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APPLICATION NUMBER:
50-797

MICROBIOLOGY REVIEW

Division of Anti-Infective Drug Products
Clinical Microbiology Review

NDA #50-797

Date Completed: May 13, 2005

Applicant:

Pfizer Global Research & Development
WorldWide Regulatory Affairs
50 Pequot Avenue
New London, CT 06320
(860) 732-6991

Therapeutic Type: Modified release dosage for the treatment of acute respiratory tract infections in adults and children

Submission Reviewed: NDA 50-797

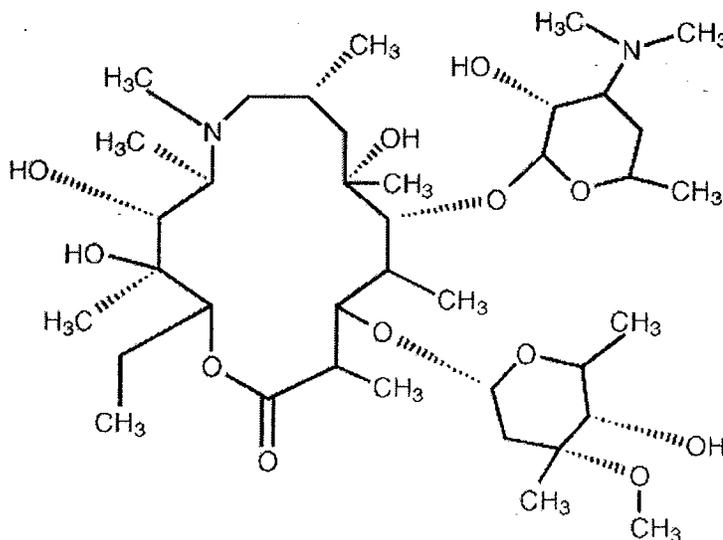
Providing for: Treatment of Community Acquired Pneumonia (CAP),
Acute Maxillary Sinusitis (ABS) in adults

Product Names:

Proprietary: Zithromax® SR
Non-proprietary: Azithromycin Sustained Release

Chemical Name: Azithromycin has the chemical name
(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-(alpha)-*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-(beta)-*D*-xylo-exopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

Structural Formula:



Molecular Formula: C₃₈H₇₂N₂O₁₂ (MW 749.0)

Dosage Form: Single dose sustained release

Route of Administration: Oral

Pharmacological Category: Antibacterial

Dispensed: Rx

Initial Submission Dates:

Received by CDER: August 12, 2004
Received by Reviewer: August 20, 2004
Review Completed: May 13, 2005

Related Documents: IND 66,194, NDA 50-670.

Remarks:

Azithromycin is an azalide antibiotic approved for the treatment of a variety of community-acquired infections, including those of the respiratory tract e.g., community-acquired pneumonia [CAP], [REDACTED] and acute bacterial sinusitis [ABS]. The recommended duration of therapy ranges from 1 to 5 days, depending on the infection being treated. Azithromycin is available in tablet, sachet, powder for oral suspension, and intravenous formulations. A sustained release formulation of azithromycin that allows single-dose therapy of a full range of infections has been developed. This formulation is supported by studies in animals with results demonstrating improved clinical and bacteriologic outcomes when a complete course of azithromycin is administered in a single dose rather than over several days,

Clinical studies to define the appropriate pharmaceutical formulation, as well as two proof of concept studies that delineated the product's pharmacokinetic and GI tolerability profile, were conducted prior to September 2002 under IND-24,999. An End-of-Phase 2 meeting was held with the Division of Anti-infective Drug Products to discuss clinical issues (October 4, 2002) and for CMC discussion (October 1, 2002). Subsequently, a new IND was submitted on November 14, 2002 (IND-66,194) to cover the Phase 3 program and any additional Phase 1 studies deemed necessary to support the program.

Dialog on trial design, comparator selection and statistical considerations was held during teleconferences on November 13 and December 20, 2002, and April 8, 2003. As a result of these discussions, the Applicant agreed to the suggestions from the Division regarding information pertinent to Microbiology: delta selection for all non-inferiority trials, [REDACTED]

Two pre-NDA interactions were held in 2003. A Pre-NDA/CMC meeting was held June 10, 2003 to discuss Pfizer's final plans in preparation for the registration of the sustained release oral powder for suspension dosage of azithromycin. An administrative Pre-NDA

teleconference was held on December 3, 2003. The purpose of the teleconference was to discuss and seek agreement on the Levofloxacin trial blinding proposal, to share examples of tables and data formats, obtain agreement on providing Clinical Study Reports for indicated studies, and to share the CTD/NDA submission plans.

Following up on the administrative Pre-NDA teleconference, a Pre-NDA meeting was held on May 19, 2004 to review issues relating to the upcoming NDA submission for Azithromycin SR. Items discussed that were pertinent to Microbiology included the following: pursuit of an azithromycin breakpoint change for *S. pneumoniae* ; [REDACTED]

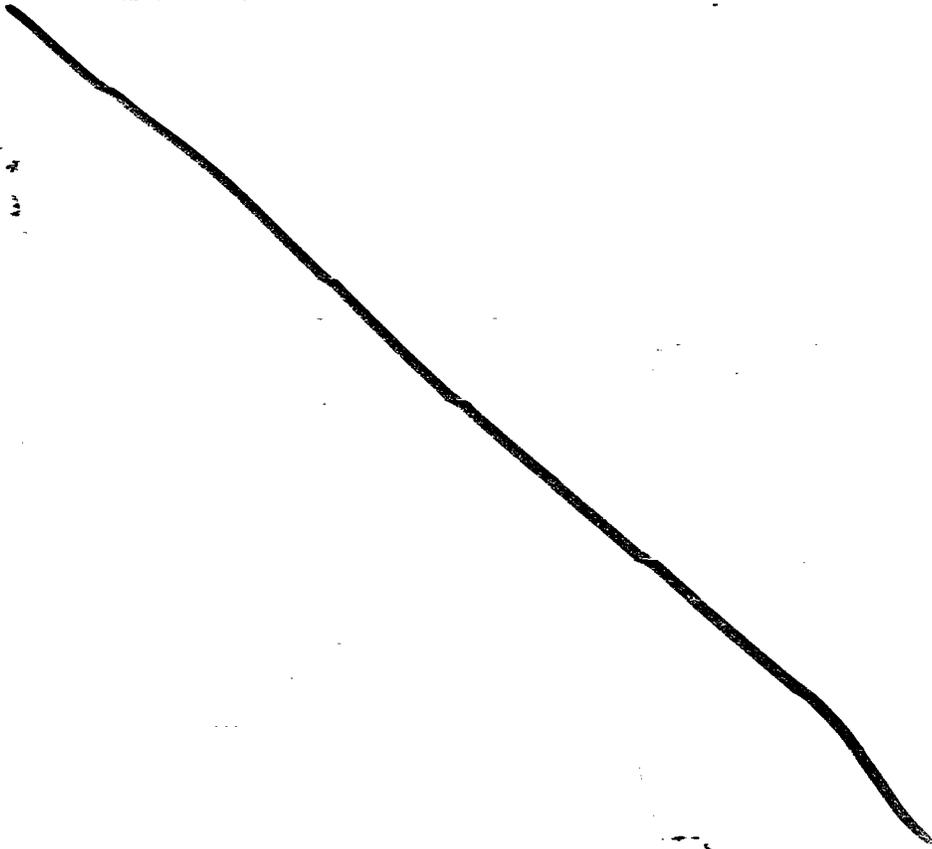
The Division could consider a change in the *S. pneumoniae* breakpoint, assuming that the MICs, PK/PD and animal studies, clinical isolates and quality controls support this change. The Division pointed to the experience with Augmentin XR and Augmentin ES as an example when the breakpoint for *S. pneumoniae* was changed for a new formulation of an existing drug. A similar experience in term of the number of isolates and clinical outcomes would be needed to support changes in the azithromycin breakpoint.

The Division inquired if a separate interpretive criterion will be published for azithromycin SR if a new breakpoint is established, and in this context, how will this information be communicated in the clinical laboratory setting and clinical practice. The Division also requested the Applicant include penicillin and erythromycin susceptibility data along with the azithromycin susceptibility data.

There was a conversation on July 21, 2004 between Judit Milstein, Project Manager in the Division, and Donald Jaffe, representing the Applicant, where the latter confirmed that [REDACTED] for adult [REDACTED] indications. The adult indications [REDACTED] (ABS, and CAP) are included in the current adult NDA submission.

Conclusion/Recommendation:

This application is **approvable** contingent upon changes to the Microbiology Section of the Package Insert as follows:



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EXECUTIVE SUMMARY

The macrolide antimicrobial agents were initially seen as an important alternative therapy to beta lactams for infected patients. While macrolides were initially seen as having activity against Gram-positive pathogens, their subsequent value for the atypical organisms expanded their utility. Further modifications to the chemical structure, as in the case of azithromycin, expanded the spectrums to include some Gram-negative pathogens suggesting a more ideal spectrum for an agent being used to treat respiratory tract infections, i.e., Gram-positive, Gram-negative, and atypical pathogens.

Azithromycin is a member of the azalide subclass of macrolide antibiotics. Similar to erythromycin in structure, it differs by a methyl-substituted nitrogen at its 15-member lactone ring. This modification increases the stability of the drug in gastric acid and thus improves absorption by the oral route. In addition to improved bioavailability, azithromycin has other advantages over erythromycin including higher tissue concentrations, fewer gastrointestinal side effects, enhanced antimicrobial activity and a longer half-life, which allows for once daily dosing.

Azithromycin inhibits protein synthesis via its interactions with the 50s subunit of the bacterial ribosome via dual mechanisms (1-6). Azithromycin binds near the entrance to the peptide passage channel primarily to residues A2058 and A2059 (*Escherichia coli* numbering) of 23s rRNA, and it appears to inhibit movement of the growing peptide chain, perturb binding of the peptide moiety of the peptidyl-tRNA, or both (7). In addition, azithromycin inhibits the assembly of nascent 50s subunits (1, 8). The Applicant presents biochemical and *in vivo* experimental data suggesting there is little potential for azithromycin to inhibit protein synthesis in mammalian cells when given in approved dosage regimens.

Macrolides are more active *in vitro* against Gram-positive and atypical agents than against Gram-negative pathogens. All macrolides are generally active against *Streptococcus pneumoniae* isolates that are susceptible to penicillin, however, significant cross-resistance occurs for pneumococcal isolates that are highly resistant to penicillin. Erythromycin is not as active against Gram-negative organisms as azithromycin and clarithromycin. The newer macrolides are more active against *H. influenzae* with azithromycin showing the greatest activity. MIC values tend to be lower for azithromycin than for the other two agents against *Moraxella catarrhalis* and *Mycoplasma pneumoniae*.

The Applicant does not present recent susceptibility data for any organisms listed in either the first or second lists found in the package insert. Microbiology recommends that recent susceptibility data (within the last 3-5 years) for 100 clinical isolates identified in the second list of the package insert be presented in the NDA. While the Applicant, has neither generated this data nor supplied this data from the literature, these data are available from the recent literature and have been provided here by the Reviewer. Data for 100 recent clinical isolates were found for the following organisms pertinent to this application: *S. pneumoniae*, *S. aureus*, *H. influenzae*, *Moraxella catarrhalis*, *S. pyogenes*,

Chlamydophila pneumoniae, and *Mycoplasma pneumoniae*. Data for less than 100 recent clinical isolates were found for *H. parainfluenzae*, viridans streptococci, Streptococci (groups C, F, G), *Bordetella pertussis*, *Haemophilus ducreyi*, *Neisseria gonorrhoeae*, *Peptostreptococcus* sp., *Prevotella bivia*, and *Chlamydia trachomatis*.

One of the main characteristics of azithromycin is the slow serum concentration but high and persistent tissue concentration (221). The pharmacokinetic profile of azithromycin suggests a rapid and extensive uptake from the circulation into interstitial compartments, subsequently followed by slow release. Azithromycin drug levels remain in pulmonary tissue for extended periods, the mean tissue half-life is between 2 – 4 days. A comparison of the pharmacokinetic parameters of the drug product for different regimens is shown in the accompanying table.

Table 9. Mean (SD) Pharmacokinetic Parameters for Azithromycin on Day 1 Following the Administration of a Single Dose of 2.0 g Azithromycin SR or 1.5 g of Azithromycin IR Tablets Given over 3 days (500 mg/day) or 5 Days (500 mg on day 1, 250 mg on days 2-5) to Healthy Subjects

Pharmacokinetic Parameter	Regimen		
	Azithromycin SR	3-day IR [†]	5-day IR [†]
	[n=41]**	[n=12]	[n=12]
C _{max} (µg/ml)	0.821	0.441	0.434
	(0.281)	(0.223)	(0.202)
	5.0	2.5	2.5
T _{max} [‡] (hr)	8.62	2.58	2.60
	(2.0-8.0)	(1.0-4.0)	(1.0-6.0)
	20.0	17.4	14.9
AUC ₀₋₂₄ (µg·hr/ml)	58.8	71.8	68.9
	(6.66)	(6.2)	(3.1)
	20.0	17.4	14.9
AUC _{0-∞} ^{***} (µg·hr/ml)	58.8	71.8	68.9
	(6.66)	(6.2)	(3.1)
	58.8	71.8	68.9
T _{1/2} (hr)	68.9	68.9	68.9
	(6.91)	(14.7)	(13.8)
	68.9	68.9	68.9

[†] SR and IR parameters obtained from separate PK studies;

[‡] Median (range);

* C_{max}, T_{max} and AUC₀₋₂₄ values for Day 1 only;

** n = 21 for AUC_{0-∞} and t_{1/2};

*** Total AUC for the 1-day, 3-day and 5-day regimens

SD = Standard deviation

C_{max} = Maximum serum concentration

T_{max} = Time to C_{max}

AUC = Area under concentration vs. time curve

t_{1/2} = terminal serum half-life

The Applicant does not seek a change in the current FDA and NCCLS breakpoints for azithromycin for streptococci and *Haemophilus* spp., as summarized in Table 11 of this review.

Table 11. Susceptibility Interpretive Criteria for Azithromycin

Pathogen	Minimum Inhibitory Concentrations (µg/ml)			Disk Diffusion (zone diameters in mm)		
	S	I	R	S	I	R
Streptococci including <i>S. pneumoniae</i> & <i>S. pyogenes</i>	< 0.5	1	> 2	≥ 18	14-17	≤ 13
<i>Haemophilus</i> spp.	≤ 4	*	*	≥ 12	*	*
<i>M. catarrhalis</i>	NA	NA	NA	NA	NA	NA
<i>Mycoplasma</i>	NA	NA	NA	NA	NA	NA
<i>Chlamydia</i>	NA	NA	NA	NA	NA	NA

The Applicant seeks approval of Zithromax SR for the treatment of respiratory infection indications: community acquired pneumonia (CAP), and acute bacterial sinusitis (ABS). A well controlled clinical trial was conducted for each indication with the exception of CAP, for which two clinical trials were conducted.

The Applicant seeks approval of Zithromax SR for the treatment of community acquired pneumonia (CAP) due to *Chlamydia pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, or *Streptococcus pneumoniae*. While the data provide evidence for efficacy of the drug against all of these organisms, some, but not all of these organisms should be approved (see the table below).

Summary of Clinical Cure Rates and Bacteriologic Cure Rates by Pathogen in Both CAP Studies.

Pathogen	No. Subjects Cured/No. Subjects with Pathogen (%)		No. Eradicated/No. Isolated (%)	
	Azithromycin SR	Comparators	Azithromycin SR	Comparators
<i>C. pneumoniae</i>	37/40 (93)	50/53 (94)	37/40 (93)	50/53 (94)
<i>S. pneumoniae</i>	28/33 (85)	35/39 (90)	29/33 (88)	36/41 (88)
<i>M. pneumoniae</i>	30/33 (91)	38/39 (97)	30/33 (91)	38/39 (97)
<i>H. influenzae</i>	28/30 (93)	31/34 (91)	28/30 (93)	31/34 (91)

The Applicant seeks approval of Zithromax SR for the treatment of acute bacterial sinusitis (ABS) due to, *Haemophilus influenzae*, *Moraxella catarrhalis*, or *Streptococcus pneumoniae*. While the data provide evidence for efficacy of the drug against all of these organisms, some, but not all of these organisms should be approved (see the table below). An inadequate number of *Moraxella catarrhalis* isolates were reported. Consequently, these organisms may not be included in the first list of the Package Insert.

Summary of Clinical Cure Rates and Bacteriologic Cure Rates by Pathogen in the ABS Study.

Pathogen	No. Subjects Cured/No. Subjects with Pathogen (%)		No. Eradicated/No. Isolated (%)	
	Azithromycin SR	Comparator	Azithromycin SR	Comparator
<i>S. pneumoniae</i>	36/37 (97)	36/39 (92)	37/37 (100)	36/39 (92)
<i>H. influenzae</i>	26/27 (96)	27/27 (100)	26/27 (96)	30/30 (100)
<i>M. catarrhalis</i>	8/8 (100)	10/11 (91)	8/8 (100)	10/11 (91)

The Applicant combined *in vitro* susceptibility data from all four studies for *S. pneumoniae*, *H. influenzae*, [REDACTED] and *M. catarrhalis*. The combined data is presented below. *In vitro* susceptibility data was not collected for *C. pneumoniae* or *M. pneumoniae*.

***In vitro* susceptibility data for pertinent respiratory pathogens.**

Organism	N	MIC ₉₀	%S
<i>S. pneumoniae</i>	221	4	83%
<i>H. influenzae</i>	179	2	100%
[REDACTED]			
<i>M. catarrhalis</i>	104	0.5	ND

%S=percent susceptible

Breakpoints for *S. pneumoniae*, *H. influenzae*, and *H. influenzae* were consistent with the established CLIS (NCCLS) breakpoints. Susceptibility data for *C. pneumoniae* and *M. pneumoniae* were not collected. Interpretative criteria for *M. catarrhalis* have not been defined at this time. The Applicant does not request any changes to the breakpoints for any of the organisms sought in the proposed indications.

From the Microbiology perspective, the crucial question raised by this submission is the following. **Can Zithromax SR be safely administered to patients without an unnecessary level of risk of development of azithromycin resistance by the etiological agent?**

Factors that may contribute to increasing macrolide resistance include patient compliance, inappropriate usage, drug pharmacokinetics and pharmacodynamics, as well as numerous intrinsic bacterial properties (201). The Applicant asserts that other factors must be involved in the development of resistance to azithromycin. *However, data presented by the Applicant indicate that pathogens treated with Zithromax SR may not be more likely to develop resistance.*

Increased patient compliance would certainly be expected in controlled clinical trials. However, in an out-patient setting, logic dictates that patient compliance would also be increased due to the low number of dosages required.

Data summarized by Carbon and Poole indicate that the prevalence of macrolide resistance is closely related to the extent to which the agents are used (202). However, in controlled clinical trials, there is less likelihood for abuse of the antimicrobial.

The pharmacokinetics and pharmacodynamics of Zithromax SR indicate that the drug concentrates in pulmonary tissues, tissues abundant in inflammatory cells. These cells may provide a mode of transport and reservoir for azithromycin to the infection site. Administering the same total dose as a single dose or in three days instead of five days will result in higher concentrations in the infected tissue at early times after treatment due to increased initial dosing and increased inflammation (157). The higher initial concentration at the infection site may help prevent less susceptible sub-populations of the pathogens initially present from becoming established. This has been proposed as the reason for less *in vivo* emergence of resistance to azithromycin compared with clarithromycin.

The persistence of azithromycin (and also macrolide resistance) is an important consideration in the epidemiology of *S. pneumoniae* infections. The increase in antimicrobial resistance in *S. pneumoniae* in recent years has been well documented in a variety of surveillance studies (21, 41, 203, 204, 205). The prevalence of penicillin resistance in *S. pneumoniae* varies on a global level and has recently been reported to range from 5% to nearly 70% (204, 205). While the data from the literature indicate about 75% of isolates susceptible to azithromycin, the Applicant's data demonstrated 83% of isolates susceptible to azithromycin. It is interesting that macrolide resistance has not developed in *M. catarrhalis*, *M. pneumoniae*, *C. pneumoniae*, and *L. pneumophila* (215).

Resistance in *S. pneumoniae* has certain intrinsic properties which increase the likelihood of developing resistance. Different levels of macrolide resistance (higher vs. lower MIC values) have been identified in *S. pneumoniae* isolates based on the mechanism of resistance (*ermB*--target site vs. *mefA*-- efflux). However, *in vitro* susceptibility testing for azithromycin susceptibility may not be an accurate predictor of clinical cure rate (19, 51, 52). That is, resistance to azithromycin may not correlate with clinical failure. Indeed, data presented by the Applicant demonstrated several resistant isolates harboring either *ermB* or *mef* that still demonstrated clinical cure. As a precaution to avoid the risks

of clinical failure, azithromycin use may need to be restricted in those geographical areas with higher prevalence of highly resistant pneumococci.

Thus, from the Microbiology perspective, the data suggest that Zithromax SR can be safely administered to patients without an unnecessary level of risk of development of azithromycin resistance.

This application is **approvable** contingent upon changes to the Microbiology Section of the Package Insert as follows:

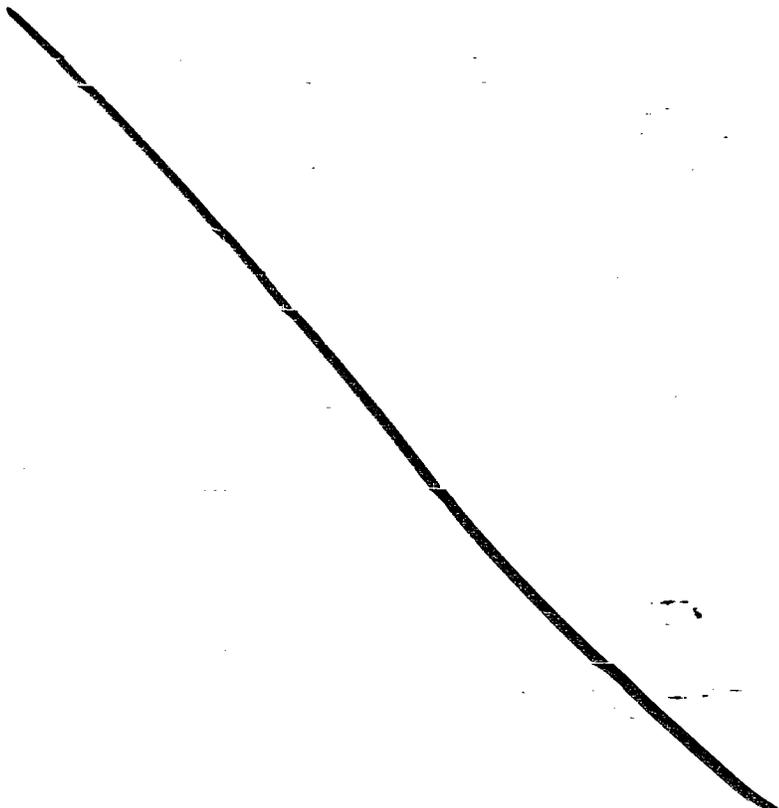


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INTRODUCTION

Azithromycin is a member of the azalide subclass of macrolide antibiotics. Similar to erythromycin in structure, it differs in having a methyl-substituted nitrogen in its 15-member lactone ring. This modification increases the stability of the drug in gastric acid and thus improves absorption by the oral route (1). In addition to improved bioavailability, azithromycin has other advantages over erythromycin including higher tissue concentrations, fewer gastrointestinal side effects, enhanced antimicrobial activity and a longer half-life, which allows for once daily dosing.

Zithromax SR is a modified release dosage form of azithromycin for the treatment of acute respiratory tract infections in adults. The Applicant states that the intent of this new formulation is to allow the administration of a complete course of azithromycin in a single dose that is both efficacious and is well tolerated. This would allow for convenience for the patient and improved compliance and would provide maximum exposure to the active drug on the first day of infection when bacterial burden is highest.

The premise for this proposal is based upon the established pharmacokinetics of azithromycin. This drug has been demonstrated to afford a therapeutic dose upon administration over five days for which the same full course of therapy may be given over shorter dosing schedules. The Applicant states that this new formulation is designed to minimize release of the drug from the matrix microspheres in the stomach via the use of an alkaline buffer that would increase gastric pH thus lowering the solubility of azithromycin and minimizing the drug's release from the matrix microspheres. Once in the duodenum, the pH would decrease allowing for slow release resulting in less chance of vomiting and nausea that is associated with single doses of current formulations.

PRECLINICAL EFFICACY-- *IN VITRO*

Mechanism of Action

Azithromycin inhibits protein synthesis via its interactions with the 50s subunit of the bacterial ribosome via dual mechanisms (1-6). Azithromycin binds near the entrance to the peptide passage channel primarily to residues A2058 and A2059 (*Escherichia coli* numbering) of 23s rRNA, and it appears to inhibit movement of the growing peptide chain, perturb binding of the peptide moiety of the peptidyl-tRNA, or both (7). In addition, azithromycin inhibits the assembly of nascent 50s subunits (1, 8).

Cell-free bacterial and mammalian coupled transcription/translation synthesis assays are able to measure the inhibitory activity of diverse protein synthesis inhibitors (9, 10). Using ribosomes prepared from a macrolide-susceptible *E. coli* strain, azithromycin inhibits 50% of the reconstituted translational activity (IC₅₀) at 0.33 μM compared to an IC₅₀ of > 150 μM in a rabbit reticulocyte translation system. In contrast, puromycin acts non-specifically in prokaryotic and eukaryotic translational systems, with IC₅₀s of 0.4--0.45 μM.

The Applicant presents results suggesting azithromycin equally binds to both mature 50s particles and the assembly intermediate, and thus may inhibit protein synthesis and ribosome assembly with equal effectiveness. The results of a recent report suggest azithromycin has a dual effect on inhibiting protein synthesis (8). Studies of the 50s ribosomal assembly has indicated in the past that erythromycin can inhibit assembly at concentrations higher than those required for protein synthesis inhibition. In the new study, it was found that azithromycin was much more effective (IC₅₀ 0.4 µg/ml vs. 1.5 µg/ml for erythromycin) at inhibiting 50s assembly of *H. influenzae* ribosomes.

The Applicant presents biochemical and *in vivo* experimental data suggesting there is little potential for azithromycin to inhibit protein synthesis in mammalian cells when given in approved dosage regimens. Previous experiments have shown that when rats are dosed orally with azithromycin at 20 or 200 mg/kg for four days, high drug concentrations are observed in liver cells (39 and 417 µg/g respectively). However, only low concentrations of azithromycin are found in the mitochondria or microsomal fractions of the liver cells, and approximately 90% of the azithromycin is associated with the lysosomal fraction [(11) and the Pharmacokinetic Section of the original application, NDA 50-670].

Antimicrobial Spectrum of Activity

The Applicant provides no spectrum of activity data generated during the past five years. Instead, the Applicant refers to NDA 50-670, the original NDA submission of April 11, 1990. The table that follows shows selected MIC data for those organisms pertinent to the current application.

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Table 1. MICs from the original azithromycin application.

Organism	N	MIC ₅₀	MIC ₉₀
<i>Haemophilus influenzae</i>	70	0.39	0.78
<i>Moraxella catarrhalis</i>	17	≤0.015	0.03
<i>Haemophilus parainfluenzae</i>	4	ND	1
<i>Bordetella pertussis</i>	34	ND	0.015
<i>Haemophilus ducreyi</i>	100	ND	0.003
<i>Legionella pneumophila</i>			
Philadelphia	1	ND	0.5*
Knoxville	1	ND	0.5*
Togus	2	ND	0.5*
Bloomington	3	ND	0.25*
Los Angeles	4	ND	1*
<i>Neisseria gonorrhoeae</i>	30	0.12	0.25
(penicillinase producing)	13	0.062	0.125
<i>Streptococcus pneumoniae</i>	10	≤0.025	0.05
<i>Staphylococcus aureus</i>	100	0.78	1.56
<i>Streptococcus agalactiae</i>	54	0.05	0.1
<i>Streptococci</i> (Groups C,F,G)	17	ND	0.25
Viridans group streptococci	13	ND	0.12
<i>Chlamydophila pneumoniae</i>	3	ND	0.125**
<i>Mycoplasma pneumoniae</i>	4	ND	<0.001**
<i>Chlamydia trachomatis</i>	1	ND	0.0125*
<i>Ureaplasma urealyticum</i>	30	ND	0.5

*not a true MIC₉₀

**MIC₁₀₀

data from tables 1, 3, 5, pp 22-30, Sub-Section 7, Microbiology Section, NDA-50-670.

Activity against Aerobic Gram-Positive Microorganisms

The Applicant presents recently published data on susceptibility of *S. pneumoniae* and *S. pyogenes* to azithromycin (or erythromycin) and relevant comparators are reviewed below. These data draw from a number of key studies, and the information for *S. pneumoniae* is summarized (Table 1, pp 7-9, Clinical Microbiology Section, this submission) and for *S. pyogenes* (Table 2, pp 11-12 Clinical Microbiology Section, this submission).

S. pneumoniae. Recent analysis of worldwide results from the PROTEKT study (1999-2000) show that 31% of *S. pneumoniae* isolates in this survey were resistant to macrolides. Overall, resistance rates varied, depending on the geographic area where the study was performed. In the US, 31% of *S. pneumoniae* strains were resistant to azithromycin (or erythromycin). Asian countries had the highest rates of macrolide resistance, averaging 80%, while the rate of macrolide resistance for the rest of the world ranged from 11.6 to 29.1% (45).

The distribution of mechanisms of resistance was determined for 1043 macrolide-resistant *S. pneumoniae* isolates from the PROTEKT 1999-2000 study and were as follows: 35.3% *mef*, 56.2% *erm*(B), 6.8% both *mef* and *erm*(B), 0.2% *erm*(TR) and 1.5%

had a mutation in one to four of the 23s rRNA alleles. The mutations were primarily A2059G, A2058G and one C2611G, and three of the isolates with A2059G mutations also had G95D in riboprotein 22 (46). Mechanisms of resistance varied widely between countries and geographic regions with *mef* predominating in North America and *erm*(B) in Europe (47).

PROTEKT US was initiated in 2000 to monitor resistant phenotypes and genotypes of the major respiratory pathogens across the US. For the 2000/2001 respiratory season the distribution of macrolide resistance mechanisms for 3,053 macrolide resistant *S. pneumoniae* isolates collected from outpatients with RTIs was 70.7% *mef*, 17.3% *erm*(B), 9.8% both mechanisms, 0.1% *erm*(TR) and for 2%, no mechanism was detected (48). The proportion of macrolide resistance due to *mef* in the US has remained constant at approximately 70% since 1994 (41).

During six respiratory disease seasons in the United States, the rate of non-susceptible *S. pneumoniae* for azithromycin/erythromycin increased from 13-14% in 1996 to 28% in 2001-2002, but remained constant at 34% for the same isolates that were non-susceptible to penicillin (n = 27,828 isolates) (44). The MIC₉₀ increased by one dilution over the 1998-2002 years to 16 µg/ml. The highest rates of resistance for azithromycin (33%) were in bacteria isolated from patients who were < 18 years old. However, this youngest patient population also experienced the highest levels of resistance for penicillin (25%), trimethoprim/sulfamethoxazole (TMP-SMX) (38%) and ceftriaxone (3%) compared to the comparable older age groups. As seen in numerous previous studies, there was a significant positive correlation between resistance to penicillin and resistance to azithromycin, TMP-SMX, ceftriaxone and levofloxacin. The rate of clindamycin resistance in these studies ranged from 4.7--7.2% (21, 42, 49).

Resistance rates of *S. pneumoniae* vary by region of the world and also within some regions as documented during 1998-2000 by scientists of the Alexander Project (19). For azithromycin, the lowest MIC₅₀₋₉₀ values were measured in Africa (0.06/0.25 µg/ml; n = 540) and Eastern Europe (0.12/0.12 µg/ml; n = 1109). The highest MIC₅₀₋₉₀ values were in the Far East (> 32 µg/ml; n = 730) where 33% of the isolates were susceptible to azithromycin, but only 19% were susceptible in Hong Kong. In Western Europe the MIC₅₀₋₉₀ values were 0.12/> 32 µg/ml (n = 3,328). However, susceptibility rates to azithromycin ranged from a low of 47% in France to a high of 96% in the Netherlands. Clindamycin susceptibility rates were similar to azithromycin susceptibility rates for all 12 Western European countries evaluated, which indicate that the methylation of the ribosome (*erm*) mechanism of resistance accounts for most of the resistance in those isolates. In contrast, in the US, 71% of 2,432 isolates were susceptible to azithromycin, but 90% were susceptible to clindamycin indicating a mixture of efflux and methylation resistance mechanisms.

In the PROTEKT surveillance conducted in Hong Kong, South Korea and Japan during 1999-2000, azithromycin susceptibility rates were relatively low (27). Twenty percent of the 515 *S. pneumoniae* isolates were susceptible to azithromycin ranging from 12%

susceptible in South Korea (n = 137), 22% susceptible in Japan (n = 308) and 29% susceptible in Hong Kong (n = 70). Of the 406 erythromycin resistant isolates, 37% had *mef*, 49% had *erm*(B), and 13% contained both *mef* and *erm*(B).

There was no difference in susceptibility between North American *S. pneumoniae* strains isolated from community-acquired respiratory tract infections (n = 5,467 from 1997-2001) or from hospitalized patients with pneumonia (n = 1,048) (50). For erythromycin, 77-79% were susceptible to erythromycin, 93-96% were susceptible to amoxicillin/clavulanate, and 99% were susceptible to levofloxacin.

The European Antimicrobial Resistance Surveillance System (EARSS) is an international network of 600 laboratories in 27 countries which collects comparable and validated antimicrobial susceptibility data for selected pathogens including invasive *S. pneumoniae*. In 2002, the overall rate of erythromycin non-susceptible isolates was 17% of the 5,816 invasive *S. pneumoniae* isolates. The highest rates of macrolide resistance were found in Italy, France and Belgium (> 30%), while the lowest rates of macrolide resistance were found in the Czech Republic (< 3%), followed by the Scandinavian countries, the Netherlands, Austria and Iceland (3-10%) (16). In most countries macrolide resistance was more prevalent among penicillin intermediate and resistant *S. pneumoniae* isolates (24). An MIC₉₀ of ≥ 8 $\mu\text{g/ml}$ was observed for three macrolides (erythromycin, clarithromycin and azithromycin) in a 1999-2000 surveillance study of 1,531 pneumococcal isolates from the US. The overall rate of macrolide resistance was 26%. Of the penicillin-resistant *S. pneumoniae* isolates, 76-78% were resistant to the macrolides (clarithromycin, erythromycin, azithromycin) while 43% of penicillin-intermediate strains were macrolide resistant (41). According to a recent surveillance report of 2,245 respiratory isolates of *S. pneumoniae*, macrolide resistance in Canada is approximately one-third (11%) of that seen in the US (51).

Several publications have appeared that compare *S. pneumoniae* resistance rates to clinical cure rates obtained with azithromycin or clarithromycin therapy (52, 53). Both studies suggest that the resistance seen in surveillance studies is not borne out by clinical failure rates for these two macrolides. There have been recent discussions regarding whether azithromycin and clarithromycin could cover the *S. pneumoniae* and *S. pyogenes* strains that harbor *mef*, a resistance determinant that mediates efflux of 14- and 15-membered macrolides (53, 54). Many of these strains have MICs of ≤ 8 $\mu\text{g/ml}$ to azithromycin. The accumulation of drug in inflammatory cells and transport of azithromycin to the site of infection may explain why the correlation between *in vitro* susceptibility testing and clinical outcome in *mef* infections may not be strong. For example, in a prior sponsor study on the single dose regimen of azithromycin for acute otitis media treatment, six of seven patients with *mef*-containing *S. pneumoniae* were clinically cured (55). Azoulay-Dupuis et al. suggested that highly resistant strains may have reduced virulence (56).

S. pyogenes. Although there has been an increase of erythromycin-resistant *S. pyogenes* in recent years, during 2000 – 2001, the frequency of erythromycin-resistant *S. pyogenes* in the U.S. remained relatively low at 9% for 484 isolates with a 1.4% resistance rate for

clindamycin (67). In Canada and the US during 2000, 5.2% of 383 isolates were not susceptible to erythromycin (22). The macrolide resistance rates for children 0 – 12 years old during 1999-2000 ranged from a high of 38% for 29 Italian children, to 20% for 59 Japanese children, to 6-10% for 138 Latin American children, to a low of 2-4% for Swiss, Canadian and American children (60). However, significant outbreaks since the 1970's have been reported in Japan, Finland, England, and Australia (68). From ten Central and Eastern European countries in 1999-2000, 124/1011 (12.3%) *S. pyogenes* isolates were not susceptible to erythromycin, in contrast to 1.1-1.5% non-susceptible to telithromycin. The primary mechanism of resistance was mediated by *erm(A)* (75/124, 60%) and by *mef* (29/124, 23%, ref 64). During 2000-2001 in Germany, 13.7% of the 307 *S. pyogenes* were resistant to erythromycin using mainly *mef* (22/42 resistant isolates) and *erm(A)* (16/42 resistant isolates, ref 26). This current resistance rate has doubled from 6.0% and 7.9% in two separate studies during 1995-1998 in Germany (cited by ref 26.) In Taiwan, during 2000-2001, 78% of the 419 *S. pyogenes* clinical isolates were not susceptible to erythromycin primarily due to *mef*, and 17% were non-susceptible to telithromycin (65). In Spain during 1999-2001, erythromycin non-susceptible isolates accounted for 23% of the 412 *S. pyogenes* isolates that were collected. *Mef* was the main mechanism in 73% of the 95 resistant isolates (62).

Critchley et al. conducted a study to determine antimicrobial susceptibilities to isolates of *S. pyogenes* collected between January and October of 1999 from the US (57). Of 2,742 *S. pyogenes* isolates, 2039 (74.4%), 405 (14.8%), and 148 (5.4%) were isolated from the upper respiratory tract, skin and soft tissue, and blood, respectively. Overall, 2,548 (92.9%) of 2,742 isolates demonstrated susceptibility to azithromycin (MIC, < 0.5 µg/ml). Furthermore, although resistance was observed in 170 (6.2%) isolates, 152 (89.4%) of these isolates displayed low-level resistance (MIC, 2-8 µg/ml). Regional variation in azithromycin resistance was minor, with rates in seven of the nine U. S. regions differing by < 1.5% (range, 6.2%-7.7%). Rates of resistance were found to differ significantly by patient age and specimen source. Overall, azithromycin resistance was significantly higher ($p < 0.001$) among patients 15-64 years (8.3%) than among patients aged < 14 years (4.3%) or > 65 years (4.6%). Furthermore, of the 2,039 upper respiratory tract isolates, 1917 (94%), 16 (0.8%), and 106 (5.2%) were susceptible, intermediate, and resistant to azithromycin, respectively.

Martin et al. examined the resistance patterns of group A streptococci to erythromycin and clindamycin in children from a single elementary school in Pittsburgh as part of an ongoing longitudinal study (69). Throat swabs were obtained for culture approximately every two weeks from each child during each school year and each time a child had a respiratory tract illness from October 1998 to May 2001. A total of 2,200 throat cultures were obtained during the first two years of the study (October 1998-May 2000); 322 cultures (15%) were positive for group A streptococci, and all isolates were sensitive to erythromycin. During the third year of the study (October 2000-May 2001), 318 isolates (18%) from 60 children were positive for group A streptococci; 153 of these isolates (48%) were resistant to erythromycin (MIC required to inhibit the growth of 50% of organisms [MIC₅₀] = 32 µg/ml, MIC₉₀ = 32 µg/ml). All isolates were sensitive to clindamycin. Of 100 isolates that were randomly selected from the surrounding

community in April, May, and June of 2001, 38% were resistant to erythromycin. Upon evaluation with field-inversion gel electrophoresis, the outbreak of resistance to erythromycin was determined to be due to a single clone. Based on results of the double-disk diffusion test, the mechanism of resistance was found to be of the M phenotype.

Activity Against Aerobic Gram-Negative Microorganisms

H. influenzae. A 2000-2001 *H. influenzae* surveillance study of 1434 respiratory isolates from the US showed that 99.7% were susceptible to azithromycin with an MIC₉₀ of 2 µg/ml, ref 70). Another large worldwide surveillance study reported the same (24). A similar surveillance study with recent US isolates of *H. influenzae* and *Moraxella catarrhalis* showed 99.8% and 100% of isolates susceptible to azithromycin, respectively (71). The azithromycin MIC₉₀ for *H. influenzae* was 2 µg/ml compared with 16 µg/ml for clarithromycin. Another US study reported 99.7% of 1032 *H. influenzae* strains susceptible to azithromycin (MIC₉₀ of 2 µg/ml, ref 72). *H. influenzae* harbor intrinsic efflux pumps homologous to the *acrAB-tolC* tripartite system seen in many Gram-negative bacteria, but no acquired resistance mechanisms to macrolides have been found in this species (73).

In 2001-02, *H. influenzae* MIC₅₀₋₉₀ values for azithromycin were 1-2 µg/ml, 0.5-1 µg/ml for amoxicillin/clavulanate, and 0.015-0.015 µg/ml for levofloxacin for 1,393 isolates from the US (74). For the 8,523 *H. influenzae* isolates collected for the Alexander Project in 1998-2000, Jacobs et al. report identical worldwide and large geographic regional susceptibility results as those reported in the US by Karlowsky et al. (19, 74). Nearly identical MIC₅₀₋₉₀ data were also reported by Johnson et al. for 9,320 *H. influenzae* isolates collected from North America, Latin America and Europe during 1997-2001 (75). In 1999-2000, 1% of the 612 *H. influenzae* isolates from Canada and the U.S. were resistant to azithromycin (22). In the PROTEKT survey during 2000-2001, the MIC₉₀ for the 2,706 US *H. influenzae* isolates from the US was 4 µg/ml for azithromycin and telithromycin (43).

M. catarrhalis. For *M. catarrhalis* in 2001-02, the MIC₅₀₋₉₀ values were 0.03 µg/ml for azithromycin, 0.12-0.25 µg/ml for amoxicillin/clavulanate, and 0.03-0.06 µg/ml for levofloxacin among 910 isolates from the US (74). Worldwide during 1998-2000, the *M. catarrhalis* susceptibility values for 874 isolates were similar to those reported by Karlowsky et al. in the US (19, 74). The MIC₅₀₋₉₀ values were 0.06-0.12 µg/ml for azithromycin, < 0.12-0.25 µg/ml for amoxicillin/clavulanate, and 0.03-0.06 µg/ml for levofloxacin. Similarly, Johnson et al. report that 4,050 *M. catarrhalis* strains from North America, Latin America and Europe during 1997-2001 were > 99% susceptible to erythromycin, amoxicillin/clavulanate, and levofloxacin (75).

Activity Against Aerobic Atypical Bacteria

At this time, there are no confirmed reports on emerging resistance to azithromycin in atypical pathogens (*Mycoplasma*, *Chlamydia*, *Legionella*). *Legionella* remain susceptible to azithromycin, with a MIC range of 0.03–0.5 µg/ml (76). Azithromycin MIC₉₀ values of ≤0.008 µg/ml, ≤0.0005 µg/ml, and ≤0.001 µg/ml were reported in three studies against

130, 41, and 97 *Mycoplasma pneumoniae* isolates, respectively (76-78). Azithromycin and erythromycin were the only two antibiotics of seven tested that did not select for macrolide resistance to *M. pneumoniae* after 50 passages in sub-inhibitory concentrations of the respective antibiotics (79). In two studies, the MIC₉₀ value for *Chlamydophila pneumoniae* was 0.25 µg/ml for 19 isolates and the MIC₉₀ value was 0.125 µg/ml for 20 isolates mainly from the US (80, 82). In a smaller study of nine isolates, the MIC values for azithromycin ranged from 0.06-0.12 µg/ml (76). Azithromycin is bactericidal against *Chlamydia* species (82-84). The MBC for 50 *Chlamydia trachomatis* isolates was < 0.25 µg/ml (81).

Reviewer comments: A summary of the *in vitro* activities of azithromycin, clarithromycin, and erythromycin against select respiratory pathogens is given in Table 2 below (194). Macrolides are more active *in vitro* against Gram-positive and atypical agents than against Gram-negative pathogens. Erythromycin tends to be two to four times more active against Gram-positive organisms than azithromycin. However, the MIC values for azithromycin against Gram-positive organisms are still within clinically achievable therapeutic levels.

Table 2. Comparative *in vitro* activity of macrolides against select pathogens.

	Azithromycin MIC ₉₀ (µg/ml)	Clarithromycin MIC ₉₀ (µg/ml)	Erythromycin MIC ₉₀ (µg/ml)
<i>H. influenzae</i>	1 - 4	8 - 16	4 - 16
β-lactam +	1 - 4	8 - 16	4 - 16
β-lactam -	1 - 4	8 - 16	4 - 18
<i>M. catarrhalis</i>	0.06	0.25	0.25
β-lactam +	2	0.19	0.25
β-lactam -	0.094 - 2	0.125	0.25
<i>S. pneumoniae</i>	0.12 - 4	0.015 - 16	0.06 - 4
Pen S	0.12 - 4	0.06 -	0.06 - 0.12
Pen I	16 - > 32	16 - > 32	8 - > 32
Pen R	16 - > 32	8 - > 32	8 - > 32
<i>S. aureus</i>			
Methicillin S	1 - 8	0.05 - > 8.7	1 - > 10.7
Methicillin R	> 27.3 - 128	> 59.9	> 64 - > 100
<i>S. pyogenes</i>	0.12 - 0.5	0.015 - 0.16	0.03 - 0.18
<i>L. pneumophila</i>	0.5 - 1.2	0.06 - 0.22	0.46 - 0.5
<i>N. gonorrhoeae</i>	0.05 - 0.25	0.25 - 2	0.25 - 2
<i>C. pneumoniae</i>	0.25 - 0.33	0.11 - 0.25	0.19 - 0.5
<i>C. trachomatis</i>	< 0.125 - 0.25	0.008 - 0.125	0.06 - 2
<i>H. ducreyi</i>	0.004	0.015	0.03
<i>M. pneumoniae</i>	0.00024 - < 0.01	0.008 - 0.5	0.011
<i>B. pertussis</i>	0.06	0.03	0.03

Source: Ref. 194

Gram-positive organisms that are resistant to erythromycin are also cross-resistant to both azithromycin and clarithromycin. All macrolides are generally active against *Streptococcus pneumoniae* isolates that are susceptible to penicillin, however, significant cross-resistance occurs for pneumococcal isolates that are highly resistant to penicillin. These characteristics are demonstrated by recent data presented in Table 3, below (195).

Table 3. Azithromycin susceptibility among respiratory pathogens.

organism	N	MIC ₅₀	MIC ₉₀	range	% susceptible
MSSA	196	0.5	>64	0.5-->64	78.1
MRSA	132	>64	>64	0.25-->64	3.8
<i>S. agalactiae</i> (Group B, BHS)	133	0.06	16	<0.06-->32	75.2
<i>S. pyogenes</i>	119	0.06	0.06	<0.06-->32	91.6
<i>S. pneumoniae</i>	417	0.06	16	<0.06-->32	74.3
<i>S. pneumoniae</i> (Pen-S)	249	0.06	0.12	<0.06-->32	94.4
<i>S. pneumoniae</i> (Pen-I)	70	0.06	>64	<0.06-->64	58.6
<i>S. pneumoniae</i> (Pen-R)	98	2	>64	<0.06-->64	34.7
<i>S. pneumoniae</i> (macrolide-R) ^b	90	8	>64	2-->64	NA
<i>S. viridans</i> ^a	92	0.06	8	<0.06-->32	68.5
<i>H. influenzae</i>	300	1	2	<0.06-->32	99
<i>H. parainfluenzae</i>	30	1	2	<0.06-->32	93.3
<i>M. catarrhalis</i>	231	0.06	0.06	<0.06--0.25	NA

^b=macrolide-R defined as azithromycin resistant >2 µg/ml

Source: Ref. 195

Erythromycin is not as active against Gram-negative organisms as azithromycin and clarithromycin. The newer macrolides are more active against *H. influenzae* with azithromycin showing the greatest activity. Clarithromycin demonstrates lower MIC values against *Legionella pneumophila* and *Chlamydia pneumoniae* than azithromycin and erythromycin, however, MIC values tend to be lower for azithromycin than for the other two agents against *Moraxella catarrhalis* and *Mycoplasma pneumoniae*.

The Applicant does not present recent susceptibility data for any organisms listed in either the first or second lists found in the package insert. Microbiology recommends that recent susceptibility data (within the last 3-5 years) for 100 clinical isolates identified in the second list of the package insert be presented in the NDA. While the Applicant, has neither generated this data nor supplied this data from the literature, these data are available from the recent literature and have been provided here by the Reviewer. Data for 100 recent clinical isolates were found for the following organisms pertinent to this application: *S. pneumoniae*, *S. aureus*, *H. influenzae*, *Moraxella catarrhalis*, *S. pyogenes*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*. Data for less than 100 recent clinical isolates were found for *H. parainfluenzae*, viridans streptococci, Streptococci (groups C, F, G), *Bordetella pertussis*, *Haemophilus ducreyi*, *Neisseria gonorrhoeae*, *Peptostreptococcus* sp., *Prevotella bivia*, and *Chlamydia trachomatis*.

Effects of Miscellaneous Factors on Activity

The original submission on oral azithromycin contained experimental data demonstrating how the *in vitro* potency of macrolides and especially azithromycin is underestimated by the lowering of pH by CO₂, which have been borne out by recent studies (p. 32-40 of Section 7, NDA 50-670; 85, 86). This is especially true when the Etest procedure is used, as incubation in CO₂ is recommended. Currently, the Etest is not acceptable for testing of macrolides under CLSI (NCCLS) guidelines (87). Another study has documented the increased potency of azithromycin against a broad spectrum of anaerobic species when

the pH of the medium is controlled. MIC's determined at acidic pHs are also not physiologically relevant as the mean pH of effusion from acute otitis media is 7.7 (88).

Effect of Medium, Temperature and Storage

The change in formulation of azithromycin will not affect the form of azithromycin that is used for the various types of susceptibility testing, including disk diffusion and liquid broth methods. Therefore, the manufacturer's suggestions for storage and expiration dates of those materials should be followed. For *in vitro* testing, the media and incubation temperatures recommended in the current CLSI (NCCLS) guidelines for testing of the various pathogens should be used (12-14).

Effect of Serum

As detailed on pages 32- 33 of Section 7, NDA 50-670, "The addition of 95% inactivated serum to the media was shown to greatly enhance the activity of azithromycin and erythromycin. However, as shown in Figure 4 of section 7, NDA 50-670, page 39, this appears to be completely explained by an increase in the pH of serum-containing media during incubation. Similar results were obtained with an *E. coli* strain. The effect is much less pronounced in the presence of 50% serum."

Effect of Inoculum

As stated on page 32 of Section 7, NDA 50-670, "In working with azithromycin, it has been found that inoculum size does not have a great effect on *in vitro* potency. Varying the inoculum from $> 5 \times 10^6 - 5 \times 10^4$ CFU/ml had no effect on azithromycin's *in vitro* potency (< 1 dilution) against three *S. aureus* and ten *Enterobacteriaceae* strains (Table 6, section 7, NDA 50-670, page 34). An inoculum effect was observed with ampicillin, especially with the *S. aureus* strains (MIC decreased from 2000 to 6.25 $\mu\text{g/ml}$). All laboratory methods described in this document used the inoculum preparation methods described by CLSI (NCCLS), unless specified otherwise.

Bactericidal Activity

The use of azithromycin to treat respiratory tract infections may have advantages over using cell wall-active antibiotics that cause lysis of cells. Although azithromycin is cidal to many *S. pneumoniae*, *M. catarrhalis*, and *H. influenzae* strains, the cells largely remain intact (89). This is in contrast to β -lactam antibiotics, which often result in cellular lysis, releasing proinflammatory components of the bacterial cell surface that have been shown to exacerbate the inflammatory response (90). Azithromycin also has intrinsic anti-inflammatory properties that may contribute to dampening the destructive immune response triggered by proinflammatory cellular components (91-93). Recent studies conducted by Pfizer scientists showed that azithromycin at 1, 4 and 8-times the MIC of 4 $\mu\text{g/ml}$ killed two clinical isolates of *mef* containing *S. pneumoniae* (2 log reduction in viable counts from the initial inoculum) within 6 to 8 h while the non-treated cells increased at least 1000-fold from their initial viable cell count (Appendix 1).

Post-antibiotic Effect (PAE) and Post-Antibiotic Sub-MIC Effects

Reports of PAEs of between 2.2 and 4.7 h have been observed for *S. pneumoniae* and

H. influenzae (94-96). One paper reported a PAE > 12 h for a penicillin-resistant isolate of *S. pneumoniae* and a mean PAE of 2 h for *M. catarrhalis* strains (97). The PAE of 5X the MIC of azithromycin against 20 pneumococcal strains ranged from 1 to 6 h. The *in vivo* azithromycin PAE was 11 h for *S. pneumoniae* using the neutropenic mouse thigh model (94). A significant decrease in the virulence of post-antibiotic-phase pneumococci was measurable by increases in LD₅₀ values (98). The sub-MIC affect of azithromycin at 0.1X, 0.2X, or 0.3X the MIC for *S. pneumoniae* was 1.7, 6.2, or 12.0 h, respectively. The *in vivo* postantibiotic effects seen with azithromycin are partly a function of its long half-life and account for significant suppression of growth for extended periods even at sub-MIC concentrations (94, 98).

Synergy Studies

Azithromycin is not part of a fixed combination of drug products. Therefore, no checkerboard assays are presented by the Applicant. Additionally, since azithromycin has been marketed, no synergistic or antagonistic combinations of azithromycin and any other antibiotics have been reported *in vitro*, *in vivo*, or in humans.

Reviewer's comments: Susceptibility testing of macrolides is influenced by a number of different factors such as pH, the addition of serum and/or incubation in a CO₂ environment. Such conditions have either increase or decrease the MIC measurement (196). The Applicant has accounted for this in the susceptibility testing.

Mechanisms of Resistance

Several reviews of macrolide resistance mechanisms in clinical strains of *S. pneumoniae* and *S. pyogenes* were authored by the Applicant's researchers (2, 5, 99). There are two predominant resistance determinants in clinical isolates of *S. pneumoniae*: *mef* and *erm*(B). *Mef* encodes an efflux pump that mediates resistance to 14- and 15-membered macrolides only. The increase of macrolide resistance in pneumococci in the US is primarily due to dissemination of strains harboring this determinant (100-103). Originally, *mef*(E) was described in *S. pneumoniae* and *mef* in *S. pyogenes* (101, 103, 104). The two genes are now collectively referred to as *mef* due to 90% identity at both the DNA and amino acid sequence levels (4). The *mef* gene has been described in a variety of other species, among which are *Streptococcus agalactiae*, *viridans* streptococci, *Streptococcus milleri*, *Streptococcus mitis*, and several other species (105-110).

A member of the *erm* gene family mediates the other widespread mechanism of resistance to macrolides. An *erm* gene encodes a 23s rRNA methyltransferase that adds methyl groups to adenine 2058 of the 23s rRNA (*E. coli* rRNA numbering system). This nucleotide is in domain V and has been found to interact with lincosamides and streptogramin B, in addition to macrolides, with methylation interfering in the binding of these compounds and resulting in a phenotype known as MLS_B resistance (2, 4-6, 99).

The distribution of *erm*(B) and *mef* varies geographically. In many countries in Europe, the macrolide-resistant *S. pneumoniae* are primarily associated with *erm*(B) (106, 111-114). In contrast, *mef* is found in over two-thirds of the macrolide-resistant *S.*

pneumoniae in the US (100, 102). A study in Japan reports that approximately 60% of erythromycin-resistant pediatric *S. pneumoniae* isolates had the *erm(B)* gene, while 40% had the *mef* gene (115). No correlation between the presence of either macrolide resistance determinant and resistance to penicillin was found in the Japanese study. Another recent study showed a correlation with penicillin G and macrolide resistance as observable by the MIC₅₀/MIC₉₀ values of $\leq 0.125/\leq 0.125$, $\leq 0.125/4$, and $1/\geq 64$ $\mu\text{g/ml}$ for penicillin-susceptible, penicillin-intermediate, and penicillin-resistant isolates, respectively, in the US (37).

Although the majority of macrolide-resistant pneumococcal strains can be accounted for by containing either *mef* or *erm(B)*, there have been recent sporadic reports of clinical isolates that have either mutations in three of the four 23s rRNA alleles at position 2059 or a mutation in a highly conserved region of ribosomal protein L4 (117). A second type of *erm* gene, *erm(A)* [subclass *erm(TR)*] has also been described in clinical isolates of *S. pneumoniae* in Greece and Spain (118, 119). Originally, this subclass of *erm(A)* was described in *S. pyogenes* and seems to be widespread in macrolide-resistant isolates from Finland (108, 120).

The frequency of macrolide resistance in *S. pyogenes* is lower than what is observed in pneumococcal strains in North America with the majority of isolates carrying *mef(A)* (58, 103, 121). The majority of erythromycin-resistant *S. pyogenes* in Western Europe also have the efflux mechanism (111, 122-125). Despite the shared ecological niches of *S. pyogenes* and *S. pneumoniae*, in some countries (Spain, Belgium, Italy, and France) the *erm* resistant determinants predominate in pneumococci while the efflux determinant is found predominantly in *S. pyogenes*.

Efflux can be mediated by either a pump with narrow specificity (e.g., Mef, Msr(A)) or a pump with broad specificity (e.g., AcrAB-TolC, MexAB-OprM, AmrAB-OprA, MtrCDE, MdfA, Cmr) (2, 5, 99, 126, 127). The AcrAB-TolC pump, or at least one homolog of this tripartite pump found exclusively in Gram-negative bacteria like *E. coli* and *H. influenzae*, is responsible for the innate resistance of Gram-negative species to macrolides and other hydrophobic compounds.

In vitro laboratory experiments indicate that resistance does not emerge rapidly to azithromycin, even when the organisms are grown in the presence of sub-MIC concentrations of azithromycin. However, resistant *S. pneumoniae* strains have been selected after very long-term passage (generally between 25-50 passages) in sub-inhibitory concentrations of macrolides (128-132). In studies where resistance development was monitored for five susceptible pneumococcal strains, more passages with azithromycin were required to observe resistance mutation emergence (average number = 28 subcultures) than with either clarithromycin (15 subcultures) or erythromycin (23 subcultures) (132). The resistance that develops in *S. pneumoniae* strains after passage results from a mutation in 23s rRNA, specifically in nucleotides 2057-2059 or 2611 in domain V, or as a consequence of mutations in ribosomal proteins L4 or L22 (128, 129, 131).

Pneumococci have four rRNA operons, and mutations in 23s rRNA or L4 protein have only recently been defined in either isolates passaged with a macrolide or in clinical strains (117, 131, 133). Although mutations in L4 ribosomal protein were determined to be the second most prevalent mechanism in pneumococcal isolates from Eastern and Central Europe, only one isolate has been described in North America (Canada) that has a mutation in L4 as its sole resistance mechanism (116, 117).

In vivo infection models designed to test the hypothesis that sustained levels of azithromycin in tissues might lead to emergence of resistant strains yielded no resistant isolates of *Staphylococcus aureus* or *Escherichia coli* (130). In mouse infection models challenged with *M. avium*, resistance to macrolides developed, but resistance appeared less frequently with azithromycin than with clarithromycin (134). The hypothesis that sustained tissue concentrations of azithromycin lead to a "selective window" for resistance emergence has not been borne out in clinical studies (135). For example, in pediatric pharyngitis/tonsillitis studies, no significant differences in susceptibility of persistent *S. pyogenes* isolates present at day 30 were observed when compared to the susceptibility of isolates prior to azithromycin therapy (5-day dosage regimen) (136). Along with the very extensive passages required for resistance selection, the positive postantibiotic and sub-MIC effects of azithromycin may also be involved in the lack of resistant isolates recovered post-treatment (94-96, 98, 137).

There have been conflicting reports on the effect of azithromycin on nasopharyngeal (NP) carriage. These conflicts may arise as a result of the differences in study designs; some studies measure the impact of antibiotic treatment within 3-4 days of initiation of treatment versus two weeks to more than one month after antibiotic treatment (136, 138-141). There are no studies in which seasonal changes in NP carriage is taken into account nor does assessing carriage in an untreated population over time control these studies. Also, an initial higher prevalence of antibiotic-resistant NP flora in a specific geographical area will impact transmission and subsequent NP colonization. Given all these limitations, the studies do emerge with one common theme - antibiotics have selective effects, killing susceptible, and in some cases, a certain percentage of nonsusceptible microorganisms. The greatest impact on the NP carriage is seen during or shortly after drug treatment (136, 138-142). For example, in patients treated with amoxicillin/clavulanate, carriage of *S. pneumoniae* significantly decreased (140). The authors conclude that a greater percentage of the remaining pneumococci were resistant, but there is no way to assess if the resistant strains were resident before drug treatment or obtained by exposure to a carrier of penicillin-resistant pneumococci.

The reduction in carriage of *S. pneumoniae* is seen in a number of studies and for multiple antibiotics, including azithromycin. In a recent study designed to evaluate the efficacy of azithromycin for eradication of oropharyngeal Group A streptococci as well as its impact on the NP colonization rate of *S. pneumoniae*, NP colonization rates for pneumococci decreased from 46% to 12% by day 17 and were 20% by day 32 (142). The prevalence of azithromycin-resistant isolates increased from 2% to 4% by day 17 and to 8% by day 32. Analyses by serotyping, genotyping, and antimicrobial susceptibility provided no evidence that any individual strain present before therapy became resistant or

was present and previously missed. Thus, it is likely that antibiotic-resistant strains were acquired by contact with other carriers. Consistent with this, the majority of studies that evaluate the resistant or persistent strains after antibiotic therapy usually find only a small proportion of the pneumococci to be clonally related to the initial isolate. One recent study found that the carriage of non-pneumococcal α -hemolytic streptococci increased after either amoxicillin/clavulanate or azithromycin treatment. However, there was no data to show that the α -hemolytic streptococci that were present before therapy became resistant after therapy. Interestingly, the more highly resistant pneumococcal strains do not appear to account for any greater proportion of acute otitis media illness despite seasonal fluctuations of NP carriage (143).

More exploration on the different populations that constitute the NP flora may be needed to adequately interpret the impact of antibiotic usage. The alteration of NP flora might permit habitation of new organisms or provide a reservoir of non-pneumococcal α -hemolytic streptococci that are antibiotic resistant.

With regard to *H. influenzae* carriage, the impact of either β -lactam or azalide treatment is less profound (140). Azithromycin used for community treatment of children with trachoma was shown to have no effect on increasing the incidence of *S. pneumoniae* resistance to azithromycin in NP cultures or in conjunctival bacterial flora (144, 145). Lower levels of resistance in *M. avium* isolates were associated with azithromycin administration, either prophylactically or therapeutically, compared to clarithromycin administration (134, 146) (also see MAC Treatment sNDA recently submitted by Applicant.)

The Applicant asserts that although some have proposed that increased usage of macrolides leads to increased resistance, this cannot be the only factor that accounts for resistance (147). Macrolide resistance in the United States rose from 1986-1997 while overall macrolide prescriptions decreased (147). The noted increase in macrolide resistance has been linked to "long-acting" macrolides; however, this correlation does not necessarily hold as increases in macrolide resistance in the US are apparent over the 1986-1997 time period, when there was little use of long-acting macrolides like clarithromycin and azithromycin (148).

In Zagreb, Croatia, the consumption of azithromycin rapidly rose from 0.43 DDD/1000 inhabitants/day in 1991 to 1.39 DDD/1000 inhabitants/day in 1996 with no increase in macrolide resistance in *S. pneumoniae* (11% and 10% macrolide resistance in 1996 and 1997, respectively) or *S. pyogenes* (9% vs. 10% in 1996 and 1997, respectively) (149). In Slovenia, as macrolide consumption doubled from 1.89 DDD/1000 inhabitants/day in 1994 to 3.84 DDD/1000 inhabitants/day in 1999, there was a statistically significant increase on the resistance rates of *S. pneumoniae* (from the upper respiratory tract) and on *S. pyogenes*, but not on respiratory tract isolates of *H. influenzae* and *M. catarrhalis* (150). These studies differ from others that have ascribed a correlation of macrolide consumption to rises in resistance rates (151, 152). One major difference in the Eastern European studies is that azithromycin accounted for $\geq 50\%$ of the prescriptions in Slovenia or Croatia whereas in other countries, azithromycin was a much smaller

component of macrolide use. The high intracellular levels and concentrations of azithromycin at the sites of infection could suppress emergence of bacterial cells with reduced susceptibility as compared to other antibiotics (52). In two studies, middle ear fluid concentrations of azithromycin were found to exceed the MIC₉₀ values for penicillin-susceptible and relatively penicillin-resistant pneumococci and the MIC₅₀ for highly penicillin-resistant pneumococci (153). In addition, 48 hours after an initial dose of 10 mg/kg, followed by one dose of 5 mg/kg, the concentration of azithromycin in middle ear fluids was 9.43 µg/ml, a value that exceeds the MIC₉₀ for *H. influenzae* and even many strains of pneumococci that harbor *mef*. This may explain the data from Eastern Europe (Croatia and Slovenia) as well as the reduced macrolide resistance that was observed in Finland when erythromycin use was decreased, but azithromycin became the leading prescribed oral macrolide (151, data on file, Pfizer Inc. NY, NY).

Methods for assessing *mef*, *erm(B)* and *erm(TR)* in the bacterial isolates collected in these trials are described in Section 5.3.5.4.1.IV.A.4. of this NDA submission.

Reviewer's comments: The two mechanisms of macrolide resistance are *altered target site* and *efflux*. The *altered target site* mechanism is mediated by the synthesis of ribosomal RNA methylases resulting in methylation of an adenine residue in the 23s ribosomal RNA of the 50s subunit. This leads to macrolide, lincosamide and streptogramin B resistance and is coded by the *ermB* gene (also referred to as *ermAB*) (197, 198). *S. pneumoniae* organisms possessing the *ermB* gene usually express high level resistance (MIC₉₀ ≥ 64 µg/ml). Results obtained by the Applicant are consistent with these observations.

Efflux has emerged as an important mechanism of resistance to the macrolides. Efflux related to the ability of organisms to pump antimicrobial agents (i.e., macrolides) out of the bacterial cells and is encoded by the *mefA* gene (198, 199). The net result is an insufficient concentration of drug within the cell to inhibit protein synthesis, thereby rendering the organism resistant. *S. pneumoniae* isolates possessing the *mefA* gene usually express low level resistance (MIC₉₀ ≥ 4 µg/ml) to macrolides but demonstrate no cross-resistance to lincosamides and streptogramin B (200).

Of the two mechanisms of resistance, *ermB* mediated resistance is more prevalent than *mef* (*mefA*) mediated resistance for *S. pneumoniae* and the documentation of the type of resistance in prevalence studies is essential both clinical (due to level of resistance) and epidemiologic.

There is little doubt that the increasing antimicrobial resistance documented globally is significant not only for the macrolides but for all other classes of antimicrobial agents as well. Many surveillance studies have repeatedly documented differences in prevalence rates for macrolide resistant pathogens in many different geographical regions of the world. Local resistance patterns could influence antimicrobial use as susceptibility rates may vary by institution, state or province, country, continent, etc. Local and recent susceptibility data may be of utmost importance when selecting antimicrobial therapy.

Factors that may contribute to increasing macrolide resistance include patient compliance, inappropriate usage, drug pharmacokinetics and pharmacodynamics, as well as numerous intrinsic bacterial properties (201). The Applicant asserts that other factors must be involved in the development of resistance to azithromycin. Data summarized by Carbon and Poole indicate that the prevalence of macrolide resistance is closely related to the extent to which the agents are used (202). These observations have been most notable for *S. pneumoniae* and *S. pyogenes*.

Resistance in *S. pneumoniae*.

The persistence of azithromycin (and also macrolide resistance) is an important consideration in the epidemiology of *S. pneumoniae* infections. The increase in antimicrobial resistance in *S. pneumoniae* in recent years has been well documented in a variety of surveillance studies (21, 41, 203, 204, 205). The prevalence of penicillin resistance in *S. pneumoniae* varies on a global level and has recently been reported to range from 5% to nearly 70% (204, 205).

Although it is difficult to determine whether resistance will develop clinically, numerous *in vitro* assays have been used to predict the rate and magnitude at which bacterial resistance may occur (203, 206). Koeth et al. conducted a study to determine the effect of repeated exposure to sub-inhibitory concentrations of azithromycin and amoxicillin/clavulanic acid on the development of resistance in *S. pneumoniae* (207). Twenty *S. pneumoniae* were passaged for nine days in the presence of sub-inhibitory concentrations of each antimicrobial agent and MICs determined. Eleven of thirteen isolates showed ≥ 4 -fold increase in MICs to azithromycin. Of the agents tested which included amoxicillin/clavulanic acid, levofloxacin, cefaclor, and azithromycin, azithromycin and levofloxacin MICs were most affected.

The Alexander Project is a multicenter, multinational surveillance program that was established in 1997 to survey and monitor changing patterns of antimicrobial resistance of respiratory pathogens to a wide variety of antimicrobial agents commonly used to treat patients in either the out-patient or in-patient setting. Blondeau and Tillotson recently reviewed the antimicrobial resistance of respiratory pathogens from a global perspective and summarized the prevalence of macrolide resistance for *S. pneumoniae* and *H. influenzae* for different regions of the world (208).

Macrolide resistant, *S. pneumoniae* are a major concern, especially as prevalence rates rose dramatically in the 1990s. Mean prevalence rates of macrolide resistant pneumococcus were 22%, varying from 78% in Hong Kong to 2% in the Czech Republic (209). While macrolide resistance is higher among penicillin-resistant strains, resistance is also problematic with penicillin-susceptible strains. Data from the Applicant substantiates these observations.

One notable trend found with the epidemiology of pneumococcal isolates is that penicillin resistance is associated with macrolide resistance. In the 1999-2000 survey of US pneumococcal isolates, the overall rate of macrolide resistance was 26%. Of the penicillin-resistant pneumococcal isolates, 76-78% were resistant to the macrolides while

43% of penicillin-intermediate strains were macrolide resistant (41). For 374 *S. pneumoniae* isolates tested, 93.1% of penicillin-susceptible isolates were susceptible to azithromycin (22). Against isolates that were highly resistant to penicillin, resistance to the macrolides was similar to that reported by Doern *et al.* (i.e., $\geq 70\%$) [33].

Hoban *et al.* studied 60 clinical pneumococcal isolates from four New York City medical centers (200). The isolates demonstrate varying degrees of penicillin resistance. The *ermB* gene was present in 22% of isolates with intermediate resistance to penicillin, compared to 38% of isolates with high penicillin resistance. In contrast, *mef* was present in 8, 11, and 19% of the pneumococcal isolates that were susceptible, intermediate, or highly penicillin-resistant, respectively. Marchese *et al.* studied pneumococcal isolates from central and northern Italy and reported that 82.6% of the macrolide resistant strains possessed the *ermB* gene while the remaining isolates were macrolide resistant due to *mef* (210). Tonoli *et al.* examined 117 penicillin susceptible macrolide resistant *S. pneumoniae* isolates and observed that 88% expressed the *ermB* gene and 12% were resistant due to *mef* (211).

In vitro susceptibility testing for azithromycin susceptibility may not be an accurate predictor of clinical cure rate (19, 51, 52). That is, resistance to azithromycin may not correlate with clinical failure. Unfortunately, studies specifically investigating the clinical activity of macrolides against organisms demonstrating various levels of resistance to these drugs have not been carried out. Against isolates highly resistant to penicillin, cross-resistance to the macrolides was 77-78%.

Data from the Applicant again, substantiates this observation. The correlation between *in vitro* susceptibility testing and clinical outcome in infections resulting from *mef* bearing strains of *S. pneumoniae* may not be strong. However, the correlation may be explained by the accumulation of the antibiotic in inflammatory cells and transport of azithromycin to the site of infection. This theory is supported by the data in Table 21 (Table 15, Combined Analysis section, current submission). These data demonstrate clinical cures in CAP, ABS, ██████████ for several *S. pneumoniae* strains having a MIC range of 2 to $>256 \mu\text{g/ml}$. The number of strains harboring *mef*, *ermB*, or *ermB*⁺*ermTR* was four, four, and one, respectively. In four strains, genotypes were not determined.

Studies characterizing the prevalence rates of antimicrobial resistant organisms are necessary for the appropriate use of antimicrobial agents in any one geographical area. Similarly, documenting the prevalence of the different mechanisms of macrolide resistance (*ermB*, *mef*) in *S. pneumoniae* is also essential given the different levels of resistance (higher and lower MIC values) and the potential impact on appropriate clinical use.

Resistance in *H. influenzae* and *M. catarrhalis*

Together with *Streptococcus pneumoniae* and *Moraxella catarrhalis*, *Haemophilus influenzae* is a major cause of community acquired respiratory infections in children and adults, including pneumonia, acute exacerbations of chronic bronchitis, sinusitis, and otitis media. The major resistance mechanism in *H. influenzae* is β -lactamase production

(TEM-1, ROB-1). A recent study in the United States has documented the incidence of β -lactamase production in 1676 *H. influenzae* strains isolated throughout the US to be 41.6% (213). It is well known that antimicrobial abuse leads to development of resistance. Baquero has postulated that abuse of azithromycin and clarithromycin has played an important part in the development of macrolide resistance in *S. pneumoniae* in Spain Italy, and France (147, 212, 213).

Clark et al. studied the abilities of amoxicillin-clavulanate, cefpodoxime, cefprozil, azithromycin, and clarithromycin to select resistant mutants of *Haemophilus influenzae* using multistep and single-step methodologies (214). In multistep studies, clarithromycin and azithromycin both gave a > 4-fold increase in eight of ten strains after 14 to 46 and 20 to 50 days respectively. Mutants selected by clarithromycin and azithromycin were associated with mutations in 23s rRNA and ribosomal proteins (L4 and L22). In single-step studies, clarithromycin and azithromycin had the highest mutation rates, while amoxicillin-clavulanate had the lowest. The MICs of azithromycin for azithromycin-resistant clones were 16 to > 128 $\mu\text{g/ml}$. After 50 daily subcultures in the presence of drugs, MICs of amoxicillin-clavulanate and cefpodoxime against *H. influenzae* did not rise more than fourfold, in contrast to cefprozil, azithromycin, and clarithromycin, whose MICs rose to variable degrees.

It is interesting that macrolide resistance has not developed in *M. catarrhalis*, *M. pneumoniae*, *C. pneumoniae*, and *L. pneumophila* (215). Blondeau et al. recently reported that 100% of 200 *M. catarrhalis* isolates collected from six Canadian medical centers were susceptible to azithromycin (199). In the same study, 99% of 566 *H. influenzae* isolates tested by the Kirby-Bauer method were susceptible to azithromycin.

PRECLINICAL EFFICACY--IN VIVO

Preclinical Pharmacokinetic/Pharmacodynamic (PK/PD) Evaluation of Azithromycin Dosing Regimens in a Gerbil Otitis Media Model with *H. influenzae* Strains

Given that azithromycin is now being developed as a single-day therapy for the treatment of community-acquired respiratory tract infections, it is imperative to differentiate the one-day dosing regimen with the sustained-release formulation from the traditional three-day and five-day regimens on a relevant scientific basis. Global PK/PD measures could not adequately describe the results across all three regimens. Developing a mechanism-based mathematical PK/PD model, which describes the relationship between exposure to an antimicrobial agent and the time course of bacterial killing, would provide better resolution of the true nature of the drug effect, and be helpful in discriminating among the three regimens.

The time courses of pharmacokinetics and pharmacodynamics of three azithromycin dosing regimens were evaluated at the threshold efficacious doses (around ED₅₀) in a gerbil otitis media model that used either of two *H. influenzae* strains (strain 54A1100: MIC = 0.5 $\mu\text{g/ml}$ and strain 54A1325: MIC = 2.0 $\mu\text{g/ml}$). The PK data were fit to a three-compartment model, and the time course of CFU was fit to a PD model with capacity-

limited bacterial growth, 1st-order rate constant for bacterial death (K_d) and a Hill-type function in which azithromycin either inhibits replication or enhances K_d . The PK and the PD parameters were fit separately for PK/PD modeling to ensure that variability in PD data do not influence PK parameters.

Qualitatively, similar results were observed for both *H. influenzae* strains. The mechanism based PK/PD modeling showed that the percent (%) increase in K_d (1st-order killing rate) and the duration of the maximum K_d determine the efficiency of antibacterial effect, which provided the support for the hypothesis that the front-loading of AUC benefits the outcome.

Simulations based on this PK/PD model illustrate the difference in the outcome of the three dosing regimens. Figure 5 (upper panel, p34 of the Microbiology Section of this submission) shows the simulated concentration versus time and \log_{10} CFU versus time profiles following one-day dosing regimen against strain 54A1325. The percent change in the baseline K_d (K_{d0}) versus time and \log_{10} CFU versus time profiles are shown in Figure 5 (lower panel, p34 of the Microbiology Section of this submission). The bacterial burden was driven below the limit of detection ($2 \log_{10}$ CFU) by approximately 10 hours, K_d was amplified four-fold for approximately 16 hours, and the K_{dmax} was achieved rapidly following dosing. Figure 6 (p35 of the Microbiology Section of this submission) depicts the simulation of PK and PD profiles following three-day dosing regimen. When the identical total exposure was delivered as the three-day regimen rather than the one-day regimen, the duration of time required to drive the bacterial burden to the limit of detection approached 30 hours. Moreover, K_d is maximized for only five hours of a 24-hour dosing interval.

There are a number of important findings of this PK/PD study. The way of delivering the dose affects the rate and extent of bacterial kill, and front-loading of AUC improves the killing efficiency in the preclinical models. Each of the three dosing regimens delivered the same total exposure, but the one-day SR regimen which front-loaded total exposure consistently outperformed the three-day and five-day regimen. In other words, front-loaded exposure resulted in more prolonged durations of K_{dmax} at high bacterial titers, which in turn resulted in faster and more complete reduction in bacterial burdens, thus optimizing the probability of positive outcomes. The unique PK characteristics of azithromycin, such as the long elimination half-life, extensive tissue distribution and the drug-loaded white blood cell (WBC) trafficking to the site of infection also support the one-day dosing regimen paradigm.

The difference in efficacy of one-day, three-day, and five-day regimens was observed at low doses ($\sim ED_{50}$) in animal models. In clinical practice, all three regimens would be expected to be in the E_{max} region of the Hill type relationship and expected to do well. Therefore, the difference in efficacy of the three regimens in clinic may be observed with resistant strains based on preclinical PK/PD results.

An important implication of one-day azithromycin therapy relates to the issue of antimicrobial resistance. Mutational events occur as a function of bacterial burden and

time. Therefore, it is important to maximize K_d at times when the burden is the greatest. Regimens achieving this goal are likely to reduce the probability of the emergence of resistance during therapy. In the current experiments, the simulated one-day regimen with sustained released formulation outperformed three-day and five-day regimens. It has recently been demonstrated that as the duration of antimicrobial therapy increases, so does the probability of the selection of preexisting antimicrobial-resistant subpopulations of bacteria (193). Based on preclinical PK/PD findings, it is likely that larger initial exposures of azithromycin will result in faster reduction in bacterial loads at the primary infection site, and therefore lessen the probability of the transfer of resistance genes among subpopulations of bacteria.

In summary, these results are supportive of the overall clinical benefit of the single dose SR regimen. The Applicant asserts that a detailed study report can be found in Appendix 4 of the Microbiology Section of this submission. However, this reference section was blank with the exception of a title: **Preclinical Pharmacokinetic/Pharmacodynamic (PK/PD) Evaluation of Azithromycin Dosing Regimens in a Gerbil Otitis Media Model with *H. influenzae* Strains.**

Pharmacodynamics in Animal Models

Using the *S. pneumoniae* neutropenic mouse thigh model, efficacy for azithromycin correlated best with the PK-PD global predictor of the ratio of the area under the serum concentration versus time curve (AUC) over the microorganism's MIC (24 h AUC/MIC ratio) [94, 191]. In contrast, efficacy with erythromycin and clarithromycin correlated best with the time that free serum levels exceed the MIC. Experiments done in the Applicant's laboratories using immediate release azithromycin therapy (Azithromycin SR formulation could not be used in preclinical models as its drug delivery characteristics were optimized for human use) with susceptible strains of *S. pneumoniae* and *S. pyogenes* in an immune-competent murine acute infection model demonstrated that efficacy (survivorship), as measured by PD_{50} , is independent of the dosing interval (Table 4 and Appendix 2, Microbiology Section of this submission). For these two data sets, the best predictor of efficacy was AUC/MIC. In E_{max} models of murine thigh or lung infections, the global PK-PD predictors of AUC/MIC or Peak/MIC correlate best with efficacy (Table 5 and Appendix 2, Microbiology Section of this submission). In a mouse pulmonary infection E_{max} model challenged with *S. pneumoniae*, static doses of 2.3, 6.4, 2.6, and 1.1 mg/kg were observed for dosing regimens of q24h, q12h, q6h, or q3h, respectively, resulting in static doses that were equivalent when 95% confidence intervals are considered. In a neutropenic thigh infection E_{max} model challenged with *S. pyogenes*, there was again no statistical difference between the different dosing regimens; the static doses required at any one regimen are equivalent when the 95% confidence intervals are considered (Table 5; Appendix 2, Microbiology Section of this submission).

Table 4. Influence of Dosing Interval on PD50 of Azithromycin in Immune-Competent Murine Peritonitis Infection Models

Pathogen	Strain	MIC (µg/ml)	Dosing Interval	Oral PD50 (mg/kg/day)
<i>S. pneumoniae</i>	02J1016	0.06	q24h	18 (17-19)
			q12h	23 (19-27)
			q6h	18 (6.5-28)
			q3h	18 (13-22)
<i>S. pyogenes</i>	02C0203	0.03	q24h	1.3 (0.8-1.8)
			q12h	1.4 (1.4-1.4)
			q6h	2.2 (1.7-2.7)
			q3h	2.2 (1.0-4.4)

Source: Appendix 2

Table 5. Influence of Dosing Interval on Static Dose of Azithromycin in Murine Pulmonary and Thigh Infection Models

Pathogen	Strain	MIC (µg/ml)	Dosing Interval	Oral Static Dose (mg/kg/day)
Immune Competent Emax Pulmonary Infection Model				
<i>S. pneumoniae</i>	02J1016	0.06	q24h	2.3
			q12h	6.4
			q6h	1.8
			q3h	1.2
Immune Compromised Emax Thigh Infection Model				
<i>S. pyogenes</i>	02C0203	0.03	q24h	3.2
			q12h	12
			q6h	7.7
			q3h	9.5

Source: Appendix 2

Furthermore, laboratory experiments with azithromycin suggest it is the total amount of drug administered rather than the interval of the dosing regimen that determines the concentration at the infection site and drives the observed efficacy (94, 157, Tables 6 and 7 below, and Appendix 3, Microbiology Section of this submission). In the acute murine models challenged with *S. pneumoniae*, *H. influenzae*, *S. pyogenes*, or *E. faecalis*, azithromycin was more potent when given as a single oral dose (relative to multi-day therapies) determined by PD₅₀ estimates. The improvement in the PD₅₀ for treatment of *H. influenzae* is especially noteworthy. It appears that the additional component of C_{max} (three-fold better when administered as a single dose vs. the same total dose administered over three days) may also factor into the effectiveness of the one-day regimen. Thus, using the same total therapeutic dose, the duration of treatment with azithromycin can be reduced and appears to be more potent (157 and Appendix 3, Microbiology Section of this submission). While these data are consistent with the 24 h AUC/MIC being the global PK-PD predictor that is highly correlated with azithromycin efficacy, the results also suggest that front-loading the dose of azithromycin increases C_{max} and provides a greater AUC/MIC during the first 24 h of therapy when bacterial burden is maximal. Coupled with the prolonged persistent effects observed with azithromycin, these results support the concept that a single dose can result in increased potency.

Table 6. The Effect of Dose Regimen on Efficacy of Azithromycin and Clarithromycin in an Acute Murine Model

Pathogen	Drug	MIC (µg/ml)	Dosing Regimen	Oral PD ₅₀ (mg/kg/day)
<i>S. pyogenes</i>	Azithromycin	0.06	3 day	3.8 (3.8-3.9)
			2 day	2.5 (1.8-3.3)
			1 day	1.0 (0.6-1.4)
<i>S. pyogenes</i>	Clarithromycin	0.06	3 day	3.1 (2.6-3.7)
			2 day	2.2 (0.9-3.6)
			1 day	11 (3.1-19)
<i>H. influenzae</i>	Azithromycin	0.5	3 day	181 (180-183)
			2 day	50 (42-59)
			1 day	25 (14-36)
<i>H. influenzae</i>	Clarithromycin	8.0	1 day	> 200
<i>E. faecalis</i>	Azithromycin	6.3	3 day	59 (28-91)
			2 day	43 (42-43)
			1 day	15 (10-20)
<i>E. faecalis</i>	Clarithromycin	3.2	3 day	19 (7.2-30)
			2 day	24 (5.4-42)
			1 day	2.2 (0.19-4.2)

Source: Appendix 3

Table 7. The Effect of Dose Regimen on Efficacy of Azithromycin and Clarithromycin in Murine Pulmonary Infection Model

Pathogen	Drug	MIC (µg/ml)	Dosing Regimen	Oral PD ₅₀ (mg/kg/day)
<i>S. pneumoniae</i>	Azithromycin	0.06	3 days	50 (28-71)
			2 days	28 (23-32)
			1 day	20 (16-24)
<i>S. pneumoniae</i>	Clarithromycin	0.06	3 days	> 200
			2 days	> 200
			1 day	> 200

Source: Appendix 3, Microbiology Section of this submission.

In additional profiling of azithromycin in the murine *S. pneumoniae* pulmonary infection model, a simulated sustained release (SSR) formulation suitable for dosing in rodents was evaluated, since the sustained release formulation for humans did not work in mice due to differences in the gastrointestinal tracts. The dose of 100 mg azithromycin/course of therapy was compared in four different regimens: 1.) as a single bolus dose, 2.) as a SSR (the dose was given at 19, 21 and 23 hours post-infection to more closely simulate the prolonged C_{max} found when the human sustained release formulation is given to humans), 3.) administered over three days, and 4.) administered over five days. At 100 mg/kg/therapy, all four formulations of azithromycin cleared the lungs of bacteria, and they remained clear for 11 days post-dosing, except for one breakthrough in one mouse on day 11 in the SSR treatment group. There was no breakthrough on day 13, however. Clarithromycin at 400 mg/kg/therapy had some breakthrough of bacteria on days 5 and 13 (Appendix 3, Microbiology Section of this submission).

As summarized in Table 8, the most potent overall dosing regimen for survival was the SSR formulation, but this was not significantly different from the q.d. x 1 group, at the

95% confidence limits at day 8, 11 or 13. The SSR group ED₅₀ value (effective dose for 50% of the mice) was different from the q.d. x 3 and q.d. x 5 groups at days 8 and 11, but there was a slight overlap with the q.d x 3 group by day 13.

Table 8. Effect of Dosing Regimen on Survival of Mice Infected with *S. pneumoniae* in a Pulmonary Infection Model.

Treatment	ED ₅₀ (95% confidence interval; mg/kg/therapy)		
	Day 8 PI*	Day 11 PI	Day 13 PI
azithromycin every 2 h x 3	12 (9 - 14)	12 (7 - 17)	12 (6 - 18)
azithromycin q.d. x 1	17 (11 - 23)	19 (13 - 24)	19 (13 - 25)
azithromycin q.d. x 3	22 (21 - 23)	23 (20 - 26)	23 (17 - 30)
azithromycin q.d. x 5	19 (17 - 20)	30 (30 - 31)	30 (30 - 31)
azithromycin human SR q.d. x 1	65 (41 - 90)	75 (59 - 92)	75 (59 - 92)
clarithromycin q.d. x 5	243 (235 - 252)	302 (241 - 363)	329 (317 - 340)

*, post-infection

Source: Appendix 3

In *in vivo* kill kinetic experiments in the gerbil model of otitis media, azithromycin was administered as one-, two-, and three-day regimens (total dose of 200 mg/kg/therapy). Comparison of the outcomes for azithromycin therapy against a penicillin-susceptible *H. influenzae* strain indicated that the one-day therapy more rapidly eradicated the pathogen from the middle ear while the multi-day therapies never cleared the pathogen (Fig. 1, p 31, Microbiology Section of this submission, 192). Against a penicillin-resistant *H. influenzae* isolate a similar profile was observed with a more rapid eradication using the one-day therapy (Fig. 2, p 30, Microbiology Section of this submission, 192). Consistent with murine models, front-loading the dose optimizes C_{max} and provides a greater 24 h AUC/MIC during maximum bacterial burden resulting in more rapid killing of *H. influenzae* relative to the two- and three-day regimens.

In the gerbil otitis media model, the penicillin-resistant strain *H. influenzae* 1218 survived in the gerbil bulla for at least seven days, which allowed for further characterization of the effects of a single, one-day dose compared to multi-day dosing of azithromycin. Administration of 200 mg active ingredient/kg/therapy of azithromycin as a SSR (i.e. dosed at 18, 20, and 22 hours post-infection) was sufficient to eliminate *H. influenzae* to below detectable levels within one day of dosing and to prevent re-growth for six days post-dosing (seven days post-infection), which was the end of the experiment (Figure 3, p31, Microbiology Section of this submission). The lower daily doses of azithromycin spread over three or five days (q.d. x 3 or q.d. x 5) were inadequate to eliminate the bacteria within one day of the first dose. In order to discriminate further among the various dosing regimens, another experiment was conducted in which the dose of azithromycin and amoxicillin/clavulanate was lowered to 100 mg active ingredient/kg/therapy.

Administration of azithromycin at 100 mg/kg/course of therapy as a SSR (i.e. dosed at 18, 20, and 22 hours post-infection) or as a one-time dose at 18 hours post-infection was sufficient to eliminate *H. influenzae* to below detectable levels (< 100 CFU/ml) at seven days post-infection (Figure 4, p31, Microbiology Section of this submission). The lower

single daily doses of azithromycin spread over three or five days (q.d. x 3 or q.d. x 5) were inadequate to eliminate *H. influenzae* from all of the gerbils at seven days post-infection. These gerbil data were used in the initial phases of the development of preclinical pharmacokinetic/pharmacodynamic (PK/PD) evaluations of azithromycin dosing regimens in the gerbil otitis media model infected with *H. influenzae* strains. Results are demonstrated by the Figures 1-4, pp 30-31 of the Microbiology Section of this submission.

Pharmacokinetics in Humans

Azithromycin SR is a sustained release formulation, which provides a full course of antibacterial therapy in a single oral dose. Data from separate pharmacokinetic (PK) studies in healthy adult subjects indicate that a higher peak serum concentration (C_{max}) and greater systemic exposure (AUC) of azithromycin are achieved on the day of dosing following a single 2.0 g dose of azithromycin SR versus 1.5 g of azithromycin immediate release (IR) given over three days (500 mg/day) or five days (500 mg on day one, 250 mg/day on days 2-5), see Table 9 [Table 5, Microbiology Section of this submission]. Consequently, due to these different PK profiles on the day of dosing, azithromycin SR and conventional three- and five-day Azithromycin Immediate Release (IR) dosing regimens are not interchangeable.

Table 9. Mean (SD) Pharmacokinetic Parameters for Azithromycin on Day 1 Following the Administration of a Single Dose of 2.0 g Azithromycin SR or 1.5 g of Azithromycin IR Tablets Given over 3 days (500 mg/day) or 5 Days (500 mg on day 1, 250 mg on days 2-5) to Healthy Subjects

Pharmacokinetic Parameter	Regimen		
	Azithromycin SR [n=41]**	3-day IR* [n=12]	5-day IR* [n=12]
C_{max} (µg/ml)	0.821	0.441	0.434
	(0.281)	(0.223)	(0.202)
	5.0	2.5	2.5
T_{max}^{\dagger} (hr)			
	(2.0-8.0)	(1.0-4.0)	(1.0-6.0)
	8.62	2.58	2.60
AUC_{0-24} (µg·hr/ml)			
	(2.34)	(0.84)	(0.71)
	20.0	17.4	14.9
$AUC_{0-\infty}^{***}$ (µg·hr/ml)			
	(6.66)	(6.2)	(3.1)
	58.8	71.8	68.9
$T_{1/2}$ (hr)			
	(6.91)	(14.7)	(13.8)

† SR and IR parameters obtained from separate PK studies;

‡ Median (range);

* C_{max} , T_{max} and AUC_{0-24} values for Day 1 only;

** n = 21 for $AUC_{0-\infty}$ and $t_{1/2}$;

*** Total AUC for the 1-day, 3-day and 5-day regimens

SD = Standard deviation

C_{\max} = Maximum serum concentration
 T_{\max} = Time to C_{\max}
AUC = Area under concentration vs. time curve
 $T_{1/2}$ = terminal serum half-life

Azithromycin SR is formulated to release azithromycin slowly in the GI tract. The single 2.0 g doses of azithromycin SR and Azithromycin POS were not bioequivalent. The bioavailability of azithromycin SR relative to the Azithromycin POS was 83%. Peak serum concentrations were achieved approximately 2.5 hours later following azithromycin SR administration compared to Azithromycin POS. T_{\max} for azithromycin SR was 4.1 hr and T_{\max} for POS was 1.6 hr.

When a 2.0 g dose of azithromycin SR was administered following a high-fat meal (150 kcal from proteins, 250 kcal from carbohydrates and 500-600 kcal from fats) to 15 healthy adult subjects, peak serum concentration was increased by 115% and systemic exposure as measured by AUC increased by 23% as compared to administration in a fasted state. When a 2.0 g dose of azithromycin SR was administered following a standard meal (56 kcal from proteins, 316 kcal from carbohydrates and 207 kcal from fats) to 88 healthy subjects, peak serum concentration was increased by 119% and systemic exposure as measured by AUC was not affected.

Tissue Distribution

The serum protein binding of azithromycin is concentration dependent, decreasing from 51% at 0.02 $\mu\text{g/ml}$ to 7% at 2.0 $\mu\text{g/ml}$. Following oral administration, azithromycin is widely distributed throughout the body with an apparent steady-state volume of distribution of 31 l/kg.

Higher azithromycin concentrations in tissues than in plasma or serum have been observed. The extensive distribution of drug to tissues may be relevant to clinical activity. The antimicrobial activity of azithromycin is pH related and appears to be reduced with decreasing pH. Hence, high tissue concentrations should not be interpreted as being quantitatively related to clinical efficacy.

Excretion

Serum azithromycin concentrations following a single 2.0 g dose of azithromycin SR declined in a polyphasic pattern with a terminal elimination half-life of 59 hours. The prolonged terminal half-life is thought to be due to a large apparent volume of distribution.

Biliary excretion of azithromycin, predominantly as unchanged drug, is a major route of elimination. Over the course of a week, approximately 6% of the administered dose appears as unchanged drug in urine. *In vitro* and *in vivo* studies to assess the metabolism of azithromycin have not been performed.

Pharmacodynamics in Humans

There are several key factors that have been responsible for the successful treatment of both extracellular and intracellular bacterial pathogens with azithromycin in humans and in animal models. *In vivo*, high and prolonged concentrations of azithromycin at the site of infection are mediated by accumulation in tissues and phagocytes, and infected tissues have significantly higher levels of azithromycin than uninfected tissues. The shorter course of therapy compared with most other antibiotics also is responsible for greater patient compliance and perhaps better outcomes. These factors are described in detail below.

After oral dosing of azithromycin, serum concentrations do not appear sufficient to account for efficacy. As described in the original application, efficacy appears to be related to the sustained high levels of azithromycin in tissues and white blood cells. The delivery and maintenance of high concentrations of azithromycin at the site of infection have been shown to be mediated by phagocytes, in their normal chemotactic response to an infection (154-162). *In vitro* experiments have documented the transport and delivery of bioactive azithromycin by human neutrophils (163). The phagocyte delivery mechanism has also been demonstrated in various rodent infection models that simulate human infections: *S. aureus* infected thigh muscle, *H. influenzae* middle ear infection, *H. influenzae* and *S. pneumoniae* infected lung, and *S. pyogenes* and *S. aureus* abscess models (156, 157, 160, 161, 162). In all examples, azithromycin concentrations at the site of infection were significantly higher relative to control non-infected tissue, and the increased drug levels correlated with increased numbers of phagocytes at the infection site. In the *S. pyogenes* abscess model, the concentration of azithromycin was increased up to 50 times in the infected compared to non-infected animals (156). In the *S. aureus* thigh abscess and *H. influenzae* lung models of infection, the half-life for azithromycin in the infected tissues was > 2 times the half-life observed in noninfected tissues (157, 160, 161, 164). High prolonged tissue concentrations result in a greater AUC for infected tissue compared with non-infected tissue, i.e., a four-fold greater AUC in the *H. influenzae* lung model (157, 160, 161, 164). The higher concentration of azithromycin correlated with potent efficacy compared with other drugs (155, 160-162). The efficacy observed is explained by the phagocyte release of azithromycin during the process of phagocytosis and degranulation at the infection foci (158, 165). For example, in the case of a *S. pneumoniae* infection, the high tissue levels as well as macrophage-mediated delivery of azithromycin at the site of infection would eliminate the focus of the infection in the lung, thereby preventing further spread of *S. pneumoniae* into the circulation (166). Any remaining *S. pneumoniae* circulating in the bloodstream would be filtered and killed in the spleen or by white blood cells or other tissues with high concentrations of azithromycin.

For intracellular pathogens, the concentration of drug in the infected macrophages is important. Infected macrophages take up more drug than non-infected cells (167). The concentration of azithromycin in the bronchoalveolar lavage from guinea pigs infected with *L. pneumophila* is significantly higher than the bronchoalveolar lavage from non-infected animals. This helps explain the superior potency of azithromycin as compared to other macrolides in the *L. pneumophila* guinea pig infection model (167). Thus the

phagocytes, in their natural process of fighting an infection, take up, transport and release azithromycin at the site of infection. Other macrolides and β -lactams did not show significantly high sustained concentrations at the infection site (157, 160-162). In contrast to azithromycin, their concentrations were reduced in the early *S. aureus* abscess model (157). Of several macrolides tested (azithromycin, erythromycin, clarithromycin, and roxithromycin), azithromycin was the only one that was efficacious after a single oral 10 mg/kg dose in a *M. pneumoniae* pulmonary infection model in hamsters (168). This efficacy correlated with the high C_{max} and prolonged exposure for azithromycin in uninfected lungs compared with other macrolides. In a mouse *Chlamydia trachomatis* salpingitis model, azithromycin (\pm an anti-inflammatory agent) was superior to doxycycline and ofloxacin combinations in preserving fertility (169).

The phagocyte delivery mechanism is fully operational even when circulating granulocytes have been reduced 70-85% by cyclophosphamide treatment in a *S. aureus* mouse thigh model (164). Equivalent efficacy was observed in leukopenic mice compared with normal mice (164). Under conditions of severe neutropenia, higher levels of azithromycin were not observed in infected versus healthy lung tissue in a *S. pneumoniae* lung infection model (162). Surprisingly, serum levels in cyclophosphamide-treated uninfected animals were less than one-fourth that of normal animals and lung tissue concentrations were less than one-half that of normal animals. Thus, there are major differences in these models relative to the degree of neutropenia and its impact on the available phagocyte population and cyclophosphamide toxicity [Discussed by Girard et al., reference 164].

Additional information on the effectiveness of azithromycin therapy on respiratory pathogens has accrued over recent years. Two meta-analysis studies of azithromycin efficacy and safety, one for upper respiratory and one for lower respiratory pathogens, have been published (170, 171). In the analysis of upper respiratory tract infections, it was concluded that azithromycin clinical failure rates in acute otitis media, sinusitis, and acute pharyngitis were similar to comparator antibiotics (e.g., amoxicillin, amoxicillin/clavulanate, cefaclor, clarithromycin, erythromycin). Azithromycin had one of the lowest discontinuation rates, and offered advantages in short term treatment leading to better compliance. In the lower respiratory tract study, the meta-analysis of studies concluded that azithromycin may be superior to other β -lactams and macrolides tested for pneumonia, but may not offer an advantage in AECB or acute bronchitis. The authors speculate that the unique azithromycin pharmacokinetics may lead to prolonged tissue levels important for successful pneumonia treatment. A recent review of the treatment of sinusitis in children reviewed several therapeutic modalities (172). The study concluded that newer macrolides were as effective as amoxicillin, but azithromycin, with a shorter course of therapy, may offer compliance advantages. Another study found that in cases where bacteria are present in nasal secretions, they might play a pathogenic role in upper respiratory infections (173). This study centered on the use of azithromycin, and in cases where *S. pneumoniae*, *H. influenzae*, or *M. catarrhalis* were present, as determined by culture, azithromycin reduced duration of symptoms and complications.

Discrepancies concerning the frequency with which bacterial species are identified in outpatient units compared to those typically used in surveillance studies may account for some of the perplexing success of azithromycin in light of a 30% non-susceptible rate for *S. pneumoniae* in the US. The majority of pathogens isolated in the outpatient clinics are bacterial species that are sensitive to azithromycin. For example, a study of the epidemiology of sinusitis (RESP) from the 1999-2000 season found that the most commonly observed pathogens were *M. catarrhalis* (28.9%), *H. influenzae* (21.7%), *S. aureus* (17.9%), PenS *S. pneumoniae* (7.2%), and Pen IR *S. pneumoniae* (4.0%) [174]. Both *Moraxella* and *Haemophilus spp.* were highly susceptible (> 99%) to azithromycin. Pneumococci were 64% susceptible. Similarly, an antimicrobial surveillance study (PROTEKT) of *H. influenzae* and *M. catarrhalis* from community respiratory infections found 99.8% of *H. influenzae* and 100% of *M. catarrhalis* susceptible to azithromycin by established MIC cutoff values (175). In a prospective study to determine the frequency of respiratory pathogen occurrence in medical offices in the United States during 1999-2000, Pfaller et al. found that *H. influenzae* was the most prevalent pathogen isolated in patients with community acquired pneumonia and acute exacerbation of bronchitis, accounting for 38% and 35% of the 1,668 cultures (176). *M. catarrhalis* was the most prevalent pathogen in the 5,491 positive cultures from patients with sinusitis. For all three indications, *S. pneumoniae* was the least prevalent of the three pathogens. Despite concerns that the rate of resistance in pathogens obtained from clinical labs over-predicts the resistance rate in patients upon initial diagnosis in the community, Pfaller et al. showed that this is not the case (176, 177). In some cases, the opposite was true. For *S. pneumoniae*, the rate of erythromycin resistance was 18% in the SENTRY (1997-1999) study and 34% in Pfaller et al.'s prospective study. Similar discrepancies were seen for clindamycin, tetracycline, and trimethoprim/sulfamethoxazole. There were no significant discrepancies in the %R for *H. influenzae* and *M. catarrhalis* in the same comparisons with seven different classes of antibiotics. Nevertheless, lower incidences of non-susceptible bacteria should favor positive clinical outcomes.

Recent papers addressing the marked effectiveness of azithromycin included an article examining the clinical effectiveness of current macrolides despite increasing levels of *in vitro* resistance (178). This article examined numerous studies of macrolide treatment in CAP, and concluded that macrolides were as effective as β -lactams or fluoroquinolones. The author also reiterates the evidence that macrolide efficacy is enhanced by favorable pharmacokinetic/pharmacodynamic parameters and the high concentration of antimicrobials at the infection site. Further, the high levels of azalides in phagocytes that are attracted to infected sites appear to deliver much higher levels of drug to the infection site. The phagocytosis of pneumococci by polymorphonuclear leukocytes may deliver high levels of drug to the intracellular bacteria. Another article reached similar conclusions that the levels of newer generation macrolides accumulate within phagocytic cells, and that pharmacokinetic and pharmacodynamic models applicable to other antibiotic classes do not adequately explain the clinical effectiveness of macrolides. When the uptake of radiolabeled azithromycin by PMNs was measured and expressed as the ratio of cellular to extracellular drug concentration (C/E), azithromycin was massively accumulated by human PMNs, (C/E = 387.2) [179]. After the last dose of a three-day

regimen of 1.5 g of azithromycin, the plasma AUC was 3.8 mg·h/L compared to PMN AUC of 6,291 mg·h/L (180). Another recent study measured the accumulation of azithromycin in plasma and tonsillar tissues removed from pediatric patients (181). Azithromycin concentrations in tonsillar tissues were over 10 times higher than in plasma, and were sustained for eight days at doses of 10 mg/kg. Higher levels were achieved with a 20 mg/kg dose. This study again indicates that plasma levels are poor predictors of clinical efficacy.

When a 1.5 gm total dose of azithromycin is given over five days or three days, the levels in tonsils of pediatric patients are projected to exceed 2 µg/ml ten days after initiation of therapy (182, 183). The mean concentration of azithromycin in tonsillar and adenoid tissue from children at 4 and 11 days after the start of a three-day 10 mg/kg dosage regimen was 10.33 and 1.49 µg/g, respectively (182). These concentrations are well above the susceptibility breakpoint for streptococci, ≤ 0.5 µg/ml. Children ages 1-6 years with secretory otitis media that received a 10 mg/kg dose of azithromycin prior to tympanostomy had mean azithromycin concentrations of 3.97 and 1.42 µg/ml at 24 h or 48 h post-administration (153). The mean concentration in uninfected lung tissue five days after a 500 mg dose of azithromycin is 3.13 µg/g; this is above the MIC₉₀ for *H. influenzae* of 2 µg/ml and susceptible streptococci (≤ 0.12 µg/ml) [184]. The mean concentration in gynecological tissue was 1.44 and 0.78 µg/g at one and four days following a 500 mg dose (185).

As discussed earlier, tissue concentrations of azithromycin would be expected to be considerably higher in infected tissue than in uninfected tissue due to the phagocyte delivery mechanism. This was observed in a clinical study in which sinus fluid levels of azithromycin 24 h after the last dose were six-fold higher in patients with acute sinusitis compared with patients with chronic sinusitis (2.33 µg/ml versus 0.38 µg/ml) [186]. Also, in children with acute otitis media, the high-sustained concentration of azithromycin in middle ear effusions 48 h post treatment (9.43 µg/ml and a plasma:effusion ratio of 1:363) are likely the result of phagocyte-mediated delivery of azithromycin into the middle ear (153).

The increased levels at the site of infection are readily explained by the concentration of azithromycin in white blood cells. When a 1.5-gram total dose of azithromycin was administered over three days or five days to healthy patients, the AUCs obtained for serum, PMNs and monocytes/lymphocytes (M/L) were equivalent for both dosage regimens (187). The median concentration in PMNs 12 days after the start of therapy of the three-day and five-day regimens was 24 and 22 µg/ml and 10 and 9 µg/ml in M/Ls. In a second healthy volunteer study, the peak concentration in granulocytes and monocytes after the third daily 500 mg azithromycin dose are 85 µg/ml and > 100 µg/ml, respectively, and both have approximately 32 µg/ml azithromycin at seven days post-last-dose (188). These concentrations greatly exceed the MIC₉₀ for community acquired-pathogens.

Pharmacokinetic studies have also been completed in non-healthy volunteers. Pharmacokinetics of azithromycin in diabetics is comparable to healthy volunteers

although accumulation of drug in macrophages was slightly lower in diabetic patients (189). In addition, there were no significant differences observed in the intracellular AUCs for PMNs and mononuclear leukocytes obtained from AIDS patients and healthy populations orally dosed with azithromycin (190).

A hypothesis to explain the transfer of azithromycin from tissue cells to non-intracellular pathogens growing in contact with the tissue cells has been proposed (166). Recently, the results from a mouse model demonstrated azithromycin administered as a single dose significantly increased survival versus a pneumococcal challenge compared with all the other dose regimens (154). This confirmed the Applicant's in-house studies discussed below. These data suggest that the high initial concentrations of azithromycin favor a good outcome.

PK/PD summary comments

Since inflammatory cells provide a mode of transport and reservoir for azithromycin to the infection site, it seems reasonable to give large azithromycin doses as early as practical during the period of maximum inflammation associated with the infection (96, 156, 158, 159, 162, 165). Administering the same total dose as a single dose or in three days instead of five days will result in higher concentrations in the infected tissue at early times after treatment due to increased initial dosing and increased inflammation (157). The higher initial concentration at the infection site may help prevent less susceptible sub-populations of the pathogens initially present from becoming established. This has been proposed as the reason for less *in vivo* emergence of resistance to azithromycin compared with clarithromycin in patients who received either drug for treatment or prophylaxis against *M. avium* (134). A shorter oral dosage regimen would also result in greater patient compliance, which should contribute to reduced emergence of less susceptible strains.

Reviewer's comments: The new macrolides, such as azithromycin, have improved pharmacokinetic properties when compared to erythromycin (194). Such properties include longer half-lives and enhanced tissue penetration. The bioavailability of azithromycin is 37% and may be reduced by 50% in the presence of food (216). Concomitant administration of aluminum and magnesium containing antacids reduces peak serum concentration of azithromycin but does not reduce total-absorption (217).

Azithromycin extensively concentrates within a wide range of cell types, with uptake being rapid and dependent on extracellular concentration, pH, viability of the cell, and concomitant administration of cytokines (218, 219). Intracellular concentrations of azithromycin in human and mouse polymorphonuclear leukocytes, human fibroblasts, murine peritoneal macrophages, and mouse and rat alveolar macrophages may be up to 226 times the extracellular concentrations (218, 220). Accumulation of azithromycin within human polymorphonuclear neutrophils (PMNs) continues for up to 24 h (220). Fibroblasts loaded with azithromycin may serve as a reservoir, thereby releasing the drug for activity against extracellular pathogens, for uptake by PMNs, or both (218). Intracellular release of azithromycin is slow (17% in 1 h) in the absence of any extracellular drug when compared to erythromycin (68% in 1 h). Azithromycin has a

long elimination half-life, averaging 68 h, an effect which is probably due to the extensive tissue uptake and subsequent slow release (221). One of the main characteristics of azithromycin is the slow serum concentration but high and persistent tissue concentration (221).

Azithromycin concentrations of up to 9 mg/kg were found in lung and tonsil tissue 12 h – 3 days after drug administration, yet the corresponding serum concentrations were below 0.1 mg/l (222). The high tissue concentrations were maintained for extended periods such that the elimination half-life for tonsillar tissue was 3.2 days.

The pharmacokinetic profile of azithromycin suggests a rapid and extensive uptake from the circulation into interstitial compartments, subsequently followed by slow release. Azithromycin drug levels remain in pulmonary tissue for extended periods, the mean tissue half-life is between 2 – 4 days. Consequently, azithromycin could be given as a single dose therapy for sexually transmitted diseases, and as a 3 – 5 day regimen for skin and soft tissue infections, and some respiratory tract infections.

Animal Prophylactic and Therapeutic Studies

The Applicant presented data on animal studies in the IND 66,194 submission but not in the NDA 50-797 submission. What follows in this section is taken from the IND 66,194 submission reviewed previously by this Reviewer.

The Applicant indicates that preclinical data suggest that optimum treatment may be achieved by administration of the entire therapeutic course of the drug in a single dose providing maximal exposure when the bacterial burden is highest. The Applicant has conducted studies with animal models of infection showing improved survivorship (mouse model of pneumococcal pneumonia) and quicker bacterial eradication (gerbil model of *H. influenzae* otitis media) with single dosing of azithromycin rather than multiple days (p 91, IND 66,194 submission). The data indicate that the higher C_{max} and/or 24 hour AUC achieved with this “front-loaded” regimen may be the reason for improved efficacy by providing higher levels of the drug in tissues early in the course of infection when bacterial load is highest (pp 91-92, IND 66,194 submission). Human data from clinical trials of acute otitis media using a single dose of Zithromax pediatric oral suspension supports this concept.

The Applicant conducted several investigations in their own laboratories to explore the possible opportunity for shortened azithromycin dosing regimens. They report that the purpose of these studies was to examine the impact of different dosing regimens on murine infection models using *H. influenzae* and *S. pneumoniae* as test pathogens and to extend previous pathogen clearance studies to include one, three, and ~~five~~ day dosing regimens. An additional study employed a single-day dosing regiment to mimic the pharmacokinetics of the human sustained release formulation in a mouse model.

In the first study, the Applicant examined the efficacies of different doses of azithromycin in a gerbil otitis media model with a non-type B strain of *H. influenzae* (pp 217-218, IND 66,194 submission). Administration of azithromycin started one day after

establishment of the infection. The study was based on a mg/kg/total regimen for azithromycin. The Reviewer agrees with the Applicant's conclusion that dosing of azithromycin in a single bolus on Day One was as effective as fractionated doses given over two or three days as exhibited by clearance of the pathogen.

The second study used a similar experimental protocol as followed in the first study except that the beginning of dosing was delayed until three days post-infection (pp 219-220, IND 66,194 submission). All three dosing regimens showed similar results such that the Applicant concluded similar efficacies for the single, two-day and three day dosing regimens.

A third study by the Applicant employed the same gerbil otitis media model with the same strain of *H. influenzae* as the first two studies however the actual rate of bacterial eradication was examined for the different dosing regimens (pp 221-223, IND 66,194 submission). These experiments had a 200 mg/kg/therapy dose given over one, two or three days. The Applicant showed that the rate of elimination of the pathogen was more rapid for the single day dose than for either of the fractionated doses. The single dose activity appeared to be cidal as there was a $< \log 2$ bacterial load at 48 hours post infection and no re-growth at 72-96 hours post infection was observed. The two-day and three-day therapies demonstrated antibacterial activity without total clearance with a possibly slight re-growth by 96 hours post-infection. The single dose therapy demonstrated the best activity with total clearance being demonstrated early in therapy. These observations have led the Applicant to suggest that the likelihood of the development of resistance would be reduced using this regimen relative to the two-three day therapy.

The Applicant conducted a fourth study in order to compare the outcome from a single-day dosing regimen with extended treatment schedules of three and five days (pp 224-232, IND 66,194 submission). The Applicant fractionated regimens of azithromycin over one, three, and five days using a mouse lung infection model with *S. pneumoniae* as the infecting agent. The Applicant states that two different single-day regimens were used, one in which the entire dose was given as a single oral bolus and another regimen in which the dose was split into three components given at two hour intervals to simulate the protracted pharmacokinetics of the human sustained release formulation. The Applicant indicates that the all regimens were effective at eradicating bacteria from the lungs of the mice when given as a 100 mg total therapeutic dose.

In a fifth study, the Applicant used the gerbil otitis media model with *H. influenzae* as pathogen to determine the rates of bacterial eradication at various times post-infection following a 200 mg/kg total therapeutic dose (pp 233-238, IND 66,194 submission). The Applicant states that bacterial clearance was quicker with the single-day dosing regimens than the three and five day regimens as seen in the previous study with the mouse lung model using *S. pneumoniae* as infecting pathogen.

In the sixth study, the Applicant conducted a similar study as was done in the fifth study with the exception that a 100 mg/kg-dosing regimen was implemented (pp 239-243, IND

66,194 submission). The Applicants observed complete bacterial eradication for the single-day dosing regimen only. The criterion for complete eradication was the number of animals in which the infected bulla had been sterilized.

The Applicant asserts that the studies presented when combined with previous data support the observation that azithromycin efficacy can be optimized by administration of the agent as a single-day dose (p 216, IND 66,194 submission). The Applicant also states that conditions in animal infection studies may be defined in which a single-day dosing regimen is superior to multiple day fractionations of the same total dose in terms of both the extent and the rate of pathogen eradication. The Reviewer agrees with both assertions of the Applicant.

CLINICAL EFFICACY

Clinical Laboratory Susceptibility Test Methods

Disk Content Studies. Antibiotic disks are commercially available and were supplied by [REDACTED]. The quality of the disks was assessed by evaluating the susceptibility response of quality control organisms as outlined in the CLSI (NCCLS) 2003 and 2004 guidelines (12-14). The QC organisms used were *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, *H. influenzae* ATCC 49766, and *S. aureus* ATCC 25923.

Comparative Studies Between Agar and Broth Dilution Methods. The susceptibility to azithromycin and comparator antibiotics for all clinical isolates of streptococci, *Haemophilus* spp., *M. catarrhalis*, and *S. aureus* were measured by both agar and broth dilution methods. Data are recorded in the patient profiles and results are presented in Appendix 5 (Microbiology Section of this submission) in scattergrams.

Using the current FDA and CLSI (NCCLS) breakpoints, lines were placed on the scattergrams indicating the breakpoints for Susceptible, Intermediate, and Resistant for *S. pneumoniae* and for the Susceptible breakpoint for *H. influenzae* and [REDACTED]. There is concordance in the interpretation of the susceptibility of the streptococci and *Haemophilus* spp. between the two methods. For all *S. pneumoniae* isolates in which azithromycin was tested (n = 221), except one in the [REDACTED] study, there were no misinterpretations of susceptibility between the two methods. The one exception occurred with an interpretation of Intermediate due to a 16 mm zone diameter and a Sensitive designation for a 0.5 µg/ml measurement by broth dilution. For the same isolates tested against the comparator antibiotics, there was one exception in the interpretation between the two methods. In the A0661075 study, one isolate was classified as Intermediate to clarithromycin (zone diameter = 18 mm) and Resistant by broth dilution (MIC = 1 µg/ml). For the *H. influenzae* isolates, all of the isolates were Susceptible to azithromycin by both methods. One of the [REDACTED] was Resistant to azithromycin by both methods (MIC > 4 µg/ml and 6 mm zone diameter). However, this subject was not part of the BPP in either arm of the study. For the comparator antibiotics, all isolates (n = 269) of both species of *Haemophilus* obtained in the A0661078, [REDACTED], and A0661103 studies were sensitive to levofloxacin by both

methods. In study A0661075 (n = 47 *H. influenzae* and n = 26 [redacted]), there were six examples of discrepancies in the interpretation of susceptibility to clarithromycin. Two isolates of *H. influenzae* were Susceptible by disk diffusion, but Intermediate by broth dilution (13 and 15 mm vs. 16 µg/ml for both). Two isolates of *H. influenzae* were Intermediate by disk diffusion (12 mm for both) and Susceptible by broth dilution (8 µg/ml for both). One [redacted] isolate with an 11 mm zone diameter was classified as Intermediate but had a Resistant broth dilution result of > 16 µg/ml. One [redacted] isolate was classified as Resistant by disk diffusion (8 mm) and as Intermediate by broth dilution (16 µg/ml).

Quality Control. The following organisms were used as quality control organisms in accordance with CLSI (NCCLS) guidelines (12-14): *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, *H. influenzae* ATCC 49766, and *S. aureus*, ATCC 25923 for disk diffusion measurements and *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, *H. influenzae* ATCC 49766, and *S. aureus* ATCC 23213 for broth dilution measurements.

Disk Diffusion Test. The CLSI (NCCLS) 2003 and 2004 guidelines were followed (12-14). In a few cases, the E-test was used to determine MIC values, and the protocol described by [redacted] was followed.

Dilution Test (Aerobic). The CLSI (NCCLS) 2003 and 2004 guidelines were followed (12-14). The MIC plates were made by [redacted].

Dilution Test (Anaerobic). MIC values were not determined for anaerobic species of bacteria.

Testing scheme of clinical isolates done by Pfizer Inc. – Groton and Ann Arbor. (Methods for each assay are described on pp 42-44, Microbiology Section of this submission.)

- Phenotype all *S. pneumoniae* and *S. pyogenes* isolates with MIC values > 0.5 µg/ml for macrolide, streptogramin, and lincosamide susceptibility/resistance patterns.
- Genotype all azithromycin-intermediate and -resistant *S. pneumoniae* from all studies (defined as any *S. pneumoniae* with azithromycin MIC > 0.5 µg/ml) for *mef*, *erm(B)*, and *erm(TR)*. If the same organism was isolated at baseline and follow-up, genotyping was done on both isolates.

Detection of *Mycoplasma pneumoniae* and *Chlamydomphila pneumoniae*. Specimens for detection of the atypical pathogens *M. pneumoniae* and *C. pneumoniae* (oropharyngeal swab and acute and convalescent sera) were sent to the [redacted] Laboratory for testing. Laboratory work-up for *M. pneumoniae* included: oropharyngeal swab for PCR detection and culture and acute and convalescent serology by immunofluorescent antibody (IFA) assay. Laboratory work-up for *C. pneumoniae* included: oropharyngeal swab for PCR detection, and acute and convalescent serology by microimmunofluorescent (MIF) antibody assay.

Provisional Susceptibility Interpretive Criteria

Population Distribution by MIC of Organism Likely to be Included in

the Indications. The population distribution by MIC of clinical isolates of *S. pneumoniae*, *H. influenzae*, [REDACTED] and *M. catarrhalis* collected from all randomized subjects without regard to treatment group who were in the [REDACTED] CAP, and ABS trials described in this document are presented in Appendix 2.7.3, Table SUSC.3.1.1.adhoc1 of this submission. The distribution of susceptibility by zone diameters for the four major pathogens listed above is tabulated in Appendix 2.7.3, Tables SUSC.3.1.2.adhoc1, SUSC.3.1.2.adhoc2, SUSC.3.1.2.adhoc3, SUSC.3.1.2.adhoc4 of this submission. For all four bacterial species, the data are presented by region and country. The cumulative susceptibility values for the overall results and the three largest geographic regions as well as the US are presented in the graphs found on pp 46-49 of this submission. Results from a recent surveillance study under the auspices of TRUST are included on the graph of *S. pneumoniae* (44).

The MIC₅₀ for azithromycin for all *S. pneumoniae* clinical isolates in the [REDACTED] CAP, and ABS trials was < 0.25 µg/ml (23 – 24 mm) for all individual regions of the world, the US (23 mm), and the overall 221 isolates (24 mm). The MIC₉₀ was 4 µg/ml for all 221 isolates. However, there were differences among the MIC₉₀ values from specific regions of the world. The MIC₉₀ for the 79 isolates from the US was 16 µg/ml (6 mm), but when 27 additional isolates from Canada were included, the MIC₉₀ dropped to 4 µg/ml for the 106 isolates from North America (6 mm). The MIC₉₀ for the isolates from Latin America (n = 63) was 2 µg/ml (9 mm), and for Europe (n = 47) it was 4 µg/ml (6 mm). The resistance levels in the TRUST study are higher for the US than observed in the clinical trials described here. This phenomenon has been noted by Felmingham et al. who acknowledge the fact that clinical isolates that are usually included in surveillance studies differ from those collected during the course of clinical trials due to the severity or recalcitrance of the illness in subjects who have their bacteria collected in clinics outside of clinical trials (177).

For *H. influenzae*, the susceptibility to azithromycin was similar throughout the three regions of the world where most of the isolates were collected from the [REDACTED] CAP, and ABS trials. The MIC₅₀/MIC₉₀ was 1.0/2.0 µg/ml (20 – 22 mm/16 – 17 mm) for the US, North America, Latin America, and Europe and for all 179 isolates. The MIC₁₀₀ was 2.0 µg/ml (14 - 15 mm) for Latin America and Europe, and it was 4 µg/ml (13 mm) for the US and North America.

For the 90 *H. parainfluenzae* isolates, the MIC₅₀ for azithromycin was 1 µg/ml (17 – 20 mm) for the overall results and for North America, US, and Europe. The MIC₉₀ was 2 µg/ml (14 mm) for the overall results, North America, and the US, and it was 4 µg/ml (14 mm) for Europe.

For the 104 *M. catarrhalis* isolates, the MIC₅₀ was < 0.25 µg/ml (22 – 27 mm) for all regions of the world, and the MIC₉₀ was < 0.25 µg/ml (22 - 25 mm) for North America, US, and Latin America and 0.5 µg/ml (19 mm) for the overall results and 1 µg/ml (16 mm) for Europe.

Regression Analysis of MICs versus Zone Diameters. The scattergrams representing the distribution of MIC values for *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae* versus zone diameters for the clinical isolates from all treated patients are attached as **Appendix 5** in this submission. Across the four clinical trials, all isolates of *H. influenzae* were susceptible to azithromycin by both methods of susceptibility testing (< 4 µg/ml). One isolate from all four trials had a MIC that exceeded the susceptibility cut-off value of 4 µg/ml. All of the others were < 4 µg/ml. Similarly for *S. pneumoniae*, there was concordance of the interpretation of susceptibility to azithromycin by both methods.

Phase II Clinical Trials. Phase II clinical trials were not conducted with azithromycin sustained-release microspheres for oral suspension.

Class Susceptibility Criteria. The susceptibility of streptococci to two other macrolides and for *Haemophilus* spp. to one other macrolide has been determined by the CLSI (NCCLS) and by submission to the Agency. The currently approved susceptibility interpretive criteria are listed immediately below in Table 10.

Table 10. Susceptibility Interpretive Criteria for Streptococci and *Haemophilus* spp. for Comparator Macrolides

Pathogen / Antibiotic	Minimum Inhibitory Concentration (g/ml)			Disk Diffusion (zone diameters in mm)		
	S	I	R	S	I	R
Streptococci* / Clarithromycin	< 0.25	0.5	> 1	≥ 21	17 - 20	≤ 16
Streptococci/Erythromycin (NCCLS)	< 0.25	0.5	> 1	≥ 21	16 - 20	≤ 15
Streptococci/Erythromycin (FDA)	< 0.5	1 - 4	> 8	≥ 23	14 - 22	≤ 13
<i>Haemophilus</i> spp. / Clarithromycin	< 8	16	> 32	≥ 13	11 - 12	≤ 10

*Streptococci include *S. pneumoniae* and *S. pyogenes*.

Provisional Susceptibility Criteria for *Haemophilus* spp. and *S. pneumoniae*

The Applicant does not seek a change in the current FDA and NCCLS breakpoints for azithromycin for streptococci and *Haemophilus* spp., as summarized in Table 11. This is because, although the formulation has changed, there has not been a change in the active ingredient, and there is insufficient clinical efficacy data from patients with *S. pneumoniae* isolates that had MIC values > 2 µg/ml. Also, there is no significant change in the distribution of MIC values among patients infected with *Haemophilus*; > 90% of the isolates had MIC values < 2 µg/ml.

Table 11. Susceptibility Interpretive Criteria for Azithromycin

Pathogen	Minimum Inhibitory Concentrations (µg/ml)			Disk Diffusion (zone diameters in mm)		
	S	I	R	S	I	R
Streptococci including <i>S. pneumoniae</i> & <i>S. pyogenes</i>	< 0.5	1	> 2	≥ 18	14-17	≤ 13
<i>Haemophilus</i> spp.	≤ 4	*	*	≥ 12	*	*
<i>M. catarrhalis</i>	NA	NA	NA	NA	NA	NA
<i>Mycoplasma</i>	NA	NA	NA	NA	NA	NA
<i>Chlamydia</i>	NA	NA	NA	NA	NA	NA

NA, not applicable

*Current absence of data on resistant strains precludes defining any resistant categories other than "susceptible" for azithromycin.

Correlation of provisional interpretive criteria with clinical and microbiological outcome

Definitions. For each of the four clinical trials, the following information is supplied in Section 13, Table 2.3.1 of this submission for each of the respective clinical trial databases for the bacteriologic per protocol patients at test of cure (TOC), as well as at other time points, e.g. Long Term Follow-Up (LTFU) and End of Treatment (EOT).

- Patient ID number which includes a code for the study center,
- Species of bacterial isolate,
- Patient-by-patient clinical evaluations including separate columns for each patient, the patient's status of microbiological eradication, and the patient's overall clinical response (e.g. cure, fail),
- Indication,
- Susceptibility testing results by diffusion methods for the test drug and the comparator drug,
- Susceptibility testing results by dilution methods for the test drug and the comparator drug, and
- Center number.

The specific references are:

A0661075 (CAP study) Section 13, Table 2.3.1

A0661103 (CAP study) Section 13, Table 2.3.1

A0661078 (ABS study) Section 13, Table 2.3.1

Clinical Outcome: The primary efficacy endpoint in both community-acquired pneumonia (CAP) trials, in the _____, and acute bacterial sinusitis (ABS) studies was sponsor assessment of clinical response for the Clinical per Protocol (CPP) population at the Test-of-Cure (TOC) visit. The TOC visit occurred on study days 14-21 for patients enrolled in both CAP studies and in the _____ study, and on study days 17-24 for patients in the sinusitis study.

Microbiological Outcome: For the four clinical trials described below, the primary microbiological outcome was *eradication* or, more commonly, *presumed eradication* assessed at the TOC visit. When results are discussed, typically the term *eradicated* is used to denote both *documented eradication* and *presumed eradication*. Current CLSI (NCCLS) approved breakpoints for MICs measured by disk diffusion and broth dilution tests for all antibiotics were applied to all clinical isolates for which CLSI (NCCLS) breakpoints exist.

Clinical Studies

All comparator antibiotics used in these clinical trials are antibiotics recommended by at least one of the following groups: the Centers for Disease Control and Prevention, Infectious Disease Society of America, American Academy of Family Physicians and the American College of Physicians – American Society of Internal Medicine and have label claims for the indications. In the clinical trials described in this application, the following comparator antibiotics were used for treatment of the indications listed. Clarithromycin extended release and levofloxacin (in two separate clinical trials) for adults with mild to moderate community-acquired pneumonia;

and in another trial for adults with acute bacterial maxillary sinusitis undergoing diagnostic sinus aspiration.

Community Acquired Pneumonia [CAP] (A0661075 and A0661103)

Brief overview of study design and results. Efficacy data and safety data using a single 2.0 gram dose of Azithromycin SR in mild-to moderate community acquired pneumonia (CAP) are contained in two independent, Phase 3, randomized, multicenter, double-blind, double-dummy, comparative international trials, A0661075 and A0661103. The *primary objective* of these two trials was to demonstrate that a single oral 2.0 g dose of Azithromycin SR is clinically non-inferior to either clarithromycin ER tablets, given at a dose of 1 g once daily for seven days (A0661075), or levofloxacin, given at a dose of 500 mg once daily for seven days (A0661103) when used as empiric monotherapy of mild to moderate CAP. One of the *secondary objectives* was to assess bacteriologic efficacy of the two treatment regimens.

Clinical Per Protocol (CPP) subjects included all treated subjects with a clinical diagnosis of CAP and a positive chest radiograph for pneumonia. In addition, subjects were required to take at least 80% of study medications and be assessed within the appropriate visit windows, except in cases of early discontinuation for treatment failure.

The *primary efficacy endpoint* in both studies was sponsor assessment of clinical response for the CPP population at the TOC visit (TOC; study days 14-21). Clinical and bacteriologic responses by baseline pathogen at TOC in the Bacteriologic Per Protocol (BPP) population were also assessed. For response assessments at the LTFU visit (Days 28-35), relapse was defined by the following criteria: (1) symptoms related to pneumonia returned after initial resolution or improvement; (2) new clinical signs or symptoms of pneumonia appeared without documentation of a new pathogen; or (3) the subject received alternate antibiotic therapy for worsening signs or symptoms or reappearance of new signs and symptoms of pneumonia. Other efficacy assessments included sponsor assessment of clinical response by baseline pathogen vs. baseline susceptibility.

For **Study A0661075**, 411 subjects from 55 centers in seven countries in North America, Latin America, Europe, and India (202 Azithromycin SR, 209 clarithromycin) were evaluated for efficacy in the primary analysis. The sponsor's assessment of clinical response rates in the CPP population at the TOC visit (14-21 days after the first dose) was 187/202 (92.6%) for Azithromycin SR and 198/209 (94.7%) for clarithromycin (A0661075 Table 5.2). The 95% confidence intervals for the difference in cure rates

(Azithromycin SR – clarithromycin) were (- 6.9, 2.6), demonstrating that Azithromycin SR is non-inferior to clarithromycin for the treatment of mild-to-moderate CAP (A0661075 Table 5.2).

Bacteriologic response rates were based primarily on clinical response at post-treatment assessment timepoints (presumed eradication or persistence for cures or failures, respectively), as sputum production in subjects with resolving pneumonia infections is often markedly reduced. However, if a post-baseline culture was obtained (e.g., in the event of clinical failure), then bacteriologic response could be documented. Clinical cure rates in the BPP population by baseline pathogen for the major respiratory organisms were as listed in the top half of the following table (Table 12). Overall bacteriologic eradication rates (combined sum of eradication plus presumed eradication) at TOC in the BPP population were similar between treatment groups [123/134 (92%) for Azithromycin SR and 153/169 (91%) for clarithromycin, 95% CI = 5.2, 7.7] (A0661075 Table 5.5 and microbiological responses of individual BPP subjects are in A0661075 Section 13, Table 2.3.1).

Of 176 Azithromycin SR subjects that had a clinical response of cure at TOC, 175 (99.4%) maintained a response of cure at the LTFU (Days 28-35). One hundred seventy-two of 177 clarithromycin subjects (97%) that were considered cures at TOC were also assessed as cured at LTFU. Five clarithromycin subjects (2.8%) were assessed as having relapsed at the LTFU evaluation (A0661075 Table 5.2).

For **Study A0661103**, 363 subjects (174 Azithromycin SR, 189 levofloxacin) from 54 centers in eight countries in North America, Latin America, Europe, and India were evaluated for efficacy in the primary analysis. The sponsor's assessment of clinical response rates in the CPP population at the TOC visit was 156/174 (90%) for Azithromycin SR and 177/189 (94%) for levofloxacin. The 95% confidence intervals on the difference in cure rates were -9.7, 1.7%, supporting the conclusion that Azithromycin SR is non-inferior to levofloxacin for the treatment of mild-to-moderate CAP (A0661103 Table 5.2).

For **Study A0661103**, clinical and bacteriologic response rates in the BPP population by baseline pathogen for the major respiratory organisms were as listed in the bottom half of Table 12 (excerpted from A0661103 Table 5.3.3). Overall bacteriologic eradication rates at TOC in the BPP population were [97/107 (90.7%) for Azithromycin SR and 120/130 (92.3%) for levofloxacin, 95% CI=[-8.8, 5.5] (A0661103 Table 5.5 and microbiological responses of individual BPP subjects are in A0661103 Section 13, Table 2.3.1).

Of 146 Azithromycin SR subjects that had a clinical response of cure at TOC, 100% maintained a response of cure at the LTFU (Days 28-35). One hundred sixty-nine of 170 levofloxacin subjects (99.4%) that were considered cured at TOC were also assessed as cured at LTFU. One levofloxacin subject (0.6%) was assessed as having relapsed at the LTFU evaluation (A0661103 Table 5.2).