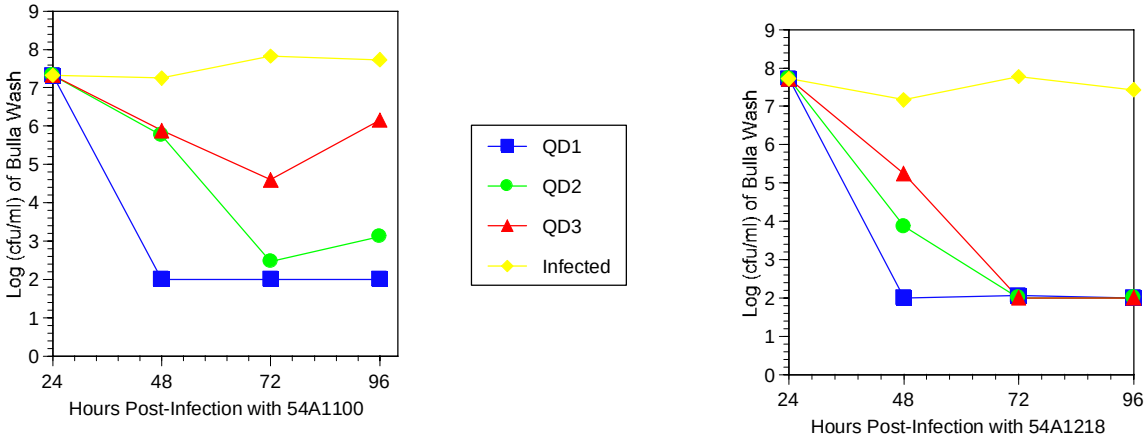
2.1. Summary and Conclusion

Laboratory experiments with azithromycin detailed in the Azithromycin Accelerated Dosing Pediatric sNDA-50-710, suggest it is the total amount of drug rather than the interval of the dosing regimen that determines the concentration at the infection site and results in efficacy. Acute murine models challenged with either *S. pneumoniae, H. influenzae, S. pyogenes*, or *E. faecalis* showed that azithromycin was superior in efficacy when given as a single oral dose as determined by PD_{50} measurements. The improvement in the PD_{50} for treatment of *H. influenzae* was especially noteworthy. These findings have been extended to a gerbil model of middle ear infection challenged with two different strains of *H. influenzae* (Table 1 and Figure 1). *H. influenzae* strains 54A1100 and 54A1218 are non-typable isolates; 54A1218 also carries a TEM-1 β-lactamase. In these experiments, colony-forming units (CFU) are assessed from the bulla wash of 5 gerbils per time point. The ED_{50} values reflect the dose in which the CFU recoverable from the bulla wash is 50% of the non-treated animals. In the first experiment, azithromycin administered as a single dose was found to be as efficacious as the same total dose given over 2 or 3 days (Table 1). However, *in vivo* kill kinetics demonstrated that the one-day dosing therapeutic regimen resulted in the most rapid eradication of the pathogens (Figure 1) and, in the case of 54A1100, was the only dosing regimen that led to complete clearance. These pre-clinical infection model data are consistent with what has been observed in clinical studies included in sNDA-50-710, supporting the notion that one-day therapy has an advantage over a more prolonged course of therapy.

Table 1. The Effect of Dose Regimen on Efficacy of Azithromycin in the Gerbil Middle Ear Infection Model.

Pathogen	Drug	MIC (μg/ml)	Dosing Regimen	ED50 (mg/kg/total regimen)
H. influenzae	Azithromycin	1	3 days	162.9 (140 – 185.9)
			2 days	138.1 (118.7 – 157.6)
			1 day	138.1 (118.7 – 157.6)

Figure 1. Eradication of *H. influenzae* after therapy of 200 mg/kg azithromycin administered over one, two or three days



2.2. Evaluation of azithromycin given as the same total dose (300 mg/kg) over 1, 2 and 3 days versus a localized infection due to Haemophilus influenzae (non-type B) (02-09-01)

Original Reports

TO: Antibacterials Biology DATE: 02-09-01

FROM: D. Girard/ C.R. Cimochowski

SUBJECT: Accelerated dosing paradigm in the gerbil middle ear infection model. *In vivo* evaluation of azithromycin vs. a localized infection of *Haemophilus influenzae* 54A1100 (non-type B) in 56.4 gram [F] Mongolian gerbils comparing equivalent therapies (same total therapeutic dose) administered over 1, 2, or 3 days.

DISCUSSION: Historically, azithromycin demonstrates potent activity in our gerbil model of otitis media vs *Haemophilus influenzae* 54A1100. Initial model development and validation experiments indicated that TID therapy for two days was required to demonstrate robust clearance for azithromycin (AEG). Therefore, the standard model protocol with *H. influenzae* utilizes this TID dosing regimen beginning 20 hours post-infection with outcome being determined on day 3. Our oral ED_{50} values are based on mg/kg/dose and typically fall between 21 and 45 mg/kg.

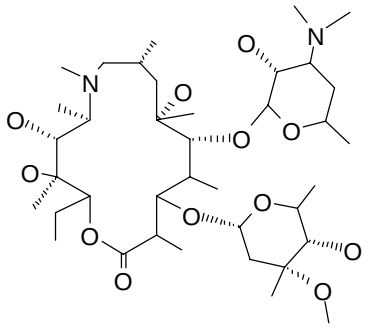
In this study based on a mg/kg/total regimen, for azithromycin- the same total therapeutic dose (i.e., 300 mg/kg/ therapeutic regimen) was administered Q.D. on day one (i.e., 300 mg/kg on day 1), Q.D. on day one and two (i.e., 150 mg/kg on day 1 and 2) or Q.D. on day one, two, and three (i.e., 100 mg/kg on day 1, 2 and 3). Another change in protocol was necessary since we included a third day of dosing and that is the clearance was determined a day later, on day 4.

SUMMARY: In this study, the outcome from the three different durations of therapy (1, 2 or 3 days of therapy) was equivalent. Accelerated dosing for one day had no impact on efficacy relative to the 2 and 3 day therapeutic regimens. All therapies had 100% clearance of organism at 300 mg/kg p.o. and 0% clearance at 75 mg/kg p.o. ; the breakpoint (i.e. that dose level at which some samples were culture negative and some were culture positive) was the same for the three therapies (150 mg/kg p.o.). Although these ED_{50} values appear to be extremely high, it has to be re-iterated that our characteristic range of ED_{50} value with azithromycin (21-45 mg/kg) using our standard protocol is on a mg/kg/dose basis and translated into mg/kg/therapy as we have done in this study would comprise a range of 126-270 mg/kg/therapy.

Compound	Dose (mg/kg/day)	% Clearance	Recoverable CFU (Bulla) (Log_{10}.Geo.Mean)	Sig.Red. CFU (P value)
CP-062993 s.c.	300	100%	<2.0000	P=0.000
(17419-064-1F)	150	60%	<4.0369	P=0.052
Q.D. [1 day]	75	0%	7.5006	P=0.677
MIC: 0.5-1 μg/ml	37.5	0%	7.0941	P=0.756
Azithromycin				
CP-062993	150	100%	<2.0000	P=0.000
(17419-064-1F)	75	60%	<3.8065	P=0.029
Q.D. [2 days]	37.5	0%	6.9299	P=0.519
Azithromycin	18.75	0%	7.3456	P=0.941
CP-062993	100	100%	<2.0000	P=0.000
(17419-064-1F)	50	40%	<3.6619	P=0.009
Q.D. [3 days]	25	0%	7.2589	P=0.911
Azithromycin	12.5	0%	6.6642	P=0.189
Untreated	----	0%	7.3091	

Bacterial challenge: 4.8×10^{3} /gerbil Ref:43970.232

The ED50's for azithromycin QD1 and QD2 were both 138.1 (118.7-157.6) mg/kg p.o.; for QD3, 162.9 (140-185.9) mg/kg p.o. Confidence limits overlapped for the three dose regimens. Note: the ED50's were all based on mg/kg/total regimen.



CP-062993

2.3. Evaluation of azithromycin given as the same total dose (200 mg/kg) over 1, 2 and 3 days versus a localized infection due to Haemophilus influenzae (non-type B) (06-25-01)

TO: Antibacterials Biology DATE: 06-25-01

FROM: D. Girard/ C. Cimochowski

SUBJECT: Accelerated dosing paradigm in the gerbil middle ear infection model. *In vivo* evaluation of azithromycin vs. a localized infection of *Haemophilus influenzae* 54A1100 (non-type B) in 50.4 gram [F] Mongolian gerbils comparing one equivalent therapy [200 mg/kg/therapy p.o.] (same total therapeutic dose) administered over 1, 2, or 3 days. CFU counts/ml. bulla wash (5 gerbils per timepoint) were done at 24, 48, 72, and 96 hours post-infection for each of the Q.D. 1, Q.D. 2, and Q.D. 3 groups.

DISCUSSION: In this study based on a mg/kg/total regimen, for azithromycin- the same total therapeutic dose (i.e., 200 mg/kg/ therapeutic regimen) was administered Q.D. on day one (i.e., 200 mg/kg on day 1), Q.D. on day one and two (i.e., 100 mg/kg on day 1 and 2) or Q.D. on day one, two, and three (i.e., 66.6 mg/kg on day 1, 2 and 3). A baseline CFU count of bulla wash was done 24 hours post-infection, before dosing began. Thereafter, samples for counts from each group were taken at 48, 72, 96 hours post-infection. By looking at the kinetics of bacterial reduction in the bulla wash, a kinetic kill curve could be constructed which hopefully would visually elucidate the observed *in vivo* therapeutic efficacy by examining the dose/ time-kill relationship.

SUMMARY: In past studies of accelerated dosing with azithromycin vs 54A1100 (in the gerbil), Q.D.1, Q.D.2, and Q.D.3 therapies had equivalent activity. (ED50's of 138.1, 138.1, and 162.7 mg/kg/total regimen, respectively. Ref: 43907.233) and it did not matter if dosing began at 24 or 72 hours post-infection. (Ref: 51931.037). This study indicates the importance of dose related (concentration) kill over time. For Q.D.1, activity appeared to be cidal at the 200 mg/kg p.o.level; (i.e. There was < log 2.0000 bacterial load at 48 hours post-infection (24 hours post-dose) and there was no re-growth at 72-96 hours post-infection). For Q.D.2, at 48 hours post-infection (24 hours post-last-dose), cfu counts were reduced relative to infected control [P=0.0250] and eradication was observed in 4/5 test subjects at 72 hours post-infection (24 hours post-last-dose, P=.0000 and at 96 hours post-infection (48 hours-post-last dose), P=0.0105. For Q.D.3, at 48 hours post-infection (24 hours post-last-dose) bacterial reduction relative to infected control was not significant (P=0.8540) and while the bacterial burden was reduced at 72 and 96 hr post-infection eradication was not observed in any test subject; 72 hours post-infection (24 hours post-last-dose), P=0.0007 and at 96 hours post-infection (24 hours post-last-dose), P=0.0902. The Q.D. 2 and Q.D.3 therapies demonstrated antibacterial activity, but not total clearance and with possibly a slight re-growth by 96 hours post-infection (see graph pg. 29). In these two groups (in

animals not cleared or ≥ log 2.0000 cfu/ml bulla wash), concern for development of bacterial resistance arises since the bacterial load that is present is exposed to implicitly sub-therapeutic concentrations of antibiotic for the duration of the therapeutic evaluation (no susceptibilities were determined to address this point). Q.D.1 at the 200 mg/kg p.o. level demonstrated the best activity with total clearance being demonstrated early on in therapy and this observation implies that the likelihood of development of resistance using this accelerated regimen would be diminished relative to the 2-3 day therapy.

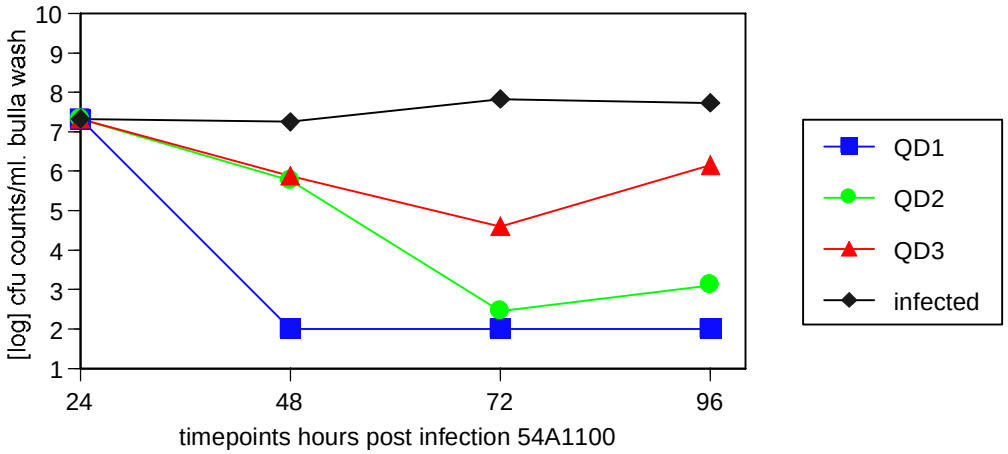
Compound	Dose (mg/kg/day) oral	Sample time [hours] post infection	Recoverable CFU (Bulla) [Log_{10}.Geo.Mean] (SEM)	P-value [2]
CP-062,993	200	24	7.3206 (0.28) [1]	
(17419-064-1F)		48	<2.0000	P=.0000
Q.D. 1 [1 day]		72	<2.0000	P=.0001
Azithromycin		96	<2.0000	P=.0001
CP-062,993	100	24	7.3206 (0.28) [1]	
Q.D.2 [2 days]		48	5.7572 (0.54)	P=.0250
Q.D.2 (continued)		72	<2.4580 (0.46)	P=.0000
		96	<3.1112 (1.11)	P=.0105
CP-062,993	66.6	24	7.3206 (0.28) [1]	
Q.D. 3 [3 days]		48	5.8817 (0.44)	P=.8540
		72	4.5981 (0.14)	P=.0007
		96	6.1520 (0.75)	P=.0902
Infected only	---	24	7.3206 (0.28) [1]	
		48	7.2491 (0.26)	
		72	7.8242 (0.41)	
		96	7.7274 (0.33)	

Bacterial challenge: 1.2×10^{4} /gerbil Ref: 51931.047

1 24 hours post infection baseline count-done before dosing begins (SEM)

2 P-value: infected/dosed compared to infected only of same timepoint

Log (Geo. mean) of cfu counts/ml bulla wash [5 animals /timepoint] at 24, 48, 72, and 96 hours post infection with H. influenzae 54A1100. Therapy at 200 mg/kg p.o. administered over one day, two days, or three days



Summary: cfu counts in bulla wash samples at 48, 72, and 96 hours post infection

Group	Hrs. post infection	Geo. Mean	SD	SEM	P-value [dosed to infected only of same timepoint group]
QD1	48	<2.0000			P=.0000
QD2	48	5.7572	1.2	0.54	P=.0250
QD3	48	5.8817	0.99	0.44	P=.8540
infected	48	7.2491	0.57	0.26	
QD1	72	<2.0000			P=.0001
QD2	72	<2.4580	1.02	0.46	P=.0000
QD3	72	4.5981	0.3	0.14	P=.0007
infected	72	7.8242	0.92	0.41	
QD1	96	<2.0000			P=.0001
QD2	96	<3.1112	2.48	1.11	P=.0105
QD3	96	6.1520	1.67	0.75	P=.0902
infected	96	7.7274	0.73	0.33	
Note: 24 hour cfu counts (before dosing began): 7.3206 [log of cfu-bulla wash/ml]					

2.4. Evaluation of azithromycin given as the same total dose (200 mg/kg) over 1, 2 and 3 days versus a localized infection due to Haemophilus influenzae (non typeable/beta lactamase +) (07-23-01)

TO: Antibacterials Biology DATE: 07-23-01

FROM: D. Girard/ C. Cimochowski

SUBJECT: Accelerated dosing paradigm in the gerbil middle ear infection model. *In vivo* evaluation of azithromycin vs. a localized infection of *Haemophilus influenzae* 54A1218 (non-typeable/ beta lactamase +) in 49.4 gram [F] Mongolian gerbils comparing one equivalent therapy [200 mg/kg/therapy p.o.] (same total therapeutic dose) administered over 1, 2, or 3 days. CFU counts/ml. bulla wash (5 gerbils per timepoint) were done at 24, 48, 72, and 96 hours post-infection for each of the Q.D. 1, Q.D. 2, and Q.D. 3 groups.

DISCUSSION: In this study based on a mg/kg/total regimen, for azithromycin- the same total therapeutic dose (i.e., 200 mg/kg/ therapeutic regimen) was administered Q.D. on day one (i.e., 200 mg/kg on day 1), Q.D. on day one and two (i.e., 100 mg/kg on day 1 and 2) or Q.D. on day one, two, and three (i.e., 66.6 mg/kg on day 1, 2 and 3). A baseline CFU count of bulla wash was done 24 hours post-infection, before dosing began. Thereafter, samples for counts from each group were taken at 48, 72, 96 hours post-infection. By looking at the kinetics of bacterial reduction in the bulla wash, a kinetic kill curve could be constructed which hopefully would visually elucidate the observed *in vivo* therapeutic efficacy by examining the dose/ time-kill relationship.

SUMMARY: In past studies of accelerated dosing with azithromycin vs 54A1218 (in the gerbil), Q.D.1, Q.D.2, and Q.D.3 therapies had equivalent activity. (ED50's of 83.8 (53.6-114.1), 53.4 (21.2-85.5), and 65.2 (51-79.4) mg/kg/total regimen, respectively. [Ref:51931.066] This study indicates the importance of dose related (concentration) kill over time. For Q.D.1, activity appeared to be cidal at the 200 mg/kg p.o.level; (i.e. There was log <2.0602 bacterial load at 48 hours post-infection (24 hours post-dose) and there was no re-growth at 72-96 hours post-infection). For Q.D.2, at 48 hours post-infection (24 hours post-last-dose), cfu counts were reduced relative to infected control [P=0.0338] and eradication was observed in at 72 and 96 hours post-infection. For Q.D.3, at 48 hours post-infection (24 hours post-last-dose) bacterial reduction relative to infected control was not significant (P=0.1889), but at 72 and 96 hr post-infection eradication was observed. Q.D.1, Q.D.2, and Q.D.3 therapies were all effective at eradicating the organism by 72 hours post infection. Q.D.1 at the 200 mg/kg p.o. level demonstrated the best activity with total clearance being demonstrated early on in therapy (48 hours post infection; 24 hours post-last-dose) and this observation implies that the likelihood of development of resistance using this accelerated regimen would be somewhat diminished relative to the 2-3 day therapy. These data are consistent with

the results that were observed vs. strain 54A1100 (non-type B *H. influenzae*) except that we see eradication with all azithromycin regimens by 72 h post-challenge with this strain.

Compound	Dose (mg/kg/day) oral	Sample time [hours] post infection	Recoverable CFU (Bulla) [Log_{10}.Geo.Mean] (SEM)	P-value [2]
CP-062,993	200	24	7.7206 (0.15) [1]	
(17419-064-1F)		48	<2.0000	P=.0000
Q.D. 1 [1 day]		72	<2.0602	P=.0000
Azithromycin		96	<2.0000	P=.0000
CP-062,993	100	24	7.7206 (0.15) [1]	
Q.D.2 [2 days]		48	<3.8587 (1.04)	P=.0338
Q.D.2 (continued)		72	<2.0000	P=.0001
		96	<2.0000	P=.0000
CP-062,993	66.6	24	7.7206 (0.15) [1]	
Q.D. 3 [3 days]		48	<5.2399 (1.21)	P=.1889
		72	<2.0000	P=.0001
Q.D.3 (continued)		96	<2.0000	P=.0000
Infected only	---	24	7.7206 (0.15) [1]	
		48	7.1691 (0.09)	
		72	7.7736 (0.2) [3]	
		96	7.4296 (0.23)	

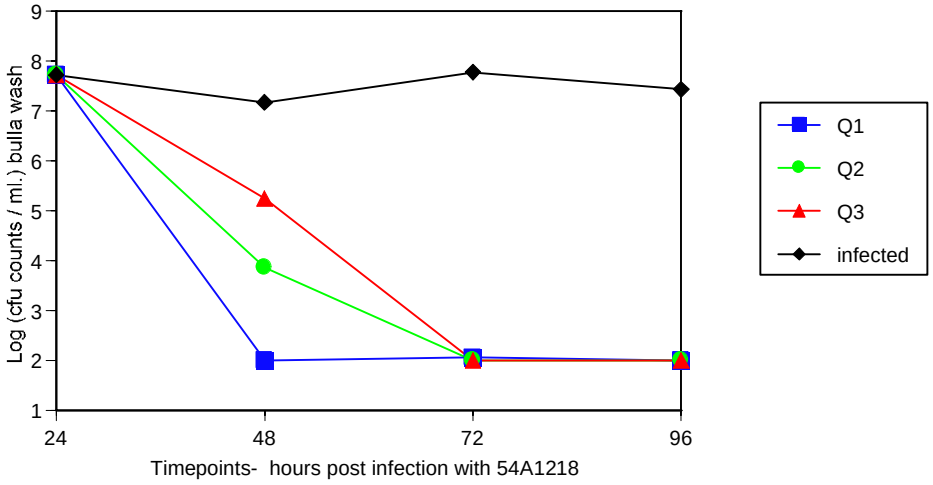
Bacterial challenge: 1.2×10^{6} /gerbil Ref: 51931.073

1 24 hours post infection baseline count-done before dosing begins (SEM)

2 P-value: infected/dosed compared to infected only of same timepoint

3 There were four gerbil samples in this group; all other groups had five samples/group

Log (geometric mean of cfu counts /ml. bulla wash) at 24, 48, 72, and 96 hours post infection with H. influenzae 54A1218. [5 animals/ timepoint] Therapy with 200 mg/kg p.o. of azithromycin administered over one day, two days, or three days



Summary: cfu counts in bulla wash samples at 48, 72, and 96 hours post infection

Group	Hrs. post infection	Geo. Mean	% * clearance of group	SD	SEM	P-value [dosed to infected only of same timepoint group]
QD1	48	<2.0000	100%			P=.0000
QD2	48	<3.8587	40%	2.32	1.04	P=.0338
QD3	48	<5.2399	40%	2.71	1.21	P=.1889
infected	48	7.1691	0%	0.22	0.098	
QD1	72	<2.0602	100%	0.13	0.06	P=.0000
QD2	72	<2.0000	100%			P=.0001
QD3	72	<2.0000	100%			P=.0001
infected	72	7.7736 **	0%	0.39	0.2	
QD1	96	<2.0000	100%			P=.0000
QD2	96	<2.0000	100%			P=.0000
QD3	96	<2.0000	100%			P=.0000
infected	96	7.4296	0%	0.51	0.23	
Note: 24 hour cfu counts (before dosing began): 7.7206 [log of cfu-bulla wash/ml]						

* $<1 \times 10^3$ cfu/ml considered negative; $\geq 1 \times 10^3$ considered positive

** 4 samples in this group; all others had 5 samples

APPENDIX II

STUDY SUMMARIES

PROTOCOL A0661014

A MULTICENTER, RANDOMIZED, DOUBLE-BLIND, DOUBLE-DUMMY COMPARATIVE TRIAL OF AZITHROMYCIN (ZITHROMAX®) VERSUS AMOXICILLIN/CLAVULANATE POTASSIUM (AUGMENTIN®) IN THE TREATMENT OF ACUTE OTITIS MEDIA IN CHILDREN

Study Publication: Abstract, 41st ICAAC, Sept, 2001

Study Dates: 4 January 2000 – 31 March 2000

Phase of Development: Phase 3

Study Objectives: (1) to test the hypothesis that azithromycin 10 mg/kg once daily for 3 days has efficacy equivalent to amoxicillin/clavulanate potassium (AUGMENTIN®) 45 mg/kg daily dose given in divided doses q12h for 10 days for the treatment of acute otitis media in children, and (2) to compare the safety and toleration of the two regimens.

Study Design: Randomized, double-blind, comparative multicenter trial of azithromycin (10 mg/kg daily as a single dose each morning, maximum 500 mg/day) for 3 days, followed by matching placebo for 7 days, versus amoxicillin/clavulanate potassium (45 mg/kg daily dose given in divided doses q12h, maximum 1575 mg/day amoxicillin) for 10 days for the treatment of pediatric subjects with acute otitis media. The study included evaluations at study day 1 (baseline), day 5 (telephone contact), day 10 ±2 (End of Therapy, EOT), and Day 24-28 ±4 (Test of Cure, TOC).

Evaluation Groups: This study enrolled 373 subjects. The number of subjects in each treatment group and their disposition are shown in the following table.

Subject Evaluation Groups		
	Azithromycin (N)	Amox/Clav (N)
Randomized to Treatment	188	185
Received Treatment (at least 1 dose)	188	185
Discontinued study	9	11
Completed study	179	174
Subjects at EOT Visit (Day 10 ±2 days)	185	181
Subjects at TOC Visit (Day 24-28 ± 4 days)	179	177
Efficacy Analysis at EOT		
MITT	188	185
Per Protocol	166	151
Efficacy at TOC Visit		
MITT	188	185
Per Protocol	179	175
Safety Analysis - Adverse Events	188	185

Amox/Clav = Amoxicillin/Clavulanate potassium

EOT = End-of-Therapy; TOC = Test of Cure; MITT = Modified Intent-to-Treat

Diagnoses and Criteria for Inclusion of Subjects: This study included 373 subjects, aged 6 months to 12 years, with signs and symptoms of acute otitis media. Overall, ear pain and/or fullness/bulging of the tympanic membrane were present in 98% of enrolled subjects at baseline.

Demographic Parameter	Azithromycin	Amox/Clav
Mean age (years)	3.5	3.4
≤2 years old (%) > 2 years old (%)	61 (32%) 127 (68%)	53 (29%) 132 (71%)
% Male % Female	51% 49%	49% 51%
% White % Black %Hispanic % Other	83% 6% 8% 3%	80% 5% 9% 5%
Mean duration of diagnosis (days)*	1.2 [range 0-9]	1.2 [range 0-8]

Amox/Clav = Amoxicillin/Clavulanate potassium; * Duration from onset of symptoms to enrollment.

Drug Administration: The following investigational drugs were used in this study:

Azithromycin

Dosing: Total daily dose: 10 mg/kg to be taken as a single dose without regard to meals. (Maximum daily dose: 500 mg)

Duration: Three consecutive days of active therapy plus 7 days of placebo

Amoxicillin/Clavulanate potassium

Dosing: Total daily dose: 45 mg/kg/day (based on amoxicillin) q12h (maximum 1575 mg/day), irrespective of food intake.

Duration: Ten consecutive days

Efficacy and Safety Evaluations: Modified Intent-to-Treat (MITT) and Per Protocol (PP).

Clinical response was assessed as follows, as defined in the protocol. Persistence of middle ear fluid was not to be taken into account when assessing clinical outcome.

Cure	Complete resolution of specific signs and symptoms of acute otitis media. (note: TOC visit has no option for "improvement")
Improvement	Partial resolution of specific signs and symptoms of acute otitis media and no use of any additional antibiotic therapy or hospitalization due to the lack of adequate clinical response. (note: applies to EOT only)
Failure	Lack of resolution of specific signs and symptoms of acute otitis media, or need of additional antibiotic therapy or hospitalization because of an inadequate response to study medication.

The efficacy analyses was carried out based on a modified intent to treat population (MITT) and, separately, based on a PP evaluable population. The MITT population included all subjects who had at least one dose of study medication and a diagnosis of acute otitis media, as indicated by one or more of the following: ear pain or ear ache, ear fullness, discharge from the external auditory canal, decreased hearing, and fever. The PP evaluable population required, in addition, that the subject received adequate study medication (80-120% of protocol specified doses), did not receive excluded concomitant medication, and had study visits within the appropriate time windows (Day 10 ± 2 days for Visit 3 and Day 24-28 ± 4 days for Visit 4). An exception to the above rule was subjects who were not doing well and given other antibiotics for failure were classified as evaluable failures.

All subjects who had received at least one dose of study medication were included in the safety analysis.

Statistical Methods: Statistical analysis of the primary efficacy variable Clinical Outcome was performed at the TOC visit. Determination of equivalence between the two groups was based on the 95% confidence interval of the difference between the cure rates. The two regimens would be considered equivalent if the 95% confidence interval contains zero and lies within certain pre-specified boundaries. Following general FDA Guidance, these pre-specified boundaries were ±10% when the higher of the 2 cure rates is above 90%, ±15% when it is between 80 to 90% and ±20% when it is below 80%. Normal approximation to the binomial distribution was used in computing the 95% confidence intervals.

Analysis of signs and symptoms, otoscopic findings, and acoustic reflectometry consisted of a display of frequencies in the different score categories and the corresponding percentages or means, as appropriate.

Efficacy Results:

Clinical Outcome—MITT Subjects

	Azithromycin N (%)	Amox/Clav N (%)	P-Value	95% CI
No. MITT Subjects	188	185		
Subjects Evaluable at EOT	185 (100%)	181 (100%)		
Success	153 (83%)	159 (88%)	0.186	-12.9%, 2.7%
Cure	100	114		
Improvement	53	45		
Failure	32 (17%)	22 (12%)		
Subjects Evaluable at TOC	182 (100%)	180 (100%)		
Success (Cure)	134 (74%)	124 (69%)	0.353	-5.2%, 14.6%
Failure	48 (26%)	56 (31%)		

Based on MITT subjects with observed values or classified as Failures due to additional antibiotics

Statistical analysis at EOT based on dichotomized categories Success and Failure

Amox/Clav = Amoxicillin/Clavulanate potassium; P-Value based on Fisher's exact test; 95% CI = 95% confidence interval based on normal approximation to the binomial; EOT = End-of-Therapy; TOC = Test of Cure; MITT = Modified Intent-to-Treat.

The primary analyses demonstrated that the number of subjects with a response of Cure was similar between the two treatment groups at the Test of Cure visit (95% Confidence Interval = -5.2%, 14.6%). The results of the clinical response outcome analyses at EOT support the findings of the primary MITT analyses.

As shown in the following table, cure rates at TOC were higher in the older subset of MITT subjects (>2 years old) than the younger subset of subjects (≤2 years old) in both treatment groups. However, the number of younger subjects ≤2 years old was small.

Clinical Cure Rates by Age at TOC – MITT Subjects

	Azithromycin		Amox/Clav		
Age Group	n/N	%	n/N	%	95% CI*
≤2 years old	35/58	60%	30/52	58%	-17.7%, 23.1%
>2 years old	99/124	80%	94/128	73%	-4.8%, 17.6%

Amox/Clav = Amoxicillin/Clavulanate potassium; CI = Confidence Interval on the difference between treatment groups (azithromycin-comparator)

The following three tables summarize the signs/symptoms and acoustic reflectometry data at baseline, EOT, and TOC.

Clinical Symptoms of Otitis Media – Clinical MITT Subjects

	Azithromycin					Amox/Clav				
		Symptoms Score*					Symptoms Score*			
Symptom	n	0	1	2	3	n	0	1	2	3
Baseline										
Ear Pain	187	16	47	79	45	185	13	49	69	54
Ear Fullness	174	59	29	62	24	168	59	28	55	26
EOT										
Ear Pain	183	159	17	6	1	178	164	10	4	0
Ear Fullness	170	153	12	5	0	166	156	7	3	0
TOC										
Ear Pain	177	169	8	0	0	175	160	11	3	1
Ear Fullness	165	160	4	0	1	161	150	5	3	3

Scores reflect the more severe symptom of either ear. Scores are: 0 = Absent, 1 = Mild, 2 = Moderate, 3 =Severe

Percent of Subjects with Signs of Otitis Media Based on Pneumatic Otoscopy – Clinical MITT Subjects

	Azithromycin			Amox/Clav		
Sign	Baseline	EOT	TOC	Baseline	EOT	TOC
Fullness/Bulging of TM	93	22	10	90	13	16
Redness of TM	97	32	12	96	25	18
Loss of normal light reflex	94	31	10	92	25	22
Loss of normal TM landmarks	95	31	11	95	20	22
Impaired TM mobility	95	39	18	95	28	26
TM perforation*	2	2	0	4	1	0

TM = Tympanic Membrane; * Prior to baseline tympanocentesis

Results of Tympanic Membrane Acoustic Reflectometry – Clinical MITT Subjects

	Azithromycin		Amox/Clav	
	Subjects Assessed	Mean Classification Level*	Subjects Assessed	Mean Classification Level*
Baseline				
Left ear	170	3.11	164	3.20
Right ear	169	3.16	174	3.42
More affected ear	183	3.92	184	4.05
EOT				
Left ear	166	1.99	157	1.88
Right ear	168	1.98	167	1.98
More affected ear	176	2.22	174	2.16
TOC				
Left ear	163	1.64	158	1.78
Right ear	163	1.63	165	2.09
More affected ear	170	1.75	170	2.19

* Study entry required classification level of ≥3 in spectral gradient angle. Results were classified as level 5 = angle <49, level 4 = angle 49-59, level 3 = angle 60-69, level 2 = angle 70-95, level 1 = angle >95.

Safety Results: Safety results (treatment-related) are summarized in the following table:

Summary of Adverse Events (Treatment-Related)

	Azithromycin	Amox/Clav
	N (%)	N (%)
Subjects		
Total number treated	188 (100.0%)	185 (100.0%)
Number of adverse events	22	45
Number with adverse events	20 (10.6%)	37 (20.0%)
Number with serious adverse events	0 (0%)	0 (0%)
Number with severe adverse events	0 (0%)	1 (0.5%)
Number discontinued due to adverse events	1 (0.5%)	2 (1.1%)
Number temporarily discontinued due to adverse events	1 (0.5%)	2 (1.1%)

Subjects may have had more than one adverse event.
Amox/Clav = Amoxicillin/clavulanate potassium

The incidence of treatment-related adverse events in the azithromycin group was about half that of the amoxicillin/clavulanate potassium group. The most commonly occurring treatment-related adverse events were gastrointestinal (diarrhea) and rash. Fewer azithromycin subjects than amoxicillin/clavulanate potassium subjects experienced treatment-related adverse events from these categories (diarrhea: azithromycin, n=11 [5.9%], versus amoxicillin/clavulanate potassium, n=27 [14.6%]; rash: azithromycin, n=0 [0.0%] versus amoxicillin/clavulanate potassium, n=8 [4.3%]).

In addition to a lower incidence of treatment-related adverse events, subjects' adverse events in the azithromycin group tended to be milder in severity. In the adverse events reported in the azithromycin group, 77% were of mild severity, versus 56% in the amoxicillin/clavulanate potassium group.

Two serious adverse events, one in each treatment group, were reported in this study. Both events were gastrointestinal and occurred several days after active study drug treatment was completed. Neither was considered to be treatment-related.
There were no deaths reported in this study.

Overall, 99% azithromycin versus 89% of amoxicillin/clavulanate subjects were complaint with their study regimen ($p<0.001$). This was based on subjects taking active drug in their study regimen.

Conclusions:

The results of this study demonstrate that, for the treatment of acute otitis media, the once-daily administration of 10 mg/kg azithromycin for three days is as safe and effective as the administration of 45 mg/kg amoxicillin/clavulanate potassium in two divided doses q12h for 10 days.

The subjects in the azithromycin group were less likely to experience treatment-related adverse events, and had milder adverse events than the comparator group.

A short course of azithromycin therapy once daily for three days was as safe and effective as a ten-day twice daily course of amoxicillin/clavulanate potassium.

PROTOCOL A0661015

A MULTICENTER, OPEN LABEL, NONCOMPARATIVE TRIAL OF A SINGLE DOSE OF 30MG/KG AZITHROMYCIN IN THE TREATMENT OF ACUTE OTITIS MEDIA IN PEDIATRIC SUBJECTS UNDERGOING DIAGNOSTIC TYMPANOCENTESIS

Study Publication: Ehrlich GD, Johnson S, Hayes J, Chow JH, Duncanson FP, Dunne MW. Polymerase chain reaction (PCR) testing of middle ear effusions in pediatric patients with acute otitis media (AOM) treated with single dose azithromycin. Abstract #108, p. 492;. 40th ICACC Abstracts, 40th Interscience Conference on Antimicrobial Agents and Chemotherapy; Toronto, Ontario, Canada; September 17-20, 2000.

Study Dates: 6 December 1999 – 9 May 2000

Phase of Development: Phase 3

Study Objectives: The primary objective of this study was to assess the clinical and bacteriologic efficacy of azithromycin suspension given as a 30 mg/kg single dose to children for treatment of acute otitis media. A secondary objective was to assess safety and toleration.

Study Design: This was an open-label, multicenter, noncomparative 28-day study in pediatric subjects. Follow-up included a phone contact on study day 5 and site visits on day 10 (End of Therapy, EOT) and on day 24-28 (Test of Cure, TOC). Pathogens of primary interest were *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae*. Samples of middle ear fluid were obtained by tympanocentesis or swab (in cases of a perforated tympanic membrane), and pathogens were isolated by culture and/or PCR testing when possible.

Evaluation Groups:

Randomized	248
Received Treatment	247*
Completed Study	237
Clinical Efficacy	
Clinical MITT	247
Bacteriologic MITT	127
Clinical Per-Protocol at TOC	229
Bacteriologic Per-Protocol at TOC	111
Assessed for Safety (Adverse Events)	248

* One subject vomited 15 minutes after the first dose and within 30 minutes after re-dose (i.e., before drug absorption), and is included in safety but excluded from efficacy assessments.

Diagnoses and Criteria for Inclusion of Subjects: This study included 248 male or female (pre-menarchal) outpatients aged 6 months to 12 years inclusive and weighing ≥4.5 kg (10 lb.) with clinical evidence of acute otitis media. Subjects with a perforated tympanic membrane were enrolled only if it occurred within <48 hours prior to entry. The actual age range of study subjects was 6 months – 12 years. Overall, ear pain and/or fullness/bulging of the tympanic membrane were present in 98% of enrolled subjects at baseline.

Demographic Parameter	Azithromycin
Mean age (years)	3.4
≤2 years old (%) > 2 years old (%)	86 (35%) 162 (65%)
% Male % Female	52% 48%
% White % Black % Hispanic % Other	46% 5% 44% 4%
Mean duration of diagnosis (days)*	2.5 [range 1-8]

*Duration from onset of symptoms to enrollment.

Drug Administration:

Dosage Form	Azithromycin powder for oral suspension (40 mg/ml) (FID #64041; Lot #N9009-G3) Dispenser (FID #BAXA; Lot #L04723-G1)
Dosing	30 mg/kg single dose (maximum 1500 mg)
Duration	1 day dosing; 28 days follow-up

Efficacy and Safety Evaluations: Analyses were a modified intent-to-treat (MITT) (had acute otitis media and received at least one dose of study drug) and a per-protocol (PP) evaluable subgroup analysis (same as MITT plus did not receive excluded concomitant medication and had study visits within appropriate time windows).

Clinical response was assessed as follows, as defined in the protocol. Persistence of middle ear fluid was not to be taken into account when assessing clinical outcome.

Cure	Complete resolution of specific signs and symptoms of acute otitis media.* (note: TOC visit has no option for "improvement")
Improvement	Partial resolution of specific signs and symptoms of acute otitis media and no use of additional antibiotic therapy or hospitalization due to the lack of adequate clinical response. (note: applies to EOT only)
Failure	Lack of resolution of specific signs and symptoms of acute otitis media, or need of additional antibiotic therapy or hospitalization for an inadequate response to study medication.

The primary endpoint was the investigator's assessment of clinical outcome (cured, failed) at the TOC visit. Clinical outcome (cured, improved, failed) at EOT was also analyzed. Clinical outcomes were also assessed in subjects by baseline pathogen, MIC, and culture versus PCR identification. Also analyzed were individual clinical signs/symptoms, and results of pneumatic otoscopy and acoustic reflectometry. Safety was assessed by the incidence of adverse events, serious adverse events and study discontinuations in subjects who received any study drug.

Statistical Methods: The percentage of subjects cured versus failed at TOC and a clinical success (cured plus improved) versus failed at EOT were determined. A 95% confidence interval was computed using the normal approximation to the binomial distribution for clinical success rates at EOT and cure rates at TOC. The percentage of subjects with individual signs/symptoms and pneumatic otoscopic findings were calculated, as were mean spectral gradient angles based on acoustic reflectometry. Culture and PCR results were cross-tabulated.

Efficacy Results:

Clinical Outcomes in Azithromycin Subjects – Observed Cases Analysis

	MITT Analysis*		
	N	(%)	95% CI***
Subjects Evaluable at EOT	240	(100%)	--
Success	213	(89%)	84.5, 93.0
Cure	139	(58%)	--
Improvement	74	(31%)	--
Failure	27	(11%)	--
Subjects Evaluable at TOC**	242	(100%)	--
Cure	206	(85%)	80.4, 89.8
Failure	36	(15%)	--

EOT=End of Therapy, TOC=Test of Cure, CI=Confidence Interval; * Total number of Clinical MITT subjects was 247; ** TOC visit had no option for "improvement";
*** Based on normal approximation to the binomial distribution

MITT Clinical Outcome by Baseline Pathogen in Subjects* Included in the Bacteriologic Analysis – Observed Cases – Based on Culture Data

EOT Assessment:						
	H. influenzae (N=42)		*M. catarrhalis* (N=10)		*S. pneumoniae* (N=76)	
Outcome	n (%)	95% CI***	n (%)	95% CI***	n (%)	95% CI***
Success	30 (71%)	56.6, 86.3	10 (100%)	95.0, 105	70 (92%)	85.4, 98.8
Cure	18 (43%)	--	5 (50%)	--	49 (64%)	--
Improvement	12 (29%)	--	5 (50%)	--	21 (28%)	--
Failure	12 (29%)	--	0 (0%)	--	6 (8%)	--
TOC Assessment:**						
	H. influenzae (N=44)		*M. catarrhalis* (N=10)		*S. pneumoniae* (N=76)	
Outcome	n (%)	95% CI***	n (%)	95% CI***	n (%)	95% CI***
Cure	28 (64%)	48.3, 79.0	10 (100%)	95.0, 105	67 (88%)	80.2, 96.1
Failure	16 (36%)	--	0 (0%)	--	9 (12%)	--

EOT=End of Therapy, TOC=Test of Cure, CI=Confidence Interval; * Some subjects are counted under more than one pathogen; ** TOC visit had no option for "improvement"; *** Based on normal approximation to the binomial distribution

In the MITT analysis, cure rates increased from just under 60% at EOT to over 80% of subjects at the primary efficacy outcome timepoint of TOC. Ear pain and fullness were the most common signs/symptoms at baseline. Clinical efficacy was seen in subjects infected with *H. influenzae, M. catarrhalis,* or *S. pneumoniae* at baseline. Of note, 86% (6 of 7) of subjects infected with *S. pneumoniae* who had had an MIC to azithromycin of 8 µg/ml, presently considered resistant pathogens, were clinical cures. All of these organisms were documented to have had an efflux-pump based mechanism of resistance.

Clinical Cure Rates by Age at TOC – MITT Subjects

Age Group	n/N	%	95% CI
≤2 years old	64/83	77%	67%, 87%
>2 years old	142/159	89%	84%, 94%

CI = Confidence Interval

Older subjects (>2 years of age) responded more favorably than younger subjects.

The following three tables summarize the signs/symptoms and acoustic reflectometry data at baseline, EOT, and TOC.

Clinical Symptoms of Otitis Media – Clinical MITT Subjects

		Symptoms Score*			
Symptom	Subjects Assessed	0 (Absent)	1 (Mild)	2 (Moderate)	3 (Severe)
Baseline					
Ear Pain	246	17	31	99	99
Ear Fullness	234	38	25	101	70
EOT					
Ear Pain	237	222	9	4	2
Ear Fullness	229	175	43	8	3
TOC					
Ear Pain	235	226	5	3	1
Fullness	232	225	1	3	3

* Scores reflect the more severe symptom of either ear.

Percent of Subjects with Signs of Otitis Media Based on Pneumatic Otoscopy – Clinical MITT Subjects

Sign	Baseline	EOT	TOC
Fullness/Bulging of TM	86%	22%	6%
Redness of TM	91%	18%	5%
Loss of normal light reflex	91%	35%	8%
Loss of normal TM landmarks	94%	18%	7%
Impaired TM mobility	93%	28%	12%
TM perforation*	20%	4%	2%

TM = Tympanic Membrane; * Prior to baseline tympanocentesis

Results of Tympanic Membrane Acoustic Reflectometry – Clinical MITT Subjects

	Subjects Assessed	Mean Classification Level*
Baseline		
Left ear	222	3.09
Right ear	215	3.13
More affected ear	233	3.89
EOT		
Left ear	227	1.85
Right ear	231	1.85
More affected ear	234	2.24
TOC		
Left ear	222	1.43
Right ear	232	1.59
More affected ear	235	1.77

* Study entry required classification level of ≥3 in spectral gradient angle. Results were classified as level 5 = angle <49, level 4 = angle 49-59, level 3 = angle 60-69, level 2 = angle 70-95, level 1 = angle >95.

Safety Results:

Number of subjects with:	Azithromycin
Treatment emergent adverse events (all causality)	90/248 (36%) [1]
Treatment emergent adverse events (treatment related)	30/248 (12%) [1]
Clinically significant laboratory test abnormalities	NA

[ ] = Resulting in discontinuation of study; NA = Not Assessed

Overall, 12.1% subjects had treatment related adverse events, with the most common adverse events involving the gastrointestinal tract (vomiting 5.6%, diarrhea 3.2%, and abdominal pain 1.6%). All treatment related adverse events were mild to moderate in severity and the majority resolved within a few days. One subject discontinued from the study due to a treatment-related adverse event (vomiting). Two subjects had serious adverse events (one had streptococcal pneumonia with subsequent development of secondary pulmonary abscess and right pleural effusion, one had an atonic seizure due to neurologic sequelae of previous encephalitis) considered unrelated to treatment, and no subjects died.

Summary and Conclusions:

In conclusion, a single 30 mg/kg dose of azithromycin as oral suspension was effective in promoting resolution of the signs and symptoms of clinically diagnosed acute otitis media. Clinical efficacy is associated with each of the three pathogens relevant to acute otitis media infection, including *S. pneumoniae*, *M. catarrhalis* and *H. influenzae*. Azithromycin was safe and generally well-tolerated.

PROTOCOL R-0581

A DOUBLE-BLIND, DOUBLE-DUMMY, MULTICENTER, RANDOMIZED TRIAL OF SINGLE-DOSE AZITHROMYCIN VERSUS AMOXICILLIN/CLAVULANATE IN THE TREATMENT OF ACUTE OTITIS MEDIA IN CHILDREN AGES 6 MONTHS TO 12 YEARS

Study Publication: Clinical Infectious Diseases, Vol.31, #1, July 2000, Abstract #174 page 243. IDSA 2000, 38th Annual Meeting, September 2000, Abstract #174, page 71.

Study Dates: 18 November 1998 – 2 December 1999

Phase of Development: 3

Study Objectives: The objective of this study was to compare the safety and efficacy of a single dose of azithromycin (30 mg/kg) to standard oral therapy with amoxicillin/clavulanate (Augmentin®) (45 mg/kg/day divided in 2 equal doses for 10 days) in the treatment of acute otitis media (AOM) in children 6 months to 12 years of age.

Study Design: This study was a double-blind, double-dummy, randomized, multicenter study comparing a single dose of azithromycin oral suspension to amoxicillin/clavulanate oral suspension administered twice a day for 10 days for the treatment of acute otitis media in children 6 months to 12 years of age. Each subject was also administered one of two placebo suspensions. Eligible subjects were randomly assigned to treatment with azithromycin or amoxicillin/clavulanate in a 1:1 ratio.

Subjects were evaluated at four visits; Baseline Day 1 (Visit 1), Day 3-5 telephone follow-up (Visit 2), Day 12-16 (End of Therapy) and Day 28-32 (Test of Cure). Additional visits could be scheduled as needed.

Evaluation Groups:

Subject Evaluation Groups	Azithromycin (N)	Amox/Clav (N)
Randomized to treatment	175	175
Treated	173	173
Completed study	137	144
Discontinued study	36	29
Efficacy MITT evaluable subjects		
EOT and TOC Visits	173	173
Efficacy Per-Protocol evaluable subjects		
EOT Visit	147	148
TOC Visit	144	141
Safety evaluable subjects	173	173

EOT = End-of-Therapy; MITT = Modified Intent-to-treat; TOC = Test of Cure

Diagnoses and Criteria for Inclusion of Subjects: This study included 346 subjects who were outpatients between 6 months to 12 years of age with acute otitis media diagnosed based on clinical signs and symptoms of middle ear effusion and acute inflammation. Overall, ear pain and/or fullness/bulging of the tympanic membrane were present in 99% of enrolled subjects at baseline. The subject's parent or guardian had given written informed consent.

Demographic Parameter	Azithromycin	Amox/Clav
Mean age	2.7	3.4
≤2 years old (%) > 2 years old (%)	75 (43%) 98 (57%)	63 (36%) 110 (64%)
% Male % Female	53% 47%	53% 47%
% White % Black %Hispanic % Other	50% 31% 16% 2%	52% 35% 13% 0%
Mean duration of symptoms (days)*	3.4 [1-18]	3.9 [1-17]

Amox/Clav = Amoxicillin/Clavulanate; * Duration from onset of symptoms to enrollment.

Drug Administration: Subjects randomized to treatment with azithromycin received the following products during this study:

Azithromycin

Dosing	30mg/kg once
Duration	1 day

Amoxicillin/clavulanate Placebo

Dosing	45 mg/kg/day divided in 2 equal doses (every 12 hours)
Duration	10 days

Subjects randomized to treatment with amoxicillin/clavulanate received the following products during this study:

Amoxicillin/clavulanate

Dosing	45 mg/kg/day in divided doses every 12 hours
Duration	10 days

Azithromycin Placebo

Dosing	30 mg/kg once
Duration	1 day

Efficacy and Safety Evaluations: Two subject populations were defined for the conduct of the efficacy analyses. The first was a modified intent-to-treat (MITT) population, and the second was the population specified by the protocol (per-protocol, PP).

Clinical response was assessed as follows, as defined in the protocol.

Cure	Complete resolution of specific signs and symptoms of acute otitis media. Middle ear effusion may or may not be present.
Improvement	Partial resolution of specific signs and symptoms, with or without persistence of middle ear effusion, without need for additional antibiotics for acute otitis media.
Failure	Worsening of signs and/or symptoms of infection or no response to therapy or requirement for additional antimicrobial therapy for acute otitis media.
Recurrence	Subject previously considered cured or improved who currently satisfy criteria for failure. (note: assessed only at TOC)

The MITT population included all subjects who had at least one dose of study medication and a diagnosis of acute otitis media. The PP evaluable population required, in addition, that the subject met all inclusion/exclusion criteria, received adequate study

medication (at least 80% of the protocol specified duration of therapy), did not receive concurrent antibiotics, and had study visits within the appropriate time windows (Day 12-16 ± 2 days for Visit 3 and Day 28-32 ± 4 days for Visit 4). Subjects who were given other antibiotics for failure were classified as evaluable failures.

The primary measure of efficacy according to the protocol was the investigator assessment of the clinical outcome at the Day 12-16 visit. However, in order to conform to recent FDA Guidance and to be consistent with other otitis media studies in this program, the term End of Therapy (EOT) was used to denote the Day 12-16 visit and the term Test of Cure (TOC) was used to denote the Day 28-32 visit. The possible clinical outcomes at EOT were Cure, Improvement, and Failure. The possible outcomes at TOC were Cure, Improvement, Failure, and Recurrence.

Secondary efficacy parameters included a clinical evaluation of signs and symptoms, acoustic reflectometry results and a parental questionnaire.

To be evaluable for safety, subjects had to have received at least one dose of the study medication. Safety data were collected from Day 1 up to 35 days following the last dose.

Statistical Methods: Statistical analysis of the primary efficacy variable Clinical Outcome was performed at the EOT and TOC visits. The number and percentage of subjects classified as Success (Cure+Improved) or Failure (Failure+Recurrence) for each treatment was calculated and displayed. Determination of equivalence between the two treatment regimens was based on the difference between the percentages of Successes and the 95% confidence interval of this difference. The two regimens were considered equivalent if the 95% confidence interval contained zero and lay within certain pre-specified boundaries. Following general FDA Guidance, these pre-specified boundaries are ± 10% when the higher of the 2 cure rates is above 90%, ± 15% when it is between 80 to 90% and ± 20% when it is below 80%. Normal approximation to the binomial distribution was used in computing the 95% confidence intervals.

In the primary MITT analysis of clinical outcome, subjects with missing observations were excluded except for evaluable failures due to use of concomitant antibiotics. Analysis of signs, symptoms, acoustic reflectometry, and parental questionnaire consisted of a display of frequencies of the different score categories and the corresponding percentages or means, as appropriate.

Efficacy Results:

Clinical Outcome at EOT and TOC—MITT Analysis			
	Azithromycin	Amox/Clav	
	N (%)	N (%)	95% CI
Total No. subjects treated	173	173	
Primary MITT analysis			
Subjects evaluable at EOT*	160	161	
Cure + Improvement	139 (87)	142 (88)	-9.2, 6.5
Cure	105 (66)	121 (75)	-20.1, 1.1
Improvement	34 (21)	21 (13)	
Failure	21 (13)	19 (12)	
Primary MITT analysis			
Subjects Evaluable at TOC*	151	154	
Cure + Improvement	114 (75)	116 (75)	-10.2, 10.5
Cure	112 (74)	108 (70)	-6.7, 14.8
Improvement	2 (1)	8 (5)	
Failure	37 (25)	38 (25)	
Failure	31 (21)	32 (21)	
Recurrence	6 (4)	6 (4)	

*Includes MITT subjects with observed values or classified as failures due to additional antibiotics
EOT = End-of-Therapy; TOC = Test of Cure; CI = Confidence interval

The number of subjects who had a satisfactory clinical response (Cure + Improvement) was similar between the two treatment groups at EOT and TOC. The number of subjects cured was similar between the azithromycin and amoxicillin/clavulanic acid groups at TOC (74% and 70%, respectively). The results of the MITT sensitivity analysis (missing values set to failure) support the findings of the primary, MITT observed case analysis at both EOT and TOC.

Clinical Cure Rates by Age at TOC – MITT Subjects					
	Azithromycin		Amox/Clav		
Age Group	n/N	%	n/N	%	95% CI*
≤2 years old	40/64	63%	27/53	51%	-8.3%, 31.4%
>2 years old	72/87	83%	81/101	80%	-9.7%, 14.8%
Amox/Clav = Amoxicillin/Clavulanate potassium; CI = Confidence Interval on the difference between treatment groups (azithromycin-comparator).					

Clinical response was also examined by age ≤ 2 years or >2 years at EOT and TOC. The proportion of subjects who had a successful clinical response (Cure + Improvement) was similar between the two treatment groups for age ≤ 2 years and >2 years. However, a larger percentage of patients in both the azithromycin and amoxicillin/clavulanic acid treatment groups had a successful clinical response or cure in the >2 year group than the ≤ 2 year group at both EOT and TOC.

The following three tables summarize the signs/symptoms and acoustic reflectometry data at baseline, EOT, and TOC.

Clinical Symptoms of Otitis Media – MITT Subjects

	Azithromycin					Amox/Clav				
		Symptoms Score*					Symptoms Score*			
	n	0	1	2	3	n	0	1	2	3
Baseline										
Ear Pain	173	43	33	67	30	173	38	36	65	34
EOT										
Ear Pain	159	137	16	6	0	157	146	9	2	0
TOC										
Ear Pain	137	130	6	1	0	145	0	12	3	1

* Pain scores reflect the more severe symptom of either ear. Scores are: 0 = Absent, 1 = Mild, 2 = Moderate, 3 =Severe

Percent of Subjects with Signs of Otitis Media – MITT Subjects

	Azithromycin			Amox/Clav		
Sign	Baseline	EOT	TOC	Baseline	EOT	TOC
Fullness/Bulging of TM	90%	16%	10%	90%	15%	13%
Redness of TM	86%	12%	5%	79%	10%	8%
Opacification of TM	84%	30%	14%	84%	18%	12%
Visible air fluid level	51%	18%	12%	49%	16%	12%
Impaired TM mobility	99%	38%	26%	95%	38%	30%
TM discoloration*	69%	19%	11%	67%	17%	18%

TM = Tympanic Membrane; * Yellow/white discoloration of the TM

Results of Tympanic Membrane Acoustic Reflectometry - MITT Subjects

	Azithromycin		Amox/Clav	
	Subjects Assessed	Mean Classification Level*	Subjects Assessed	Mean Classification Level*
Baseline				
Left ear	169	3.15	170	3.15
Right ear	167	3.32	168	3.26
More affected ear	172	3.99	173	4.06
EOT				
Left ear	153	2.02	153	1.82
Right ear	154	2.06	149	1.91
More affected ear	157	2.46	154	2.26
TOC				
Left ear	135	1.87	134	2.01
Right ear	135	1.85	134	1.92
More affected ear	136	2.22	140	2.30

* Study entry required classification level of ≥3 in spectral gradient angle. Results were classified as level 5 = angle <49, level 4 = angle 49-59, level 3 = angle 60-69, level 2 = angle 70-95, level 1 = angle >95.

Clinical Evaluations:

Signs, symptoms and spectral gradient angle levels decreased from baseline in both treatment groups. The incidence of symptoms and positive otoscopic findings were slightly less in the amoxicillin/clavulanic acid group than the azithromycin group at EOT, whereas at TOC, the trend was reversed, with lower incidence of positive otoscopic findings in the azithromycin group compared to the amoxicillin/clavulanic acid group. Mean scores for all of the questions on the parental questionnaire showed improvement over time and were similar between the treatment groups at baseline and at each of the study visits (see Appendix III to the Briefing Document).

Safety Results: Safety results are summarized in the following table.

Summary of Safety Results

	Azithromycin	Amox/Clav
	N (%)	N (%)
Total Treated	173 (100.0)	173 (100.0)
Number with Adverse Events (All Causality)	94 (54.3)	110 (63.6)
Number with Treatment-Related Adverse Events	29 (16.8)	39 (22.5)
Number with Serious Adverse Events	1 (0.6)	0 (0)
Number of Discontinuations Due to Adverse Events	5 (2.9)	11 (6.4)
Number of Temporary Discontinuations Due to Adverse Events	4 (2.3)	6 (3.5)

Slightly more amoxicillin/clavulanic acid subjects experienced one or more treatment-emergent adverse events (all causalities) than azithromycin subjects. The majority of adverse events in both the azithromycin and amoxicillin/clavulanic acid groups were mild (67% and 55%, respectively) in severity, with fewer events moderate (27% and 40%, respectively) and severe (6% and 6%, respectively). The incidence of diarrhea in the amoxicillin/clavulanic acid group (18.5%) was twice that of the azithromycin group (9.2%). Of the adverse events occurring in at least 5% of subjects in either treatment group, there was a greater incidence of vomiting, increased cough, rash and ear pain in the amoxicillin/clavulanic acid group compared to the azithromycin group. The incidence of fever, respiratory tract infection and rhinitis were similar between the treatment groups. Slightly more amoxicillin/clavulanic acid-treated subjects (N=39; 22.5%) experienced one or more treatment-related adverse events than azithromycin-treated subjects (N=29; 16.8%). Diarrhea (12.7% vs. 6.4%) and rash (5.2% vs. 1.7%) were more common in the comparator than the azithromycin group, respectively.

There was one serious adverse event reported in this study. Subject no. 0009-0422, a 28-month-old-male subject randomized to azithromycin, was hospitalized for diarrhea and vomiting on Day 4 of the study. The cause of the diarrhea and vomiting was viral gastroenteritis caused by rotavirus (not treatment related).

Five azithromycin and 11 amoxicillin/clavulanic acid-treated subjects discontinued treatment due to adverse events. Of these 16 subjects, one azithromycin-treated subject and five amoxicillin/clavulanic acid-treated subjects also discontinued the study. Treatment discontinuations were due to rash (two subjects), diarrhea, stomach pain and streptococcal pharyngitis in the azithromycin-treated subjects. Treatment discontinuations were due to rash (three subjects), diarrhea (two subjects), severe gastroenteritis (two subjects), diarrhea and vomiting, vomiting, refractory otitis media, and constipation (one subject each) in the amoxicillin/clavulanic acid-treated subjects.

Overall, 100% of azithromycin single dose and 84% of amoxicillin/clavulanate subjects were compliant with their treatment regimens ($p<0.001$). This was based on subjects taking active drug in their study regimen.

Summary and Conclusions: The results of this study in 346 pediatric subjects showed that, for the treatment of otitis media, the administration of a single dose of azithromycin oral suspension (30 mg/kg) was as safe and effective as standard oral therapy with amoxicillin/clavulanic acid (45 mg/kg divided in 2 equal doses [one dose every 12 hours]) over a 10-day period.

PROTOCOL AZM-NY-95-001

A SINGLE BLIND COMPARATIVE STUDY OF THE EFFICACY, SAFETY AND TOLERATION OF ORAL SINGLE DOSE AZITHROMYCIN SUSPENSION 30 MG/KG, ORAL AZITHROMYCIN SUSPENSION 10 MG/KG/DAY FOR 3 DAYS AND INTRAMUSCULAR SINGLE-DOSE CEFTRIAXONE (50 MG/KG) IN THE TREATMENT OF PEDIATRIC PATIENTS WITH DOCUMENTED ACUTE OTITIS MEDIA

Study Publication:
Arguedas, A; Loaiza, C; Perez, A; Vargas, F; Herrera, M; Rodriguez, et al. Microbiology of acute otitis media in Costa Rican children. *Pediatr Infec Dis J*, 1998; 17:680-9.
Arguedas, A; Loaiza, C; Perez, A, Guttierrez, A. Single dose azithromycin versus Single dose intramuscular (IM) certriaxone (CTX) versus 3-day oral AZM in the treatment of acute otitis media (AOM). Fourth International Conference on the Macrolides, Azalides, Streptogramins & Ketolides, Barcelona Spain, January 21-23, 1998. Poster Abstract # 6.10, p. 59.

Study Dates: 11 September 95 - 6 May 97

Phase of Development: 4

Study Objectives: To compare the efficacy, safety, and toleration of a single oral dose of azithromycin suspension (30 mg/kg), conventional dosing of oral azithromycin suspension (10 mg/kg/day for 3 days), and a single dose of intramuscular ceftriaxone (50 mg/kg) in the treatment of pediatric patients with documented acute otitis media.

Study Design: This was a randomized, single-blind, parallel group study. Eligible subjects were randomly assigned in a 1:1:1 ratio, to one of three treatment groups: 1) azithromycin single oral dose (30 mg/kg), 2) azithromycin 3-day oral dosing (10 mg/kg/day), 3) ceftriaxone single dose IM (50 mg/kg). Day 1 was the first day of treatment. Subjects were evaluated on Days 7 to 8, 14 to 15 (End of Therapy), 28 to 30 (Follow-up), or at the time of early withdrawal. In subjects with evidence of middle ear effusion at the Days 28 to 30 visit, a further visit at Days 55 to 60 (Late Follow-up) was required. Clinical efficacy was assessed at visit Days 14 to 15, 28 to 30, and 55 to 60. At each visit, graded clinical, microbiological, and safety assessments were performed.

Evaluation Groups: One hundred ninety-eight subjects (66 azithromycin single dose; 66 azithromycin 3-day; 66 ceftriaxone) were randomized to treatment and received study drug. The following table shows the number of subjects evaluable for efficacy and safety analysis by treatment group.

Subject Evaluation Groups			
	AZM Single	AZM 3-Day	Ceftriaxone
Randomized	66	66	66
Treated	66	66	66
Completed Study	61	61	63
Discontinued Treatment	0	0	0
Discontinued Study	5	5	3
Efficacy MITT Evaluable Subjects			
EOT and Follow-up (LOCF)			
Clinical	66	66	66
Bacteriological	30	35	33
Late Follow-up (Days 55-60)			
Clinical	7	9	13
Bacteriological	3	4	5
Efficacy Per-protocol Evaluable Subjects			
EOT			
Clinical	62	61	57
Bacteriological	38	34	34
Follow-up			
Clinical	59	58	55
Bacteriological	35	32	32
Evaluated for Safety			
Adverse Events	66	66	66
Laboratory Tests	66	66	66

MITT = modified intent-to-treat; EOT = end of therapy; LOCF = last observation carried forward.

Diagnoses and Criteria for Inclusion of Subjects: The study was to include pediatric outpatients between the ages of 6 months and 5 years (actual upper limit was up to 6.5 years), weighing between 6 and 20 kg, with middle ear effusion and intact eardrum, and with an investigator's diagnosis of acute otitis media based on graded clinical signs and symptoms. Overall, ear pain and/or fullness/bulging of the tympanic membrane were present in 100% of enrolled subjects at baseline.

Demographic Parameter	AZM Single	AZM 3-Day	Ceftriaxone
Mean age (years)	2.5	2.7	2.3
≤2 years old (%)	26 (39%)	27 (41%)	35 (53%)
> 2 years old (%)	40 (61%)	39 (59%)	31 (47%)
% Male	56%	55%	48%
% Female	44%	45%	52%
% White	97%	95%	98%
% Other	3%	5%	2%
Mean duration of diagnosis (days)*	1.5 [range 1-6]	2.4 [range 1-24]	1.7 [range 1-9]

AZM = Azithromycin; * Duration from onset of symptoms to enrollment.

Drug Administration:

Azithromycin for Oral Suspension

Dosing Single oral dose of 30 mg/kg, or 10 mg/kg/day for 3 days

Duration Single dose or 3 days

Ceftriaxone Disodium for Injection

Dosing Single dose of 50 mg/kg, IM

Duration Single dose

Placebo for Azithromycin for Oral Suspension

Dosing Single oral dose of 30 mg/kg, or 10 mg/kg/day for 3 days

Duration Single dose or 3 days

Placebo for Ceftriaxone Disodium for Injection

Dosing Single IM injection

Duration Single dose

Efficacy and Safety Evaluations:

Efficacy Evaluations: Two subject populations were defined for the conduct of efficacy analyses. The first consists of the modified intent-to-treat (MITT) subjects and the second consists of the per-protocol evaluable subjects. Within each population, subjects were further defined to be evaluable for the clinical outcome and evaluable for the bacteriological outcome.

Clinical response was assessed as follows, as defined in the protocol. It should be noted that in this study, effusion was listed in the signs and symptoms block of the case record.

Cure	Disappearance of all pretreatment signs and symptoms of infection.
Improvement	Improvement in or partial disappearance of pretreatment signs and symptoms.
Failure	No change in or worsening of signs and symptoms. In defining "failure", the CRF further specified no requirement for additional antibiotic for acute otitis media.
Relapse*	Improvement or disappearance of pretreatment signs and symptoms followed by their worsening or reappearance. (note: assessed at TOC only)
Indeterminate*	The CRF included an option of "Indeterminate – Specify reason why unable to evaluate clinical efficacy."

* Collapsed with Failure in the efficacy analysis.

Clinical MITT subjects were required to have taken at least one dose of study medication and have a diagnosis of acute otitis media at baseline. Bacteriological

MITT subjects were required to be clinically MITT evaluable and, in addition, have a positive culture from middle ear effusions for *S. pneumoniae*, *H. influenzae*, or *M. catarrhalis*, at baseline.

Clinical per protocol evaluable subjects were required to have been evaluated at least once after initiating treatment. Subjects who had, in addition, a baseline pathogen isolated from middle ear effusion culture that was susceptible to the assigned treatment, were defined to be per-protocol bacteriologically evaluable.

Efficacy was assessed by clinical and bacteriological response at the End of Therapy visit (EOT) (Visit 3, Days 14 to 15) and at the Follow-up visit (Visit 4, Days 28 to 30). Clinical effectiveness, categorized as "Cure", "Improvement", "Failure", "Relapse" (only for those patients who returned after Visit 3 due to recurrence of symptoms), or "Indeterminate" was based on graded clinical signs and symptoms (fever, lethargy, irritability, earache, tugging of ear, erythema and/or bulging of the tympanic membrane, loss of tympanic landmarks and presence of fluid as assessed by pneumatic otoscopy or tympanometry). Bacteriological assessment was based on clinical outcome because only 7 subjects had a repeat tympanocentesis.

Safety Evaluations: All subjects who took at least one dose of study medication were included in the safety analyses. Safety was assessed by adverse event reporting and routine laboratory tests. Safety evaluations included medical history, physical examination, and vital signs.

Statistical Methods:

Efficacy—MITT Analyses

Statistical comparisons of the three treatment groups used hypothesis testing as well as interval estimation methods. The chi-square test was used to compare the frequencies of satisfactory (Cure + Improvement) responses versus Failures, and, separately, of Cure versus Failure (Improvement + Failure). The normal approximation to the binomial distribution was used to compute the 95% confidence intervals of the differences between the satisfactory response rates, and, separately, between the Cure rates.

The Follow-up assessment of efficacy, corresponding to each analysis at EOT, was done using the method of last observation carried forward (LOCF) for missing values. The frequencies of outcomes, observed and imputed by LOCF, are clearly noted and displayed in the tables.

No statistical analysis of bacteriological results was done as no second tympanocentesis (except for 7 individual cases) was performed. However, a display by baseline pathogen of the clinical outcome of the bacteriological MITT subjects is provided.

Efficacy—Per-Protocol Analyses

Clinical response at EOT (Days 14 to 15) and Follow-up (Days 28 to 30) was compared between treatment groups using Fisher's Exact Test. Subjects were considered evaluable for microbiological efficacy only if a pathogen was isolated at baseline by tympanocentesis. Eradication rates on a per pathogen basis was analyzed using Fisher's exact test. All statistical tests were two-tailed and performed at the 5% significance level.

Safety

Descriptive statistics were used to summarize the safety data. All subjects who took at least one dose of study medication were included in the safety analysis.

Efficacy Results:

Clinical Outcomes - MITT Analysis

	AZM SD	AZM 3D	CX	95 % CI	
	N	N	N	AZM 3D-CX	AZM SD-CX
Observed Case MITT Analysis*					
Clinical Outcome at EOT					
Total Evaluable	64	63	62		
Cure + Improved	96.9%	95.2%	98.4%	-10.9%, 4.6%	-8.4%, 5.4%
Clinical Outcome at F/U					
Total Evaluable	65	66	64		
Cure + Improved	93.8%	92.4%	96.9%	-13.7%, 4.8%	-11.9%, 5.8%
Cure	86.2%	77.3%	76.6%	-15.4%, 16.9%	-5.4%, 24.6%
Improvement	7.7%	15.2%	20.3%		
Failure	6.2%	7.6%	3.1%		

*Includes MITT subjects with observed values or classified as failures due to additional antibiotics;
AZM SD = azithromycin single dose; AZM-3D = azithromycin 3-Day; CX = ceftriaxone.
EOT = End of Therapy (Days 14–15); F/U = Follow-Up (Days 28–30).
Missing values at Follow-up were computed using LOCF.

Sixty-five azithromycin treated subjects and 33 ceftriaxone treated subjects had an appropriate pathogen (*H. influenzae*, *M. catarrhalis*, or *H. influenzae*) isolated at baseline, and were evaluable for clinical response at EOT and Follow-up by baseline pathogen. No subject had more than 1 pathogen isolated at baseline. *S. pneumoniae* (39 of 65 isolates in the azithromycin groups; 23 of 33 isolates in the ceftriaxone group) was the most common pathogen isolated at baseline in both treatment groups.

Overall, for MITT evaluable subjects in the azithromycin 1-day group, clinical cure by baseline pathogen at the TOC visit occurred in 7 of 8 (88%) subjects infected with *H. influenzae* and 17 of 20 (85%) infected with *S. pneumoniae*. The corresponding rates in the azithromycin 3-day group were 9 of 13 (69%) *H. influenzae* and 16 of 17 (94%) *S. pneumoniae*, and in the comparator group were 8 of 9 *H. influenzae* (89%) and 19 of 23 (83%) *S. pneumoniae*.

Clinical Cure Rates at TOC – MITT Subjects						
	AZM Single		AZM 3-Day		Ceftriaxone	
Age Group	n/N	%	n/N	%	n/N	%
≤2 years old (%)	19/25	76%	18/27	67%	23/33	70%
> 2 years old (%)	37/40	93%	33/39	85%	26/31	84%
	95% CI: ≤2 years old:		95% CI >2 years old:			
AZM Single –AZM 3-Day	-19.4%, 38.1%		-8.8%, 24.6%			
AZM Single – Comparator	-20.6%, 33.2%		-9.8%, 27.0%			
AZM 3-Day – Comparator	-30.5%, 24.5%		-19.6%, 21.1%			

AZM = Azithromycin

Cure rates at TOC were higher in the older subset of MITT subjects (>2 years old) than the younger subset of subjects (≤2 years old) in all three treatment groups.

The following tables summarize the signs/symptoms data at baseline, EOT and TOC.

Clinical Symptoms of Otitis Media – Clinical MITT Subjects in Study AZM-NY-95-001					
Treatment group					
Timepoint		Symptom Score			
Symptom	Subjects Assessed	0 (Absent)	1 (Mild)	2 (Moderate)	3 (Severe)
Azithromycin 1 day (30 mg/kg/day)					
Baseline					
Ear Pain					
Right Ear	66	18	1	6	41
Left Ear	66	25	2	5	34
Tugging at Ear					
Right Ear	66	27	2	5	32
Left Ear	66	20	1	6	39
EOT					
Ear Pain					
Right Ear	64	61	2	0	1
Left Ear	64	62	2	0	0
Tugging at Ear					
Right Ear	64	61	2	1	0
Left Ear	64	61	2	1	0
TOC*					
Ear Pain					
Right Ear	62	61	0	1	0
Left Ear	62	61	0	1	0
Tugging at Ear					
Right Ear	62	61	0	1	0
Left Ear	62	61	0	1	0
Azithromycin 3 day (10 mg/kg/day)					
Baseline					
Ear Pain					
Right Ear	61	18	3	1	44
Left Ear	66	22	2	7	35
Tugging at Ear					
Right Ear	66	24	1	8	33
Left Ear	66	21	2	2	41
EOT					
Ear Pain					
Right Ear	61	57	3	0	1
Left Ear	61	56	4	0	1
Tugging at Ear					
Right Ear	61	58	2	0	1
Left Ear	61	59	1	0	1
TOC*					
Ear Pain					
Right Ear	62	61	0	0	1
Left Ear	61	60	0	0	1
Tugging at Ear					
Right Ear	61	60	0	0	1
Left Ear	62	61	0	0	1

Clinical Symptoms of Otitis Media – Clinical MITT Subjects in Study AZM-NY-95-001					
Treatment group					
Timepoint		Symptom Score			
Symptom	Subjects Assessed	0 (Absent)	1 (Mild)	2 (Moderate)	3 (Severe)
Ceftriaxone (50 mg/kg single dose)					
Baseline					
Ear Pain					
Right Ear	66	22	2	2	40
Left Ear	66	25	3	3	35
Tugging at Ear					
Right Ear	66	28	1	3	34
Left Ear	66	24	1	2	39
EOT					
Ear Pain					
Right Ear	62	60	1	0	1
Left Ear	62	60	0	1	1
Tugging at Ear					
Right Ear	62	60	0	2	0
Left Ear	62	61	1	0	0
TOC*					
Ear Pain					
Right Ear	63	62	1	0	0
Left Ear	63	63	0	0	0
Tugging at Ear					
Right Ear	63	62	1	0	0
Left Ear	63	61	2	0	0

*Follow-up visit (scheduled for Day 28 - 30).
Ref: Study Report AZM-NY-95-001, Addendum Table 9

Percent of Subjects with Signs of Otitis Media Based on Pneumatic Otoscopy or Tympanometry – Clinical MITT Subjects in Study AZM-NY-95-001

	Treatment Group								
	Azithromycin 1 day (30 mg/kg/day)			**Azithromycin 3 day (10 mg/kg/day)**			**Ceftriaxone (50 mg/kg single dose)**		
Symptom	BL*	EOT	TOC**	BL	EOT	TOC*	BL	EOT	TOC*
Erythema of Eardrum									
Right	73%	19%	2%	74%	21%	5%	73%	21%	6%
Left	64%	12%	2%	70%	20%	5%	67%	16%	6%
Bulging of TM***									
Right	71%	9%	2%	74%	5%	3%	73%	10%	2%
Left	64%	5%	2%	70%	5%	3%	68%	11%	3%
Loss of TM landmarks									
Right	73%	16%	3%	74%	21%	5%	74%	14%	3%
Left	64%	11%	3%	70%	20%	7%	68%	14%	5%

*BL = Baseline **TOC = Follow-up visit (scheduled for Day 28 30). ***TM = Tympanic Membrane
Ref: Study Report AZM-NY-95-001, Addendum Table 9

Safety Results: The safety results are summarized in the following table.

Summary of Safety Results

	AZM Single N (%)	AZM 3-Day N (%)	Ceftriaxone N (%)
Total Treated	66 (100.0)	66 (100.0)	66 (100.0)
Number With Adverse Events (all Causality)	9 (13.6)	10 (15.2)	13 (19.7)
Number With Treatment-related Adverse Events	7 (10.6)	6 (9.1)	6 (9.1)
Number With Serious Adverse Events	0 (0.0)	0 (0.0)	0 (0.0)
Number With Clinically Significant Laboratory Abnormalities	21 (32.8)	24 (38.7)	19 (30.2)

The incidence of adverse events, irrespective of relationship to study drug, was higher in the ceftriaxone treatment group (19.7%) than in either of the azithromycin treatment groups (13.6% azithromycin single dose; 15.2% azithromycin 3-day). The most frequently occurring adverse event was diarrhea and occurred at a higher rate in ceftriaxone treated subjects (15.2%) than in either of the azithromycin treatment groups (6.1% in both azithromycin treatment groups). Incidence of vomiting was higher in the azithromycin single dose group (7.6%) than in either the azithromycin 3-day group (3.0%) or in the ceftriaxone group (1.5%). Severe rash occurred in 3 ceftriaxone treated subjects.

The incidence of treatment related adverse events was similar among the three treatment groups. Diarrhea, rash, and vomiting were the only treatment related adverse events that occurred in 2 or more subjects. Similar to what was observed in the all causality adverse events, diarrhea and rash occurred more frequently in the ceftriaxone group than in the two azithromycin groups, and vomiting was more frequent in the single dose azithromycin group than in the 3-day azithromycin or ceftriaxone group.

There were no deaths or other serious adverse events during the study. No subjects discontinued study drug due to an adverse event or laboratory abnormality.

All subjects completed treatment in this study.

Summary and Conclusions: In this study of 198 subjects, a single oral dose of azithromycin suspension (30 mg/kg), three day dosing of oral azithromycin suspension (10 mg/kg/day for 3 days), or a single dose of ceftriaxone (50 mg/kg) intramuscularly had comparable safety and effectiveness in the treatment of acute otitis media.

APPENDIX III

RESULTS OF AZITHROMYCIN STUDY R-0581
PARENTAL QUESTIONNAIRE

TABLE 5.6
AZITHROMYCIN PROTOCOL R-0581
PARENTAL QUESTIONNAIRE - MITT SUBJECTS

	AZITHROMYCIN							AMOX/CLAV						
	N	1	2	3	4	5	MEAN	N	1	2	3	4	5	MEAN
BASELINE														
HOW SICK OVERALL HAS CHILD BEEN	169	4	29	71	57	8	3.21	172	7	39	78	44	4	2.99
HOW MUCH TIME DID CHILD:														
SEEM CONTENT/CHEERFUL	169	60	66	33	10		1.96	172	55	75	34	8		1.97
SEEM LIVELY/ENERGETIC	169	65	60	35	9		1.93	172	56	69	36	11		2.01
HAVE TROUBLE FALLING ASLEEP	168	25	52	20	71		2.82	172	27	48	34	63		2.77
WAKE UP DURING THE NIGHT	169	32	49	32	56		2.66	172	26	54	34	58		2.72
HAVE USUAL APPETITE	169	69	42	35	23		2.07	172	73	38	39	22		2.06
DAY 3-5 TELEPHONE CONTACT														
HOW SICK OVERALL HAS CHILD BEEN	165	0	5	16	84	60	4.21	170	3	4	32	72	59	4.06
HOW MUCH TIME DID CHILD:														
SEEM CONTENT/CHEERFUL	165	120	40	4	1		1.31	170	118	45	6	1		1.35
SEEM LIVELY/ENERGETIC	165	125	34	5	1		1.28	170	125	34	8	3		1.35
HAVE TROUBLE FALLING ASLEEP	165	10	16	12	127		3.55	169	5	17	20	127		3.59
WAKE UP DURING THE NIGHT	165	8	15	26	116		3.52	170	8	13	15	134		3.62
HAVE USUAL APPETITE	165	106	34	20	5		1.54	169	123	32	10	4		1.38
EOT														
HOW SICK OVERALL HAS CHILD BEEN	156	2	1	7	38	108	4.60	154	0	2	8	44	100	4.57
HOW MUCH TIME DID CHILD:														
SEEM CONTENT/CHEERFUL	156	140	15	0	1		1.12	154	131	21	2	0		1.16
SEEM LIVELY/ENERGETIC	156	143	11	2	0		1.10	154	135	16	3	0		1.14
HAVE TROUBLE FALLING ASLEEP	156	5	15	14	122		3.62	154	6	9	17	122		3.66
WAKE UP DURING THE NIGHT	156	7	8	16	125		3.66	154	3	8	19	124		3.71
HAVE USUAL APPETITE	156	130	17	5	4		1.25	154	126	20	7	1		1.24
TOC														
HOW SICK OVERALL HAS CHILD BEEN	136	0	2	8	18	108	4.71	141	1	2	12	30	96	4.55
HOW MUCH TIME DID CHILD:														
SEEM CONTENT/CHEERFUL	136	123	11	2	0		1.11	142	125	12	5	0		1.15
SEEM LIVELY/ENERGETIC	136	131	4	1	0		1.04	142	131	9	2	0		1.09
HAVE TROUBLE FALLING ASLEEP	136	4	9	10	113		3.71	142	6	11	13	112		3.63
WAKE UP DURING THE NIGHT	136	3	10	17	106		3.66	142	6	10	14	112		3.63
HAVE USUAL APPETITE	136	120	10	4	2		1.18	142	119	15	7	1		1.23

DATA FOR 1 SUBJECT (#0354 AT TOC) WAS TAKEN OVER THE TELEPHONE
SCORE FOR OVERALL SICK QUESTION: 1=EXTREMELY SICK; 2=QUITE SICK; 3=MODERATELY SICK; 4=SLIGHTLY SICK; 5=NOT AT ALL SICK
SCORE FOR TIME SPENT QUESTIONS: 1=ALL OR MOST OF THE TIME; 2=SOME OF THE TIME; 3=A LITTLE OF THE TIME; 4=NONE OF THE TIME

FILE: T:\CLINI\A581\SAS\T0506.SAS DATE: 07JUL00

APPENDIX IV

CLINICAL SIGNS AND SYMPTOMS AND ACOUSTIC REFLECTOMETRY

Persistent effusion in the middle ear suggests persistent inflammation. Data pooled from Pfizer studies A0661015[1], A0661014[2], R-0581[3] and AZM-NY-95-001[4] are presented below and reflect trends seen in each of the individual study reports. "Fullness/Bulging of TM" (tympanic membrane) and "Impaired TM Mobility" are indicators of middle ear effusion.

Signs & Symptoms of Middle Ear Effusion

	Azithromycin 10 mg/kg/d x 3d	**Azithromycin 30 mg/kg x 1d**	**Comparator**	**p-value**
End –of –Therapy (EOT)				
Fullness/Bulging of TM				
	43/244 (18%)	84/460 (18%)	54/397 (14%)	
Az30-Comparator				0.064
Az10-Comparator				0.168
Impaired TM Mobility				
	67/170 (39%)	126/394 (32%)	105/318 (33%)	
Az30-Comparator				0.768
Az10-Comparator				0.159
Test-of Cure (TOC)				
Fullness/Bulging of TM				
	20/239 (8%)	29/435 (7%)	50/380 (13%)	
Az30-Comparator				0.002
Az10-Comparator				0.067
Impaired TM Mobility				
	29/164 (18%)	64/371 (17%)	84/298 (28%)	
Az30-Comparator				0.001
Az10-Comparator				0.012

TM = Tympanic Membrane; Az = Azithromycin; d = day

Acoustic reflectometry scores, which use the gradient angle of the tympanic membrane as a measure of middle ear effusion, follow a similar pattern, as seen in the table below.

Acoustic Reflectometry

	Azithromycin 10 mg/kg/d x 3d	**Azithromycin 30 mg/kg x 1d**	**Comparator**	**p-value**
End –of –Therapy (EOT)				
Subjects with Normal Gradient Angle*	65/168 (39%)	93/338 (28%)	109/304 (36%)	
Az30-Comparator				0.023
Az10-Comparator				0.541
Test-of Cure (TOC)				
Subjects with Normal Gradient Angle*	93/170 (55%)	160/345 (46%)	117/309 (38%)	
Az30-Comparator				0.028
Az10-Comparator				0.001

Az = Azithromycin; d= day; * Normal gradient angle defined as >95 degrees; angle ≤69 degrees required for study entry.

Overall, signs, symptoms, and acoustic reflectometry scores decreased for each parameter tested from Baseline values to End of Therapy values to TOC values. In each study, the numerical values at Day 14 were consistently more favorable for Augmentin® than for azithromycin. At Test of Cure, however, the numerical values were consistently and statistically significantly more favorable for azithromycin than for Augmentin®, suggesting a more favorable impact on the host inflammatory response.

These clinical findings are consistent with recent discussions around the impact of mechanism of bacterial killing on the host response to infection. These discussions have been focused primarily on the role of antibiotics in treatment of meningitis. But, by extension, the impact in other potential closed space infections should be considered. Acute otitis media may be one such closed space infection.

Beta-lactams and cephalosporins, while being rapidly cidal, may also amplify the inflammatory response by enhancing bacterial cell wall component release. In studies of meningitis caused by *H. influenzae type b* (Hib), rapidly cidal antibiotics increased the release of bacterial cell wall components (e.g. endotoxin, teichoic

acid, peptidoglycan fragments), amplifying production of inflammatory mediators such as tumor necrosis factor (TNF) and Interleukin-1 beta (IL-1 beta), resulting in increased inflammation. Inflammation, in turn, is linked to long-term sequelae, such as hearing loss[5, 6, 7]. This has lead to the widespread use of dexamethasone as an important adjunct to antimicrobials in the treatment of Hib meningitis[8, 9,10].

Fewer studies have explored the specific inflammatory response to gram positive cell wall components and the role of cidal antibiotics in amplifying that response. In experimental animal models, instillation of gram-positive cell wall components into sterile sites produced signs of meningitis, shock, and multi-organ system failure[11,12]. In humans, lipoteichoic and teichoic acid concentrations in CSF were significantly associated with neurologic sequelae and mortality in *S. pneumoniae* meningitis[13]. Accordingly, dexamethasone has been used to dampen the inflammatory response in *S. pneumoniae* meningitis[8, 10].

Quinolones, which are bactericidal without affecting cell wall synthesis, have been shown to attenuate the inflammatory burst in the subarachnoid space that occurs after initiation of penicillins and cephalosporins in the treatment of experimental pneumococcal meningitis. Indeed, trovafloxacin delayed the release of the pro-inflammatory cytokines TNF and IL-1 beta in pneumococcal meningitis in rabbits, while ceftriaxone appeared to have the opposite effect[14]. Macrolides, which act by inhibiting bacterial protein synthesis, may also kill without interrupting the pathogen's cell membrane[15,16,17]. Could this different mechanism of action result in different host response characteristics than those observed with beta-lactams? Recent work would suggest that this is the case.

In a recently published paper, murine macrophages exposed to pneumococci produced significantly more inducible nitric oxide synthase (iNOS) and tumor necrosis factor (TNF) in the presence of oxacillin than in the presence of clindamycin[18]. These experiments were also repeated with cefotaxime compared to chloramphenicol and similar results were found. The effect of azithromycin in this model has not been studied but should be similar to that of the other two protein synthesis inhibitors (clindamycin and chloramphenicol).

While the pathophysiology has not yet been established, fewer signs and symptoms of effusion at later follow-up visits may have clinical consequences. Persistence of middle ear fluid drives the subsequent (unnecessary) administration of antibiotics to children who have been adequately treated for acute otitis media. Driven by concerns around overuse of antibiotics, recent Centers for Disease Control guidelines have attempted to address the issue of post-treatment effusions[19]. Based on the observations from the studies in this application, the use of azithromycin for the treatment of acute otitis media may decrease the likelihood of unnecessary re-treatment, as it appears to decrease the presence of post-treatment effusion observed during late post-therapy visits. This is one way that the judicious use of azithromycin can help control the growing problem of antimicrobial resistance.

Of course, it would be helpful to determine which subjects have otitis media with effusion (OME) versus acute otitis media (AOM) *before* antimicrobial treatment has been initiated. Short of tapping every tympanic membrane in a child with suspected AOM, this distinction is left in the hands of the clinician skilled in otoscopy. Other systemic signs of acute infection, such as fever, do little in aiding the clinician in making the diagnosis.

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APPENDIX V

H. INFLUENZAE AND *S. PNEUMONIAE* RESPONSE RATES TO AZITHROMYCIN REGIMENS AND OTHER ANTIBIOTICS

H. influenzae **Response Rates to Azithromycin Regimens and Other Antibiotics**				
Primary Antibiotic	Response	95% Confidence Interval	Endpoint	Follow-up as long as:
Azithromycin (1-day)* Study A0661015 Study AZM-NY-95-001	35/52 (67%) 28/44 (64%) 7/8 (88%)	54.6, 80.1	Clinical Cure at TOC Studies A0661015, AZM-NY-95-001 (MITT)	 Day 32 Day 30
Azithromycin (3-day)*	9/13 (69%)	41.3, 88.7	Clinical Cure at follow-up Study AZM-NY-95-001 (MITT)	Day 30
Azithromycin (5-day)**	30/47 (64%)	50.1, 77.6	Cured/Improved Study 066-176 (evaluable group)	Day 35
Cefpodoxime proxetil 5-day**	50/76 (66%)	55.1, 76.5	Cured/Improved Based on controlled studies. (Label indicated strict evaluability criteria used)	Day 26
Ceftriaxone - single dose IM**	22/31 (71%)	55.0, 86.9	Cured (Per Protocol)	Day 32
Amoxicillin/ Clavulanate Potassium 10-day (ES-600)***	106/156 (68%)	60.6, 75.3	Cured/Improved (Per Protocol)	Day 28
Cefprozil 10-day+	17/33 (52%)	34.5, 68.6	Cured (evaluable subjects)	Day 26 (~ 2 wk. post-therapy)
Cefixime 10-day++	61/81 (75%)	65.9, 84.7	Cure/Improved Based on controlled studies. (Label indicated strict evaluability criteria were used)***	Day 31
Cefdinir 5-day+++	27/44 (61%)	47.0, 75.8	Cured (evaluable group) (Study 983-65)	Day 21

* Azithromycin Pediatric Supplemental NDA-50-710 (Overall Summary of Efficacy Table B.7, A0661015 Study Report Table 5.3.1, AZM-NY-95-001 Study Report Addendum Table 8a); ** Taken from 2001 Physicians' Desk Reference (PDR), and Summary Basis of Approval (SBA) also consulted;*** Recently approved, so label used was not in 2001 PDR; + Label consulted, but taken primarily from Medical Officer review in SBA; ++ Cefixime data taken from cefpodoxime proxetil label; +++ From study 983-65 in sponsor submission to FDA; MITT = Modified Intent-to-Treat; TOC=Test of Cure.

***S. pneumoniae* Response Rates to Azithromycin Regimens and Other Antibiotics**				
Primary Antibiotic	Response	95% Confidence Interval	Endpoint	Follow-up as long as:
Azithromycin (1-day)* Study A0661015 Study AZM-NY-95-001	84/96 (88%) 67/76 (88%) 17/20 (85%)	80.9, 94.1	Clinical Cure at TOC Studies A0661015, AZM-NY-95-001 (MITT)	 Day 32 Day 30
Azithromycin (3-day)*	16/17 (94%)	72.2, 99.7	Clinical Cure at follow-up Study AZM-NY-95-001 (MITT)	Day 30
Azithromycin (5-day)**	40/56 (71%)	59.6, 83.3	Cured/Improved Study 066-176 (evaluable group)	Day 35
Cefpodoxime proxetil 5-day**	88/122 (72%)	64.2, 80.1	Cured/Improved Based on controlled studies. (Label indicated strict evaluability criteria used)	Day 26
Ceftriaxone - single dose IM**	25/35 (71%)	56.5, 86.4	Cured (Per Protocol)	Day 32
Amoxicillin/ Clavulanate Potassium 10-day (ES-600)***	95/136 (70%)	62.1, 77.6	Cured/Improved (Per Protocol)	Day 28
Cefprozil 10-day+	41/49 (84%)	73.3, 94.0	Cured (evaluable subjects)	Day 26 (~ 2 wk. post-therapy)
Cefixime 10-day++	72/124 (58%)	49.4, 66.7	Cure/Improved Based on controlled studies. (Label indicated strict evaluability criteria were used)***	Day 31
Cefdinir 5-day+++	28/49 (57%)	43.3, 71.0	Cure (evaluable group) (Study 983-65))	Day 21

* Azithromycin Pediatric Supplemental NDA-50-710 (Overall Summary of Efficacy Table B.7, A0661015 Study Report Table 5.3.1, AZM-NY-95-001 Study Report Addendum Table 8a); ** Taken from 2001 Physicians' Desk Reference (PDR), and Summary Basis of Approval (SBA) also consulted;*** Recently approved, so label used was not in 2001 PDR; + Label consulted, but taken primarily from Medical Officer review in SBA; ++ Cefixime data taken from cefpodoxime proxetil label; +++ From study 983-65 in sponsor submission to FDA; MITT = Modified Intent-to-Treat; TOC=Test of Cure.

APPENDIX VI

ALL CAUSALITY ADVERSE EVENTS

Summary of the Most Commonly Reported Adverse Events (≥2% of Subjects in Either Azithromycin or Comparator Group, All Causality) by Body System			
	Number (%) of Subjects		
	Azithromycin		
	10 mg/kg x 3D	**30 mg/kg –SD**	**Comparator**
Number of Subjects	1729	487	1897
Subjects with at least one AE	382 (22.1)	193 (39.6)	550 (29.0)
Body System			
Event (preferred term)			
Body as a Whole			
Abdominal pain	39 (2.3)	15 (3.1)	37 (2.0)
Fever	34 (2.0)	20 (4.1)	41 (2.2)
Headache	10 (0.6)	6 (1.2)	21 (1.1)
Digestive			
Diarrhea	72 (4.2)	40 (8.2)	169 (8.9)
Nausea	11 (0.6)	11 (2.3)	22 (1.2)
Vomiting	68 (3.9)	52 (10.7)	92 (4.8)
Respiratory			
Cough increased	28 (1.6)	21 (4.3)	55 (2.9)
Pharyngitis	27 (1.6)	7 (1.4)	32 (1.7)
Respiratory Tract Infection	20 (1.2)	28 (5.7)	45 (2.4)
Rhinitis	33 (1.9)	19 (3.9)	45 (2.4)
Skin and Appendages			
Rash	24 (1.4)	14 (2.9)	57 (3.0)
AE = Adverse Event; 3D = 3 days; SD = Single Dose			