

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
050814Orig1s000

MICROBIOLOGY REVIEW(S)

Division of Anti-Infective and Ophthalmology Products

NDA 50-814 SN040
Aztreonam Lysine
Gilead Sciences

Clinical Microbiology Review #2
Peter Coderre, PhD
29 December 2009

APPLICANT:

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SUBMISSION REVIEWED: NDA 50-814 SN040

PROVIDING FOR: The management of cystic fibrosis (CF) patients with *Pseudomonas aeruginosa* to improve respiratory symptoms.

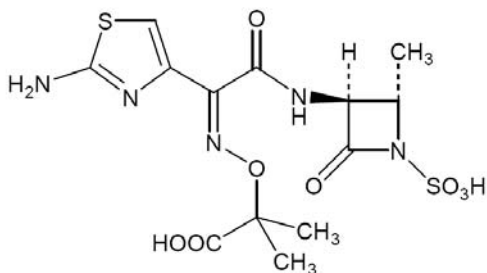
PRODUCT NAME:

Proprietary: CAYSTON
Non-proprietary: Aztreonam Lysine for Inhalation

CHEMICAL NAME: (Z)-2-[[[(2-Amino-4-thiazolyl)[[(2S, 3S)-2 methyl-4-oxo-1-sulfo-3-azetidiny]carbomoyl]methylene]amino]oxy]-2-ethylpropionic acid

MOLECULAR FORMULA: C₁₃H₁₇N₅O₈S₂, MW 453.43 (anhydrous, β-form)

STRUCTURAL FORMULA:



ROUTE OF ADMINISTRATION, DOSAGE AND FORMULATION:

- **Dosage Form:** Aerosol (nebulization) NOTE: A device called the PARI eFlow will be used to deliver the dose. Patients will receive either 75 mg aztreonam for inhalation (AI) [1 ml] twice daily for 14 days.
- **Formulation:** Aztreonam for inhalation (75 mg/ml pyrogen-free aztreonam lysinate) dissolved in sterile 0.17% saline to result in a pH of 4.2—7.0 and osmolarity of 300—550 mOsmol/kg

PHARMACOLOGICAL CATEGORY: Antimicrobial

DISPENSED: Rx

INITIAL SUBMISSION DATES:

Received by CDER:	11 August 2009
Received by Reviewer:	13 August 2009
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REMARKS:

The Applicant submits a resubmission intended to be a complete response to the deficiencies outlined by the Division in the Complete Response Letter (CRL). In addition, the Applicant requested this NDA 50-814 be presented to the Anti-Infective Drugs Advisory Committee as part of the review. This request was granted.

On 10 December 2009, the Anti-Infective Drugs Advisory Committee convened to discuss aztreonam, the subject of this NDA. Presentations were made by the Applicant, the Agency and the Public. It should be noted that one of the three public speakers was a 44-year old attorney afflicted with cystic fibrosis who is being treated with AZLI via an EAP program. This speaker made a compelling, emotional plea for approval of AZLI due to the lack of antimicrobials available for cystic fibrosis.

Following the presentations by the Applicant, the Agency and Public individuals, the committee addressed the following two questions posed by the Agency.

1. Has the Applicant provided substantial evidence of the efficacy and safety of 75 mg three times daily of AZLI for the requested indication of improvement of respiratory symptoms and pulmonary function in cystic fibrosis patients with *Pseudomonas aeruginosa*? In your response, discuss the rationale for your answer.
 - a. If you voted YES, are there any specific issues that should be addressed in labeling?
 - b. If you voted NO, what additional information is necessary?
2. Has the Applicant identified the correct dose and regimen for AZLI for the requested indication? In your response, discuss the rationale for your answer and discuss the rationale for your answer and discuss if there is any additional information that should be generated regarding the dose and regimen.

In response to question #1 regarding evidence of efficacy and safety, the committee voted as follows:

YES—15 members

NO — 2 members

The two dissenting voters did not elaborate on the rationale for their dissent other than saying the Applicant did not show sufficient data for efficacy, which did not show statistical significance. The other member believed that AZLI was not "durable" and that aztreonam resistance could be a potential safety problem.

While there were a variety of reasons given, many of the 15 assenting voters gave similar rationale for their vote.

- The bar should be low for approval for this drug due to the lack of alternative drugs for the treatment of this disease.
- New drugs are needed for this disease.
- The risk to benefit ratio is low for this drug.
- There was overriding evidence for the safety and efficacy of this drug.

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- The [CP-AI-006](#) study presented the necessary evidence to demonstrate safety and efficacy of this drug.

Some members of the committee did express concerns. One member was concerned about the time-to-need (TTN) data suffering from the lack of data. By far, the most common concern was the “durability” of AZLI compared to that of TOBI.

Members expressed concerns about the approval of the drug that should be addressed in the labeling of the drug. Due to the concern regarding durability of AZLI, the development of AZLI resistance should be tracked over time. This is based on the belief that the 28 day treatment period was not sufficient to address the chronic effect on resistance. Another committee member stated that the label should be crafted such that only the device described in this application could be used to deliver AZLI to cystic fibrosis patients.

In response to question #2 regarding identification of the correct dose and regimen for AZLI, the committee voted as follows:

YES—17 members

NO — 0 members

There was less discussion of the rationale for the answers given by the committee for this question as compared to the first question. Several members favored the TID regimen, due to the favorable time above MIC for the TID regimen versus the BID regimen. One member would consider the BID regimen as appropriate treatment while another member was skeptical that the BID regimen was an appropriate treatment.

The recommendations of the Anti-Infective Advisory Committee were considered along with the microbiological outcomes in the determination of a recommendation by this Reviewer.

This review describes the findings and recommendations of the Clinical Microbiology Reviewer. These recommendations are for evaluation by the Division Director for the determination of a decision regarding the approval of this new drug application.

CONCLUSION AND RECOMMENDATIONS:

The Applicant seeks approval of AZLI for the improvement of respiratory symptoms and pulmonary function in CF patients with *Pseudomonas aeruginosa*.

AI is intended as a suppressive therapy and is not expected to eradicate *P. aeruginosa* in CF patients with established airway infections. Consequently, in the Phase 2 and three Phase 3 clinical trials, there are no definitive endpoints for microbiological eradication or clinical cure. Under these circumstances, the objective of the review team is to determine **what constitutes microbiological or clinical success?** While it is not the place for this Reviewer to define clinical success, he shall attempt to define microbiological success.

There are two ways to evaluate the data from this submission. First, the microbiological efficacy endpoints were compared to the most common clinical efficacy endpoint, improvement in FEV₁. Second, the results of the microbiological

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and primary clinical efficacy endpoint data were compared to the corresponding data derived from the package insert for TOBI Solution for Inhalation (TSI), a drug for which AI is intended to be an alternative treatment for CF. TOBI is the only other Agency-approved drug for the management of cystic fibrosis and also is administered in a similar manner.

There are three measures of microbiological outcome:

1. Change in log₁₀ *P. aeruginosa* CFUs/g sputum specimens from patients;
2. Appearance of other pathogens, specifically *S. aureus*, *B. cepacia*, *S. maltophilia* or *A. xylosoxidans* in CF patients; and
3. Changes in aztreonam MIC50s and MIC90s of *P. aeruginosa* isolates from CF patients.

However, none of the data from the three microbiology outcomes correlated with clinical outcomes.

In the original NDA submission, to investigate the possible correlation between microbiological and clinical endpoints, scatterplots were produced. Scatterplots examining these endpoints showed the following:

- Changes in numbers of PA in sputum were not associated with changes in FEV₁ or aztreonam concentrations in study CP-AI-003, CP-AI-005 and slightly negatively associated in study CP-AI-007.
- Changes in numbers of PA in sputum were not associated with changes in aztreonam concentrations in study CP-AI-003.
- Changes in aztreonam MIC for the PA isolate with the highest MIC from each patient were slightly positively associated with changes in FEV₁ in study CP-AI-003 but slightly negatively associated in study CP-AI-007.
- Changes in numbers of PA in sputum were not associated with changes in aztreonam MIC for the PA isolate with the highest MIC in study CP-AI-005 and CP-AI-007.
- Changes in numbers of PA in sputum were not associated with changes in CFQ-R respiratory domain score in study CP-AI-007.
- The Applicant did not present scattergrams to explain relationships between microbiological to clinical outcomes.

The lack of correlation of microbiology outcomes with clinical outcomes for the treatment of cystic fibrosis with aztreonam is not unprecedented. In the Microbiology subsection of the package insert for TOBI for the management of cystic fibrosis patients with *P. aeruginosa*, the Agency states "The relationship between *in vitro* susceptibility test results and clinical outcome with TOBI therapy is not clear".

The efficacy of AZLI for CF caused by *P. aeruginosa* is not entirely clear in terms of clinical outcomes. *While none of the three microbiology responses correlated with clinical responses, AZLI treated patients demonstrated positive microbiological outcomes by all three measures.* In addition, the Applicant has demonstrated the safety of AZLI from the Microbiology standpoint as demonstrated by the lack of the development of aztreonam resistance in patients during therapy.

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This Reviewer recommends that CAYSTON be APPROVED for the treatment of cystic fibrosis in patients infected with *Pseudomonas aeruginosa* based on the following:

- the medical need for new antibiotics to treat cystic fibrosis,
- the demonstrated safety of the drug from the Microbiology standpoint, and
- the drug is efficacious from the Microbiology standpoint,
- the overwhelming vote by the Anti-Infective Advisory Committee for the approval of CAYSTON at the prescribed dose suggested this drug is needed by cystic fibrosis patients.

One major concern expressed by the Advisory Committee was the potential for the development of aztreonam resistance in *P. aeruginosa* isolates harbored in cystic fibrosis patients. *In vitro* data does support this concern.

Table A. Aztreonam Susceptibility Trends in CF Isolates of *P. aeruginosa*

Reference	Region	No. of Isolates (Patients)	MIC ₅₀	MIC ₉₀	Range	%S	%R
1995-1996 ⁶³	US	1240 (508 pts)	≤ 2	32	≤ 2-> 512	80.0	11.9
2004-2005	US	242 (105 pts)	≤ 1	64	≤ 1-> 2048	78.9	10.3
Phase 2 AI baseline data ⁶⁴							
1993-1997 ⁶⁷	Italy	1267 (224 pts)	NR	NR		NR	19.7
2004-2005 ⁶⁶	Europe	150	8	256	0.125-> 512	56	30
2006 ⁶⁵	US	1826	8	≥ 32		68.6	23.1

NR = Not reported

Source: Table 6, NDA 50-814 Microbiology review.

Notice in [Table A](#) that MIC90 values for *P. aeruginosa* isolates increased from 32 µg/ml in 1995—96 to 64 µg/ml in 2004—05 to ≥ 32 µg/ml in 2006. While this *in vitro* data suggests the potential for development of resistance to aztreonam, patients did not develop aztreonam resistance during therapy.

To address the concerns of the Advisory Committee, this Reviewer recommends the Applicant be subject to a **Phase 4 commitment**.

This commitment shall include the monitoring of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients for aztreonam and tobramycin resistance for a period of not less than three years. The aztreonam and tobramycin susceptibilities from these isolates should be taken from the Cystic Fibrosis Foundation Registry and reported to the Agency on a yearly basis.

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EXECUTIVE SUMMARY

Aztreonam is a synthetic bactericidal monobactam antibiotic that was originally isolated from *Chromobacterium violaceum*. Aztreonam (Azactam®) is the only monobactam approved for clinical use in the US.

The Applicant seeks the use of Aztreonam for Inhalation (AI) for the improvement of respiratory symptoms and pulmonary function in cystic fibrosis (CF) patients over 6 years of age with *Pseudomonas aeruginosa*.

What follows is a summary of the *in vitro* preclinical, *in vivo* preclinical and clinical efficacy data for the use of aztreonam for the improvement of symptoms of CF.

The ***in vitro* preclinical efficacy** is determined by: the mechanism of action, spectrum of activity, mechanism of resistance, cross-resistance, post-antibiotic effect and other miscellaneous studies.

Mechanism of Action. Aztreonam, like beta-lactam antibiotics, inhibits bacterial cell wall biosynthesis, resulting in cell lysis and death through irreversible inhibition of penicillin-binding proteins (PBPs), enzymes that catalyze the elongation and crosslinking of the peptidoglycan chains. Aztreonam binds preferentially and with high affinity to PBP3 of aerobic Gram-negative bacteria, accounting for its potent antibacterial activity against *P. aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter cloacae* and *Klebsiella pneumoniae*. In contrast, aztreonam has poor affinity for the PBP3 of Gram-positive and anaerobic bacteria and consequently is inactive against these bacteria. Binding of aztreonam to PBP3 results in filamentation and cell lysis, an effect similar to that observed with cephalosporins.

Aztreonam, like beta-lactam antibiotics, demonstrates time-dependent killing against sensitive Gram-negative bacteria; increasing the concentration of aztreonam above the minimum inhibitory concentration (MIC) of the test organism does not substantially increase the speed or degree of bacterial killing.

Spectrum of Activity. Aztreonam is active against several aerobic Gram-negative pathogens, including many *Enterobacteriaceae* as well as *P. aeruginosa*, but is relatively inactive against Gram-positive and anaerobic organisms.

Other aerobic Gram-negative bacteria show *in vitro* susceptibility to aztreonam, although the clinical significance of these organisms in CF is unknown, and data regarding clinical efficacy is not available. Susceptible bacteria are typically killed at concentrations one to four times their MICs. However, in some cases, the concentration of aztreonam required to achieve bactericidal killing of *P. aeruginosa* has been reported to be 4 to 16 times the MIC. Aztreonam exhibits MICs of ≤ 8 $\mu\text{g/mL}$ against at least 90% of the following aerobic Gram-negative bacteria: *Aeromonas hydrophila*, *Morganella morganii*, *Neisseria gonorrhoeae* (including penicillinase-producing strains), *Pasteurella multocida*, *Proteus vulgaris*, *Providencia stuartii*, *Providencia rettgeri*, and *Yersinia enterocolitica*. The safety and efficacy of aztreonam in treating clinical infections due to these microorganisms, however, have not been established in clinical trials.

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Recent data regarding the activity of aztreonam compared with other antibiotics commonly used to treat Gram-negative infections has been examined. Overall, aztreonam is less active than other commonly used antibiotics in Asia, Europe, Latin America and North America.

Antibiotic susceptibility trends in bacterial isolates collected from CF patients may differ from the general patient population due to the chronic nature of antibiotic therapy in CF patients. In the US, susceptibility of *P. aeruginosa* to aztreonam has gradually decreased within both the CF and non-CF populations over the last 10 years with 80% of *P. aeruginosa* isolates susceptible ($\text{MIC} \leq 8 \mu\text{g/mL}$) to aztreonam in 1995, 78.9 % susceptible in 2004, and 68.6% susceptible in 2006. Aztreonam has been shown to have *in vitro* activity against tobramycin-resistant and multidrug-resistant *P. aeruginosa* isolates from CF patients.

Mechanisms of Resistance. Multiple mechanisms of bacterial resistance to aztreonam and beta-lactam antibiotics have been identified. These mechanisms include the derepression of chromosomal beta-lactamases, the acquisition of beta-lactamase encoding plasmids, overexpression of efflux pump systems, impermeability of the outer membrane, and alteration of the drug target (PBPs).

In Gram-negative bacteria the prevalence of various resistance mechanisms varies by organism and two or more mechanisms may contribute to the overall resistance profile. In contrast, for *P. aeruginosa* isolated from CF patients, resistance to beta-lactam antibiotics was primarily due to overproduction of a constitutive cephalosporinase (18.6%), and rarely due to production of a transferable beta-lactamase (1.4%). However, researchers also reported that susceptibility to aminoglycosides and fluoroquinolones was lower in isolates producing beta-lactamases, thereby suggesting involvement of other mechanisms.

Aztreonam is a poor inducer of beta-lactamase production and, although not used as such, is an inhibitor of many beta-lactamases.

The beta-lactamases are classified using the Ambler system into four groups (A, B, C, and D) according to their amino acid sequences. Classes A, C, and D beta-lactamases use a catalytically active serine molecule for inactivation of the beta-lactam drug, while Class B enzymes are metallo-enzymes requiring zinc for activity.

Of most concern among the Class A beta-lactamases are the clavulanic acid-inhibited Ambler Class A ESBLs that are broadly active against most beta-lactams, as well as aztreonam. The TEM- and SHV-type ESBLs have been extensively reported in *Enterobacteriaceae* but have been only rarely reported from *P. aeruginosa*.

The class B metallo-beta-lactamases (MBLs) hydrolyze a wide variety of beta-lactams, including carbapenems, but do not hydrolyze aztreonam. While there have been rare reports of organisms with MBLs that are also resistant to aztreonam [VIM-5 (*K. pneumoniae* and *P. aeruginosa*-Turkey), VIM-6 (*P. putida*-Singapore) and VIM-7 (*P. aeruginosa*-United States)], it is possible that these organisms were expressing multiple resistance mechanisms.

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Recently, a strain of *P. aeruginosa* was found to express an ESBL, GES-9 which differs from GES-1 by a Gly243Ser substitution. This ESBL hydrolyzes aztreonam but is inhibited by clavulanic acid and imipenem. The gene for GES-9 is located inside a class 1 integron structure containing two copies of an insertion sequence.

AmpC, the best characterized enzyme of Class C beta-lactamases, is normally produced at low levels in clinical isolates of *P. aeruginosa* and *Enterobacteriaceae* but production can be induced to high levels by the presence of beta-lactams, especially cefoxitin and imipenem. High-level production of both chromosomal- and plasmid-encoded AmpC is responsible for cross-resistance to aztreonam and to multiple beta-lactam antibiotics.

The OXA-type carbapenemases (Class D beta-lactamases) generally have little or no activity against aztreonam, but there have been rare reports of oxacillinases from clinical isolates of *P. aeruginosa* (OXA-11 [Turkey] and OXA-45 [United States]) that have hydrolyzed aztreonam.

Some porins provide entry for a wide variety of drugs and others are specific for a single drug allowing permeation through the outer membrane. In *P. aeruginosa*, porins have extremely low permeability to most beta-lactams and thereby play a major role in intrinsic resistance. Due to variability in porin specificity, alterations may lead to resistance to one or more antibiotics. An example of specific drug resistance is seen in loss of the OprD porin, which can result in resistance of *P. aeruginosa* to carbapenems but does not confer cross-resistance to non-carbapenem beta-lactams or aztreonam.

Several chromosomally-encoded efflux systems play an important role in *P. aeruginosa* antibiotic resistance. The MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM systems extrude a wide variety of antibiotics including beta-lactams. Most beta-lactams, (e.g., penicillins, cepheems, and meropenem) are pump substrates for all of these efflux systems. In contrast, aztreonam can only be extruded by the MexAB-OprM pump system.

Clinical resistance associated with alteration of PBPs is exceedingly rare in *P. aeruginosa*, but cannot be ruled out.

In vitro resistance frequencies for *P. aeruginosa* ATCC 27853 were low, less than 1×10^{-8} in the presence or absence of aztreonam (500 µg/mL). A modified Szybalski method was used to demonstrate that aztreonam, at four times the MIC, selected resistant variants of 10 *P. aeruginosa* isolates (two lab isolates, eight isolated from blood of cancer patients) *in vitro*. Mutants resistant to aztreonam were generated at a frequency of approximately 1×10^{-6} to 1×10^{-7} . Some mutants produced large amounts of cephalosporinase; however, aztreonam was stable to hydrolysis by this enzyme.

Cross resistance. Aztreonam is not antagonistic with the antibiotics commonly used on- and off-label to treat respiratory infections in CF patients, suggesting that clinical use of aztreonam lysine in conjunction with another antibiotic to treat CF respiratory infections will not reduce the antibacterial activity of either drug due to antagonistic interactions.

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When *P. aeruginosa* or *Enterobacteriaceae* are treated with aztreonam in combination with beta-lactam antibiotics, the interactions are typically indifferent or synergistic. In a study of 14 multidrug-resistant isolates of *Enterobacteriaceae* (N = 10) and *P. aeruginosa* (N = 4 [ATCC 27853 and three clinical isolates]), synergy was noted with 42% (6 of 14) of the organisms when aztreonam was combined with any of the beta-lactam antibiotics tested (piperacillin, moxalactam, cefotaxime, or ceftazidime); indifferent effects were observed with all other organisms.

Investigators have demonstrated that combinations of aztreonam plus cefepime, piperacillin or imipenem resulted in indifference against *P. aeruginosa* isolates. Interactions with cephalosporins were predominantly partially synergistic.

Combinations of aztreonam with quinolones sometimes result in synergy, but indifferent or additive effects are more commonly observed. Combinations of aztreonam plus ciprofloxacin generally resulted in indifference against *P. aeruginosa* isolates. However, one investigator examined interactions between aztreonam and ciprofloxacin, levofloxacin, gatifloxacin or moxifloxacin using the checkerboard method. Interactions were evaluated against four isolates each of *P. aeruginosa*, *B. cepacia*, *E. cloacae*, *K. pneumoniae*, *E. coli* and *P. mirabilis*. All combinations demonstrated uniformly additive activity against *P. aeruginosa*.

A recent report by Gasink et al. concluded that curbing the use of fluoroquinolones and antimicrobials with activity against anaerobes may be an effective strategy to limit the emergence of aztreonam-resistant *P. aeruginosa*. These investigators identified risk factors for infection or colonization with aztreonam-resistant *P. aeruginosa*. Nosocomial mortality was greater among patients infected or colonized with aztreonam-resistant *P. aeruginosa* compared with those infected with aztreonam-susceptible *P. aeruginosa*.

In vitro interactions between aztreonam and aminoglycosides have been shown to be synergistic against *P. aeruginosa*, *Enterobacteriaceae*, and other Gram-negative aerobic bacilli. These aminoglycosides included: amikacin, gentamicin, tobramycin or netilmicin.

PAE. Post antibiotic effect (PAE) refers to the suppression of bacterial growth that persists after short-term exposure to an antibiotic. Aztreonam, like beta-lactam antibiotics, has minimal or no persistent effects on most organisms. The duration of PAE for aztreonam against *P. aeruginosa* ATCC 27853 is less than 0.5 hour when tested at concentrations equivalent to four times the MIC. Because aztreonam does not have a demonstrable PAE for *P. aeruginosa*, the dosing schedule was determined on the basis of the pharmacokinetic (PK) parameters.

Miscellaneous Studies. A number of miscellaneous studies were conducted to examine the effects of aztreonam in a pharmacodynamic model, effects on phagocytic cells, effects on gastrointestinal flora, effects on biofilms and the effects of nebulization on aztreonam potency.

A pharmacodynamic model was developed to facilitate evaluation of aztreonam activity when delivered to an *in vitro*, mucin-covered epithelial cell surface. Although the model's reproducibility limited its utility, a significant decrease in CFUs was

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observed by 6 to 12 hours for both aztreonam and tobramycin with regrowth at 24 hours.

Exposure of bacteria to aztreonam can modify the interactions between Gram-negative bacteria and phagocytic cells, resulting in enhanced phagocytic killing due to aztreonam-induced alterations in the bacterial cells. Sub-MIC concentrations of aztreonam enhanced the phagocytosis and killing of *P. aeruginosa* K1 by a murine macrophage cell line. Enhanced phagocytic killing was attributed to modification of bacterial cell surface structures upon exposure to aztreonam.

Aztreonam has the potential to alter the normal gastrointestinal flora, thereby decreasing patients' resistance to colonization with other pathogens such as *Candida* and *Clostridium* spp. However, in the clinical trials conducted for this NDA, systemic exposure to aztreonam is very low and is unlikely to lead to pseudomembranous colitis.

In chronic airway infections in CF patients, *P. aeruginosa* is likely to exist in both planktonic and biofilm phases. The presence of a biofilm in CF helps to explain why *P. aeruginosa* lung infections are rarely eradicated with antibiotic therapy, regardless of the organism's reported *in vitro* susceptibility. Recently a methodology to test antibiotic susceptibility within a biofilm was developed with the hope that it might be more predictive. As with beta-lactams, biofilm inhibitory concentrations (BICs) for aztreonam are much higher than conventionally determined MICs. Against 85 clinical isolates of *P. aeruginosa* from the US, the aztreonam, the MIC₉₀ was 32 µg/mL, whereas the BIC₉₀ was > 128 µg/mL.

To determine whether nebulization impacts the potency of aztreonam, the *in vitro* activity of aztreonam lysine was tested before and after nebulization against 161 clinical isolates of *B. cepacia* complex, *S. maltophilia*, *A. xylosoxidans*, *S. aureus*, and *P. aeruginosa* from CF patients. In aggregate, these results support the physical finding that the antimicrobial activity of aztreonam is not significantly reduced during nebulization by the eFlow device.

Pharmacokinetics. AI is delivered using the PARI eFlow electronic nebulizer (eFlow) which produces an aerosol with a relatively monodispersed particle spectrum which is well-suited for lower airway drug deposition. Thus, this drug is essentially a topical antibiotic and does not have the same pharmacokinetic properties of a paternally administered antibiotic.

Sputum provides a growth environment for bacteria and can significantly inhibit the activity of antibiotics. Investigators evaluated aztreonam lysine time-kill kinetics for *P. aeruginosa* ATCC 27853 using aztreonam concentrations equivalent to 0.1 times, 1 time, and 10 times the MIC. No killing was observed at 0.1 times the aztreonam MIC. Bactericidal levels of killing (= 3 log₁₀) were reached by five hours, and the speed and degree of killing were comparable at aztreonam concentrations of 1 time and 10 times the MIC. The killing kinetics of aztreonam against *P. aeruginosa* ATCC 27853 in the presence of 1% CF sputum and 10% porcine mucin were nearly identical to those conducted in media alone. By comparison, the activity of aminoglycosides is known to be antagonized by sputum.

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Similar time-kill studies were conducted with 20 CF *P. aeruginosa* isolates using 10% porcine gastric mucin as a model for the protein-binding component of sputum. Similar results were found with the gastric mucin model. The sputum and mucin kill-curve data suggest that aztreonam administered as an aerosol to the CF lungs will not be significantly inhibited by sputum.

Clinical Laboratory Susceptibility Testing. The approved antimicrobial susceptibility test methods and quality control parameters used for parenteral aztreonam therapy were used to monitor the activity of AI against *P. aeruginosa* isolates from CF patients. It should be noted that susceptibility testing of *P. aeruginosa* isolates from CF patients, in some cases, required extended incubation up to 24 hours for accurate determination of MIC values.

Quality Control. Aztreonam has been approved by the Agency for other indications. In addition, as AI is a topically administered antibiotic, interpretive criteria will not be determined. Therefore, quality control parameters were not determined.

Provisional Interpretive Criteria. Aztreonam has been approved by the Agency for other indications. Therefore, provisional interpretive criteria were not determined.

Clinical Efficacy. The Applicant presents the protocols and results from four clinical trials: one phase 2 trial, [Study AI-003](#), and three phase 3 trials. Among the three phase 3 trials were two pivotal studies, [Study AI-005](#) and [Study AI-007](#), as well as a follow-on study, [AI-006](#), which will not be discussed in detail.

Study AI-003 was a blinded, multicenter, randomized, placebo-controlled phase 2 trial with aztreonam for inhalation (AI) in CF patients with lung disease due to *P. aeruginosa* infection. The trial was designed to assess the safety and efficacy of 14-day treatment with AI at two dosage levels compared to placebo in CF patients with pulmonary *P. aeruginosa* (PA) infections.

Clinical Results. There was an increase in adjusted mean percent change in FEV₁ (L) from Day 0 to Day 14 in all treatment groups (4.08% in the placebo group, 6.20% in the 75 mg AI group, and 7.71% in the 225 mg AI group). There was no significant difference in the mean percent change in FEV₁ from Day 0 to Day 14 between the 225 mg AI and placebo treatment groups, either overall (treatment difference: 3.62%; CI: -1.50, 8.75) or for any of the subsets tested (disease severity [< 60 or $\geq 60\%$ FEV₁ percent predicted], age, gender, and MIC category [≥ 8 µg/mL]) for the ITT or PP datasets.

There was an increase in adjusted mean percent change in FEV₁ from Day 0 to Day 7 in all treatment groups (5.03% in the placebo group, 7.59% in the 75 mg AI group, and 9.74% in the 225 mg AI group). The difference of 4.71% between the 225 mg AI and placebo groups was statistically significant (CI: 0.06, 9.35), however, it was not confirmed by the PP dataset.

None of the results for the remaining spirometry tests (FEV₁, FVC, and FEF₂₅₋₇₅) was statistically significant at any time point. For FEV₁ and FVC, mean absolute and percent changes were greater at Day 7 than they were at Day 14 for all treatment

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groups. For FEF₂₅₋₇₅, the mean percent change from Day 0 at Day 7 was similar to that at Day 14 for the 75 mg AI group, and slightly smaller for the 225 mg AI group.

There were no trends in the numbers of patients who required hospitalization, emergency treatment or anti-PA antibiotics during the trial. There was no statistically significant difference between the placebo and 225 mg AI groups in the numbers of patients unable to expectorate sputum at Day 14.

There was a potential loss of power in the trial because there were only 105 evaluable patients in the ITT dataset, which was less than the planned sample size of 138, and this may have contributed to the lack of statistical significance shown in the results.

Microbiology Results: There was a mean reduction in log₁₀ PA CFUs at Days 7 and 14 in both AI treatment groups, and the mean reductions in the 225 mg group were slightly larger than those in the 75 mg AI group. The difference (CI) between the 225 mg AI and placebo groups in change in log₁₀ PA CFUs from Days 0 to 14 of -2.146 (-3.029, -1.263) was highly statistically significant (p-value < 0.0001).

The majority of patients in each treatment group showed no changes in the presence or absence of the pathogens tested. After dosing, PA infection disappeared from two patients in the 75 mg AI group (Day 28), and three and seven patients in the 225 mg AI group (Days 7 and 14, respectively). PA infection did not disappear at any postdosing day for any patients in the placebo group. There were no notable differences between treatments in the numbers of patients becoming positive after dosing for any of the pathogens tested (*S. aureus*, *B. cepacia*, *S. maltophilia*, or *A. xylosoxidans*). There were no notable differences between treatment groups in the numbers of patients who were positive at Day 0 and became negative for any of the other pathogens tested.

There were no notable increases from Day 0 in MIC₅₀ or MIC₉₀ (using all PA isolates and the PA isolate with the highest MIC from each patient) in any treatment group, indicating there was no evidence of reduced susceptibility of PA to aztreonam. There were no notable increases from Day 0 in the numbers of patients whose PA isolate with the highest MIC was above the parenteral susceptibility breakpoint for aztreonam (≥ 8 µg/mL) in either AI treatment group.

Thus, from the microbiological viewpoint, the results of **Study AI-003** were encouraging since treatment arms showed statistically significant log₁₀ reductions of *P. aeruginosa*, there were few changes in the appearance of new pathogens and there were no notable increases in MIC₉₀ values from baseline to TOC, thus no reduced susceptibility of the drug on therapy.

Study AI-005 was a phase 3 clinical trial with AI in CF patients with pulmonary *P. aeruginosa* requiring frequent antibiotics. This trial was designed to assess the safety and efficacy of a 28-day treatment with Aztreonam Lysine for Inhalation (AI) and the ability of AI to maintain or improve clinical status following a 28-day course of Tobramycin Inhalation Solution (TSI, TOBI®) therapy in cystic fibrosis (CF) patients with pulmonary *Pseudomonas aeruginosa* (PA).

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Clinical Results. The median time to need for inhaled or IV anti-pseudomonal antibiotics due to pre-defined symptoms (*primary efficacy endpoint*) was 21 days longer in the pooled AI group (92 days) than in the pooled placebo group (71 days) ($p = 0.0070$). The median time to antibiotic need for the AI BID group was significantly different than that in the pooled placebo group ($p = 0.0019$; median time not estimable), and the median time to antibiotic need was 87 days for the AI TID group ($p = 0.1816$). There was a strong suggestion of a regimen need for inhaled or IV antibiotics was different between the pooled BID and pooled TID regimens ($p = 0.0012$). The regimen effect was not seen for other efficacy endpoints.

Microbiology Results. As expected, dosing of AI by inhalation generally gave a much higher ($\sim 1,000$ -fold) exposure in sputum than in plasma. There was a large degree of individual patient variation in aztreonam concentrations in both plasma and sputum. After dosing, the majority of patients continued to have positive sputum culture results for PA infection.

P. aeruginosa CFU/g in sputum decreased among patients who received 75 mg AI BID or TID for 28 days. However, the log₁₀ reductions in the treatment arms were lower than the reductions in *P. aeruginosa* CFU/g in sputum seen in patients from [Study AI-003](#).

The difference between the pooled AI and pooled placebo groups for change in log₁₀ PA CFUs in sputum approached statistical significance at Day 14 (-0.490 , $p = 0.0586$) and achieved significance at Day 28 (-0.66 , $p = 0.0059$). From Day 0 to Day 28, log₁₀ PA CFU sputum density decreased (improved) by 0.43 in the pooled AI group, and increased (deteriorated) by 0.23 in the pooled placebo group. The change in log₁₀ PA CFU density was also significantly different for the AI BID (-0.72 , $p = 0.011$) and TID (-0.60 , $p = 0.031$) groups compared with the pooled placebo group. After cessation of therapy (Day 28), log₁₀ PA CFU density among AI-treated patients was greater than baseline at Days 42 and 56.

A higher number of patients had *S. aureus* isolated intermittently in the pooled AI group (eight [6%] patients) than in the pooled placebo group (two [2.8%] patients). While there was no overall difference in persistent isolation of *S. aureus* between the pooled AI and pooled placebo groups, persistent isolation of *S. aureus* was higher in the AI TID group than in the AI BID group (eight [12%] patients vs. four [6%] patients). The small number of patients who persistently tested positive was not sufficient to determine a dose-response effect. There were no notable effects of AI on the isolation of any of the other pathogens tested (*B. cepacia*, *S. maltophilia*, and *A. xylosoxidans*).

MIC₅₀ and MIC₉₀ of aztreonam for all PA isolates, and for the isolates with the highest MIC from each patient generally remained unchanged for all treatment groups. In the AI TID group, for all PA isolates, there was a transient increase from Day 0 in MIC₅₀ and MIC₉₀ at Day 14 (from 2 to 8 $\mu\text{g/mL}$, and from 32 to 128 $\mu\text{g/mL}$), which at the subsequent time point (Day 28) had each returned to unchanged from Day 0. For PA isolates with the highest MIC from each patient, the observed ranges of MIC₅₀ and MIC₉₀ values were generally not different among the AI BID, AI TID, and pooled placebo groups between Day -28 and Day 56, with no

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consistent pattern of increase above pre-drug levels observed after administration of AI.

The percentages of patients whose highest aztreonam MICs increased after dosing were higher in the AI BID and TID groups than in the pooled placebo group at all time points. The differences were statistically significant at Day 28, where 38 (37%) patients in the pooled AI group had an increase in their highest MICs, compared with 13 (21%) patients in the pooled placebo group ($p = 0.0320$). When separated by regimen, the result was significant for the AI TID group ($p = 0.0463$), and trended toward significance for the AI BID group (0.0932). The differences were not significant at Days 42 or 56, likely indicating a transient increase in MICs during treatment that is reversible when AI is discontinued. The percentage of patients whose PA isolate with the highest MIC of aztreonam was above the parenteral breakpoint (8 $\mu\text{g/mL}$) increased from Day 0 to Days 14 and 28 in the pooled AI group, but not in the pooled placebo group. The largest increase from Day 0 occurred at Day 28, where 47 (44%) patients in the pooled AI group had their highest MICs $\geq 8 \mu\text{g/mL}$, compared with 37 (30%) patients at Day 0. Within the AI TID group, percentages returned to near Day 0 values after Day 28, whereas within the AI BID group, percentages remained relatively high compared with Day 0. There were no differences between pooled treatment groups in change in MIC of tobramycin from Day 0 for the PA isolate with the highest MIC from each patient or in the percentage of patients with MIC of tobramycin $= 8 \mu\text{g/mL}$ for the PA isolate with the highest MIC. There were no notable increases in MIC50 or MIC90 of other antibiotics tested against PA after treatment with AI.

Study AI-007 was a phase 3, double-blind, multicenter, multinational, randomized, placebo-controlled trial evaluating aztreonam for Inhalation in CF patients with pulmonary *P. aeruginosa* infection. This trial was designed to assess the safety and efficacy of a 28-day treatment with aztreonam lysine for inhalation (AI) (75 mg three times daily [TID]) compared to placebo.

Clinical Results: Patients in the AI group had a clinically significant improvement in respiratory symptoms as measured by the CFQ-R respiratory domain score for Child/Teen/Adult combined. The adjusted mean change from Day 0 at Day 28 was 7.08 compared with -2.63 in the placebo group. The adjusted mean difference (9.71) between AI and placebo at this time point was statistically significant ($p = 0.0005$). Significant differences were also observed between the treatments at Day 14 (7.98, $p = 0.0006$) and Day 42 (6.33, $p = 0.0154$). A greater proportion of patients in the AI group showed clinically meaningful improvement in CFQ-R respiratory domain score at Day 28 (56%) compared with patients in the placebo group (37%), $p = 0.0055$.

Microbiology Results. As expected, dosing of AI by inhalation generally gave a much higher (more than 1,000-fold) exposure in sputum than in plasma. There was a large degree of individual patient variation in aztreonam concentrations in both plasma and sputum. After dosing, the majority of patients continued to have positive sputum culture results for PA.

At baseline (Day 0), mean log₁₀ PA CFU sputum density values were nearly equivalent in the AI and placebo groups. In the AI group, log₁₀ PA CFU sputum

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density had decreased by more than 1 log₁₀ CFU at Day 14 and by more than 1.5 log₁₀ CFUs at Day 28. Log₁₀ PA CFU sputum density in the placebo group remained near baseline values at Days 14 and 28. At Day 42, two weeks after cessation of therapy, log₁₀ PA CFU sputum density had returned to near baseline values in both the AI and placebo groups.

Fewer patients in the AI group compared with the placebo group had intermittent isolation of *S. aureus* (four [5%] patients receiving AI vs. seven [9%] patients receiving placebo) and persistent isolation of *S. aureus* (two [2.7%] patients receiving AI vs. five [6%] patients receiving placebo). There was no difference between the AI and placebo groups in the percentage of patients with intermittent isolation of *S. maltophilia*. Two (2.7%) patients in the AI group had persistent isolation of *S. maltophilia* compared to zero patients in the placebo group. Zero patients in the AI group had intermittent isolation of *A. xylosoxidans* compared to two (2.5%) patients in the placebo group. There was no difference between the AI and placebo groups in the percentage of patients with persistent isolation of *A. xylosoxidans*. There was no intermittent or persistent isolation of *B. cepacia* from any patient.

The MIC₅₀ and MIC₉₀ of aztreonam for all PA isolates, and for the isolate with the highest MIC from each patient generally remained unchanged for both treatment groups. The only notable effects were reported for MIC₉₀ after dosing in the AI group; for all PA isolates, there was a transient increase in MIC₉₀ from Day 0 to Day 14 (from 128 to 512 µg/mL), which returned to unchanged from Day 0 at the subsequent time point (Day 28). At Days 28 and 42 the percentage of patients whose highest MICs increased >4-fold was higher in the AI group than in the placebo group. At Day 28, 16 (28%) patients in the AI group had an increase in their highest MICs, compared with 8 (12%) patients in the placebo group; this difference approached statistical significance based on a CMH test ($p = 0.0804$). The difference between the AI and placebo groups was not statistically significant at Day 42 and the percentage of patients with increases in their highest MICs had not increased in the AI group relative to Day 28. However, the percentage of patients with increases in their highest MICs remained higher in the AI group than in the placebo group (14 [25%] patients treated with AI vs. 7 [13%] patients treated with placebo). There were no notable changes from Day 0 in either treatment group in percentages of patients with MICs of aztreonam above the parenteral breakpoint (≥ 8 µg/mL) for the PA isolate with the highest MIC. There was no difference between the AI and placebo treatment groups in the change in MIC of tobramycin from Day 0 for the PA isolate with the highest MIC from each patient.

There was no difference between the AI and placebo treatment groups in the percentage of patients with MIC of tobramycin ≥ 8 µg/mL for the PA isolate with the highest MIC. There were no notable increases in MIC₅₀ or MIC₉₀ of other antibiotics tested against PA after treatment with AI for 28 days.

No correlations were found between highest baseline aztreonam MIC for PA and three key efficacy parameters (CFQ-R respiratory domain score, FEV₁, and log₁₀ PA CFU density).

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Study AI-006 was an open-label, multicenter follow-on study of multiple courses of AI designed to assess safety and disease-related outcomes following repeated exposure of up to nine courses of AI in CF patients who participated in either **AI-005** or **AI-007**. Patients enrolled into the **AI-006** study receive 75 mg AI in the same treatment regimen, BID or TID, to which they were randomized in **AI-005** or **AI-007** for 28-day AI courses with 25 days between courses. The original open-label follow-on study protocol was designed to have patients receive two AI treatment courses with an optional third course.

Log₁₀ *Pseudomonas aeruginosa* (PA) CFUs in sputum generally decreased from the start of each course to the end of each course, and the decrease in log₁₀ PA CFUs was generally larger in TID-treated than BID-treated patients. Log₁₀ PA CFUs in sputum decreased from Visit 1 at each visit (Visits 2 through 20) in both the BID and TID groups, and the changes at the end of each on treatment interval were generally greater for patients in the TID group.

During the off-treatment intervals, log₁₀ PA CFUs increased between visits; these increases were all slightly higher for the TID regimen compared with the BID regimen. However, during the on-treatment intervals, log₁₀ PA CFUs decreases were slightly greater in the TID cohort.

Log₁₀ PA CFUs in sputum decreased from Visit 1 over the nine treatment courses in patients with FEV₁ ≤ 50% of predicted at baseline and the decreases were greater than in patients with FEV₁ > 50% of predicted. An overall decline in log₁₀ PA CFUs was anticipated, given that the use of other antibiotics was allowed during study participation and prior to the study. However, in addition to suppression of log₁₀ PA CFUs, AZLI-treated patients demonstrated improvements in FEV₁ and respiratory symptoms, as measured by the CFQ-R Respiratory symptoms domain score.

As expected, over the nine treatment courses both on- and off-treatment, the vast majority of BID- and TID-treated patients continued to have positive sputum cultures for *Pseudomonas aeruginosa*.

There were slight fluctuations over time in the percentages of patients testing positive for methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) following repeat dosing with up to nine courses of AZLI treatment in both treatment regimens. Acquisition or clearance of MRSA occurred in such a small percentage of the patient population throughout the study that AZLI therapy does not appear to be associated with or responsible for MRSA emergence.

There were no notable changes in the percentages of patients testing positive for *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans* following repeat dosing with up to nine courses of AZLI treatment in the BID or TID groups.

Burkholderia cepacia complex (BCC) was isolated from five patients, all in the TID group. One of these patients tested positive at baseline (Visit 1) and withdrew after the second treatment course. Two of the patients tested positive for BCC intermittently during the study period but completed all nine treatment courses. Another patient had tested positive at baseline in a previous study, an exclusion

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criteria, and subsequently tested positive in this study at an EOT Visit following study withdrawal for noncompliance. The fifth patient had intermittent positive cultures for BCC, withdrew early from the study but had four negative cultures prior to early study withdrawal.

The percentage of patients with *Aspergillus* spp. at the end of the study was nearly identical to the baseline value. The high prevalence of *Candida* spp. observed in this study is consistent with a recent report demonstrating detection of *Candida albicans* in sputum from 76% of adult patients with CF.

For all *PA* isolates, the MIC₅₀ of aztreonam increased 4-fold at 8 visits in the BID group and at all other visits the MIC₅₀ remained unchanged (± 2 fold change). In the TID group, the MIC₅₀ of aztreonam remained unchanged (± 2 -fold change) from Visit 1. Similarly, there were fewer increases in the MIC₉₀ of aztreonam from Visit 1 for all *PA* isolates in the TID regimen than in the BID regimen throughout nine 28-day on/off courses of AZLI therapy.

For the *PA* isolate with the highest MIC from each patient, the MIC₅₀ of aztreonam remained unchanged (± 2 -fold change) from Visit 1 for the BID and TID regimens. In the BID regimen, the MIC₉₀ for the *PA* isolate with the highest MIC from each patient increased greater than 2-fold above the baseline value for the majority of visits but returned to baseline value (± 2 -fold change) at Visit 20. In the TID regimen, the MIC₉₀ for the *PA* isolate with the highest MIC from each patient remained unchanged (± 2 -fold change) over the course of the nine treatments with the exception of two visits (Visits 13 and 19).

In the BID group, the MIC of aztreonam for the *PA* isolate with the highest MIC remained unchanged from Visit 1 in the majority ($> 50\%$) of patients over nine treatment courses of AZLI, except at Visit 19. In the TID group, the MIC of aztreonam for the *PA* isolate with the highest MIC remained unchanged from Visit 1 in the majority ($> 50\%$) of patients over nine treatment courses of AZLI, except at Visits 11, 13, 15, and 19. At all visits, more patients demonstrated an increase in the MIC of aztreonam from Visit 1 for the *PA* isolate with the highest MIC than a decrease in the TID group. The same is true for the BID group with the exception of Visits 3, 6, and 20.

At Visit 1, the percentage of patients with a *PA* isolate with aztreonam MIC > 8 $\mu\text{g/mL}$ was 40% for the BID regimen and 42% for the TID regimen. Fluctuations in these percentages occurred throughout the study; the largest increases were to 61% in the BID regimen at Visits 13 and 17 and to 55% at Visit 6 in the TID regimen. Following nine 28-day on/off AZLI treatment courses (Visit 20), the percentage of patients with MIC > 8 $\mu\text{g/mL}$ for the *PA* isolate with the highest MIC was 47.5% for the BID regimen and 47% for the TID regimen.

Patients treated with AZLI TID showed improvements in FEV₁ percent of predicted, CFQ-R Respiratory symptoms domain score, and decreases in log₁₀ *PA* CFUs in sputum regardless of baseline aztreonam MIC for *PA*, suggesting there is no association between AZLI efficacy and *PA* susceptibility to aztreonam.

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No correlations were found between highest baseline aztreonam MIC for PA and three key efficacy parameters (CFQ-R respiratory domain score, FEV₁, and log₁₀ PA CFU density).

There were no notable increases in the MIC50 or MIC90 of any of the other antibiotics tested against PA after treatment with AZLI. There were notable decreases in the MIC90 of tobramycin in the BID and TID groups.

Microbiology results from the individual clinical studies of AI in the treatment of CF patients with pulmonary *P. aeruginosa* are summarized in [Table I](#).

Table I. Microbiology Results from Individual Clinical Studies of AI.

	CP-AI-003 (N=105)			CP-AI-005 (N=211)			CP-AI-007 (N=164)		CP-AI-006 (N=274)	
	AI			AI			AI		AI	
	Placebo	75 mg BID	225 mg BID	Placebo	75 mg BID	75 mg TID	Placebo	75 mg TID	75 mg BID	75 mg TID
	(N=32)	(N=38)	(N=35)	(N=76)	(N=69)	(N=66)	(N=84)	(N=80)	(N=85)	(N=189)
Change from baseline in log₁₀ <i>P. aeruginosa</i> CFU/g, Mean (SD)										
Mid-Treatment (1 course)*	0.014 (1.595)	-1.509 (1.453)	-2.080 (1.542)	0.239 (1.505)	-0.055 (1.407)	-0.479 (1.999)	0.032 (1.135)	-0.813 (2.104)	--	--
End-Treatment (1 course)*	0.025 (1.313)	-1.453 (1.575)	-2.109 (1.99)	0.284 (1.167)	-0.387 (1.333)	-0.222 (1.691)	-0.025 (0.786)	-1.476 (2.54)	-0.200 (1.476)	-0.700 (1.651)
End-Treatment (3 courses)*	--	--	--	--	--	--	--	--	-0.430 (1.360)	-0.530 (2.121)
Treatment-emergent persistent isolation of pathogens, n (%)										
<i>S. aureus</i>	--	--	--	6 (8.5)	4 (6.1)	8 (12.3)	5 (6.2)	2 (2.7)	0	0
<i>S. maltophilia</i>	--	--	--	1 (1.4)	2 (3.0)	3 (4.6)	0	2 (2.7)	0	0
<i>A. xylosoxidans</i>	--	--	--	2 (2.8)	1 (1.5)	0	2 (2.5)	1 (1.4)	0	0
<i>B. cepacia</i>	--	--	--	0	0	0	0	0	0	4 (2.1)
MIC50 and MIC90 (mcg/ml) of aztreonam for all <i>P. aeruginosa</i> isolates										
Baseline	≤ 1, 16	2, 64	≤ 1, 128	≤ 1, 64	2, 64	2, 32	2, 64	4, 128	4, 128	4, 128
End-Treatment (1 course)	≤ 1, 16	≤ 1, 64	4, 128	≤ 1, 64	4, 128	4, 64	2, 64	8, 128	4, 256	8, 256
End-Treatment (3 courses)	--	--	--	--	--	--	--	--	8, 256	8, 256
End-Treatment (Visit 20)	--	--	--	--	--	--	--	--	4, 128	8, 256
MIC50 and MIC90 (mcg/ml) of aztreonam for <i>P. aeruginosa</i> isolates with the highest MIC from each patient										
Baseline	4, 64	8, 128	4, 256	4, 128	4, 128	4, 32	8, 128	8, 256	8, 128	8, 256
End-Treatment (1 course)	4, 128	4, 128	4, 256	4, 128	8, 256	8, 128	4, 64	8, 256	8, 512	8, 256
End-Treatment (3 courses)	--	--	--	--	--	--	--	--	16, 512	8, 256
End-Treatment (Visit 20)	--	--	--	--	--	--	--	--	8, 256	8, 512

*Mid-treatment (1 course) data represent Day 7 for CP-AI-003 and Day 14 for CP-AI-005 and CP-AI-007.
End-treatment (1 course) data represent Day 14 for CP-AI-003 and Day 28 for CP-AI-005, -006 and -007.
End-treatment (3 courses) data represent Day 140 (end of treatment course 3) for CP-AI-006.
-- Data not available.

The Applicant seeks approval of AZLI for the improvement of respiratory symptoms and pulmonary function in CF patients with *Pseudomonas aeruginosa*. AI is intended as a suppressive therapy and is not expected to eradicate *P. aeruginosa* in CF patients with established airway infections. Consequently, in the Phase 2 and three

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Phase 3 clinical trials, there are no definitive endpoints for microbiological eradication or clinical cure. Under these circumstances, the objective of the review team is to determine ***what constitutes microbiological or clinical success?*** While it is not the place for this Reviewer to define clinical success, he shall attempt to define microbiological success.

There are two ways to evaluate the data from this submission. First, the microbiological efficacy endpoints were compared to the most common clinical efficacy endpoint, improvement in FEV₁. Second, the results of the microbiological and primary clinical efficacy endpoint data were compared to the corresponding data derived from the package insert for TOBI Solution for Inhalation (TSI), a drug for which AI is intended to be an alternative treatment for CF. TOBI is the only other Agency-approved drug for the management of cystic fibrosis and also is administered in a similar manner.

In the original NDA submission, to investigate the possible correlation between microbiological and clinical endpoints, scatterplots were produced. Scatterplots examining these endpoints showed the following:

- Changes in numbers of PA in sputum were not associated with changes in FEV₁ or aztreonam concentrations in study CP-AI-003, CP-AI-005 and slightly negatively associated in study CP-AI-007.
- Changes in numbers of PA in sputum were not associated with changes in aztreonam concentrations in study CP-AI-003.
- Changes in aztreonam MIC for the PA isolate with the highest MIC from each patient were slightly positively associated with changes in FEV₁ in study CP-AI-003 but slightly negatively associated in study CP-AI-007.
- Changes in numbers of PA in sputum were not associated with changes in aztreonam MIC for the PA isolate with the highest MIC in study CP-AI-005 and CP-AI-007.
- Changes in numbers of PA in sputum were not associated with changes in CFQ-R respiratory domain score in study CP-AI-007.
- The Applicant did not present scattergrams to explain relationships between microbiological to clinical outcomes.

There were three measures of microbiological outcome:

1. Change in log₁₀ *P. aeruginosa* CFUs/g sputum specimens from patients;
2. Appearance of other pathogens, specifically *S. aureus*, *B. cepacia*, *S. maltophilia* or *A. xylosoxidans* in CF patients; and
3. Changes in aztreonam MIC₅₀s and MIC₉₀s of *P. aeruginosa* isolates from CF patients.

The lack of correlation of microbiology outcomes with clinical outcomes for the treatment of cystic fibrosis with aztreonam is not unprecedented. In the Microbiology subsection of the package insert for TOBI for the management of cystic fibrosis patients with *P. aeruginosa*, the Agency states "The relationship between *in vitro* susceptibility test results and clinical outcome with TOBI therapy is not clear". The efficacy of AZLI for CF caused by *P. aeruginosa* is not entirely clear in terms of clinical outcomes. *While none of the three microbiology responses correlated with clinical responses, AZLI treated patients demonstrated positive microbiological outcomes by all three measures.* In addition, the Applicant has demonstrated the

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safety of AZLI from the Microbiology standpoint as demonstrated by the lack of the development of aztreonam resistance in patients during therapy.

This Reviewer recommends that CAYSTON be APPROVED for the treatment of cystic fibrosis in patients infected with *Pseudomonas aeruginosa* based on the following:

- the medical need for new antibiotics to treat cystic fibrosis,
- the demonstrated safety of the drug from the Microbiology standpoint, and
- the drug is efficacious from the Microbiology standpoint,
- the overwhelming vote by the Anti-Infective Advisory Committee for the approval of CAYSTON at the prescribed dose suggested this drug is needed by cystic fibrosis patients.

One major concern expressed by the Advisory Committee was the potential for the development of aztreonam resistance in *P. aeruginosa* isolates harbored in cystic fibrosis patients. *In vitro* data does support this concern.

Table II. Aztreonam Susceptibility Trends in CF Isolates of *P. aeruginosa*

Reference	Region	No. of Isolates (Patients)	MIC ₅₀	MIC ₉₀	Range	%S	%R
1995-1996 ⁶³	US	1240 (508 pts)	≤ 2	32	≤ 2-> 512	80.0	11.9
2004-2005 Phase 2 AI baseline data ⁶⁴	US	242 (105 pts)	≤ 1	64	≤ 1-> 2048	78.9	10.3
1993-1997 ⁶⁷	Italy	1267 (224 pts)	NR	NR		NR	19.7
2004-2005 ⁶⁶	Europe	150	8	256	0.125-> 512	56	30
2006 ⁶⁵	US	1826	8	≥ 32		68.6	23.1

NR = Not reported

Source: Table 6, NDA 50-814 Microbiology review.

Notice in [Table II](#) that MIC90 values for *P. aeruginosa* isolates increased from 32 µg/ml in 1995–96 to 64 µg/ml in 2004–05 to ≥ 32 µg/ml in 2006. While this *in vitro* data suggests the potential for development of resistance to aztreonam, patients did not develop aztreonam resistance during therapy.

To address the concerns of the Advisory Committee, this Reviewer recommends the Applicant be subject to a **Phase 4 commitment**.

This commitment shall include the monitoring of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients for aztreonam and tobramycin resistance for a period of not less than three years. The aztreonam and tobramycin susceptibilities from these isolates should be taken from the Cystic Fibrosis Foundation Registry and reported to the Agency on a yearly basis.

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MICROBIOLOGY SUBSECTION OF THE PACKAGE INSERT**12 CLINICAL PHARMACOLOGY****12.1 Mechanism of Action (see Pharmacology 12.4)****12.4 Microbiology****Mechanism of Action**

Aztreonam exhibits activity *in vitro* against Gram-negative aerobic pathogens including *P. aeruginosa*. Aztreonam binds to penicillin-binding proteins of susceptible bacteria, which leads to inhibition of bacterial cell wall synthesis leading to death of the cell. Aztreonam activity is not decreased in the presence of cystic fibrosis lung secretions.

Susceptibility Testing

A single sputum sample from a cystic fibrosis patient may contain multiple morphotypes of *P. aeruginosa* and each morphotype may be susceptible to a different concentration of aztreonam. The *in vitro* antimicrobial susceptibility test methods used for parenteral aztreonam therapy can be used to monitor the susceptibility of *P. aeruginosa* isolated from cystic fibrosis patients (1).

Cross Resistance

No cross-resistance to other antibiotics, including aminoglycosides, quinolones, and beta-lactams, has been observed following up to six 28-day courses of 75 mg CAYSTON administered three times a day.

Other

No changes in the susceptibility of *P. aeruginosa* to aztreonam were observed following a 28-day course of CAYSTON in the Phase 3 placebo-controlled studies.

No trends in the treatment-emergent isolation of other bacterial respiratory pathogens (*Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, and *Staphylococcus aureus*). There was a slight increase in the isolation of *Candida* spp. following up to six 28-day courses of aztreonam inhalation therapy.

REFERENCE

1. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically – Eighth Edition; Approved Standard. CLSI Document M7-A8. CLSI, 940 West Valley Rd., Suite 1400, Wayne, PA 19087 January, 2009.

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INTRODUCTION

Background

Cystic fibrosis (CF) affects approximately 24,500 people in the US, 50,000 in Europe, 3500 in Canada, 2350 in Australia, and 100,000 worldwide. CF continues to be the most common life-shortening genetic disorder in Caucasians, with an estimated median survival age of 37.4 years in the US. CF is an autosomal recessive disease characterized by progressive, obstructive pulmonary disease. Patients with CF are particularly susceptible to pulmonary infections with organisms including *Pseudomonas aeruginosa* (*PA*), *Burkholderia cepacia*, and *Stenotrophomonas maltophilia*.

The most significant bacterial pathogen associated with CF pulmonary disease is *PA*. *PA* infection is a significant predictor of mortality, and has also been associated with higher rates of pulmonary function decline. By age 24, approximately 75% of patients with CF are infected with *PA*; overall, 54.4% of patients with CF are infected. Once established, these infections are rarely eradicated despite aggressive antibiotic therapy. Infected patients experience progressive obstruction of the airways and loss of lung function. Loss of pulmonary function is the primary cause of death in patients with CF. *PA*-infected patients also experience episodes of acute pulmonary exacerbation, which are characterized by worsening respiratory symptoms and an acute decline in lung function. The primary reason for CF patient hospitalization is the need for intravenous (IV) antibiotic treatment for potentially life-threatening events of acute exacerbation, which was experienced by 30% of pediatric patients and 48% of adult patients with CF in the US in 2005.

Inhaled Antibiotic Therapy

Inhaled antibiotics offer advantages over IV therapy because relatively high drug concentrations can be delivered to the site of infection with minimal systemic absorption, thus reducing the risk of adverse effects associated with IV exposure. In the US, the most widely used aerosolized antibiotic for treatment of patients with CF is tobramycin, which has been shown to produce substantial improvements in pulmonary function and other clinical parameters. In 1997, PathoGenesis Corporation (now Novartis) received approval by the Agency to market Tobramycin Solution for Inhalation (TSI), now called Tobramycin Inhalation Solution (TIS), for the management of patients with CF and *PA*. This approval was based on Phase 3 clinical studies that demonstrated significant improvements in pulmonary function as well as reductions in sputum *PA* colony-forming unit (CFU) densities and the need for hospitalization and IV drug therapy.

Despite the proven efficacy of TIS, a clinical need exists for another treatment option in aerosol antibiotic therapy. The primary therapy for pulmonary exacerbations is IV tobramycin, and frequent aerosol use of this agent may lead to selective pressure favoring resistant *PA* strains, which is a growing concern in the CF community. One investigator reported that 21% of patients screened from 69 different CF treatment centers for the Phase 3 TIS studies and 20% of patients in a 2008 study with the same entrance criteria

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had *PA* isolates considered to be resistant to tobramycin according to the breakpoint established for parenteral administration [minimum inhibitory concentration (MIC) $\geq$ 16 microgram per milliliter ($\mu\text{g/mL}$)]. Accordingly, many clinicians are reluctant to use aerosolized tobramycin for chronic suppressive therapy, fearing that long-term exposure could further promote resistance and diminish the effectiveness of IV aminoglycoside therapy. In order to reduce the risk of treatment-emergent resistance, TIS treatment has been restricted to alternating cycles of 28 days on drug followed by 28 days off drug. Additionally, aminoglycosides have nephrotoxic and ototoxic effects that require routine monitoring of serum concentrations when administered intravenously. Aminoglycoside toxicity is cumulative, and long-term, repeated administration by any route raises concern about total lifetime exposure to these agents, especially as the life expectancy of patients with CF is increasing.

Aztreonam for Inhalation Solution

The Applicant is developing AZLI, a unique lyophilized formulation of the monobactam antibiotic aztreonam for the treatment of patients with CF and *PA*. AZLI offers a potential treatment option for CF lung infections. Part of its appeal is that it belongs to a different antibiotic class than aminoglycosides. Although aztreonam's spectrum of activity is similar to that of tobramycin and gentamicin, its mechanism of action is different than that of the aminoglycosides. Thus, aztreonam should not contribute to the emergence of aminoglycoside-resistant organisms. Furthermore, aztreonam has been shown to have *in vitro* activity against aminoglycoside-resistant CF pathogens and is not inhibited in the presence of sputum.

Aztreonam for injection (Azactam®) has been approved for IV administration in the US since 1986. The approved IV formulation contains, as a buffer, approximately 780 milligram (mg) arginine per gram of aztreonam. Aerosolized arginine has been tested as a mucolytic in patients with CF and was shown to cause airway inflammation. The AZLI formulation does not contain arginine, but instead incorporates lysine, which eliminates a potentially pro-inflammatory component of the IV formulation. Additionally, the pH and osmolality of AZLI solution are adjusted within parameters known to be generally safe and tolerable in the airway.

AZLI is a reconstituted product that consists of the lyophilized aztreonam (75 mg) mixed with 1 mL of sterile, 0.17% saline diluent immediately before aerosolization.

PRECLINICAL EFFICACY

New *in vitro* or *in vivo* data are not presented. Please see this Reviewer's review of NDA 50-814 for details of the following: **Mechanism of Action, Antimicrobial Spectrum of Activity, Surveillance Studies, Mechanisms of Resistance, Post Antibiotic Effect, Miscellaneous Studies, Animal Therapeutic Studies, Animal Pharmacokinetics, Clinical Susceptibility Test Methods, Quality Control Studies, and Provisional Susceptibility Interpretive Criteria.**

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CLINICAL LABORATORY SUSCEPTIBILITY TEST METHODS

A single sputum sample from a CF patient may contain multiple morphotypes of *P. aeruginosa* and each morphotype may have a different level of *in vitro* susceptibility. The approved antimicrobial susceptibility test methods and quality control parameters used for parenteral aztreonam therapy were used to monitor the activity of AI against *P. aeruginosa* isolates from CF patients. It should be noted that susceptibility testing of *P. aeruginosa* isolates from CF patients may require extended incubation up to 24 hours for accurate determination of MIC values.

CLINICAL MICROBIOLOGY METHODS

Microbiology data were collected in four studies of AI in the treatment of CF patients with pulmonary *P. aeruginosa*: one Phase 2 placebo-controlled study (CP-AI-003), two Phase 3 placebo-controlled studies (CP-AI-005 and CP-AI-007), and one open-label follow-on study (CP-AI-006). Extensive microbiology methodology is found in the study reports included in this submission.

Sputa were collected at all patient visits of each study for analysis of microbiology endpoints. Sputum samples were processed for qualitative and quantitative culture of *P. aeruginosa* (each morphotype), *S. aureus*, *S. maltophilia*, *A. xylosoxidans* and *B. cepacia* complex. In addition, qualitative and quantitative culture data for *Candida* spp. and *Aspergillus* spp. were collected in the CP-AI-006 study. The *in vitro* susceptibility of all *P. aeruginosa* and *B. cepacia* isolates to aztreonam and other selected antibiotics was determined using standard susceptibility testing methods. Established susceptibility breakpoints for parenteral administration of aztreonam using agar or broth dilution are ≤ 8 $\mu\text{g/mL}$ (susceptible), 16 $\mu\text{g/mL}$ (intermediate), and ≥ 32 $\mu\text{g/mL}$ (resistant) [3].

Sputum Collection

Sputum samples (minimum of 0.5 mL) were collected at each patient visit in sterile 50 mL tubes; if the patient was unable to produce sputum, an oropharyngeal swab was collected instead. Since collection of a swab rather than sputum significantly limits microbiology testing, every effort was made to collect expectorated (not induced) sputum.

Sputum for microbiology testing was collected at least four hours after the most recent dose of AI. The date and time of sputum collection were recorded. After collection, a sterile swab was inserted into the most purulent section of sputum and a small amount of the sputum was spread onto a glass slide. This procedure was repeated twice in order to prepare two slides.

Sputum Storage and Transport

Sputum was generally shipped for microbiology testing on the day of collection. Sputum was stored refrigerated (2 – 8°C) and swabs were stored at room temperature prior to shipment to the central laboratory. Sputum samples were sent on frozen gel packs and

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swabs on ambient gel packs by overnight mail. Upon receipt, the shipping time and conditions were recorded. Sputum samples were rejected if they met any one of the following criteria: sample was more than 48 hours old upon arrival, sample arrived with no gel pack or a warm gel pack, sample was leaking or frozen, or quantity of sample was not sufficient for microbiology testing.

Pathogen Isolation and Susceptibility Testing

Gram stains were performed on the two sputum slides to assess the quality of the sample. The macroscopic appearance of the sputum was recorded as mucoid or non-mucoid and purulent (containing pus) or nonpurulent (no pus present).

Biochemical identification and quantitation of *P. aeruginosa* (each morphotype), *S. aureus*, *S. maltophilia*, *A. xylosoxidans* and *B. cepacia* were performed in accordance with current clinical laboratory standards [2].

In vitro susceptibility testing was performed on all *P. aeruginosa* and *B. cepacia* isolates using custom minimum inhibitory concentration (MIC) panels containing the following antibiotics: aztreonam, tobramycin, amikacin, cefepime, ceftazidime, ciprofloxacin, gentamicin, meropenem, piperacillin, and ticarcillin/clavulanate [3]. Quality control (QC) organisms (*Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212) were also tested against appropriate concentrations of the selected antibiotics according to standard procedures for testing patient isolates [1]. Susceptibility of each pathogen to the antibiotics tested was assessed using established breakpoints [2].

QUALITY CONTROL

Aztreonam (as AZACTAM) has been approved by the Agency for treatment of the following indications caused by *Pseudomonas aeruginosa*: urinary tract infection, lower respiratory tract infection, septicemia, skin and soft structure infection and intra-abdominal infection. In addition, *since AI is a topically administered antibiotic, interpretive criteria were not determined. Therefore, quality control parameters were not determined.*

PROVISIONAL INTERPRETIVE CRITERIA

Aztreonam has been *approved* by the Agency for *other indications* as noted above. Therefore, provisional interpretive criteria were not determined.

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CLINICAL EFFICACY

STUDY PROTOCOL FOR CP-AI-006 (AIR-CF3)

Title

A Phase 3, Open-label, Follow-on Study of Multiple Courses of Aztreonam Lysinate for Inhalation (AZLI) in Cystic Fibrosis Patients (AIR-CF3)

Study Objectives

The **primary objective** of the study was to evaluate the safety of repeated exposure to AZLI in patients with CF.

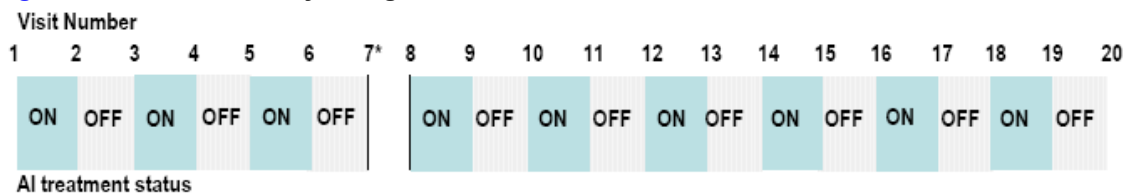
The **secondary objectives** included evaluation of the effects of repeated exposure to AZLI on disease-related endpoints and microbiology.

Study Design

This was a Phase 3, open-label, follow-on study that was conducted at 71 CF centers in the US, Canada, Australia, and New Zealand. Up to 400 patients with CF who previously participated in either of the Phase 3 studies (CP-AI-005 or CP-AI-007) were eligible to enroll in Study CP-AI-006.

Each patient could receive up to nine courses of AZLI (Figure 1). Each 28-day course of AZLI was followed by a 28-day off-treatment interval. Patients attended up to 20 scheduled clinic visits and they received the first and last doses of each treatment course at the clinic. Patients from CP-AI-005 received open-label AZLI (75 mg) in the same regimen (twice daily [BID] or three times daily [TID]) to which they were previously randomized, and patients from CP-AI-007 received open-label AZLI (75 mg) TID, the regimen used in CP-AI-007. Patients attended a follow-up visit 28 days after completing the last course of AZLI.

Figure 1. CP-AI-006 Study Design.



*Visit 7 occurred only for patients enrolled under Protocol Versions 2 or 3 who discontinued the study early or who completed the first three courses of AZLI treatment (i.e., completed the study) before Protocol Version 4 was implemented (See Section 9.1.2, this submission). All other patients skip Visit 7 and continue to Visit 8. Patients who attend for Visit 7 and continue in the study also attend Visit 8 to begin Course 4. Some patients also received additional courses between Visit 6 and Visit 8 (See Section 9.8.1, this submission).

Source: Figure 1, this submission.

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Number of Subjects

274 patients were enrolled in CP-AI-006. A total of 274 patients received study drug and had at least one post baseline assessment, including 85 in the AZLI BID regimen and 189 in the AZLI TID regimen. There were 163 patients enrolled from CP-AI-005 and 111 patients enrolled from CP-AI-007.

Inclusion Criteria (*microbiologically pertinent*)

Patients who met all of the following inclusion criteria were eligible for participation in this study:

- Complied with CP-AI-005 or CP-AI-007 by taking at least 50% of expected study medication as determined by vial count (adjusting for time on study if treatment was stopped early for AEs or for receiving prohibited antibiotics)
- Patient completed CP-AI-005/007 or was withdrawn from CP-AI-005/007 due to need for antipseudomonal antibiotics or for AE unrelated to study medication intolerance
- Male or female CF patient aged ≥ 6 years
- Ability to provide written informed consent/assent prior to initiation of study-related procedures (patient or guardian, as appropriate)
- Ability to perform reproducible pulmonary function tests

Exclusion Criteria (*microbiologically pertinent*)

Patients who met any of the following criteria were excluded from enrollment into this study:

- Use of any investigational medication or device between the last visit of CP-AI-005/007 and Visit 1 of CP-AI-006
- Concurrent participation in a study of another investigational medication or device
- Current use of oral corticosteroids in doses exceeding the equivalent of 10 mg prednisone a day or 20 mg prednisone every other day
- History of sputum or throat swab culture yielding *B. cepacia* in the previous 2 years
- History of daily continuous oxygen supplementation or requirement for more than 2 liter (L)/minute at night
- Inability to tolerate study medication in Study CP-AI-005 and CP-AI-007
- Known local or systemic hypersensitivity to aztreonam
- Inability to tolerate inhalation of a short-acting β_2 agonist
- Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) > 5 times upper limit of normal at most recent test
- Female of child-bearing potential who is pregnant or lactating or is not (in the opinion of the investigator) practicing an acceptable method of birth control
- Any serious or active medical or psychiatric illness which in the opinion of the investigator would interfere with patient treatment, assessment, or compliance with the protocol

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Test Product

AZLI (75 mg/ml aztreonam lysine when reconstituted in diluent [0.17% saline]; sterile, pH 4.4 to 5.4, and osmolality 300 to 550 mOsmol/kg). AZLI was self-administered by inhalation using the PARI eFlow® Electronic Nebulizer (eFlow).

Reference Therapy: None

Duration of Treatment

The total time a patient was enrolled in the study was approximately 18 months.

Criteria for Evaluation of Safety

- Extent of exposure
- AEs
- Vital signs
- Airway reactivity
- Clinical laboratory evaluation

Criteria for Evaluation of Microbiology

Microbiology analyses were conducted on the Safety population. Data collected at Visit 1 served as the baseline for microbiology parameters.

- Presence or absence of pathogens
- Minimum inhibitory concentrations (MICs) of aztreonam for *Pseudomonas aeruginosa*

Presence or Absence of Pathogens

Presence or absence of pathogens, including *SA*, *Burkholderia cepacia* complex (BCC), *S. maltophilia*, *Achromobacter xylosoxidans*, *Candida* spp., and *Aspergillus* spp., and change from baseline (appearance, no change and disappearance) were summarized at each visit for the study as a whole, as well as for patients completing nine courses of AZLI. Additionally, presence of MRSA and MSSA and shift from baseline were summarized at each visit for the study as a whole, as well as for patients completing nine courses of AZLI.

The number of patients with treatment emergence of *S. aureus*, BCC, *S. maltophilia*, *A. xylosoxidans* along with fungal organisms *Aspergillus* and *Candida* spp. were summarized as intermittent or persistent by treatment group for the study as a whole, as well as for the subset of patients completing at least three courses of AZLI, for the subset of patients completing at least six courses of AZLI and for the subset of patients completing nine courses of AZLI. A patient was considered to have persistent treatment-emergent isolation if the organism was not present at Visit 1 but was isolated from at least three consecutive specimens on subsequent visits. A patient was considered to have treatment-emergent intermittent isolation if the organism was not present at Visit 1, but

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was isolated after Visit 1 and if the patient did not meet the criteria for persistent isolation.

Minimum Inhibitory Concentrations for PA

MIC₅₀ and MIC₉₀ of aztreonam for all *PA* isolates (µg/mL) at each visit were presented. MIC₅₀ and MIC₉₀ of aztreonam for the *PA* isolate with the highest MIC from each patient at each visit were presented.

The MIC₅₀ and MIC₉₀ of other antibiotics for all *PA* isolates at each visit were summarized by treatment group. MIC₅₀ and MIC₉₀ of other antibiotics for the *PA* isolate with the highest MIC for each antibiotic at each visit was summarized by treatment group.

MIC of aztreonam for all *PA* isolates was summarized by visit, with percentages displayed cumulatively. MIC of aztreonam for the *PA* isolate with the highest MIC from each patient was summarized by visit, with percentages displayed cumulatively.

Change in MIC of aztreonam (increased, unchanged, or decreased) from Visit 1 to all visits for the *PA* isolate with the highest MIC from each patient was summarized, where 'increased' included patients with an equal to or greater than 4-fold increase in MIC, 'unchanged' included patients with MIC values $\pm$ a 2-fold change, and 'decreased' included patients with an equal to or greater than 4-fold decrease in MIC.

Patients with a MIC of aztreonam greater than 8 µg/mL for the *PA* isolate with the highest MIC at each visit were summarized. The change from baseline in the number of patients having *PA* isolates with the highest aztreonam MIC of greater than 8 µg/mL was also summarized.

Criteria for Evaluation of Disease-Related Endpoints

- Change in forced expiratory volume in one second (FEV₁ [L]), FEV₁ percent of predicted, forced vital capacity (FVC), forced expiratory flow from 25% to 75% (FEF₂₅₋₇₅) of the FVC
- Change in clinical symptoms as assessed by the Cystic Fibrosis Questionnaire-Revised (CFQ-R) Respiratory symptoms domain
- Change in clinical symptoms as assessed by the CFQ-R non-respiratory symptoms domains
- PA colony-forming units (CFUs) in sputum
- Percent of days and number of days hospitalized
- Missed school, work days or both, due to CF
- Percent change in weight
- Change in body mass index (BMI)
- Physician assessment of symptoms
- Time to intravenous antibiotics

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CORRELATION OF *IN VITRO* SUSCEPTIBILITY AND CLINICAL OUTCOME

Change in log₁₀ *PA* CFUs in Sputum

The mean and standard deviation (SD) of log₁₀ *PA* CFUs in sputum for the nine treatment courses are presented in [Table 1](#).

Table 1. Mean (SD) Log₁₀ *PA* CFUs in Sputum, Treatment Courses 1 to 9: ITT Population

AZLI Regimen			
Treatment Course	BID* (N = 85)	TID* (N = 189)	Total (N = 274)
Start Treatment Course 1 (Visit 1)			
n	68	155	223
Mean (SD)	5.71 (2.249)	6.16 (1.894)	6.02 (2.014)
End Treatment Course 1 (Visit 2)			
n	69	150	219
Mean (SD)	5.50 (1.904)	5.41 (2.039)	5.44 (1.994)
Start Treatment Course 2 (Visit 3)			
n	71	153	224
Mean (SD)	5.38 (2.157)	5.85 (2.190)	5.70 (2.186)
End Treatment Course 2 (Visit 4)			
n	68	146	214
Mean (SD)	5.05 (2.181)	5.21 (2.306)	5.16 (2.263)
Start Treatment Course 3 (Visit 5)			
n	61	143	204
Mean (SD)	5.35 (2.372)	5.88 (2.094)	5.72 (2.188)
End Treatment Course 3 (Visit 6)			
n	70	132	202
Mean (SD)	5.01 (2.464)	5.17 (2.381)	5.12 (2.405)
Start Treatment Course 4 (Visit 7/8)			
n	65	130	195
Mean (SD)	5.25 (2.398)	6.05 (2.254)	5.78 (2.327)
End Treatment Course 4 (Visit 9)			
n	45	119	164
Mean (SD)	5.00 (2.628)	5.19 (2.418)	5.14 (2.471)
Start Treatment Course 5 (Visit 10)			
n	48	127	175
Mean (SD)	5.57 (2.285)	5.66 (2.318)	5.63 (2.303)
End Treatment Course 5 (Visit 11)			
n	50	120	170
Mean (SD)	5.44 (2.125)	5.33 (2.350)	5.36 (2.280)
Start Treatment Course 6 (Visit 12)			
n	45	116	161
Mean (SD)	5.31 (2.704)	5.60 (2.427)	5.52 (2.503)
End Treatment Course 6 (Visit 13)			
n	42	101	143
Mean (SD)	5.20 (2.528)	5.38 (2.261)	5.33 (2.335)
Start Treatment Course 7 (Visit 14)			
n	40	104	144
Mean (SD)	5.47 (2.633)	5.56 (2.403)	5.54 (2.460)

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End Treatment Course 7 (Visit 15)			
n	43	105	148
Mean (SD)	5.21 (2.345)	5.17 (2.382)	5.18 (2.363)
Start Treatment Course 8 (Visit 16)			
n	44	103	147
Mean (SD)	5.53 (2.724)	5.63 (2.456)	5.60 (2.530)
End Treatment Course 8 (Visit 17)			
n	46	101	147
Mean (SD)	5.47 (2.349)	5.16 (2.374)	5.25 (2.363)
Start Treatment Course 9 (Visit 18)			
n	43	99	142
Mean (SD)	5.49 (2.585)	5.63 (2.323)	5.58 (2.397)
End Treatment Course 9 (Visit 19)			
n	41	93	134
Mean (SD)	5.39 (2.560)	5.58 (2.176)	5.52 (2.292)
Follow-up			
n	42	99	141
Mean (SD)	5.57 (2.627)	5.59 (2.460)	5.58 (2.501)

*The BID group includes patients from CP-AI-005; the TID group includes patients from CP-AI-005 and -007.

Source: Table 100, this submission.

At the start of treatment (Visit 1, baseline), the mean log₁₀ *PA* CFUs in patient sputum was higher in the TID group than in the BID group ([Table 1](#)). Over the nine treatment courses, for both the BID and TID groups, the mean log₁₀ *PA* CFUs decreased from the start of treatment during each on-treatment course.

Changes in log₁₀ *PA* CFUs in sputum from Visit 1 are summarized for the initial treatment courses in [Table 2](#), for all treatment courses in [Table 3](#), and within visit intervals in [Table 4](#).

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Table 2. Change from Visit 1 in Log10 *PA* CFUs in Sputum, Initial Three Treatment Courses on Study: ITT Population

Treatment Course	AZLI Regimen						
	BID*			TID*			
	Prev. Placebo (N = 29)	Prev. AZLI (N = 56)	Total BID (N = 85)	Prev. Placebo (N = 85)	Prev. AZLI (N = 104)	Total TID (N = 189)	Total (N = 274)
End Treatment Course 1							
n	20	38	58	57	72	129	187
Mean	-0.70	0.07	-0.20	-0.96	-0.70	-0.81	-0.62
(SD)	(1.473)	(1.424)	(1.476)	(1.902)	(1.651)	(1.764)	(1.700)
Start Treatment Course 2							
n	21	39	60	61	72	133	193
Mean	-0.25	-0.22	-0.23	-0.42	-0.15	-0.28	-0.26
(SD)	(1.306)	(1.872)	(1.684)	(1.516)	(1.990)	(1.786)	(1.751)
End Treatment Course 2							
n	21	38	59	56	68	124	183
Mean	-1.02	-0.37	-0.60	-0.99	-0.66	-0.81	-0.74
(SD)	(1.436)	(1.860)	(1.737)	(2.207)	(2.214)	(2.208)	(2.065)
Start Treatment Course 3							
n	18	34	52	55	72	127	179
Mean	-0.73	-0.63	-0.67	0.02	-0.20	-0.10	-0.27
(SD)	(1.503)	(1.712)	(1.629)	(1.568)	(1.819)	(1.712)	(1.703)
End Treatment Course 3							
n	20	36	56	52	59	111	167
Mean	-1.10	-0.06	-0.43	-0.75	-0.34	-0.53	-0.50
(SD)	(1.081)	(1.370)	(1.360)	(2.376)	(1.868)	(2.121)	(1.896)

*The BID group includes patients from CP-AI-005; the TID group includes patients from CP-AI-005 and CP-AI-007.

Source: Table 101, this submission.

For the initial three treatment courses, log10 *PA* CFUs in sputum decreased from Visit 1 at each visit (end of Treatment Course 1 through end of Treatment Course 3 [Visits 2 through 6]) for both the BID and TID groups (Table 2). In both the BID group and the TID group, patients who were previously treated with placebo had greater differences in the changes in log10 *PA* CFUs in sputum than patients previously treated with AZLI.

Table 3. Change from Visit 1 in Log10 *PA* CFUs in Sputum, Treatment Courses 1 to 9: ITT Population

Population:					
Treatment Course	BID*	TID*			Total (N = 274)
	Total BID (N = 85)	Prev. CP-AI-005 (N = 78)	Prev. CP-AI-007 (N = 111)	Total TID (N = 189)	
End Treatment Course 1 (Visit 2)					
n	58	57	72	129	187
Mean (SD)	-0.20 (1.476)	-0.71 (1.467)	-0.89 (1.974)	-0.81 (1.764)	-0.62 (1.700)
Start Treatment Course 2 (Visit 3)					
n	60	55	78	133	193
Mean (SD)	-0.23 (1.684)	-0.24 (1.710)	-0.31 (1.849)	-0.28 (1.786)	-0.26 (1.751)

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End Treatment Course 2 (Visit 4)					
n	59	51	73	124	183
Mean (SD)	-0.60 (1.737)	-0.30 (1.649)	-1.16 (2.476)	-0.81 (2.208)	-0.74 (2.065)
Start Treatment Course 3 (Visit 5)					
n	52	55	72	127	179
Mean (SD)	-0.67 (1.629)	-0.17 (1.489)	-0.05 (1.873)	-0.10 (1.712)	-0.27 (1.703)
End Treatment Course 3 (Visit 6)					
n	56	48	63	111	167
Mean (SD)	-0.43 (1.360)	-0.35 (1.847)	-0.67 (2.314)	-0.53 (2.121)	-0.50 (1.896)
Start Treatment Course 4 (Visit 7/8)					
n	53	49	66	115	168
Mean (SD)	-0.36 (2.029)	-0.17 (1.339)	0.01 (2.126)	-0.07 (1.828)	-0.16 (1.892)
End Treatment Course 4 (Visit 9)					
n	39	44	62	106	145
Mean (SD)	-0.68 (1.953)	-0.42 (2.068)	-0.95 (2.153)	-0.73 (2.125)	-0.71 (2.073)
Start Treatment Course 5 (Visit 10)					
n	43	45	68	113	156
Mean (SD)	-0.29 (1.658)	0.04 (1.081)	-0.55 (2.061)	-0.31 (1.756)	-0.31 (1.724)
End Treatment Course 5 (Visit 11)					
n	42	39	65	104	146
Mean (SD)	-0.45 (2.041)	0.08 (1.585)	-0.90 (2.068)	-0.53 (1.953)	-0.51 (1.972)
Start Treatment Course 6 (Visit 12)					
n	41	39	61	100	141
Mean (SD)	-0.37 (1.990)	-0.04 (1.473)	-0.38 (2.058)	-0.25 (1.851)	-0.28 (1.886)
End Treatment Course 6 (Visit 13)					
n	37	32	54	86	123
Mean (SD)	-0.54 (1.878)	-0.25 (1.784)	-0.72 (2.107)	-0.55 (1.995)	-0.54 (1.953)
Start Treatment Course 7 (Visit 14)					
n	34	35	57	92	126
Mean (SD)	-0.32 (1.936)	-0.27 (1.830)	-0.31 (2.097)	-0.29 (1.989)	-0.30 (1.967)
End Treatment Course 7 (Visit 15)					
n	39	33	59	92	131
Mean (SD)	-0.63 (1.753)	-0.33 (2.025)	-0.93 (2.394)	-0.71 (2.276)	-0.69 (2.127)
Start Treatment Course 8 (Visit 16)					
n	39	34	59	93	132
Mean (SD)	-0.31 (2.296)	0.07 (1.668)	-0.53 (2.216)	-0.31 (2.044)	-0.31 (2.112)
End Treatment Course 8 (Visit 17)					
n	41	34	55	89	130
Mean (SD)	-0.40 (1.761)	-0.22 (1.861)	-1.03 (2.289)	-0.72 (2.161)	-0.62 (2.042)
Start Treatment Course 9 (Visit 18)					
n	39	35	51	86	125
Mean (SD)	-0.41 (2.270)	-0.13 (1.894)	-0.67 (1.755)	-0.45 (1.821)	-0.44 (1.963)
End Treatment Course 9 (Visit 19)					
n	36	33	52	85	121
Mean (SD)	-0.47 (1.899)	0.12 (1.644)	-1.05 (2.187)	-0.60 (2.065)	-0.56 (2.010)
Follow-up (Visit 20)					
n	37	36	50	86	123
Mean (SD)	-0.37 (1.872)	-0.25 (1.938)	-0.64 (2.449)	-0.48 (2.245)	-0.45 (2.132)

*The BID group includes patients from CP-AI-005; the TID group includes patients from CP-AI-005 and -007.
Source: Table 102, this submission.

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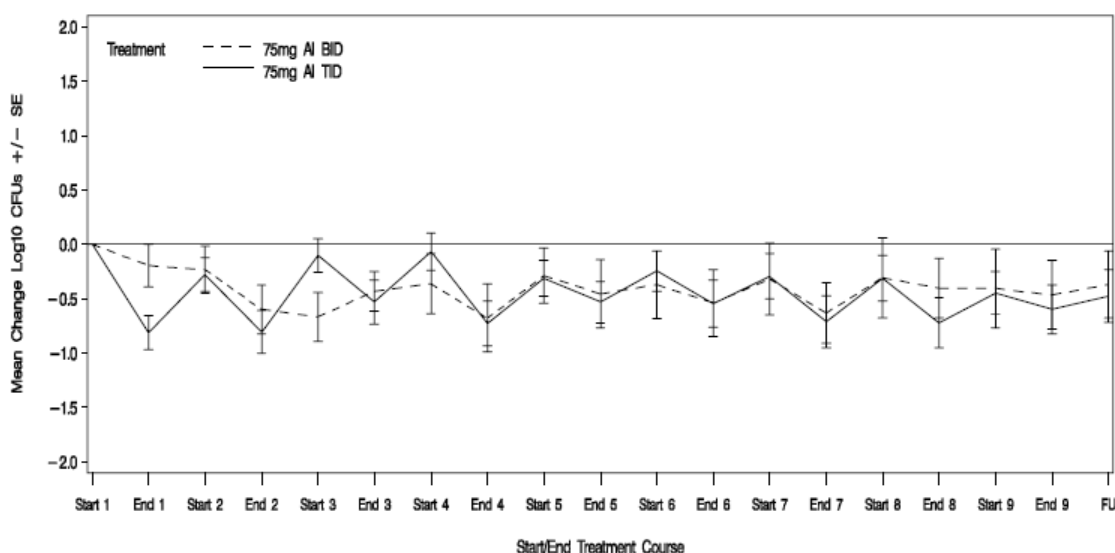
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Log₁₀ *PA* CFUs in sputum decreased from Visit 1 over the nine treatment courses (Visits 2 through 20) in both the BID and TID groups, and the changes at the end of each treatment course were generally greater for patients in the TID group (Table 3). Changes in the mean log₁₀ *PA* CFUs in sputum ranged from –0.2 to –0.68 in the BID arm and from –0.07 to –0.81 in the TID arm. In the TID group, patients enrolled from Study CP-AI-007 generally had greater changes in log₁₀ *PA* CFUs in sputum versus patients from Study CP-AI-005.

Figure 2 illustrates the absolute mean changes in log₁₀ *PA* CFUs in sputum from Visit 1 (baseline) for the BID and TID groups by study visit over the nine treatment courses (Visits 2 through 20).

Figure 2. Absolute Mean Change from Visit 1 in Log₁₀ *PA* CFUs for AZLI BID and AZLI TID vs. Visit: ITT Population



Source: Figure 7, this submission.

Both the BID and TID groups showed decreased CFUs throughout the study; in general, the TID group showed greater decreases during the on-treatment courses than the BID group.

Changes in log₁₀ *PA* CFUs in sputum from Visit 1 are summarized for the initial treatment courses within visit intervals in Table 4.

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Table 4. Change within Visit Interval in Sputum Log₁₀ PA CFUs, Treatment Courses 1 to 9: ITT Population

Treatment Course		BID*		TID*		
		Total BID (N = 85)	Prev. CP-AI-005 (N = 78)	Prev. CP-AI-007 (N = 111)	Total TID (N = 189)	Total (N = 274)
Visit 1 to 2, Treatment Course 1						
n	58	57	72	129	187	
Mean (SD)	-0.20 (1.476)	-0.71 (1.467)	-0.89 (1.974)	-0.81 (1.764)	-0.62 (1.700)	
Visit 2 to 3, Treatment Course 1 off-treatment						
n	63	56	73	129	192	
Mean (SD)	-0.03 (1.669)	0.29 (1.645)	0.54 (2.199)	0.43 (1.974)	0.28 (1.888)	
Visit 3 to 4, Treatment Course 2						
n	63	53	79	132	195	
Mean (SD)	-0.30 (1.598)	-0.20 (1.609)	-0.98 (2.147)	-0.66 (1.980)	-0.55 (1.869)	
Visit 4 to 5, Treatment Course 2 off-treatment						
n	56	56	71	127	183	
Mean (SD)	0.34 (1.478)	0.07 (1.632)	0.98 (2.554)	0.58 (2.234)	0.51 (2.032)	
Visit 5 to 6, Treatment Course 3						
n	58	55	64	119	177	
Mean (SD)	-0.15 (1.948)	-0.19 (1.413)	-0.56 (2.381)	-0.39 (1.994)	-0.31 (1.977)	
Visit 6-Visit 7/8a , Treatment Course 3 off-treatment						
n	56	48	61	109	165	
Mean (SD)	0.18 (1.606)	0.33 (1.718)	0.65 (2.161)	0.51 (1.976)	0.40 (1.861)	
Visit 8 to 9, Treatment Course 4						
n	36	44	61	105	141	
Mean (SD)	-0.27 (2.213)	-0.35 (1.710)	-0.92 (1.640)	-0.68 (1.685)	-0.58 (1.834)	
Visit 9 to 10, Treatment Course 4 off-treatment						
n	40	44	64	108	148	
Mean (SD)	0.41 (1.999)	0.11 (1.165)	0.39 (1.384)	0.28 (1.301)	0.31 (1.515)	
Visit 10 to 11, Treatment Course 5						
n	46	43	69	112	158	
Mean (SD)	-0.16 (1.599)	-0.04 (1.475)	-0.26 (1.685)	-0.18 (1.604)	-0.17 (1.598)	
Visit 11 to 12, Treatment Course 5 off-treatment						
n	42	42	60	102	144	
Mean (SD)	0.01 (1.830)	0.18 (1.884)	0.60 (2.225)	0.43 (2.092)	0.31 (2.022)	
Visit 12 to 13, Treatment Course 6						
n	37	35	57	92	129	
Mean (SD)	-0.22 (1.979)	-0.24 (1.816)	-0.38 (1.574)	-0.33 (1.662)	-0.30 (1.751)	
Visit 13 to 14, Treatment Course 6 off-treatment						
n	35	33	54	87	122	
Mean (SD)	0.25 (0.892)	-0.16 (2.327)	0.18 (1.749)	0.05 (1.982)	0.11 (1.739)	
Visit 14 to 15, Treatment Course 7						
n	35	35	56	91	126	
Mean (SD)	-0.36 (1.268)	-0.09 (1.979)	-0.54 (1.726)	-0.37 (1.830)	-0.36 (1.688)	

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Visit 15 to 16, Treatment Course 7					
off-treatment					
n	39	35	55	90	129
Mean (SD)	0.36 (1.667)	0.76 (2.092)	0.37 (2.408)	0.52 (2.286)	0.47 (2.113)
Visit 16 to 17, Treatment Course 8					
n	43	38	54	92	135
Mean (SD)	-0.03 (1.620)	-0.36 (2.315)	-0.48 (1.780)	-0.43 (2.007)	-0.30 (1.896)
Visit 17 to 18, Treatment Course 9					
off-treatment					
n	42	38	49	87	129
Mean (SD)	-0.02 (1.822)	0.17 (1.971)	0.09 (2.062)	0.12 (2.012)	0.08 (1.946)
Visit 18 to 19, Treatment Course 9					
n	39	36	51	87	126
Mean (SD)	-0.03 (1.984)	0.14 (1.725)	-0.15 (1.932)	-0.03 (1.844)	-0.03 (1.881)
Visit 19 to 20, Treatment Course 9					
off-treatment (follow-up)					
n	37	35	47	82	119
Mean (SD)	-0.00 (1.841)	-0.21 (1.625)	0.07 (2.551)	-0.05 (2.196)	-0.04 (2.085)

* Visit 7 occurred only for patients who were enrolled under [Protocol Version 2](#) or [3](#) and who discontinued the study early or completed the first three courses of AZLI treatment (i.e., completed the study) before [Protocol Version 4](#) was implemented. All other patients skipped Visit 7 and continued to Visit 8.

Source: Table 103, this submission.

Within visit intervals, the log₁₀ *PA* CFUs tended to increase during off-treatment intervals ([Table 4](#)). Over nine treatment courses, log₁₀ *PA* CFUs decreased from the start of each treatment course to the end of each treatment course, and the decreases in log₁₀ *PA* CFUs at the end of each treatment course were generally greater for patients in the TID group. *It should be noted that non-study antibiotics were allowed by the Applicant during the study so the variability observed in log₁₀ *PA* CFU changes, i.e., the lack of a return to baseline, during some of the off-treatment intervals, may reflect the use of additional therapies.*

Change in log₁₀ *PA* CFUs in Sputum by On-study Tobramycin Use

The mean change in log₁₀ *PA* CFUs in sputum by on-study inhaled tobramycin use is summarized for non-users (patients who did not use a ≥ 250 mg dose of inhaled tobramycin [at least once a day] during the off-treatment periods), for infrequent users (patients who used a ≥ 250 mg dose of inhaled tobramycin [at least once a day] during less than half of the off-treatment periods), and for frequent users (patients who used a ≥ 250 mg dose of inhaled tobramycin [at least once a day] during at least half of the off-treatment periods) in [Table 5](#).

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Table 5. Change from Visit 1 in Log10 PA CFUs in Sputum by On-study Tobramycin Use, Treatment Courses 1 to 9: ITT Population

Treatment Course	Non-users*			Infrequent Users**			Frequent Users [†]		
	BID Pooled (N=49)	TID Pooled (N=114)	Total (N=163)	BID Pooled (N=20)	TID Pooled (N=48)	Total (N=68)	BID Pooled (N=16)	TID Pooled (N=27)	Total (N=43)
End Treatment Course 1									
n	32	73	105	14	35	49	12	21	33
Mean (SD)	-0.18 (1.757)	-0.89 (1.609)	-0.67 (1.679)	-0.28 (1.094)	-0.90 (2.239)	-0.72 (1.989)	-0.13 (1.076)	-0.40 (1.366)	-0.30 (1.257)
Start Treatment Course 2									
n	34	77	111	14	36	50	12	20	32
Mean (SD)	-0.21 (1.902)	-0.45 (1.905)	-0.38 (1.899)	-0.28 (1.408)	-0.14 (1.248)	-0.18 (1.282)	-0.25 (1.416)	0.15 (2.107)	-0.00 (1.862)
End Treatment Course 2									
n	32	73	105	15	37	52	12	14	26
Mean (SD)	-0.27 (1.561)	-1.07 (2.323)	-0.83 (2.144)	-1.27 (2.115)	-0.49 (2.015)	-0.72 (2.054)	-0.65 (1.556)	-0.27 (2.008)	-0.44 (1.789)
Start Treatment Course 3									
n	26	70	96	15	38	53	11	19	30
Mean (SD)	-0.38 (1.346)	-0.03 (1.766)	-0.13 (1.663)	-1.21 (2.214)	-0.27 (1.852)	-0.54 (1.986)	-0.61 (1.208)	-0.01 (1.190)	-0.23 (1.212)
End Treatment Course 3									
n	30	58	88	14	35	49	12	18	30
Mean (SD)	-0.64 (1.576)	-0.53 (2.190)	-0.57 (1.993)	-0.34 (1.178)	-0.72 (2.386)	-0.61 (2.107)	-0.01 (0.859)	-0.18 (1.198)	-0.11 (1.062)
Start Treatment Course 4									
n	29	64	93	14	34	48	10	17	27
Mean (SD)	0.05 (1.999)	-0.07 (2.037)	-0.03 (2.015)	-0.88 (2.022)	-0.11 (1.831)	-0.34 (1.900)	-0.83 (2.055)	0.02 (0.737)	-0.30 (1.404)

* Includes patients who did not use a ≥ 250 mg dose of inhaled tobramycin (at least once a day) during the off-treatment periods.

** Includes patients who used a ≥ 250 mg dose of inhaled tobramycin (at least once a day) during less than half of the off-treatment periods.

† Includes patients who used a ≥ 250 mg dose of inhaled tobramycin (at least once a day) during at least half of the off-treatment periods.

Source: Table 104, this submission.

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Table 5. Change from Visit 1 in Log10 PA CFUs in Sputum by On-study Tobramycin Use, Treatment Courses 1 to 9: ITT Population (continued)

Treatment Course	Non-users*			Infrequent Users**			Frequent Users †		
	BID Pooled (N=49)	TID Pooled (N=114)	Total (N=163)	BID Pooled (N=20)	TID Pooled (N=48)	Total (N=68)	BID Pooled (N=16)	TID Pooled (N=27)	Total (N=43)
End Treatment Course 4									
n	16	55	71	14	35	49	9	16	25
Mean (SD)	-0.19 (1.142)	-0.68 (1.624)	-0.57 (1.535)	-1.77 (2.532)	-0.93 (2.721)	-1.17 (2.670)	0.15 (1.390)	-0.47 (2.269)	-0.25 (1.988)
Start Treatment Course 5									
n	17	62	79	17	35	52	9	16	25
Mean (SD)	0.09 (1.771)	-0.25 (1.443)	-0.17 (1.514)	-0.66 (1.668)	-0.52 (2.399)	-0.57 (2.171)	-0.30 (1.411)	-0.13 (1.165)	-0.19 (1.232)
End Treatment Course 5									
n	17	56	73	16	33	49	9	15	24
Mean (SD)	0.23 (1.665)	-0.67 (1.964)	-0.46 (1.926)	-1.29 (2.188)	-0.47 (2.179)	-0.74 (2.194)	-0.27 (2.094)	-0.14 (1.338)	-0.19 (1.619)
Start Treatment Course 6									
n	16	54	70	14	32	46	11	14	25
Mean (SD)	-0.38 (2.259)	-0.05 (1.879)	-0.12 (1.960)	-0.46 (1.546)	-0.47 (1.897)	-0.47 (1.781)	-0.25 (2.246)	-0.50 (1.658)	-0.39 (1.900)
End Treatment Course 6									
n	15	45	60	12	28	40	10	13	23
Mean (SD)	-0.49 (2.410)	-0.45 (1.875)	-0.46 (2.000)	-0.92 (1.518)	-0.83 (2.534)	-0.86 (2.257)	-0.16 (1.375)	-0.27 (0.749)	-0.22 (1.041)
Start Treatment Course 7									
n	15	48	63	10	30	40	9	14	23
Mean (SD)	-0.36 (2.392)	-0.27 (1.888)	-0.29 (1.999)	-0.87 (1.848)	-0.36 (1.920)	-0.49 (1.892)	0.36 (0.836)	-0.24 (2.563)	-0.01 (2.056)

* Includes patients who did not use a ≥ 250 mg dose of inhaled tobramycin (at least once a day) during the off-treatment periods.

** Includes patients who used a ≥ 250 mg dose of inhaled tobramycin (at least once a day) during less than half of the off-treatment periods.

† Includes patients who used a ≥ 250 mg dose of inhaled tobramycin (at least once a day) during at least half of the off-treatment periods.

Source: Table 104, this submission.

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Table 5. Change from Visit 1 in Log10 PA CFUs in Sputum by On-study Tobramycin Use, Treatment Courses 1 to 9: ITT Population (continued)

Treatment Course	Non-users*			Infrequent Users**			Frequent Users†		
	BID Pooled (N=49)	TID Pooled (N=114)	Total (N=163)	BID Pooled (N=20)	TID Pooled (N=48)	Total (N=68)	BID Pooled (N=16)	TID Pooled (N=27)	Total (N=43)
End Treatment Course 7									
n	15	49	64	14	30	44	10	13	23
Mean (SD)	-0.18 (1.604)	-0.75 (2.126)	-0.61 (2.018)	-1.28 (1.547)	-0.82 (2.695)	-0.96 (2.382)	-0.39 (2.110)	-0.35 (1.864)	-0.37 (1.928)
Start Treatment Course 8									
n	16	51	67	13	29	42	10	13	23
Mean (SD)	-0.37 (2.732)	-0.04 (1.791)	-0.11 (2.036)	-0.71 (2.282)	-0.97 (2.636)	-0.89 (2.507)	0.31 (1.486)	0.05 (0.970)	0.16 (1.198)
End Treatment Course 8									
n	17	47	64	14	29	43	10	13	23
Mean (SD)	-0.49 (2.115)	-0.91 (1.925)	-0.80 (1.970)	-0.56 (1.669)	-0.57 (2.436)	-0.57 (2.195)	-0.05 (1.268)	-0.36 (2.417)	-0.23 (1.967)
Start Treatment Course 9									
n	15	43	58	15	29	44	9	14	23
Mean (SD)	0.32 (1.717)	-0.36 (1.535)	-0.18 (1.597)	-1.63 (2.810)	-0.66 (2.043)	-0.99 (2.347)	0.43 (0.945)	-0.29 (2.219)	-0.01 (1.834)
End Treatment Course 9									
n	13	44	57	14	30	44	9	11	20
Mean (SD)	-0.26 (1.154)	-0.87 (2.072)	-0.73 (1.910)	-0.50 (1.869)	-0.45 (2.207)	-0.47 (2.083)	-0.71 (2.822)	0.07 (1.533)	-0.28 (2.179)
Follow-up									
n	15	43	58	13	31	44	9	12	21
Mean (SD)	-0.04 (1.130)	-0.28 (1.764)	-0.22 (1.618)	-0.67 (2.239)	-1.09 (2.575)	-0.97 (2.463)	-0.50 (2.374)	0.42 (2.623)	0.03 (2.501)

* Includes patients who did not use a ≥ 250 mg dose of inhaled tobramycin (at least once a day) during the off-treatment periods.

** Includes patients who used a ≥ 250 mg dose of inhaled tobramycin (at least once a day) during less than half of the off-treatment periods.

† Includes patients who used a ≥ 250 mg dose of inhaled tobramycin (at least once a day) during at least half of the off-treatment periods.

Source: Table 104, this submission.

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Reductions in log₁₀ *PA* CFUs in sputum were observed at nearly every visit for all tobramycin groups, and *the largest declines were consistently seen in the infrequent users, regardless of BID or TID dosing of aztreonam (Table 5).*

Change in log₁₀ *PA* CFUs in Sputum by Disease Severity

The mean change in log₁₀ *PA* CFUs in sputum by disease severity is summarized for patients with FEV₁ ≤ 50% of predicted at baseline and for patients with FEV₁ > 50% of predicted at baseline in [Table 6](#).

Mean decreases in log₁₀ *PA* CFUs in sputum from Visit 1 were observed at almost every visit regardless of disease severity ([Table 6](#)). *Patients with FEV₁ ≤ 50% of predicted at baseline tended to have larger decreases in log₁₀ *PA* CFUs in sputum than patients with FEV₁ > 50% of predicted at baseline.*

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Table 6. Change from Visit 1 in Log10 PA CFUs in Sputum by Disease Severity; Treatment Courses 1 to 9: ITT Population

Treatment Course	Patients with FEV ₁ ≤ 50% of Predicted			Patients with FEV ₁ > 50% of Predicted		
	BID Pooled (N=36)	TID Pooled (N=74)	Total (N=110)	BID Pooled (N=49)	TID Pooled (N=114)	Total (N=163)
End Treatment Course 1						
n	26	54	80	32	75	107
Mean (SD)	-0.31 (1.236)	-1.24 (1.819)	-0.94 (1.701)	-0.10 (1.659)	-0.51 (1.669)	-0.39 (1.669)
Start Treatment Course 2						
n	26	58	84	34	74	108
Mean (SD)	-0.37 (1.666)	-0.67 (1.907)	-0.57 (1.831)	-0.12 (1.714)	0.03 (1.648)	-0.02 (1.663)
End Treatment Course 2						
n	28	55	83	31	69	100
Mean (SD)	-0.74 (1.594)	-0.87 (2.302)	-0.83 (2.081)	-0.47 (1.872)	-0.75 (2.146)	-0.67 (2.060)
Start Treatment Course 3						
n	22	55	77	30	71	101
Mean (SD)	-0.61 (1.921)	-0.28 (1.517)	-0.37 (1.636)	-0.71 (1.410)	0.03 (1.858)	-0.19 (1.763)
End Treatment Course 3						
n	22	45	67	34	65	99
Mean (SD)	-0.25 (1.214)	-1.00 (2.356)	-0.75 (2.072)	-0.55 (1.452)	-0.22 (1.912)	-0.33 (1.767)
Start Treatment Course 4						
n	23	48	71	30	66	96
Mean (SD)	-0.61 (1.777)	-0.50 (2.459)	-0.54 (2.249)	-0.17 (2.213)	0.24 (1.116)	0.11 (1.543)
End Treatment Course 4						
n	15	43	58	24	62	86
Mean (SD)	-1.12 (2.156)	-0.94 (1.901)	-0.99 (1.952)	-0.40 (1.808)	-0.61 (2.274)	-0.55 (2.146)
Start Treatment Course 5						
n	17	46	63	26	66	92
Mean (SD)	0.13 (1.342)	-0.73 (1.706)	-0.50 (1.652)	-0.56 (1.807)	-0.03 (1.757)	-0.18 (1.778)
End Treatment Course 5						
n	16	43	59	26	60	86
Mean (SD)	0.12 (1.292)	-1.10 (1.734)	-0.77 (1.705)	-0.81 (2.342)	-0.13 (2.028)	-0.34 (2.137)

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Table 6. Change from Visit 1 in Log10 PA CFUs in Sputum by Disease Severity Treatment Courses 1 to 9: ITT Population (continued)

Treatment Course	Patients with FEV ₁ ≤ 50% of Predicted			Patients with FEV ₁ > 50% of Predicted		
	BID Pooled (N=36)	TID Pooled (N=74)	Total (N=110)	BID Pooled (N=49)	TID Pooled (N=114)	Total (N=163)
Start Treatment Course 6						
n	16	41	57	25	58	83
Mean (SD)	0.07 (1.258)	-0.82 (2.111)	-0.57 (1.942)	-0.65 (2.323)	0.14 (1.552)	-0.10 (1.841)
End Treatment Course 6						
n	14	39	53	23	46	69
Mean (SD)	-0.13 (1.019)	-0.87 (1.843)	-0.67 (1.688)	-0.79 (2.232)	-0.29 (2.117)	-0.46 (2.153)
Start Treatment Course 7						
n	14	38	52	20	53	73
Mean (SD)	-0.26 (1.061)	-0.88 (2.068)	-0.71 (1.862)	-0.36 (2.395)	0.12 (1.859)	-0.01 (2.013)
End Treatment Course 7						
n	16	40	56	23	51	74
Mean (SD)	-0.25 (1.211)	-1.37 (2.169)	-1.05 (2.000)	-0.90 (2.031)	-0.23 (2.262)	-0.44 (2.201)
Start Treatment Course 8						
n	17	40	57	22	52	74
Mean (SD)	0.24 (0.969)	-1.06 (2.378)	-0.67 (2.137)	-0.73 (2.897)	0.24 (1.562)	-0.05 (2.078)
End Treatment Course 8						
n	16	41	57	25	47	72
Mean (SD)	-0.11 (1.101)	-1.28 (2.351)	-0.95 (2.133)	-0.59 (2.077)	-0.28 (1.891)	-0.39 (1.949)
Start Treatment Course 9						
n	15	37	52	24	48	72
Mean (SD)	-0.48 (1.954)	-0.81 (1.831)	-0.71 (1.854)	-0.36 (2.486)	-0.20 (1.796)	-0.25 (2.035)
End Treatment Course 9						
n	14	36	50	22	48	70
Mean (SD)	-0.16 (1.333)	-1.25 (1.862)	-0.94 (1.786)	-0.66 (2.193)	-0.15 (2.107)	-0.31 (2.132)
Follow-up						
n	15	36	51	22	49	71
Mean (SD)	0.09 (1.250)	-0.77 (2.148)	-0.52 (1.956)	-0.68 (2.170)	-0.27 (2.333)	-0.40 (2.276)

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Source: Table 105, this submission.

Presence or Absence of Pathogens

Pseudomonas aeruginosa

The presence or absence of *Pseudomonas aeruginosa* (PA) is presented in [Table 7](#).

Table 7. Presence or Absence of PA: ITT Population

Treatment	Visit	N*	Result		Change		
			Presence n (%)	Absence n (%)	Appear n (%)	None n (%)	Disappear n (%)
AZLI BID (N = 85)	Visit 1	84	79 (94.0)	5 (6.0)	-	-	-
	Visit 2	82	77 (93.9)	5 (6.1)	4 (4.9)	73 (90.1)	4 (4.9)
	Visit 3	82	76 (92.7)	6 (7.3)	3 (3.7)	74 (91.4)	4 (4.9)
	Visit 4	80	72 (90.0)	8 (10.0)	2 (2.5)	72 (91.1)	5 (6.3)
	Visit 5	74	68 (91.9)	6 (8.1)	2 (2.7)	67 (91.8)	4 (5.5)
	Visit 6	75	65 (86.7)	10 (13.3)	2 (2.7)	65 (87.8)	7 (9.5)
	Visit 7/8	76	68 (89.5)	8 (10.5)	3 (4.0)	66 (88.0)	6 (8.0)
	Visit 9	55	47 (85.5)	8 (14.5)	0(0.0)	48 (88.9)	6 (11.1)
	Visit 10	55	51 (92.7)	4 (7.3)	1 (1.9)	50 (92.6)	3 (5.6)
	Visit 11	52	49 (94.2)	3 (5.8)	2 (3.9)	46 (90.2)	3 (5.9)
	Visit 12	53	45 (84.9)	8 (15.1)	0(0.0)	46 (88.5)	6 (11.5)
	Visit 13	50	44 (88.0)	6 (12.0)	1 (2.0)	44 (88.0)	5 (10.0)
	Visit 14	49	43 (87.8)	6 (12.2)	1 (2.1)	42 (87.5)	5 (10.4)
	Visit 15	51	45 (88.2)	6 (11.8)	1 (2.0)	44 (88.0)	5 (10.0)
	Visit 16	49	42 (85.7)	7 (14.3)	1 (2.1)	41 (85.4)	6 (12.5)
	Visit 17	49	44 (89.8)	5 (10.2)	1 (2.1)	43 (89.6)	4 (8.3)
	Visit 18	47	41 (87.2)	6 (12.8)	2 (4.3)	38 (82.6)	6 (13.0)
	Visit 19	45	39 (86.7)	6 (13.3)	0(0.0)	40 (90.9)	4 (9.1)
	Visit 20	46	40 (87.0)	6 (13.0)	1 (2.2)	39 (86.7)	5 (11.1)
AZLI TID (N = 189)	Visit 1	185	176 (95.1)	9 (4.9)	-	-	-
	Visit 2	186	172 (92.5)	14 (7.5)	4 (2.2)	169 (92.3)	10 (5.5)
	Visit 3	177	163 (92.1)	14 (7.9)	1 (0.6)	165 (94.8)	8 (4.6)
	Visit 4	176	157 (89.2)	19 (10.8)	3 (1.7)	156 (90.2)	14 (8.1)
	Visit 5	172	161 (93.6)	11 (6.4)	3 (1.8)	160 (94.7)	6 (3.6)
	Visit 6	163	144 (88.3)	19 (11.7)	4 (2.5)	142 (88.2)	15 (9.3)
	Visit 7/8	160	148 (92.5)	12 (7.5)	4 (2.5)	146 (92.4)	8 (5.1)
	Visit 9	146	128 (87.7)	18 (12.3)	2 (1.4)	129 (89.6)	13 (9.0)
	Visit 10	148	133 (89.9)	15 (10.1)	1 (0.7)	136 (93.2)	9 (6.2)
	Visit 11	143	127 (88.8)	16 (11.2)	2 (1.4)	128 (90.8)	11 (7.8)
	Visit 12	136	121 (89.0)	15 (11.0)	2 (1.5)	121 (90.3)	11 (8.2)
	Visit 13	132	119 (90.2)	13 (9.8)	2 (1.5)	117 (90.0)	11 (8.5)
	Visit 14	131	118 (90.1)	13 (9.9)	1 (0.8)	120 (93.0)	8 (6.2)
	Visit 15	130	115 (88.5)	15 (11.5)	2 (1.6)	114 (89.1)	12 (9.4)
	Visit 16	129	117 (90.7)	12 (9.3)	2 (1.6)	116 (91.3)	9 (7.1)
	Visit 17	124	110 (88.7)	14 (11.3)	2 (1.6)	108 (88.5)	12 (9.8)
	Visit 18	122	111 (91.0)	11 (9.0)	0(0.0)	113 (94.2)	7 (5.8)

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	Visit 19	121	111 (91.7)	10 (8.3)	1 (0.8)	110 (92.4)	8 (6.7)
	Visit 20	121	107 (88.4)	14 (11.6)	1 (0.8)	106 (89.1)	12 (10.1)
AZLI Total (N = 274)	Visit 1	269	255 (94.8)	14 (5.2)	-	-	-
	Visit 2	268	249 (92.9)	19 (7.1)	8 (3.0)	242 (91.7)	14 (5.3)
	Visit 3	259	239 (92.3)	20 (7.7)	4 (1.6)	239 (93.7)	12 (4.7)
	Visit 4	256	229 (89.5)	27 (10.5)	5 (2.0)	228 (90.5)	19 (7.5)
	Visit 5	246	229 (93.1)	17 (6.9)	5 (2.1)	227 (93.8)	10 (4.1)
	Visit 6	238	209 (87.8)	29 (12.2)	6 (2.6)	207 (88.1)	22 (9.4)
	Visit 7/8	236	216 (91.5)	20 (8.5)	7 (3.0)	212 (91.0)	14 (6.0)
	Visit 9	201	175 (87.1)	26 (12.9)	2 (1.0)	177 (89.4)	19 (9.6)
	Visit 10	203	184 (90.6)	19 (9.4)	2 (1.0)	186 (93.0)	12 (6.0)
	Visit 11	195	176 (90.3)	19 (9.7)	4 (2.1)	174 (90.6)	14 (7.3)
	Visit 12	189	166 (87.8)	23 (12.2)	2 (1.1)	167 (89.8)	17 (9.1)
	Visit 13	182	163 (89.6)	19 (10.4)	3 (1.7)	161 (89.4)	16 (8.9)
	Visit 14	180	161 (89.4)	19 (10.6)	2 (1.1)	162 (91.5)	13 (7.3)
	Visit 15	181	160 (88.4)	21 (11.6)	3 (1.7)	158 (88.8)	17 (9.6)
	Visit 16	178	159 (89.3)	19 (10.7)	3 (1.7)	157 (89.7)	15 (8.6)
	Visit 17	173	154 (89.0)	19 (11.0)	3 (1.8)	151 (88.8)	16 (9.4)
	Visit 18	169	152 (89.9)	17 (10.1)	2 (1.2)	151 (91.0)	13 (7.8)
	Visit 19	166	150 (90.4)	16 (9.6)	1 (0.6)	150 (92.0)	12 (7.4)
	Visit 20	167	147 (88.0)	20 (12.0)	2 (1.2)	145 (88.4)	17 (10.4)

* The number of patients with available data.

- Not applicable.

Source: [Table 68](#), this submission.

Overall, *PA* was isolated from 94.8% of patients at baseline (Visit 1; the number of patients with available data = 269), with percentages being similar for the BID and TID groups at 94.0% (n = 84) and 95.1% (n = 185), respectively. Over the nine courses, both on and off treatment, (Visits 2 through 20; n [total] = 268 to 167), *PA* was isolated from at least 88% of patients at each visit. *Eradication of established PA infection is not expected for suppressive therapy, and the presence or absence of PA is more likely to be attributable to variations in sampling technique or to the patients' ability to produce sputum, than to the disappearance of PA in individual patients.*

Other Pathogens

Staphylococcus aureus

The presence or absence of MSSA and MRSA was summarized for the Safety population as well as the population of patients who completed all nine treatment courses ([Table 8](#)).

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Table 8. Percentage of Patients with MSSA and MRSA: ITT population

	AZLI BID (N = 85)	AZLI TID (N = 189)	AZLI Total (N = 274)
Baseline, Visit 1	n = 84	n = 185	n = 269
Patients with <i>S. aureus</i> , n (%)	33 (39.3)	65 (35.1)	98 (36.4)
Patient isolates tested for ceftiofur resistance, n (%) ^a	33 (100.0)	57 (87.7)	90 (91.8)
Patients with MSSA, n (%) ^b	26 (31.0)	32 (19.7) ^d	58 (23.5) ^d
Patient with MRSA, n (%) ^c	7 (8.3)	25 (15.4) ^d	32 (13.0) ^d
End of Treatment Course 1, Visit 2	n = 82	n = 186	n = 268
Patients with <i>S. aureus</i> , n (%)	34 (41.5)	66 (35.5)	100 (37.3)
Patient isolates tested for ceftiofur resistance, n (%) ^a	34 (100.0)	57 (86.4)	91 (91.0)
Patients with MSSA, n (%) ^b	27 (32.9)	32 (19.9) ^d	59 (24.2) ^d
Patient with MRSA, n (%) ^c	7 (8.5)	25 (15.6) ^d	32 (13.1) ^d
End of Treatment Course 3, Visit 7/8	n = 75	n = 163	n = 238
Patients with <i>S. aureus</i> , n (%)	41 (54.7)	68 (41.7)	109 (45.8)
Patient isolates tested for ceftiofur resistance, n (%) ^a	40 (97.6)	61 (89.7)	101 (92.7)
Patients with MSSA, n (%) ^b	31 (42.3) ^d	35 (23.9) ^d	66 (29.9) ^d
Patient with MRSA, n (%) ^c	9 (12.3) ^d	26 (17.8) ^d	35 (15.9) ^d
End of Treatment Course 6, Visit 13	n = 50	n = 132	n = 182
Patients with <i>S. aureus</i> , n (%)	17 (34.0)	51 (38.6)	68 (37.4)
Patient isolates tested for ceftiofur resistance, n (%) ^a	17 (100.0)	51 (100.0)	68 (100.0)
Patients with MSSA, n (%) ^b	11 (22.0)	28 (21.2)	39 (21.4)
Patient with MRSA, n (%) ^c	6 (12.0)	23 (17.4)	29 (15.9)
End of Treatment Course 9, Visit 19	n = 45	n = 121	n = 166
Patients with <i>S. aureus</i> , n (%)	15 (33.3)	51 (42.1)	66 (39.8)
Patient isolates tested for ceftiofur resistance, n (%) ^a	15 (100.0)	50 (98.0)	65 (98.5)
Patients with MSSA, n (%) ^b	10 (22.2)	31 (26.1) ^d	41 (25.1) ^d
Patient with MRSA, n (%) ^c	5 (11.1)	19 (16.0) ^d	24 (14.7) ^d
Follow-up, Visit 20	n = 46	n = 121	n = 167
Patients with <i>S. aureus</i> , n (%)	16 (34.8)	49 (40.5)	65 (38.9)
Patient isolates tested for ceftiofur resistance, n (%) ^a	16 (100.0)	49 (100.0)	65 (100.0)
Patients with MSSA, n (%) ^b	11 (23.9)	32 (26.4)	43 (25.7)
Patient with MRSA, n (%) ^c	5 (10.9)	17 (14.0)	22 (13.2)

n = patients with culture data

a Percent of sample size tested was determined by dividing the number of patient isolates tested for resistance to ceftiofur by the number of patients with *S. aureus* isolates.

b Percentage calculated as the number of patients with *S. aureus* sensitive to ceftiofur divided by the number of patients with culture data.

c Percentage calculated as the number of patients with *S. aureus* resistant to ceftiofur divided by the number of patients with culture data.

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d In cases where not all *S. aureus* isolates were tested for ceftiofur resistance, the percentage of patients with MSSA (or MRSA) is calculated by the number of patients with *S. aureus* sensitive (or resistant) to ceftiofur divided by a denominator consisting of the number of patients with culture data multiplied by the percentage of patients whose *S. aureus* isolate was tested for ceftiofur resistance.

Source: [Table 69](#), this submission.

There were slight fluctuations over time in the percentages of patients testing positive for *S. aureus* following repeat dosing with up to nine courses of AZLI in both treatment groups, and the increases were generally greater in the BID group than in the TID group. In both regimens combined, *S. aureus* was present in 36.4% of patients at Visit 1, with 33.3% to 45.8% testing positive during the nine treatment courses. *S. aureus* was present at Visit 1 in 39.3% of BID-treated patients, with a range of 32.1% to 54.7% testing positive during the nine courses. In the TID regimen, *S. aureus* was isolated at Visit 1 in 35.1% of patients, with a range of 33.6% to 43.1% testing positive during the nine treatment courses. At the end of the ninth treatment course (Visit 19), *S. aureus* appeared in 33.3% of patients in the BID regimen compared to 39.3% at Visit 1 and 42.1% of patients in the TID regimen compared to 35.1% at Visit 1.

MSSA and MRSA

The antibacterial spectrum of aztreonam typically excludes Gram-positive organisms like MSSA and MRSA whereas it typically demonstrates antibacterial activity against Gram-negative organisms like *PA*. There is a theoretical concern that AZLI therapy in patients with CF could provide a growth advantage for MSSA and MRSA over that of *PA* in the lung. In order to monitor for this type of treatment-emergent infection, *S. aureus* was regularly cultured for over nine courses of AZLI treatment. Colonization of MRSA was identified by growing cultured *S. aureus* using the ceftiofur disk diffusion method. Testing for MRSA was initiated after patients had begun participation in the study; therefore, data are not available at all time points for some patients with positive *S. aureus* cultures. While not every *S. aureus* isolate was tested, the sampling percentage was over 90% early in the study and 100% at the end of the study ([Table 8](#)).

At baseline (Visit 1) of the study, culture data for patients in the Safety population were available for 269 patients, 84 from the AZLI BID treatment group and 185 from the AZLI TID treatment group. Of these 269 patients, 98 (36.4%) tested positive for *S. aureus*. Of these 98 patients, the presence of a MSSA or MRSA infection was determined in 90 (91.8%) patients. Overall, 23.5% of the patient population at baseline (Visit 1) in the study was culture positive for MSSA while 13.0% of the patient population was culture positive for MRSA. Notably, these percentages are lower than what was reported in the 2008 Cystic Fibrosis Registry. In the Safety population, following Treatment Course 9 of AZLI therapy, the percentage of patients with MSSA and MRSA was similar to that of Visit 1; 25.1% of the patient population was culture positive for MSSA while 14.7% of the patient population was culture positive for MRSA.

Treatment emergence of MSSA and MRSA was monitored during the study and has been reported at four timepoints spaced evenly over the nine treatment courses. At no time

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following Treatment Courses 1, 3, 6, and 9 of AZLI therapy, BID and TID, did the percentage of patients with MSSA and MRSA deviate significantly from baseline. *This analysis suggests that emergence of MSSA and MRSA did not occur as a result or in association with AZLI therapy (Table 8).*

In the Safety population at baseline, the BID treatment group had a higher percentage of patients with MSSA (31.0%) than the TID group (19.7%), while the TID treatment group had a higher percentage of patients with MRSA (15.4%) than the BID group (8.3%). Of the 84 BID-treated patients with culture data, 33 (39.3%) patients tested positive for *S. aureus*. Of the 185 TID-treated patients with culture data, 65 (35%) patients tested positive for *S. aureus* (Table 8).

Following nine courses of AZLI BID therapy (Visit 19), the percentage of patients with MSSA was 22.2%, and thus lower than that of Visit 1 (31.0%), while the percentage of patients with MRSA increased slightly, from 8.3% at Visit 1 to 11.1% at Visit 19. Following nine courses of AZLI TID therapy, the percentage of patients with MSSA was 26.1%, higher than that of Visit 1 (19.7%), while the percentage of patients with MRSA remained nearly identical, at 15.4% and 16.0% at Visits 1 and 19, respectively (Table 8).

From these data, it appears that emergence of MSSA and MRSA did not occur as a result of or in association with AZLI therapy. Further, it appears that AZLI therapy did not profoundly affect shifts in MRSA status. Throughout the open label study, dramatic shifts in acquisition or loss of MRSA from baseline were not observed. More than 90% of the total patient population did not change MRSA status from baseline to the end of Treatment Courses 1, 3, 6, 9 or at the end-of-study visit. The observation that changes in MRSA status occurred in such a small percentage of the patient population throughout the study suggests that AZLI therapy was not associated with or responsible for MRSA emergence or treatment.

***Burkholderia cepacia* Complex**

B. cepacia complex (BCC) was not present in any AZLI BID-treated patients at Visit 1 and did not appear in this treatment group during the study. BCC was present in one AZLI TID-treated patient at Visit 1. Treatment-emergent intermittent isolation of BCC occurred in 4 other patients.

Overall, BCC was isolated from 5 patients: Patients 019601, 019602, 104608, 152611 and 160603 in the TID treatment group:

- **Patient 019601:** The sputum sample from Visit 1 tested positive for BCC. This patient continued the study through the second treatment course and then was withdrawn. Sputum samples at Visits 2, 3 and EOT were negative, positive and positive, respectively for BCC. This patient entered this study after completing Study CP-AI-007 where he had been randomized to receive AZLI TID. The patient had tested negative for BCC at all timepoints in CP-AI-007.

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- **Patient 019602:** The sputum samples from Visit 1-4 tested negative for BCC. Sputum samples from Visits 5 and 6 tested positive for BCC. This patient continued in the study through the fifth treatment course and then was withdrawn. Sputum samples from Visits 8, 9, 10 and ET tested negative for BCC.
- **Patient 104608:** The patient was withdrawn from the previous study, [CP-AI-005](#), at Day -28 after testing positive for BCC. He was subsequently enrolled in [CP-AI-006](#) and was withdrawn for noncompliance with study visits and study procedures. His sputum culture from the ET Visit (after the second treatment course) tested positive for BCC.
- **Patient 152611:** The sputum sample from Visit 10 tested positive for BCC. No other sputum samples tested positive throughout the study and the patient completed the entire study.
- **Patient 160603:** Sputum samples from Visits 5, 12, 14, and 20 tested positive for BCC. Sputum samples for all other visits tested negative for this pathogen and the patient continued in the study. The patient entered this study after completing Study [CP-AI-007](#) where he had been randomized to receive AZLI TID. His test results from the Screening visit of [CP-AI-007](#) were positive for BCC. All subsequent tests for BCC in [CP-AI-007](#) were negative.

Stenotrophomonas maltophilia

There was no notable change in the percentages of patients testing positive for *S. maltophilia* following repeat dosing over nine treatment courses of AZLI treatment in the BID or TID group. Overall, *S. maltophilia* was present in 10.0% of patients at Visit 1, with a range of 9.6% to 13.3% testing positive during the nine treatment courses. *S. maltophilia* was present at Visit 1 in 15.5% of BID-treated patients, with a range of 7.8% to 16.2% testing positive during the nine treatment courses. In the TID regimen, 7.6% of patients tested positive for *S. maltophilia* at Visit 1 with a range of 7.6% to 13.8% testing positive during the nine treatment courses. A small increase in the percentages of patients in the TID regimen was observed but the percentage of patients with *S. maltophilia* at Visit 1 in the TID group was half that of the BID group value. Thus, the observed increase more likely represents a return to levels more commonly observed in patients with CF ([Table 14.8.1.1, this submission, not shown](#)).

Achromobacter xylosoxidans

There was no notable change in the percentages of patients testing positive for *A. xylosoxidans* following repeat dosing with up to nine courses of AZLI treatment in either regimen. Overall, 7.8% of patients were positive for *A. xylosoxidans* at Visit 1, with a range of 5.6% to 10.1% testing positive during the nine treatment courses. *A. xylosoxidans* was present at Visit 1 in 9.5% of BID-treated patients, with a range of 5.7% to 10.9% testing positive during the nine treatment courses. In the TID regimen, 7.0% of patients tested positive for *A. xylosoxidans* at Visit 1 with a range of 5.0% to 11.6% of patients testing positive during the nine treatment courses ([Table 14.8.1.1, this submission, not shown](#)).

Candida spp.

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Candida spp. were isolated from 186 patients (69.1%) at baseline (60.7% BID vs. 73.0% TID). At the end of Treatment Course 9, 83.7% of patients (84.4% BID vs. 83.5% TID) had a positive culture for *Candida* spp. The high prevalence of *Candida* spp. observed in this study is consistent with a recent report demonstrating detection of *Candida albicans* in sputum from 76% of adult patients with CF. Overall increases in the prevalence of *Candida* spp. were observed over repeated courses of AZLI; however, the majority of patients experienced no change in the presence or absence of *Candida* spp. through the nine courses. At the individual visits, the proportions of patients with no change in the presence or absence of *Candida* spp. ranged from 52.3% to 71.6% for patients in the BID group and from 70.6% to 81.2% for patients in the TID group ([Table 14.8.1.1, this submission, not shown](#)).

A total of 51 patients, 20 in the BID group and 31 in the TID group, had treatment-emergent persistent isolation of *Candida* spp. during the study. Treatment-emergent persistent isolation was defined as those patients who did not have *Candida* spp. in their sputum culture at baseline, but who had positive cultures at a minimum of three consecutive visits after Visit 1. Steroid use, which is a known risk factor for fungal infection, is provided for patients with treatment-emergent persistent isolation of *Candida* spp. Of the 51 patients with treatment-emergent persistent isolation, 35 (68.6%) patients were prescribed steroid medication prior to receiving AZLI therapy or at the start of treatment.

***Aspergillus* spp.**

Aspergillus spp. were isolated from 56 patients (20.8%) at baseline (17.9% BID vs. 22.2% TID). At the end of Treatment Course 9, 21.7% of patients (20.0% BID vs. 22.3% TID) had a positive culture for *Aspergillus* spp. The prevalence of *Aspergillus* spp. observed in this study is consistent with reports showing detection of *Aspergillus* spp. in sputum from 1% to 57% of patients with CF.

The majority of patients experienced no change in the presence or absence of *Aspergillus* spp. during the nine treatment courses. At the individual visits, the proportions of patients with no change in the presence or absence of *Aspergillus* spp. ranged from 73.9% to 85.2% for patients in the BID group and from 75.9% to 86.7% for patients in the TID group ([Table 14.8.1.1, this submission, not shown](#)).

Over the course of the entire study, 170 patients had a positive laboratory culture for *Aspergillus* spp. at one or more study visits. Of these 170 patients, 11 were prescribed an anti-fungal medication to treat an *Aspergillus* infection. Since fungal laboratory culture results are not routinely reported to clinical sites, the fact that such a small percentage of patients were prescribed anti-fungal medication suggests that sputum colonization with fungal organisms often does not result in clinically significant infection.

A total of 32 patients, 12 in the BID group and 20 in the TID group, had treatment-emergent persistent isolation of an *Aspergillus* spp. during the study. Treatment-

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emergent persistent isolation was defined as those patients who did not have *Aspergillus* spp. in their sputum culture at baseline, but who had positive cultures at a minimum of three consecutive visits after Visit 1. Of the 32 patients with treatment-emergent persistent isolation, 20 (62.5%) patients were prescribed steroid medication prior to receiving AZLI therapy or at the start of treatment and 22 (68.8%) patients were prescribed steroid medication during study participation that was not prescribed prior to, or at the start of, the study.

Minimum Inhibitory Concentrations for PA

Changes in MIC50 and MIC90 of aztreonam for all *PA* isolates are summarized in [Table 9](#).

Table 9. MIC50 and MIC90 of Aztreonam for All *PA* Isolates (µg/mL), Treatment Courses 1 to 9: ITT Population

Treatment	Visit	Result (µg/mL)					Change from Visit 1	
		MIC50	MIC90	n	Min	Max	MIC50	MIC90
AZLI BID (N = 85)	Visit 1	4	128	131	≤1	2048	-	-
	Visit 2	4	256	120	≤1	>2048	Unchanged	Unchanged
	Visit 3	4	128	124	≤1	>2048	Unchanged	Unchanged
	Visit 4	8	256	108	≤1	2048	Unchanged	Unchanged
	Visit 5	8	256	114	≤1	>2048	Unchanged	Unchanged
	Visit 6	8	256	104	≤1	2048	Unchanged	Unchanged
	Visit 7/8	8	256	107	≤1	>2048	Unchanged	Unchanged
	Visit 9	16	512	77	≤1	>2048	Increased	Increased
	Visit 10	8	512	79	≤1	>2048	Unchanged	Increased
	Visit 11	16	256	79	≤1	>2048	Increased	Unchanged
	Visit 12	16	256	72	≤1	2048	Increased	Unchanged
	Visit 13	16	128	70	≤1	1024	Increased	Unchanged
	Visit 14	16	512	67	≤1	>2048	Increased	Increased
	Visit 15	16	512	68	≤1	>2048	Increased	Increased
	Visit 16	8	128	64	≤1	2048	Unchanged	Unchanged
	Visit 17	16	256	66	≤1	2048	Increased	Unchanged
	Visit 18	8	256	69	≤1	2048	Unchanged	Unchanged
	Visit 19	16	512	64	≤1	>2048	Increased	Increased
	Visit 20	4	128	64	≤1	2048	Unchanged	Unchanged
AZLI TID (N = 189)	Visit 1	4	128	288	≤1	>2048	-	-
	Visit 2	8	256	275	≤1	>2048	Unchanged	Unchanged
	Visit 3	4	128	279	≤1	>2048	Unchanged	Unchanged
	Visit 4	4	256	256	≤1	>2048	Unchanged	Unchanged
	Visit 5	8	256	264	≤1	2048	Unchanged	Unchanged
	Visit 6	8	256	236	≤1	>2048	Unchanged	Unchanged
	Visit 7/8	8	256	242	≤1	>2048	Unchanged	Unchanged
	Visit 9	8	512	209	≤1	>2048	Unchanged	Increased
	Visit 10	4	256	214	≤1	>2048	Unchanged	Unchanged
	Visit 11	8	256	200	≤1	>2048	Unchanged	Unchanged

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	Visit 12	8	256	194	≤1	>2048	Unchanged	Unchanged
	Visit 13	8	512	190	≤1	>2048	Unchanged	Increased
	Visit 14	4	256	182	≤1	>2048	Unchanged	Unchanged
	Visit 15	8	256	171	≤1	>2048	Unchanged	Unchanged
	Visit 16	8	128	170	≤1	>2048	Unchanged	Unchanged
	Visit 17	8	256	165	≤1	>2048	Unchanged	Unchanged
	Visit 18	8	256	176	≤1	>2048	Unchanged	Unchanged
	Visit 19	8	512	169	≤1	>2048	Unchanged	Increased
	Visit 20	8	256	168	≤1	>2048	Unchanged	Unchanged
AZLI Total (N = 274)	Visit 1	4	128	419	≤1	>2048	-	-
	Visit 2	8	256	395	≤1	>2048	Unchanged	Unchanged
	Visit 3	4	128	403	≤1	>2048	Unchanged	Unchanged
	Visit 4	8	256	364	≤1	>2048	Unchanged	Unchanged
	Visit 5	8	256	378	≤1	>2048	Unchanged	Unchanged
	Visit 6	8	256	340	≤1	>2048	Unchanged	Unchanged
	Visit 7/8	8	256	349	≤1	>2048	Unchanged	Unchanged
	Visit 9	8	512	286	≤1	>2048	Unchanged	Increased
	Visit 10	4	256	293	≤1	>2048	Unchanged	Unchanged
	Visit 11	8	256	279	≤1	>2048	Unchanged	Unchanged
	Visit 12	8	256	266	≤1	>2048	Unchanged	Unchanged
	Visit 13	8	256	260	≤1	>2048	Unchanged	Unchanged
	Visit 14	8	256	249	≤1	>2048	Unchanged	Unchanged
	Visit 15	8	256	239	≤1	>2048	Unchanged	Unchanged
	Visit 16	8	128	234	≤1	>2048	Unchanged	Unchanged
	Visit 17	8	256	231	≤1	>2048	Unchanged	Unchanged
	Visit 18	8	256	245	≤1	>2048	Unchanged	Unchanged
	Visit 19	8	512	233	≤1	>2048	Unchanged	Increased
	Visit 20	8	256	232	≤1	>2048	Unchanged	Unchanged

n = number of isolates.

- Not applicable.

Change categories: 'Increased' is defined as ≥ 4-fold increase in MIC, 'Unchanged' is defined as MIC values +/- a 2-fold change, 'Decreased' is defined as ≥ 4-fold decrease in MIC.

Source: [Table 70](#) this submission.

The MIC₅₀ of aztreonam for all *PA* isolates was 4 µg/mL at Visit 1 for both the total BID and TID groups. The MIC₉₀ of aztreonam for all *PA* isolates at Visit 1 was 128 µg/mL for both BID and TID groups.

In the BID group, the MIC₅₀ value increased 4-fold from 4 µg/mL to 16 µg/mL at Visit 9 over the course of eight treatments. At all other visits, the MIC₅₀ remained unchanged (± 2 fold change). At Visit 20, the MIC₅₀ returned to the baseline value of 4 µg/mL. In the BID group, the MIC₉₀ increased from 128 µg/mL to 512 µg/mL at Visit 9 over the course of eight treatments. At all other visits the MIC₉₀ remained unchanged (±2 fold change). At visit 20, the MIC₉₀ returned to the baseline value of 128 µg/mL.

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In the TID group, the MIC50 remained unchanged ($\pm$ 2-fold change) from Visit 1. The MIC90 increased by 4-fold from Visit 1 to Visits 9, 13 and 19 but returned to within a 2-fold change from baseline by Visit 20.

MIC50 and MIC90 values of aztreonam for the *PA* isolate with the highest MIC from each patient are summarized in [Table 10](#).

Table 10. MIC50 and MIC90 of Aztreonam for *PA* Isolate with the Highest MIC from Each Patient: ITT Population

Treatment	Visit	Result ($\mu\text{g/mL}$)				
		MIC50	MIC90	n	Min	Max
AZLI BID (N = 85)	Visit 1	8	128	76	≤ 1	2048
	Visit 2	8	512	76	≤ 1	>2048
	Visit 3	4	256	76	≤ 1	>2048
	Visit 4	8	512	72	≤ 1	2048
	Visit 5	8	512	68	≤ 1	>2048
	Visit 6	8	256	65	≤ 1	2048
	Visit 7/8	16	512	67	≤ 1	>2048
	Visit 9	16	512	47	≤ 1	>2048
	Visit 10	8	256	51	≤ 1	>2048
	Visit 11	16	512	49	≤ 1	>2048
	Visit 12	16	1024	45	≤ 1	2048
	Visit 13	16	512	44	≤ 1	1024
	Visit 14	16	512	43	≤ 1	>2048
	Visit 15	16	1024	44	≤ 1	>2048
	Visit 16	16	128	42	≤ 1	2048
	Visit 17	16	256	44	≤ 1	2048
	Visit 18	16	512	41	≤ 1	2048
	Visit 19	16	1024	38	≤ 1	>2048
	Visit 20	8	256	40	≤ 1	2048
AZLI TID (N = 189)	Visit 1	8	256	171	≤ 1	>2048
	Visit 2	8	256	171	≤ 1	>2048
	Visit 3	8	256	163	≤ 1	>2048
	Visit 4	8	512	157	≤ 1	>2048
	Visit 5	8	256	161	≤ 1	2048
	Visit 6	16	512	144	≤ 1	>2048
	Visit 7/8	8	256	147	≤ 1	>2048
	Visit 9	8	512	128	≤ 1	>2048
	Visit 10	8	512	133	≤ 1	>2048
	Visit 11	8	512	127	≤ 1	>2048
	Visit 12	8	256	121	≤ 1	>2048
	Visit 13	16	1024	119	≤ 1	>2048
	Visit 14	8	512	118	≤ 1	>2048
	Visit 15	8	512	115	≤ 1	>2048

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	Visit 16	8	256	117	≤1	>2048
	Visit 17	8	512	110	≤1	>2048
	Visit 18	16	512	111	≤1	>2048
	Visit 19	8	1024	110	≤1	>2048
	Visit 20	8	512	107	≤1	>2048

n = number of patients with data.

Shading indicates a ≥ 4-fold increase from Visit 1.

Source: [Table 71](#) this submission.

The MIC₅₀ of aztreonam for the *PA* isolate with the highest MIC was 8 µg/mL at Visit 1 for both the BID and TID groups. The MIC₉₀ of aztreonam for the *PA* isolate with the highest MIC at Visit 1 was within one dilution for the treatment groups (BID: MIC₉₀ = 128 µg/mL; TID: MIC₉₀ = 256 µg/mL).

In the BID and TID groups, the MIC₅₀ remained unchanged (± 2-fold change) at all visits in comparison with Visit 1. In the BID group, the MIC₉₀ for the *PA* isolate with the highest MIC from each patient increased greater than 2-fold (to 512 µg/mL or 1024 µg/mL) above the baseline value except at Visits 3, 6, 10, 16, and 17, but returned to the baseline value (± 2-fold change) at Visit 20. In the TID group, the MIC₉₀ increased intermittently at Visits 13 and 19 and returned to within 2-fold change of the baseline value at Visit 20.

Individual Patient Changes in MIC of Aztreonam for *PA*

Change from Visit 1 in MIC of aztreonam for *PA* isolate with the highest MIC from each patient is summarized in [Table 11](#).

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Table 11. Change from Visit 1 in MIC of Aztreonam for PA Isolate with the Highest MIC from Each Patient: ITT Population

Visit Change in MIC	AZLI BID (N = 85)		AZLI TID (N = 189)		AZLI Total (N = 274)	
	n	%	n	%	n	%
Visit 2 (End Treatment Course 1)	68		160		228	
Increased	16	23.5	44	27.5	60	26.3
Unchanged	43	63.2	86	53.8	129	56.6
Decreased	9	13.2	30	18.8	39	17.1
Visit 3 (End Treatment Course 1 off-treatment)	69		154		223	
Increased	10	14.5	38	24.7	48	21.5
Unchanged	46	66.7	81	52.6	127	57.0
Decreased	13	18.8	35	22.7	48	21.5
Visit 4 (End Treatment Course 2)	67		146		213	
Increased	13	19.4	43	29.5	56	26.3
Unchanged	45	67.2	74	50.7	119	55.9
Decreased	9	13.4	29	19.9	38	17.8
Visit 5 (End Treatment Course 2 off-treatment)	62		150		212	
Increased	14	22.6	41	27.3	55	25.9
Unchanged	37	59.7	84	56.0	121	57.1
Decreased	11	17.7	25	16.7	36	17.0
Visit 6 (End Treatment Course 3)	59		133		192	
Increased	11	18.6	45	33.8	56	29.2
Unchanged	37	62.7	70	52.6	107	55.7
Decreased	11	18.6	18	13.5	29	15.1
Visit 7/8 (End Treatment Course 3 off-treatment)	61		136		197	
Increased	12	19.7	43	31.6	55	27.9
Unchanged	45	73.8	71	52.2	116	58.9
Decreased	4	6.6	22	16.2	26	13.2

Increased = patients with ≥ 4 -fold increase in MIC.

Unchanged = patients with ± 2 -fold change in MIC.

Decreased = patients with ≥ 4 -fold decrease in MIC.

Source: [Table 72](#) this submission.

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Table 11. Change from Visit 1 in MIC of Aztreonam for PA Isolate with the Highest MIC from Each Patient: ITT Population (continued)

Visit	AZLI BID (N = 85)		AZLI TID (N = 189)		AZLI Total (N = 274)	
	n	%	n	%	n	%
Change in MIC						
Visit 9 (End Treatment Course 4) Increased	45		121		166	
		31.1	38	31.4	52	31.3
Unchanged	24	53.3	64	52.9	88	53.0
Decreased	7	15.6	19	15.7	26	15.7
Visit 10 (End Treatment Course 4 off-treatment)	47		126		173	
Increased	10	21.3	35	27.8	45	26.0
Unchanged	32	68.1	69	54.8	101	58.4
Decreased	5	10.6	22	17.5	27	15.6
Visit 11 (End Treatment Course 5)	45		120		165	
Increased	10	22.2	47	39.2	57	34.5
Unchanged	34	75.6	57	47.5	91	55.2
Decreased	1	2.2	16	13.3	17	10.3
Visit 12 (End Treatment Course 5 off-treatment)	43		113		156	
Increased	8	18.6	32	28.3	40	25.6
Unchanged	29	67.4	60	53.1	89	57.1
Decreased	6	14.0	21	18.6	27	17.3
Visit 13 (End Treatment Course 6)	42		111		153	
Increased	12	28.6	41	36.9	56	34.6
Unchanged	25	59.5	51	45.9	76	49.7
Decreased	5	11.9	19	17.1	24	15.7
Visit 14 (End Treatment Course 6 off-treatment)	40		112		152	
Increased	12	30.0	31	27.7	43	28.3
Unchanged	21	52.5	63	56.3	84	55.3
Decreased	7	17.5	18	16.1	25	16.4

Increased = patients with ≥ 4 -fold increase in MIC.

Unchanged = patients with ± 2 -fold change in MIC.

Decreased = patients with ≥ 4 -fold decrease in MIC.

Source: [Table 72](#) this submission.

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Table 11. Change from Visit 1 in MIC of Aztreonam for *PA* Isolate with the Highest MIC from Each Patient: ITT Population (continued)

Visit Change in MIC	AZLI BID (N = 85) n %		AZLI TID (N = 189) n %		AZLI Total (N = 274) n %	
Visit 15 (End Treatment Course 7)						
Increased	41		108		149	
	12	29.3	35	32.4	47	31.5
Unchanged	22	53.7	53	49.1	75	50.3
Decreased	7	17.1	20	18.5	27	18.1
Visit 16 (End Treatment Course 7 off-treatment)						
Increased	40		111		151	
	9	22.5	33	29.7	42	27.8
Unchanged	28	70.0	56	50.5	84	55.6
Decreased	3	7.5	22	19.8	25	16.6
Visit 17 (End Treatment Course 8)						
Increased	41		104		145	
	14	34.1	33	31.7	47	32.4
Unchanged	22	53.7	53	51.0	75	51.7
Decreased	5	12.2	18	17.3	23	15.9
Visit 18 (End Treatment Course 8 off-treatment)						
Increased	38		106		144	
	13	34.2	38	35.8	51	35.4
Unchanged	20	52.6	56	52.8	76	52.8
Decreased	5	13.2	12	11.3	17	11.8
Visit 19 (End Treatment Course 9)						
Increased	37		105		142	
	17	45.9	37	35.2	54	38.0
Unchanged	15	40.5	44	41.9	59	41.5
Decreased	5	13.5	24	22.9	29	20.4
Visit 20 (End Treatment Course 9 off-treatment)						
Increased	36		102		138	
	8	22.2	30	29.4	38	27.5
Unchanged	20	55.6	61	59.8	81	58.7
Decreased	8	22.2	11	10.8	19	13.8

Increased = patients with ≥ 4 -fold increase in MIC.

Unchanged = patients with ± 2 -fold change in MIC.

Decreased = patients with ≥ 4 -fold decrease in MIC.

Source: [Table 72](#) this submission.

In the BID group, the MIC of aztreonam for the *PA* isolate with the highest MIC remained unchanged from Visit 1 in the majority ($> 50\%$) of patients over nine courses of treatment, except at Visit 19. In the TID group, the MIC of aztreonam for the *PA* isolate with the highest MIC remained unchanged from Visit 1 in the majority ($> 50\%$) of patients over nine courses of treatment, except at Visits 11, 13, 15 and 19. During Visit 2 through 20, 40.5% to 75.6% of patients in the BID group, and 41.9% to 59.8% of patients in the TID group, had unchanged MICs compared to Visit 1. At all visits, more patients demonstrated an increase in the MIC of aztreonam from Visit 1 for the *PA* isolate with

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the highest MIC than a decrease in the MIC in the TID group. The same is true for the BID group except for Visits 3, 6, and 20.

For the BID group, 2.2% to 22.2% of patients had decreased MICs, while 14.5% to 45.9% of patients had increased MICs compared with Visit 1. In the TID group, 10.8% to 22.9% of patients had decreased MICs, while 24.7% to 39.2% of patients had increased MICs compared with Visit 1.

Patients with a MIC of aztreonam > 8 $\mu\text{g/mL}$ for *PA* isolate with the highest MIC is summarized in [Table 12](#).

Table 12. Patients with MIC of Aztreonam > 8 $\mu\text{g/mL}$ for *PA* Isolate with the Highest MIC: ITT Population

Treatment	Visit	N*	Aztreonam MIC > 8 $\mu\text{g/mL}$ n (%)
AZLI BID (N = 85)	Visit 1	76	30 (39.5)
	Visit 2	76	34 (44.7)
	Visit 3	76	29 (38.2)
	Visit 4	72	33 (45.8)
	Visit 5	68	32 (47.1)
	Visit 6	65	27 (41.5)
	Visit 7/8	67	37 (55.2)
	Visit 9	47	24 (51.1)
	Visit 10	51	25 (49.0)
	Visit 11	49	28 (57.1)
	Visit 12	45	23 (51.1)
	Visit 13	44	27 (61.4)
	Visit 14	43	26 (60.5)
	Visit 15	44	24 (54.5)
	Visit 16	42	23 (54.8)
	Visit 17	44	27 (61.4)
	Visit 18	41	22 (53.7)
	Visit 19	38	21 (55.3)
	Visit 20	40	19 (47.5)
AZLI TID (N = 189)	Visit 1	171	72 (42.1)
	Visit 2	171	79 (46.2)
	Visit 3	163	68 (41.7)
	Visit 4	157	70 (44.6)
	Visit 5	161	71 (44.1)
	Visit 6	144	79 (54.9)
	Visit 7/8	147	72 (49.0)
	Visit 9	128	63 (49.2)
	Visit 10	133	60 (45.1)
	Visit 11	127	63 (49.6)
	Visit 12	121	47 (38.8)
	Visit 13	119	60 (50.4)

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	Visit 14	118	51 (43.2)
	Visit 15	115	57 (49.6)
	Visit 16	117	49 (41.9)
	Visit 17	110	53 (48.2)
	Visit 18	111	56 (50.5)
	Visit 19	110	46 (41.8)
	Visit 20	107	50 (46.7)
AZLI Total (N = 274)	Visit 1	247	102 (41.3)
	Visit 2	247	113 (45.7)
	Visit 3	239	97 (40.6)
	Visit 4	229	103 (45.0)
	Visit 5	229	103 (45.0)
	Visit 6	209	106 (50.7)
	Visit 7/8	214	109 (50.9)
	Visit 9	175	87 (49.7)
	Visit 10	184	85 (46.2)
	Visit 11	176	91 (51.7)
	Visit 12	166	70 (42.2)
	Visit 13	163	87 (53.4)
	Visit 14	161	77 (47.8)
	Visit 15	159	81 (50.9)
	Visit 16	159	72 (45.3)
	Visit 17	154	80 (51.9)
	Visit 18	152	78 (51.3)
	Visit 19	148	67 (45.3)
	Visit 20	147	69 (46.9)

* the number of patients with available data.

Source: Table 73, this submission.

In the BID group, 39.5% of patients had a *PA* isolate with an aztreonam MIC > 8 µg/mL at Visit 1; the percentage of patients with a *PA* isolate with an aztreonam MIC > 8 µg/mL ranged from 38.2% at Visit 3 to 61.4% at Visit 13 and Visit 17. *In the BID group, 47.5% of patients had a PA isolate with an aztreonam MIC > 8 µg/mL at Visit 20, an increase of 8.0% of patients from Visit 1.* In the TID group, 42.1% of patients had a *PA* isolate with an aztreonam MIC > 8 µg/mL at Visit 1; the percentages of patients with a *PA* isolate with an aztreonam MIC > 8 µg/mL ranged from 38.8% at Visit 12 to 54.9% at Visit 6. *In the TID group, 46.7% of patients had a PA isolate with an aztreonam MIC > 8 µg/mL at Visit 20, an increase of 4.6% of patients from Visit 1;* the percentages of patients with a *PA* isolate with an aztreonam MIC > 8 µg/mL ranged from 38.8% at Visit 12 to 54.9% at Visit 6. The percentage of patients in which the *PA* isolate with the highest aztreonam MIC was > 8 µg/mL did not consistently cycle with the on- and off-treatment intervals in the BID group but did cycle with the on- and off-treatment intervals in the TID group. Overall, in the combined BID- and TID-treated patients, 41.3% of patients had *PA* with aztreonam MIC > 8 µg/mL at Visit 1; the percentages ranged from 40.6% at Visit 3 to 53.4% at Visit 13. *Overall, in the combined BID- and TID-treated patients, 46.9% of*

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patients had PA with aztreonam MIC > 8 µg/mL at Visit 20, an increase of 5.6% of patients from Visit 1.

Disease related outcomes by MIC for the PA isolate with the highest aztreonam MIC

Change from baseline in FEV₁ percent of predicted, CFQ-R Respiratory symptoms domain score, and log₁₀ PA CFU in sputum at the end of 3, 6, and 9 AZLI treatment courses is summarized by MIC for the PA isolate with the highest aztreonam MIC at baseline in [Table 13](#). Further, analyses are presented for patients whose least susceptible PA isolate had an aztreonam MIC value categorized into ≤ 8 or > 8 µg/mL and ≤ 128 or > 128 µg/mL.

Theoretically, disease related outcomes for patients with PA isolates exhibiting high levels of resistance to aztreonam could be diminished in comparison with patients who have PA isolates that are susceptible or exhibit lower levels of resistance to aztreonam. However, there were no consistent trends over nine 28-day on/off treatment courses of AZLI therapy to suggest an association between aztreonam susceptibility (as established by parenteral breakpoints) and clinical disease related outcomes ([Table 13](#)).

For both the BID and TID groups, efficacy did not diminish among patients with decreased PA susceptibility to aztreonam at the end of 3, 6, and 9 treatment courses. Patients in the TID group with PA isolates whose MIC of aztreonam was > 8 µg/mL showed improvements in FEV₁ percent of predicted and CFQ-R Respiratory symptoms domain score as well as reductions in log₁₀ PA CFUs, with the exception of the end of Treatment Course 3. Patients in the BID group with PA isolates whose MIC of aztreonam was > 8 µg/mL showed reductions in log₁₀ PA CFUs; *however, improvements in FEV₁ percent of predicted and CFQ-R Respiratory symptoms domain score were greater in the TID group than in the BID group.*

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Table 13. Disease related outcomes by MIC for the PA isolate with the Highest Aztreonam MIC ($\mu\text{g/mL}$) at the End of Treatment Courses 3, 6, and 9: ITT Population

MIC for Isolate with Highest Aztreonam MIC for PA ($\mu\text{g mL}$) at Day 0	AZLI BID						AZLI TID					
	FEV ₁ % Pred., Percent Change		CFQ-R Respiratory Domain, Change		Log10 PA CFUs in Sputum, Change		FEV ₁ % Pred., Percent Change		CFQ-R Respiratory Domain, Change		Log10 PA CFUs in Sputum, Change	
	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n
End Treatment Course 3 (Visit 6)												
$\leq 8 \mu\text{g/mL}$	3.46 (13.3)	38	0.29 (20.05)	38	-0.53 (1.35)	29	6.74 (18.06)	85	7.97 (17.9)	85	-1.3 (2.12)	63
$> 8 \mu\text{g/mL}$	4.1 (12.26)	26	-0.43 (20.66)	26	-0.43 (0.89)	20	5.61 (15.06)	61	8.52 (19.6)	59	0.02 (0.94)	36
$\leq 128 \mu\text{g/mL}$	3.38 (12.62)	59	-0.47 (20.64)	59	-0.56 (1.17)	45	6.83 (16.88)	129	8.44 (18.9)	127	-0.9 (1.91)	90
$> 128 \mu\text{g/mL}$	7.8 (15.73)	5	5.56 (13.03)	5	0.29 (0.98)	4	2.03 (16.28)	17	6.37 (16.5)	17	0.16 (1.35)	9
End Treatment Course 6 (Visit 13)												
$\leq 8 \mu\text{g/mL}$	6.49 (12.96)	26	6.84 (18.55)	26	-0.45 (2.22)	21	6.5 (17.8)	69	6.47 (19.5)	70	-1.1 (2.12)	49
$> 8 \mu\text{g/mL}$	3.15 (11.53)	20	3.17 (18.14)	21	-0.71 (1.46)	14	4.12 (19.41)	51	4.74 (17.8)	51	-0.2 (1.13)	33
$\leq 128 \mu\text{g/mL}$	4.63 (11.73)	42	5.42 (18.91)	42	-0.59 (2.01)	32	6.17 (18.85)	106	6.33 (19.2)	107	-0.7 (1.89)	74
$> 128 \mu\text{g/mL}$	9.39 (19.34)	4	3.33 (12.79)	5	-0.1 (0.47)	3	0.33 (14.71)	14	1.19 (14.6)	14	-0.4 (1.17)	8
End Treatment Course 9 (Visit 19)												
$\leq 8 \mu\text{g/mL}$	3.76 (16.29)	24	1.16 (16.3)	24	-0.87 (2.41)	18	3.15 (17.88)	64	5.26 (16.8)	65	-0.9 (1.88)	49
$> 8 \mu\text{g/mL}$	-1.91 (12.55)	16	-0.98 (15.62)	17	-0.05 (1.23)	15	4.63 (16.3)	45	8.27 (18.5)	44	-0.3 (2.02)	29
$\leq 128 \mu\text{g/mL}$	1.26 (15.15)	36	1.35 (15.74)	37	-0.61 (2.05)	30	4.68 (17.18)	98	7.17 (16.9)	98	-0.7 (1.96)	72
$> 128 \mu\text{g/mL}$	3.6 (15.54)	4	-9.72 (15.3)	4	0.58 (0.34)	3	-4.49 (15.62)	11	0.25 (22.0)	11	0.52 (1.46)	6

Source: Table 74, this submission.

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MICs of other Antibiotics for *PA*

Apart from transient changes, there were *no notable increases* in the MIC50 or MIC90 of any of the other antibiotics tested against *PA* (amikacin, cefepime, ceftazidime, ciprofloxacin, gentamicin, meropenem, piperacillin, tobramycin, or ticarcillin/clavulanate) after treatment with AZLI. *However, there were notable decreases in the MIC90 of tobramycin for all PA isolates tested and PA isolates with the highest MICs.* While all MIC50 data remained unchanged for tobramycin, persistent decreases in the MIC90 were observed in the BID group for all *PA* isolates tested and *PA* isolates with the highest MIC. In the TID group, decreases in the MIC90 of tobramycin were persistent for all *PA* isolates tested and transient for the *PA* isolates with the highest MICs ([Table 14.8.6.1 to 14.8.6.9](#) and [Table 14.8.7.1 to 14.8.7.9](#), this submission, not shown).

For nearly all antibiotics tested, the MIC50 values were equal to or below the parenteral therapeutic breakpoint for all *PA* isolates and for the *PA* isolates with the highest MIC at Visit 1. Notably, the MIC50 values for amikacin, gentamicin and ciprofloxacin for the *PA* isolate with the highest MIC were higher than the established therapeutic breakpoints at Visit 1. Increases in the MIC50 values of the other antibiotics tested could predict a decrease in the efficacy of these antibiotics when administered parenterally. However, there were no concerning increases in the MIC50 values of any of the other antibiotics tested against *PA* isolates from patients in the TID treatment group.

The percentages of patients whose least susceptible *PA* isolates had MICs above the parenteral breakpoints for all other antibiotics tested are summarized below.

For the aminoglycosides tested, at baseline in the BID group, the percentages of patients whose least susceptible *PA* isolates had MICs above the parenteral breakpoints of amikacin (> 16 µg/mL), gentamicin (> 4 µg/mL), and tobramycin (> 4 µg/mL) were 52.6%, 71.1%, and 42.1%, respectively. At baseline in the TID group, the percentages of patients whose least susceptible *PA* isolates had MICs above the parenteral breakpoints of amikacin, gentamicin, and tobramycin were 53.8%, 65.5%, and 40.4%, respectively. Throughout the nine courses of AZLI therapy, the percentages of patients whose least susceptible *PA* isolates had MICs above the parenteral breakpoints for the aminoglycosides fluctuated around the baseline value but generally trended below the baseline value. The ranges in fluctuations by antibiotic were:

- Amikacin: 38.1% to 55.8% for BID patients; 40.8% to 53.5% for TID patients
- Gentamicin: 48.8% to 71.1% for BID patients; 47.8% to 68.1% for TID patients
- Tobramycin: 27.3% to 42.1% for BID patients; 26.9% to 38.5% for TID patients

At the end of the 18-month study (Visit 20) in the BID group, the percentages of patients whose least susceptible *PA* isolates had MICs above the parenteral breakpoints of amikacin, gentamicin, and tobramycin were 50.0%, 65.0%, and 37.5%, respectively. At Visit 20 in the TID group, the percentages of patients whose least susceptible *PA* isolates had MICs above the parenteral breakpoints of amikacin, gentamicin and tobramycin were 49.5%, 58.9%, and 36.4%, respectively.

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For ciprofloxacin, at baseline, the percentage of patients whose least susceptible *PA* isolate had an MIC above the parenteral breakpoint of ciprofloxacin ($> 1 \mu\text{g/mL}$) was 51.3% in the BID group and 54.4% in the TID group. Throughout the nine courses of AZLI therapy, the percentage of patients whose least susceptible *PA* isolate had an MIC above the parenteral breakpoint of ciprofloxacin fluctuated around the baseline value and within the following ranges: 47.4% to 79.1% for the BID group and 53.4% to 63.2% for the TID group. At Visit 20, the percentage of patients whose least susceptible *PA* isolate had an MIC above the parenteral breakpoint of ciprofloxacin was 55% in the BID group and 62% in the TID group.

For the beta-lactams tested, at baseline, the percentages of patients whose least susceptible *PA* isolates had MICs above the parenteral breakpoints were lower for all beta-lactams, with the exception of cefepime, than for ciprofloxacin or the aminoglycosides. At baseline, the percentage of patients whose least susceptible *PA* isolate had an MIC above the parenteral breakpoint of cefepime ($> 8 \mu\text{g/mL}$) was 57% in the BID group and 54% in the TID group. Throughout the nine courses of AZLI therapy, the percentage of patients whose least susceptible *PA* isolate had a MIC above the parenteral breakpoint of cefepime fluctuated around the baseline value and within the following ranges: 44.1% to 65.9% for the BID group and 46.6% to 60.4% for the TID group. At Visit 20, the percentage of patients whose least susceptible *PA* isolate had an MIC above the parenteral breakpoint of cefepime was essentially unchanged (55% in the BID group and 59% in the TID group). Regarding the four other beta-lactams tested, at baseline in the BID group, the percentages of patients whose least susceptible *PA* isolates had MICs above the parenteral breakpoints of ceftazidime ($> 8 \mu\text{g/mL}$), meropenem ($> 4 \mu\text{g/mL}$), piperacillin ($> 64 \mu\text{g/mL}$), and ticarcillin/clavulanate ($> 64/2 \mu\text{g/mL}$) were 27.6%, 25%, 25%, and 32.9%, respectively. As stated previously, these percentages are significantly lower than those observed for all other antibiotics tested, including cefepime. At baseline in the TID group, the percentages of patients whose least susceptible *PA* isolates had MICs above the parenteral breakpoints of ceftazidime, meropenem, piperacillin, and ticarcillin/clavulanate were 32.2%, 24.6%, 26.9%, and 30.4%, respectively. Throughout the nine courses of AZLI therapy, the percentages of patients whose least susceptible *PA* isolates had MICs above the parenteral breakpoints of these four beta-lactams fluctuated, but a general trend showing 5% to 10% increases was observed. At Visit 20 in the BID group, the percentages of patients whose least susceptible *PA* isolates had MICs above the parenteral breakpoints of ceftazidime, meropenem, piperacillin, and ticarcillin/clavulanate were 37.5%, 30%, 32%, and 42.5%, respectively. At Visit 20 in the TID group, the percentages of patients whose least susceptible *PA* isolates had MICs above the parenteral breakpoints of ceftazidime, meropenem, piperacillin, and ticarcillin/clavulanate were 42.1%, 32.7%, 37.4%, and 41.1%, respectively. The ranges in fluctuations by antibiotic were:

- Ceftazidime: 32.9% to 51.2% for BID patients; 36.8% to 45.6% for TID patients
- Meropenem: 23.1% to 42.1% for BID patients; 26.8% to 36.9% for TID patients
- Piperacillin: 29.2% to 48.9% for BID patients; 30.6% to 41.7% for TID patients

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- Ticarcillin/clavulanate: 35.3% to 55.3% for BID patients; 33.1% to 47.7% for TID patients

*The use of non-study antibiotics was allowed during the study and usage may have contributed to the fluctuations in antibiotic susceptibility. Concomitant non-study antibiotics used by 10% or more of patients are presented in Table 14. Various classes of antibiotics were used concomitantly, including: azalides, sulfonamides, polymyxin, aminoglycosides, beta-lactams and quinolones. Tobramycin and ciprofloxacin were used by more than two-thirds of the patients. Of the beta-lactams used concomitantly, ceftazidime, meropenem, and galenic/ piperacillin/ tazobactam were used most frequently. Notably, transient decreases in the susceptibility of *PA* to these beta-lactams were observed during the study, while increases in tobramycin susceptibility were observed, as described above.*

Table 14. Non-study Antibiotics Used Concomitantly by $\geq 10\%$ of the Total ITT Population

	AZLI BID (N = 85) n (%)	AZLI TID (N = 189) n (%)	AZLI Total (N = 274) n (%)
Antibacterials for systemic use	73 (85.9)	178 (94.2)	251 (91.6)
Azithromycin	14 (16.5)	58 (30.7)	72 (26.3)
Cefepime	9 (10.6)	30 (15.9)	39 (14.2)
Ceftazidime	25 (29.4)	59 (31.2)	84 (30.7)
Ciprofloxacin	54 (63.5)	129 (68.3)	183 (66.8)
Co-Trimoxazole	20 (23.5)	57 (30.2)	77 (28.1)
Colistin	14 (16.5)	34 (18.0)	48 (17.5)
Galenic/Piperacillin/Tazobactam	10 (11.8)	28 (14.8)	38 (13.9)
Levofloxacin	29 (34.1)	47 (24.9)	76 (27.7)
Meropenem	25 (29.4)	48 (25.4)	73 (26.6)
Tobramycin	58 (68.2)	141 (74.6)	199 (72.6)

Source: Table 75, this submission.

Change from baseline in CFQ-R Respiratory symptoms domain score, FEV₁ percent of predicted, and log₁₀ *PA* CFUs at the end of 3, 6 and 9 courses is summarized by breakpoint antibiotic concentration for each of the nine other antibiotics tested in Tables 14.2.10.8 to 14.2.10.10 (this submission, not shown). For tobramycin, analyses are presented for patients whose least susceptible *PA* isolate had a tobramycin MIC value categorized into ≤ 4 or > 4 $\mu\text{g/mL}$ and ≤ 64 or > 64 $\mu\text{g/mL}$. Theoretically, the efficacy of AZLI therapy should not be affected by the presence or absence of *PA* isolates with high levels of resistance to tobramycin or other antibiotics. Consistent with this hypothesis, there were no trends to suggest an association between the parenteral breakpoints of antibiotics other than aztreonam and the efficacy of AZLI therapy.

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BREAKPOINT DISCUSSION

Aztreonam (as AZACTAM) has been approved by the Agency for treatment of the following indications caused by *Pseudomonas aeruginosa*: urinary tract infection, lower respiratory tract infection, septicemia, skin and soft structure infection and intra-abdominal infection. In addition, *since AI is a topically administered antibiotic, interpretive criteria will not be determined.*

MICROBIOLOGY SUBSECTION OF THE PACKAGE INSERT

Reviewer's Note: In addition to the Microbiology section, sections from other disciplines were reviewed if they contained pertinent Microbiology. Only the Microbiology in these sections was reviewed. These sections were: INDICATIONS AND USAGE, DRUG INTERACTIONS and CLINICAL STUDIES. Modifications to the Package Insert are designated as follows: additions are indicated by blue font and underlined; ~~deletions are indicated by red font and strikethrough.~~

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action (see Pharmacology 12.4)

12.4 Microbiology

Mechanism of Action

Aztreonam exhibits activity *in vitro* against (b) (4) Gram-negative aerobic pathogens including *P. aeruginosa*. Aztreonam binds to penicillin-binding proteins of susceptible bacteria, which leads to inhibition of bacterial cell wall synthesis (b) (4) death of the cell.

Aztreonam activity is not decreased in the presence of cystic fibrosis lung secretions.

Reviewer's note: Aztreonam is active against Gram-negative but not Gram-positive bacteria; thus (b) (4)

Susceptibility Testing

A single sputum sample from a (b) (4) patient may contain multiple morphotypes (b) (4) of *P. aeruginosa* and each morphotype (b) (4) may have a different level of *in vitro* susceptibility to aztreonam. (b) (4)

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Reviewer's note:

(b) (4)

Development of Resistance

No changes in the susceptibility of *P. aeruginosa* to aztreonam were observed following a 28-day course of CAYSTON in the (b) (4) placebo-controlled studies. (b) (4)

(b) (4)

Reviewer's note:

(b) (4)

Development of Resistance

(b) (4)

No changes in the susceptibility of *P. aeruginosa* to aztreonam were observed following a 28-day course of CAYSTON in the (b) (4) placebo-controlled studies.

(b) (4)

Cross Resistance

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(b) (4)

Other

No trends in the treatment-emergent isolation of other bacterial respiratory pathogens (*Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, and *Staphylococcus aureus*). There was a slight increase in the isolation of *Candida* spp. following up to (b) (4) 28-day courses of aztreonam inhalation therapy.

Reviewer's note: Abbreviations should be avoided in the interest of clarity; therefore, all names of organisms should be spelled out including the genus name. The text has been subdivided into the appropriate subsections.

REFERENCES

1. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically – Eighth Edition; Approved Standard. CLSI Document M7-A8. CLSI, 940 West Valley Rd., Suite 1400, Wayne, PA 19087 January, 2009.
2. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 19th Informational Supplement. CLSI document M100-S19. CLSI, Wayne, PA 19087 January, 2009.
3. Manual of Clinical Microbiology, in Manual of Clinical Microbiology, P.R. Murray, E.J. Baron, and J.H. Jorgensen, Editors. 2003. p. 1074-1127.

Peter Coderre, PhD
Microbiology Reviewer
29 December 2009

For concurrence only:
Microbiology Team Leader
Frederic Marsik, PhD
14 Jan 10 FIN FJM

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-50814	ORIG-1	GILEAD SCIENCES INC	CAYSTON(AZTREONAM FOR INHALATION SOL)

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/s/

PETER E CODERRE
01/14/2010

FREDERIC J MARSIK
01/14/2010

Product Quality Microbiology Review

November 04, 2009

NDA: 50-814

Drug Product Name

Proprietary: CAYSTON

Non-proprietary: Aztreonam Lysine

Review Number: 2

Dates of Submission(s) Covered by this Review

Letter	Stamp	Review Request	Assigned to Reviewer
August 11, 2009	August 26, 2009	August 26, 2009	August 27, 2009

Submission History (for amendments only)

Submission Date(s)	Microbiology Review #	Review Date(s)
September 13, 2007	1	August 27, 2008

Applicant/Sponsor

Name: Gilead Sciences, Inc.

Address: 2025 1st Ave., Suite 800, Seattle, WA 98121

Representative: Jennifer Stephens, Director, RA

Telephone: 206-792-3020

Name of Reviewer: Vinayak B. Pawar, Ph.D.

Conclusion: The application is recommended for approval from microbiology product quality standpoint.

Product Quality Microbiology Data Sheet

- A. 1. **TYPE OF SUBMISSION:** Original NDA
2. **SUBMISSION PROVIDES FOR:** An inhalation drug product Aztreonam Lysine.
3. **MANUFACTURING SITE:**
Drug Product: 1. (b) (4)
2. Gilead Sciences, Inc., San Dimas, CA 91773
Diluent: (b) (4)
4. **DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:** 75 mg Aztreonam lysine for inhalation, nebulized using the eFlow® electronic nebulizer.
5. **METHOD(S) OF STERILIZATION:** Drug is (b) (4)
6. **PHARMACOLOGICAL CATEGORY:** Treatment of Cystic Fibrosis with *Pseudomonas aeruginosa*.
- B. **SUPPORTING/RELATED DOCUMENTS:** Electronic Document.
- C. **REMARKS:** The original NDA 50-814 application for the drug product Cayston (Aztreonam lysine) for inhalation was a rolling electronic submission which began in September 2007. This review pertains to the re-submission of NDA 50-814 for the drug product Cayston (Aztreonam lysine) for inhalation.

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Executive Summary

I. Recommendations

- A. Recommendation on Approvability** – Based on the sterility assurance provided by the sponsor, the application is recommended for approval.
- B. Recommendations on Phase 4 Commitments and/or Agreements, if Approvable** – N/A

II. Summary of Microbiology Assessments

- A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology** - The manufacturing procedure for drug product Aztreonam lysine consists of preparation of components, manufacture of Aztreonam lysine solution, (b) (4) The drug manufacturing processes at (b) (4) and Gilead are identical. The manufacturing process for the drug product diluent involves preparation of a 0.17% w/v sodium chloride solution, sterile filtration, and filling into individual (b) (4) ampoules.
- B. Brief Description of Microbiology Deficiencies** – N/A
- C. Assessment of Risk Due to Microbiology Deficiencies** – N/A

III. Administrative

- A. Reviewer's Signature** _____
Vinayak B. Pawar, Ph.D.
- B. Endorsement Block** _____
Stephen E. Langille, Ph.D.
- C. CC Block**
N/A

19 Pages have been Withheld in Full as b4 (CCI/TS) immediately following this page.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-50814	ORIG-1	GILEAD SCIENCES INC	CAYSTON(AZTREONAM FOR INHALATION SOL)

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

VINAYAK B PAWAR
11/09/2009

STEPHEN E LANGILLE
11/09/2009

Division of Anti-Infective and Ophthalmology Products

NDA 50-814 SN033
Aztreonam Lysine
Gilead Sciences

Clinical Microbiology Review #1
Peter Coderre, PhD
28 April 2009

APPLICANT:

Gilead Sciences, Inc.
2025 1st Avenue, Suite 800
Seattle, WA 98121

CONTACT:

Melissa Yeager, JD
Vice President, Regulatory Affairs
Tel: (206) 728-5090
Fax: (206) 728-5095

SUBMISSION REVIEWED: NDA 50-814 SN033

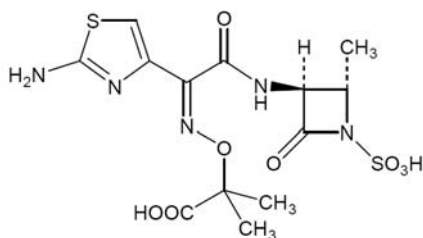
PROVIDING FOR: The management of cystic fibrosis (CF) patients with *Pseudomonas aeruginosa* to improve respiratory symptoms.

PRODUCT NAME:

Proprietary: CAYSTON
Non-proprietary: Aztreonam Lysine for Inhalation

CHEMICAL NAME: (Z)-2-[[[(2-Amino-4-thiazolyl)[[(2S, 3S)-2 methyl-4-oxo-1-sulfo-3-azetidiny]carbomoyl]methylene]amino]oxy]-2-ethylpropionic acid

MOLECULAR FORMULA: C₁₃H₁₇N₅O₈S₂, MW 453.43 (anhydrous, -form)

STRUCTURAL FORMULA:**ROUTE OF ADMINISTRATION, DOSAGE AND FORMULATION:**

- **Dosage Form:** Aerosol (nebulization) NOTE: A device called the PARI eFlow will be used to deliver the dose. Patients will receive either 75 mg aztreonam for inhalation (AI) [1 ml] twice daily for 14 days.
- **Formulation:** Aztreonam for inhalation (75 mg/ml pyrogen-free aztreonam lysinate) dissolved in sterile 0.17% saline to result in a pH of 4.2—7.0 and osmolarity of 300—550 mOsmol/kg

PHARMACOLOGICAL CATEGORY: Antimicrobial

DISPENSED: Rx

INITIAL SUBMISSION DATES:

Received by CDER:	03 April 2009
Received by Reviewer:	16 April 2009
Review Completed:	28 April 2009

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28 April 2009

REMARKS:

Reference is made to NDA 50-814 for aztreonam for inhalation solution (AZLI), filed by the Applicant (Gilead Sciences, Inc.) on 16 November 2007. Reference is also made to the complete response letter issued by the Division on 16 September 2008 and to the Formal Dispute Resolution response issued by the Office of Antimicrobial Products on 18 February 2009, both of which stipulated that it will be necessary to perform an additional adequate and well-controlled Phase 3 study before NDA 50-814 can be approved. Pursuant to 21 CFR 312.47 and the Guidance for Industry: Formal Meetings with Sponsors and Applicants for PDUFA Products (February 2000), the Applicant hereby provides background materials for the Type B meeting scheduled for 30 April 2009. The Applicant presents several questions for discussion at the proposed meeting.

This review describes the findings and recommendations of the Clinical Microbiology Reviewer. These recommendations are for evaluation by the Division Director for the determination of a decision whether to determine the suitability of the proposed clinical trial.

APPLICANT QUESTIONS:

1. Gilead's opinion is that the two randomized, placebo-controlled and long term extension trials (CP-AI-005, CP-AI-007, and CP-AI-006) in the AZLI NDA 50-814 demonstrate the effectiveness of AZLI in treating patients with CF. The Division holds the position that the regimen effect between the placebo BID and placebo TID groups in Study CP-AI-005 invalidated the study (Section 6.1) and has required another trial to evaluate time to need for IV or inhaled antibiotics and secondary endpoints of CFQ-R respiratory Symptoms scores and FEV1. Gilead has several concerns about conducting another pivotal Phase 3 study with time to need for IV or inhaled antibiotics as the primary endpoint. Gilead believes that the Cystic Fibrosis Questionnaire - Revised (CFQ-R) Respiratory Symptoms score is a validated and clinically relevant primary endpoint that provides a directly interpretable measure of treatment benefit (Section 6.2) as was demonstrated in CP-AI-007.

1a. Would the Division accept the change in CFQ-R Respiratory Symptoms score at Day 28 from Day 0 as the primary endpoint for a second pivotal Phase 3 study?

1b. Does the Division concur that the 2-week recall period, which has been validated as part of the CFQ-R, is acceptable?

Reviewer's response: The question is clinical in nature. Consequently, this Reviewer defers to the expertise of the Medical Officer.

2. Gilead believes that ongoing Study GS-US-205-0117 with the CFQ-R Respiratory Symptoms score as the primary endpoint is an additional adequate and well-controlled Phase 3 study (Section 9). Would Study GS-US-205-0117, along with final 18 month data from Study CP-AI-006, fulfill the Division's request for an additional Phase 3 study and provide the additional information to address the Division's concern that there is sparse data for the TID regimen?

Reviewer's response: The question is clinical in nature. Consequently, this Reviewer defers to the expertise of the Medical Officer.

3. Multiple AZLI doses have been evaluated during the AZLI clinical development program. AZLI 75 mg TID is a safe and effective dosing

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regimen based upon data from both placebo controlled and long-term clinical studies (Section 7). Gilead believes that a 150 mg AZLI BID treatment regimen is likely to demonstrate a decreased safety and tolerability profile, as well as increased drug administration time, thus resulting in an unfavorable risk-benefit profile. Does the Division concur that it is not necessary to include a 150 mg AZLI BID treatment arm in an additional Phase 3 study necessary for approval of the NDA given the acceptable risk-benefit profile of 75 mg TID therapy?

Reviewer's response: The question is oriented toward clinical and clinical pharmacology. Consequently, this Reviewer defers to the expertise of the Medical Officer and the Clinical Pharmacology Reviewer.

4. Gilead conducted multiple time point sampling of sputum aztreonam concentrations during a Phase Ib study (Section 8). Given that AZLI is rapidly cleared from the airways, Gilead believes that further sputum sampling at multiple time points is not warranted in a Phase 3 study. Does the Division concur that sufficient sputum sampling has been conducted during the AZLI clinical development program?

Reviewer's response: The question is clinical in nature. Consequently, this Reviewer defers to the expertise of the Medical Officer.

CONCLUSION AND RECOMMENDATION:

The Applicant presents a protocol and several questions for evaluation by the Division. This Reviewer deems the protocol to be adequate. However, this Reviewer makes the following recommendation to be conveyed to the Applicant:

1. As was performed in the original NDA, the Applicant is recommended to include log₁₀ reduction in *P. aeruginosa* as a microbiological assessment.

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Study Title: A Double-Blind, Multicenter, Multinational, Randomized, Placebo-Controlled Trial Evaluating Aztreonam Lysine for Inhalation in Patients with Cystic Fibrosis, Mild Lung Disease, and *P. aeruginosa* (AIR-CF4)

Study Centers Planned: Approximately 45 centers in the United States
Approximately 3 centers in Canada
Approximately 8 centers in Australia

Objectives: The primary objective of this study is as follows:

The **primary objective** of this study is to assess the safety and efficacy of a 28-day course of aztreonam lysine for inhalation (AZLI) in patients with cystic fibrosis (CF), mild lung disease (forced expiratory volume in one second [FEV₁] > 75% predicted), and *Pseudomonas aeruginosa* (PA).

Study Design: Double-blind, multicenter, multinational, randomized, placebo controlled study. Patients will receive 28 days of treatment with 75 mg AZLI or volume-matched placebo administered three times daily by the eFlow® Electronic Nebulizer (eFlow).

Number of Patients Planned: Approximately 140 randomized patients

Target Population: Patients aged >6 years with CF, pulmonary PA, and mild lung disease (FEV₁ > 75% predicted at Visit 1), who have not used antipseudomonal antibiotics at least 28 days prior to randomization.

Duration of Treatment: Study treatment will occur over a period of 28 days.

Diagnosis and Main Eligibility Criteria: Male or female patients aged ≥ 6 years with CF, FEV₁ > 75% predicted at Visit 1, and PA present in expectorated sputum or throat swab culture at Visit 1 OR documented PA in 2 expectorated sputum or throat swab cultures within the 12 months prior to Visit 1. One of the previous PA positive cultures must be no more than three months prior to Visit 1.

Patients with changes in chronic use of azithromycin, hypertonic saline, or dornase alpha regimens within 28 days prior to Visit 1 will be excluded.

Study Procedures/ Frequency: There will be five scheduled clinic visits:

Visit 1 - Screening (Day -14)

After obtaining informed consent, patient eligibility will be assessed. For patients with documented PA in two expectorated sputum or throat swab cultures within the 12 months prior to Visit 1 (with one of the previous PA positive cultures no more than 3 months prior to Visit 1), the Screening and Baseline visits may be conducted on the same day. For patients who do not have two PA positive sputum/throat swab cultures within the previous 12 months, schedule Visit 2 within 14 days.

Visit 2 - Baseline (Day 0)

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At Visit 2, patients still meeting entry criteria will be randomized into the study and will begin 28 days of treatment with AZLI or placebo three times daily.

Visit 3 (Day 14) and Visit 4 (Day 28) - On-Treatment

Patients will return 14 days after start of treatment for a mid treatment assessment and again at Day 28 for an End-of-Treatment assessment.

Visit 5 - End-of-Study (Day 42)

Patients will return 14 days after the last treatment date for a follow up assessment. This will be the End-of-Study Visit for all patients who complete all scheduled study visits.

Early Termination Visit

Patients who wish to discontinue the study prior to Visit 5 must return for an Early Termination (ET) Visit.

Key Assessment Parameters:

- Cystic Fibrosis Questionnaire - Revised (CFQ-R)
- Need for additional systemic or inhaled antipseudomonal antibiotics
- Hospitalizations
- Spirometry
- Adverse events (AEs)

Test Product, Dose, and Mode of Administration: Aztreonam lysine for inhalation, 75 mg (1 mL), administered three times daily (at least 4 hours apart) for 28 days via the eFlow.

Reference Therapy, Dose, and Mode of Administration: Placebo (1 mL), administered three times daily (at least 4 hours apart) for 28 days, via the eFlow.

Criteria for Evaluation:

Safety: Safety endpoints will include AEs, airway reactivity, vital signs, serum chemistry, and hematology.

Efficacy: The **primary endpoint** is change at Day 28 from baseline in the respiratory symptoms domain of the CFQ-R.

Secondary endpoints are:

- Change from baseline in clinical symptoms as assessed by the respiratory symptoms domain of the CFQ-R at Days 14 and 42
- Change from baseline in clinical symptoms as assessed by the non-respiratory domains of the CFQ-R at Day 28
- Use of additional (non-protocol specified) antipseudomonal antibiotics during the course of the study
- Proportion of patients hospitalized and the number of days patients were hospitalized during the study

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- Change from baseline in PA colony-forming units (CFUs) in sputum at Day 28
- Relative change from baseline FEV₁ percent of predicted at Day 28

Additional efficacy endpoints to be evaluated are:

- Changes from baseline in FEV₁, FVC and FEF₂₅₋₇₅ at Day 28
- Changes from baseline in weight and Body Mass Index (BMI) at Day 28
- Missed school/work days during the course of the study

Microbiology: Microbiology assessments will include:

- Disappearance or appearance of other respiratory pathogens, including *Staphylococcus aureus* (methicillin-resistant [MRSA] or methicillin-sensitive [MSSA]), *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, and *Aspergillus* spp.
- Change in minimum inhibitory concentration (MIC) of aztreonam for PA

Reviewer's comment: The Applicant presents two microbiological assessments, both of which were previously included in the original NDA. As with the original NDA, the Applicant is recommended to include log10 reduction in *P. aeruginosa* as a microbiological assessment.

Peter Coderre, PhD

For concurrence only:

Frederic Marsik, PhD.
Micro Team Leader
HFD520
29 Apr 09 FIN FJM

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/s/

Peter Coderre
5/28/2009 03:14:29 PM
MICROBIOLOGIST

Frederic Marsik
5/28/2009 04:10:03 PM
MICROBIOLOGIST

Product Quality Microbiology Review

27 August 2008

NDA: 50-814

Drug Product Name

Proprietary: CAYSTON

Non-proprietary: Aztreonam Lysine

Drug Product Priority Classification: S1

Review Number: 1

Dates of Submission(s) Covered by this Review

Letter	Stamp	Review Request	Assigned to Reviewer
September 13, 2007	September 14, 2007	November 29, 2007	December 03, 2007

Submission History (for amendments only) – N/A

Applicant/Sponsor

Name: Gilead Sciences, Inc.

Address: 2025 1st Ave., Suite 800, Seattle, WA 98121

Representative: Melissa A. Yeager, J.D., Vice President, RA

Telephone: 206-792-3015

Name of Reviewer: Vinayak B. Pawar, Ph.D.

Conclusion: The application is approvable pending resolution of the items listed in Section H of this review.

Product Quality Microbiology Data Sheet

- A. 1. **TYPE OF SUBMISSION:** Original NDA
2. **SUBMISSION PROVIDES FOR:** An inhalation drug product Aztreonam Lysine.
3. **MANUFACTURING SITE:**
Drug Product: 1. (b) (4)
2. Gilead Sciences, Inc., San Dimas, CA 91773
Diluent: (b) (4)
4. **DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:** 75 mg Aztreonam lysine for inhalation, nebulized using the eFlow® electronic nebulizer.
5. **METHOD(S) OF STERILIZATION:** (b) (4)
6. **PHARMACOLOGICAL CATEGORY:** Treatment of Cystic Fibrosis with *Pseudomonas aeruginosa*.
- B. **SUPPORTING/RELATED DOCUMENTS:** Electronic Document.
- C. **REMARKS:** The consult requests review of NDA 50-814 for the drug product Cayston (Aztreonam lysine) for inhalation. A rolling submission of the application began in September 2007 in an electronic format. (b) (4)
(b) (4) is Gilead's drug product manufacturer for Aztreonam lysine for inhalation. In the February 2008 meeting with Gilead, the Division of Anti-Infective Drug & Ophthalmologic Products informed Gilead that (b) (4) was issued a warning letter concerning compliance issues which could affect the approval of this drug product. As a result Gilead requested the Agency to include their San Dimas facility as an additional manufacturing site. This request was granted by the FDA. In addition, (b) (4) and (b) (4) were designated diluent manufacturers. However, Gilead in their March 25, 2008 amendment informed FDA that the diluent will be manufactured at (b) (4). Initial Quality Assessment was filed by Rapti Madurawe on January 18, 2008.

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Executive Summary

I. Recommendations

- A. Recommendation on Approvability** – Based on the sterility assurance provided by the sponsor, the application is approvable pending resolution of the issues listed in section H of this review.
- B. Recommendations on Phase 4 Commitments and/or Agreements, if Approvable** – N/A

II. Summary of Microbiology Assessments

- A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology** - The manufacturing procedure for drug product Aztreonam lysine consists of preparation of components, manufacture of Aztreonam lysine solution, (b) (4) The drug manufacturing processes at (b) (4) and Gilead are identical. The manufacturing process for the drug product diluent involves preparation of a 0.17% w/v sodium chloride solution, sterile filtration, and filling into individual (b) (4) ampoules.
- B. Brief Description of Microbiology Deficiencies** – There are several sterility assurance related issues to be resolved at two drug manufacturing sites and one diluent manufacturing site.
- C. Assessment of Risk Due to Microbiology Deficiencies** - High

III. Administrative

- A. Reviewer's Signature** _____
Vinayak B. Pawar, Ph.D.
- B. Endorsement Block** _____
Bryan S. Riley, Ph.D.
- C. CC Block**
N/A

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this page is the manifestation of the electronic signature.**

/s/

Vinayak Pawar
8/27/2008 03:24:27 PM
MICROBIOLOGIST

The application is approvable pending resolution of items listed
in Section H of this review.

Bryan Riley
8/27/2008 06:46:26 PM
MICROBIOLOGIST
I concur.

Division of Anti-Infective and Ophthalmology Products

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 Review Completed: 31 July 2008

APPLICANT:

Gilead Sciences, Inc.
 2025 1st Avenue, Suite 800
 Seattle, WA 98121

CONTACT:

Melissa Yeager, JD
 Vice President, Regulatory Affairs
 Tel: (206) 792-3015

SUBMISSION REVIEWED: NDA 50-814 SN003

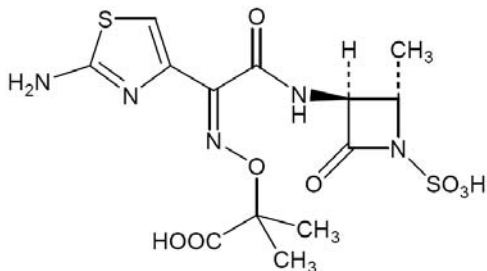
PROVIDING FOR: The management of cystic fibrosis (CF) patients with *Pseudomonas aeruginosa* to improve respiratory symptoms.

PRODUCT NAME:

Proprietary: CAYSTON
 Non-proprietary: Aztreonam Lysine for Inhalation

CHEMICAL NAME: (Z)-2-[[[(2-Amino-4-thiazolyl)[[(2S, 3S)-2 methyl-4-oxo-1-sulfo-3-azetidiny]carbomoyl]methylene]amino]oxy]-2-ethylpropionic acid

MOLECULAR FORMULA: C₁₃H₁₇N₅O₈S₂, MW 453.43 (anhydrous, □-form)

STRUCTURAL FORMULA:**ROUTE OF ADMINISTRATION, DOSAGE AND FORMULATION:**

- **Dosage Form:** Aerosol (nebulization) NOTE: A device called the PARI eFlow will be used to deliver the dose. Patients will receive either 75 mg aztreonam for inhalation (AI) [1 ml] twice daily for 14 days.
- **Formulation:** Aztreonam for inhalation (75 mg/ml pyrogen-free aztreonam lysinate) dissolved in sterile 0.17% saline to result in a pH of 4.2—7.0 and osmolarity of 300—550 mOsmol/kg

PHARMACOLOGICAL CATEGORY: Antimicrobial**DISPENSED: Rx****INITIAL SUBMISSION DATES:**

Received by CDER: 16 November 2007
 Received by Reviewer: 28 November 2007
 Review Completed: 31 July 2008

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REMARKS:

The Applicant submits the final portion of the NDA for aztreonam lysine for inhalation, AI or Cayston™. The Applicant submits the NDA as a rolling submission with the nonclinical portion of the NDA submitted 14 July 2006 (SN000) and the quality portion of the NDA submitted 13 September 2007 (SN002). This is a 505(b)(2) application with the reference listed drug product as Azactam® by Bristol Myers Squibb (NDA 50-580).

The Applicant requested a priority review status for AI; however, the Agency denied this request.

This review describes the findings and recommendations of the Clinical Microbiology Reviewer. These recommendations are for evaluation by the Division Director for the determination of a decision whether to approve this drug application.

CONCLUSION AND RECOMMENDATIONS:

The Applicant seeks the use of Aztreonam for Inhalation (AI) for the improvement of respiratory symptoms and pulmonary function in cystic fibrosis (CF) patients over 6 years of age with *Pseudomonas aeruginosa*.

After a thorough examination and analysis of this submission, **this Reviewer makes no recommendation** regarding approval of this application. Instead, **this Reviewer defers to the expertise of the Medical Officer and Statistical Reviewer.**

The basis of this decision rests on the microbiological data and its relationship to clinical outcome.

In this submission, there were three measures of microbiological outcome: change in log₁₀ *P. aeruginosa* CFUs/g sputum specimens from patients; appearance of other pathogens, specifically *S. aureus*, *B. cepacia*, *S. maltophilia* or *A. xylosoxidans* in CF patients; and changes in aztreonam MIC₅₀s and MIC₉₀s of *P. aeruginosa* isolates from CF patients.

Briefly, patients in the treatment arms of the four clinical studies showed clinical outcomes that were comparable or better than placebo-controlled patients. Patients in the treatment arm had lower or comparable PA loads in sputum, showed no increases in other pathogens associated with CF and had pathogens with similar MIC₉₀s at baseline and end of treatment, that is there were no changes in susceptibility to aztreonam in *P. aeruginosa* isolates.

However, none of the three microbiology responses correlated with clinical responses.

To investigate the possible correlation between microbiological and clinical endpoints, scatterplots were produced by the Applicant. Scatterplots examining these endpoints showed the following:

- Changes in numbers of PA in sputum were not associated with changes in FEV₁ or aztreonam concentrations in study [CP-AI-003](#), [CP-AI-005](#) and slightly negatively associated in study [CP-AI-007](#).

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- Changes in numbers of PA in sputum were not associated with changes in aztreonam concentrations in study [CP-AI-003](#).
- Changes in aztreonam MIC for the PA isolate with the highest MIC from each patient were slightly positively associated with changes in FEV₁ in study [CP-AI-003](#) but slightly negatively associated in study [CP-AI-007](#).
- Changes in numbers of PA in sputum were not associated with changes in aztreonam MIC for the PA isolate with the highest MIC in study [CP-AI-005](#) and [CP-AI-007](#).
- Changes in numbers of PA in sputum were not associated with changes in CFQ-R respiratory domain score in study [CP-AI-007](#).

The lack of correlation of microbiology outcomes with clinical outcomes is not unprecedented. In the Microbiology subsection of the package insert for TOBI for the management of cystic fibrosis patients with *P. aeruginosa*, the Agency states "The relationship between in vitro susceptibility test results and clinical outcome with TOBI therapy is not clear".

This Reviewer was unable to correlate microbiological outcomes with clinical outcomes. As none of the examined microbiology endpoints correlate with clinical outcomes, this Reviewer believes the clinical endpoints should be the deciding factors in the determination of clinical efficacy of AI. **Consequently, this Reviewer makes no recommendation. This Reviewer defers to the expertise of the Medical Officer and the Statistical Reviewer.** According to these reviewers, an additional clinical trial must be conducted with an additional arm using another dosing regimen. For details of the recommendations by the Medical Officer and the Statistical Reviewer, please see the reviews by Dr. Menfo Imiosili and Dr. Christopher Khedouri, respectively. For recommendations regarding the dosing regimen, please see the review of the Clinical Pharmacology Reviewer, Dr. Sara Robertson.

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EXECUTIVE SUMMARY

Aztreonam is a synthetic bactericidal monobactam antibiotic that was originally isolated from *Chromobacterium violaceum*. Aztreonam (Azactam®) is the only monobactam approved for clinical use in the US.

The Applicant seeks the use of Aztreonam for Inhalation (AI) for the improvement of respiratory symptoms and pulmonary function in cystic fibrosis (CF) patients over 6 years of age with *Pseudomonas aeruginosa*.

What follows is a summary of the *in vitro* preclinical, *in vivo* preclinical and clinical efficacy data for the use of aztreonam for the improvement of symptoms of CF.

The ***in vitro* preclinical efficacy** is determined by: the mechanism of action, spectrum of activity, mechanism of resistance, cross-resistance, post-antibiotic effect and other miscellaneous studies.

Mechanism of Action. Aztreonam, like beta-lactam antibiotics, inhibits bacterial cell wall biosynthesis, resulting in cell lysis and death through irreversible inhibition of penicillin-binding proteins (PBPs), enzymes that catalyze the elongation and crosslinking of the peptidoglycan chains. Aztreonam binds preferentially and with high affinity to PBP3 of aerobic Gram-negative bacteria, accounting for its potent antibacterial activity against *P. aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter cloacae* and *Klebsiella pneumoniae*. In contrast, aztreonam has poor affinity for the PBP3 of Gram-positive and anaerobic bacteria and consequently is inactive against these bacteria. Binding of aztreonam to PBP3 results in filamentation and cell lysis, an effect similar to that observed with cephalosporins.

Aztreonam, like beta-lactam antibiotics, demonstrates time-dependent killing against sensitive Gram-negative bacteria; increasing the concentration of aztreonam above the minimum inhibitory concentration (MIC) of the test organism does not substantially increase the speed or degree of bacterial killing.

Spectrum of Activity. Aztreonam is active against several aerobic Gram-negative pathogens, including many *Enterobacteriaceae* as well as *P. aeruginosa*, but is relatively inactive against Gram-positive and anaerobic organisms.

Other aerobic Gram-negative bacteria show *in vitro* susceptibility to aztreonam, although the clinical significance of these organisms in CF is unknown, and data regarding clinical efficacy is not available. Susceptible bacteria are typically killed at concentrations one to four times their MICs. However, in some cases, the concentration of aztreonam required to achieve bactericidal killing of *P. aeruginosa* has been reported to be 4 to 16 times the MIC. Aztreonam exhibits MICs of ≤ 8 $\mu\text{g/mL}$ against at least 90% of the following aerobic Gram-negative bacteria: *Aeromonas hydrophila*, *Morganella morganii*, *Neisseria gonorrhoeae* (including penicillinase-producing strains), *Pasturella multocida*, *P. vulgaris*, *Providencia stuartii*, *Providencia rettgeri*, and *Yersinia enterocolitica*. The safety and efficacy of aztreonam in treating clinical infections due to these microorganisms, however, have not been established in clinical trials.

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Recent data regarding the activity of aztreonam compared with other antibiotics commonly used to treat Gram-negative infections has been examined. Overall, aztreonam is less active than other commonly used antibiotics in Asia, Europe, Latin America and North America.

Antibiotic susceptibility trends in bacterial isolates collected from CF patients may differ from the general patient population due to the chronic nature of antibiotic therapy in CF patients. In the US, susceptibility of *P. aeruginosa* to aztreonam has gradually decreased within both the CF and non-CF populations over the last 10 years with 80% of *P. aeruginosa* isolates susceptible ($\text{MIC} \leq 8 \mu\text{g/mL}$) to aztreonam in 1995, 78.9 % susceptible in 2004, and 68.6% susceptible in 2006. Aztreonam has been shown to have *in vitro* activity against tobramycin-resistant and multidrug-resistant *P. aeruginosa* isolates from CF patients.

Mechanisms of Resistance. Multiple mechanisms of bacterial resistance to aztreonam and beta-lactam antibiotics have been identified. These mechanisms include the derepression of chromosomal beta-lactamases, the acquisition of beta-lactamase encoding plasmids, overexpression of efflux pump systems, impermeability of the outer membrane, and alteration of the drug target (PBPs).

In Gram-negative bacteria the prevalence of various resistance mechanisms varies by organism and two or more mechanisms may contribute to the overall resistance profile. In contrast, for *P. aeruginosa* isolated from CF patients, resistance to beta-lactam antibiotics was primarily due to overproduction of a constitutive cephalosporinase (18.6%), and rarely due to production of a transferable beta-lactamase (1.4%). However, researchers also reported that susceptibility to aminoglycosides and fluoroquinolones was lower in isolates producing beta-lactamases, thereby suggesting involvement of other mechanisms.

Aztreonam is a poor inducer of beta-lactamase production and, although not used as such, is an inhibitor of many beta-lactamases.

The beta-lactamases are classified using the Ambler system into four groups (A, B, C, and D) according to their amino acid sequences. Classes A, C, and D beta-lactamases use a catalytically active serine molecule for inactivation of the beta-lactam drug, while Class B enzymes are metallo-enzymes requiring zinc for activity.

Of most concern among the Class A beta-lactamases are the clavulanic acid-inhibited Ambler Class A ESBLs that are broadly active against most beta-lactams, as well as aztreonam. The TEM- and SHV-type ESBLs have been extensively reported in *Enterobacteriaceae* but have been only rarely reported from *P. aeruginosa*.

The class B metallo-beta-lactamases (MBLs) hydrolyze a wide variety of beta-lactams, including carbapenems, but do not hydrolyze aztreonam. While there have been rare reports of organisms with MBLs that are also resistant to aztreonam [VIM-5 (*K. pneumoniae* and *P. aeruginosa*-Turkey), VIM-6 (*P. putida*-Singapore) and VIM-7 (*P. aeruginosa*-United States)], it is possible that these organisms were expressing multiple resistance mechanisms.

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Recently, a strain of *P. aeruginosa* was found to express an ESBL, GES-9 which differs from GES-1 by a Gly243Ser substitution [1]. This ESBL hydrolyzes aztreonam but is inhibited by clavulanic acid and imipenem. The gene for GES-9 is located inside a class 1 integron structure containing two copies of an insertion sequence.

AmpC, the best characterized enzyme of Class C beta-lactamases, is normally produced at low levels in clinical isolates of *P. aeruginosa* and *Enterobacteriaceae* but production can be induced to high levels by the presence of beta-lactams, especially cefoxitin and imipenem. High-level production of both chromosomal- and plasmid-encoded AmpC is responsible for cross-resistance to aztreonam and to multiple beta-lactam antibiotics.

The OXA-type carbapenemases (Class D beta-lactamases) generally have little or no activity against aztreonam, but there have been rare reports of oxacillinases from clinical isolates of *P. aeruginosa* (OXA-11 [Turkey] and OXA-45 [United States]) that have hydrolyzed aztreonam.

Some porins provide entry for a wide variety of drugs and others are specific for a single drug allowing permeation through the outer membrane. In *P. aeruginosa*, porins have extremely low permeability to most beta-lactams and thereby play a major role in intrinsic resistance. Due to variability in porin specificity, alterations may lead to resistance to one or more antibiotics. An example of specific drug resistance is seen in loss of the OprD porin, which can result in resistance of *P. aeruginosa* to carbapenems but does not confer cross-resistance to non-carbapenem beta-lactams or aztreonam.

Several chromosomally-encoded efflux systems play an important role in *P. aeruginosa* antibiotic resistance. The MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM systems extrude a wide variety of antibiotics including beta-lactams. Most beta-lactams, (e.g., penicillins, cepheems, and meropenem) are pump substrates for all of these efflux systems. In contrast, aztreonam can only be extruded by the MexAB-OprM pump system.

Clinical resistance associated with alteration of PBPs is exceedingly rare in *P. aeruginosa*, but cannot be ruled out.

In vitro resistance frequencies for *P. aeruginosa* ATCC 27853 were low, less than 1×10^{-8} in the presence or absence of aztreonam (500 µg/mL). A modified Szybalski method was used to demonstrate that aztreonam, at four times the MIC, selected resistant variants of 10 *P. aeruginosa* isolates (two lab isolates, eight isolated from blood of cancer patients) *in vitro*. Mutants resistant to aztreonam were generated at a frequency of approximately 1×10^{-6} to 1×10^{-7} . Some mutants produced large amounts of cephalosporinase; however, aztreonam was stable to hydrolysis by this enzyme.

Cross resistance. Aztreonam is not antagonistic with the antibiotics commonly used on- and off-label to treat respiratory infections in CF patients, suggesting that clinical use of aztreonam lysine in conjunction with another antibiotic to treat CF respiratory infections will not reduce the antibacterial activity of either drug due to antagonistic interactions.

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When *P. aeruginosa* or *Enterobacteriaceae* are treated with aztreonam in combination with beta-lactam antibiotics, the interactions are typically indifferent or synergistic. In a study of 14 multidrug-resistant isolates of *Enterobacteriaceae* (N = 10) and *P. aeruginosa* (N = 4 [ATCC 27853 and three clinical isolates]), synergy was noted with 42% (6 of 14) of the organisms when aztreonam was combined with any of the beta-lactam antibiotics tested (piperacillin, moxalactam, cefotaxime, or ceftazidime); indifferent effects were observed with all other organisms.

Investigators have demonstrated that combinations of aztreonam plus cefepime, piperacillin or imipenem resulted in indifference against *P. aeruginosa* isolates. Interactions with cephalosporins were predominantly partially synergistic.

Combinations of aztreonam with quinolones sometimes result in synergy, but indifferent or additive effects are more commonly observed. Combinations of aztreonam plus ciprofloxacin generally resulted in indifference against *P. aeruginosa* isolates. However, one investigator examined interactions between aztreonam and ciprofloxacin, levofloxacin, gatifloxacin or moxifloxacin using the checkerboard method. Interactions were evaluated against four isolates each of *P. aeruginosa*, *B. cepacia*, *E. cloacae*, *K. pneumoniae*, *E. coli* and *P. mirabilis*. All combinations demonstrated uniformly additive activity against *P. aeruginosa*.

A recent report by Gasink et al. concluded that curbing the use of fluoroquinolones and antimicrobials with activity against anaerobes may be an effective strategy to limit the emergence of aztreonam-resistant *P. aeruginosa* [2]. These investigators identified risk factors for infection or colonization with aztreonam-resistant *P. aeruginosa*. Nosocomial mortality was greater among patients infected or colonized with aztreonam-resistant *P. aeruginosa* compared with those infected with aztreonam-susceptible *P. aeruginosa*.

In vitro interactions between aztreonam and aminoglycosides have been shown to be synergistic against *P. aeruginosa*, *Enterobacteriaceae*, and other Gram-negative aerobic bacilli. These aminoglycosides included: amikacin, gentamicin, tobramycin or netilmicin.

PAE. Post antibiotic effect (PAE) refers to the suppression of bacterial growth that persists after short-term exposure to an antibiotic. Aztreonam, like beta-lactam antibiotics, has minimal or no persistent effects on most organisms. The duration of PAE for aztreonam against *P. aeruginosa* ATCC 27853 is less than 0.5 hour when tested at concentrations equivalent to four times the MIC. Because aztreonam does not have a demonstrable PAE for *P. aeruginosa*, the dosing schedule was determined on the basis of the pharmacokinetic (PK) parameters.

Miscellaneous Studies. A number of miscellaneous studies were conducted to examine the effects of aztreonam in a pharmacodynamic model, effects on phagocytic cells, effects on gastrointestinal flora, effects on biofilms and the effects of nebulization on aztreonam potency.

A pharmacodynamic model was developed to facilitate evaluation of aztreonam activity when delivered to an *in vitro*, mucin-covered epithelial cell surface. Although the model's reproducibility limited its utility, a significant decrease in CFUs was

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observed by 6 to 12 hours for both aztreonam and tobramycin with regrowth at 24 hours.

Exposure of bacteria to aztreonam can modify the interactions between Gram-negative bacteria and phagocytic cells, resulting in enhanced phagocytic killing due to aztreonam-induced alterations in the bacterial cells. Sub-MIC concentrations of aztreonam enhanced the phagocytosis and killing of *P. aeruginosa* K1 by a murine macrophage cell line. Enhanced phagocytic killing was attributed to modification of bacterial cell surface structures upon exposure to aztreonam.

Aztreonam has the potential to alter the normal gastrointestinal flora, thereby decreasing patients' resistance to colonization with other pathogens such as *Candida* and *Clostridium* spp. However, in the clinical trials conducted for this NDA, systemic exposure to aztreonam is very low and is unlikely to lead to pseudomembranous colitis.

In chronic airway infections in CF patients, *P. aeruginosa* is likely to exist in both planktonic and biofilm phases. The presence of a biofilm in CF helps to explain why *P. aeruginosa* lung infections are rarely eradicated with antibiotic therapy, regardless of the organism's reported *in vitro* susceptibility. Recently a methodology to test antibiotic susceptibility within a biofilm was developed with the hope that it might be more predictive. As with beta-lactams, biofilm inhibitory concentrations (BICs) for aztreonam are much higher than conventionally determined MICs. Against 85 clinical isolates of *P. aeruginosa* from the US, the aztreonam, the MIC₉₀ was 32 µg/mL, whereas the BIC₉₀ was > 128 µg/mL.

To determine whether nebulization impacts the potency of aztreonam, the *in vitro* activity of aztreonam lysine was tested before and after nebulization against 161 clinical isolates of *B. cepacia* complex, *S. maltophilia*, *A. xylosoxidans*, *S. aureus*, and *P. aeruginosa* from CF patients. In aggregate, these results support the physical finding that the antimicrobial activity of aztreonam is not significantly reduced during nebulization by the eFlow device.

Pharmacokinetics. AI is delivered using the PARI eFlow electronic nebulizer (eFlow) which produces an aerosol with a relatively monodispersed particle spectrum which is well-suited for lower airway drug deposition. Thus, this drug is essentially a topical antibiotic and does not have the same pharmacokinetic properties of a paternally administered antibiotic.

Sputum provides a growth environment for bacteria and can significantly inhibit the activity of antibiotics. Investigators evaluated aztreonam lysine time-kill kinetics for *P. aeruginosa* ATCC 27853 using aztreonam concentrations equivalent to 0.1 times, 1 time, and 10 times the MIC. No killing was observed at 0.1 times the aztreonam MIC. Bactericidal levels of killing (= 3 log₁₀) were reached by five hours, and the speed and degree of killing were comparable at aztreonam concentrations of 1 time and 10 times the MIC. The killing kinetics of aztreonam against *P. aeruginosa* ATCC 27853 in the presence of 1% CF sputum and 10% porcine mucin were nearly identical to those conducted in media alone. By comparison, the activity of aminoglycosides is known to be antagonized by sputum.

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Similar time-kill studies were conducted with 20 CF *P. aeruginosa* isolates using 10% porcine gastric mucin as a model for the protein-binding component of sputum. Similar results were found with the gastric mucin model. The sputum and mucin kill-curve data suggest that aztreonam administered as an aerosol to the CF lungs will not be significantly inhibited by sputum.

Clinical Laboratory Susceptibility Testing. The approved antimicrobial susceptibility test methods and quality control parameters used for parenteral aztreonam therapy were used to monitor the activity of AI against *P. aeruginosa* isolates from CF patients. It should be noted that susceptibility testing of *P. aeruginosa* isolates from CF patients, in some cases, required extended incubation up to 24 hours for accurate determination of MIC values.

Quality Control. Aztreonam has been approved by the Agency for other indications. In addition, as AI is a topically administered antibiotic, interpretive criteria will not be determined. Therefore, quality control parameters were not determined.

Provisional Interpretive Criteria. Aztreonam has been approved by the Agency for other indications. Therefore, provisional interpretive criteria were not determined.

Clinical Efficacy. The Applicant presents the protocols and results from four clinical trials: one phase 2 trial, [Study AI-003](#), and three phase 3 trials. Among the three phase 3 trials were two pivotal studies, [Study AI-005](#) and [Study AI-007](#), as well as a follow-on study, [AI-006](#), which will not be discussed in detail.

Study AI-003 was a blinded, multicenter, randomized, placebo-controlled phase 2 trial with aztreonam for inhalation (AI) in CF patients with lung disease due to *P. aeruginosa* infection. The trial was designed to assess the safety and efficacy of 14-day treatment with AI at two dosage levels compared to placebo in CF patients with pulmonary *P. aeruginosa* (PA) infections.

Clinical Results. There was an increase in adjusted mean percent change in FEV₁ (L) from Day 0 to Day 14 in all treatment groups (4.08% in the placebo group, 6.20% in the 75 mg AI group, and 7.71% in the 225 mg AI group). There was no significant difference in the mean percent change in FEV₁ from Day 0 to Day 14 between the 225 mg AI and placebo treatment groups, either overall (treatment difference: 3.62%; CI: -1.50, 8.75) or for any of the subsets tested (disease severity [< 60 or $\geq 60\%$ FEV₁ percent predicted], age, gender, and MIC category [≥ 8 µg/mL]) for the ITT or PP datasets.

There was an increase in adjusted mean percent change in FEV₁ from Day 0 to Day 7 in all treatment groups (5.03% in the placebo group, 7.59% in the 75 mg AI group, and 9.74% in the 225 mg AI group). The difference of 4.71% between the 225 mg AI and placebo groups was statistically significant (CI: 0.06, 9.35), however, it was not confirmed by the PP dataset.

None of the results for the remaining spirometry tests (FEV₁, FVC, and FEF₂₅₋₇₅) was statistically significant at any time point. For FEV₁ and FVC, mean absolute and percent changes were greater at Day 7 than they were at Day 14 for all treatment

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groups. For FEF₂₅₋₇₅, the mean percent change from Day 0 at Day 7 was similar to that at Day 14 for the 75 mg AI group, and slightly smaller for the 225 mg AI group.

There were no trends in the numbers of patients who required hospitalization, emergency treatment or anti-PA antibiotics during the trial. There was no statistically significant difference between the placebo and 225 mg AI groups in the numbers of patients unable to expectorate sputum at Day 14.

There was a potential loss of power in the trial because there were only 105 evaluable patients in the ITT dataset, which was less than the planned sample size of 138, and this may have contributed to the lack of statistical significance shown in the results.

Microbiology Results: There was a mean reduction in log₁₀ PA CFUs at Days 7 and 14 in both AI treatment groups, and the mean reductions in the 225 mg group were slightly larger than those in the 75 mg AI group. The difference (CI) between the 225 mg AI and placebo groups in change in log₁₀ PA CFUs from Days 0 to 14 of -2.146 (-3.029, -1.263) was highly statistically significant (p-value < 0.0001).

The majority of patients in each treatment group showed no changes in the presence or absence of the pathogens tested. After dosing, PA infection disappeared from two patients in the 75 mg AI group (Day 28), and three and seven patients in the 225 mg AI group (Days 7 and 14, respectively). PA infection did not disappear at any postdosing day for any patients in the placebo group. There were no notable differences between treatments in the numbers of patients becoming positive after dosing for any of the pathogens tested (*S. aureus*, *B. cepacia*, *S. maltophilia*, or *A. xylosoxidans*). There were no notable differences between treatment groups in the numbers of patients who were positive at Day 0 and became negative for any of the other pathogens tested.

There were no notable increases from Day 0 in MIC₅₀ or MIC₉₀ (using all PA isolates and the PA isolate with the highest MIC from each patient) in any treatment group, indicating there was no evidence of reduced susceptibility of PA to aztreonam. There were no notable increases from Day 0 in the numbers of patients whose PA isolate with the highest MIC was above the parenteral susceptibility breakpoint for aztreonam ($\geq 8 \mu\text{g/mL}$) in either AI treatment group.

Thus, from the microbiological viewpoint, the results of **Study AI-003** were encouraging since treatment arms showed statistically significant log₁₀ reductions of *P. aeruginosa*, there were few changes in the appearance of new pathogens and there were no notable increases in MIC₉₀ values from baseline to TOC, thus no reduced susceptibility of the drug on therapy.

Study AI-005 was a phase 3 clinical trial with AI in CF patients with pulmonary *P. aeruginosa* requiring frequent antibiotics. This trial was designed to assess the safety and efficacy of a 28-day treatment with Aztreonam Lysine for Inhalation (AI) and the ability of AI to maintain or improve clinical status following a 28-day course of Tobramycin Inhalation Solution (TSI, TOBI®) therapy in cystic fibrosis (CF) patients with pulmonary *Pseudomonas aeruginosa* (PA).

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Clinical Results. The median time to need for inhaled or IV anti-pseudomonal antibiotics due to pre-defined symptoms (*primary efficacy endpoint*) was 21 days longer in the pooled AI group (92 days) than in the pooled placebo group (71 days) ($p = 0.0070$). The median time to antibiotic need for the AI BID group was significantly different than that in the pooled placebo group ($p = 0.0019$; median time not estimable), and the median time to antibiotic need was 87 days for the AI TID group ($p = 0.1816$). There was a strong suggestion of a regimen need for inhaled or IV antibiotics was different between the pooled BID and pooled TID regimens ($p = 0.0012$). The regimen effect was not seen for other efficacy endpoints.

Microbiology Results. As expected, dosing of AI by inhalation generally gave a much higher ($\sim 1,000$ -fold) exposure in sputum than in plasma. There was a large degree of individual patient variation in aztreonam concentrations in both plasma and sputum. After dosing, the majority of patients continued to have positive sputum culture results for PA infection.

P. aeruginosa CFU/g in sputum decreased among patients who received 75 mg AI BID or TID for 28 days. However, the log₁₀ reductions in the treatment arms were lower than the reductions in *P. aeruginosa* CFU/g in sputum seen in patients from [Study AI-003](#).

The difference between the pooled AI and pooled placebo groups for change in log₁₀ PA CFUs in sputum approached statistical significance at Day 14 (-0.490 , $p = 0.0586$) and achieved significance at Day 28 (-0.66 , $p = 0.0059$). From Day 0 to Day 28, log₁₀ PA CFU sputum density decreased (improved) by 0.43 in the pooled AI group, and increased (deteriorated) by 0.23 in the pooled placebo group. The change in log₁₀ PA CFU density was also significantly different for the AI BID (-0.72 , $p = 0.011$) and TID (-0.60 , $p = 0.031$) groups compared with the pooled placebo group. After cessation of therapy (Day 28), log₁₀ PA CFU density among AI-treated patients was greater than baseline at Days 42 and 56.

A higher number of patients had *S. aureus* isolated intermittently in the pooled AI group (eight [6%] patients) than in the pooled placebo group (two [2.8%] patients). While there was no overall difference in persistent isolation of *S. aureus* between the pooled AI and pooled placebo groups, persistent isolation of *S. aureus* was higher in the AI TID group than in the AI BID group (eight [12%] patients vs. four [6%] patients). The small number of patients who persistently tested positive was not sufficient to determine a dose-response effect. There were no notable effects of AI on the isolation of any of the other pathogens tested (*B. cepacia*, *S. maltophilia*, and *A. xylosoxidans*).

MIC₅₀ and MIC₉₀ of aztreonam for all PA isolates, and for the isolates with the highest MIC from each patient generally remained unchanged for all treatment groups. In the AI TID group, for all PA isolates, there was a transient increase from Day 0 in MIC₅₀ and MIC₉₀ at Day 14 (from 2 to 8 $\mu\text{g/mL}$, and from 32 to 128 $\mu\text{g/mL}$), which at the subsequent time point (Day 28) had each returned to unchanged from Day 0. For PA isolates with the highest MIC from each patient, the observed ranges of MIC₅₀ and MIC₉₀ values were generally not different among the AI BID, AI TID, and pooled placebo groups between Day -28 and Day 56, with no

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consistent pattern of increase above pre-drug levels observed after administration of AI.

The percentages of patients whose highest aztreonam MICs increased after dosing were higher in the AI BID and TID groups than in the pooled placebo group at all time points. The differences were statistically significant at Day 28, where 38 (37%) patients in the pooled AI group had an increase in their highest MICs, compared with 13 (21%) patients in the pooled placebo group ($p = 0.0320$). When separated by regimen, the result was significant for the AI TID group ($p = 0.0463$), and trended toward significance for the AI BID group (0.0932). The differences were not significant at Days 42 or 56, likely indicating a transient increase in MICs during treatment that is reversible when AI is discontinued. The percentage of patients whose PA isolate with the highest MIC of aztreonam was above the parenteral breakpoint (8 µg/mL) increased from Day 0 to Days 14 and 28 in the pooled AI group, but not in the pooled placebo group. The largest increase from Day 0 occurred at Day 28, where 47 (44%) patients in the pooled AI group had their highest MICs ≥ 8 µg/mL, compared with 37 (30%) patients at Day 0. Within the AI TID group, percentages returned to near Day 0 values after Day 28, whereas within the AI BID group, percentages remained relatively high compared with Day 0. There were no differences between pooled treatment groups in change in MIC of tobramycin from Day 0 for the PA isolate with the highest MIC from each patient or in the percentage of patients with MIC of tobramycin = 8 µg/mL for the PA isolate with the highest MIC. There were no notable increases in MIC50 or MIC90 of other antibiotics tested against PA after treatment with AI.

Study AI-007 was a phase 3, double-blind, multicenter, multinational, randomized, placebo-controlled trial evaluating aztreonam for Inhalation in CF patients with pulmonary *P. aeruginosa* infection. This trial was designed to assess the safety and efficacy of a 28-day treatment with aztreonam lysine for inhalation (AI) (75 mg three times daily [TID]) compared to placebo.

Clinical Results: Patients in the AI group had a clinically significant improvement in respiratory symptoms as measured by the CFQ-R respiratory domain score for Child/Teen/Adult combined. The adjusted mean change from Day 0 at Day 28 was 7.08 compared with -2.63 in the placebo group. The adjusted mean difference (9.71) between AI and placebo at this time point was statistically significant ($p = 0.0005$). Significant differences were also observed between the treatments at Day 14 (7.98, $p = 0.0006$) and Day 42 (6.33, $p = 0.0154$). A greater proportion of patients in the AI group showed clinically meaningful improvement in CFQ-R respiratory domain score at Day 28 (56%) compared with patients in the placebo group (37%), $p = 0.0055$.

Microbiology Results. As expected, dosing of AI by inhalation generally gave a much higher (more than 1,000-fold) exposure in sputum than in plasma. There was a large degree of individual patient variation in aztreonam concentrations in both plasma and sputum. After dosing, the majority of patients continued to have positive sputum culture results for PA.

At baseline (Day 0), mean log₁₀ PA CFU sputum density values were nearly equivalent in the AI and placebo groups. In the AI group, log₁₀ PA CFU sputum

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density had decreased by more than 1 log₁₀ CFU at Day 14 and by more than 1.5 log₁₀ CFUs at Day 28. Log₁₀ PA CFU sputum density in the placebo group remained near baseline values at Days 14 and 28. At Day 42, two weeks after cessation of therapy, log₁₀ PA CFU sputum density had returned to near baseline values in both the AI and placebo groups.

Fewer patients in the AI group compared with the placebo group had intermittent isolation of *S. aureus* (four [5%] patients receiving AI vs. seven [9%] patients receiving placebo) and persistent isolation of *S. aureus* (two [2.7%] patients receiving AI vs. five [6%] patients receiving placebo). There was no difference between the AI and placebo groups in the percentage of patients with intermittent isolation of *S. maltophilia*. Two (2.7%) patients in the AI group had persistent isolation of *S. maltophilia* compared to zero patients in the placebo group. Zero patients in the AI group had intermittent isolation of *A. xylosoxidans* compared to two (2.5%) patients in the placebo group. There was no difference between the AI and placebo groups in the percentage of patients with persistent isolation of *A. xylosoxidans*. There was no intermittent or persistent isolation of *B. cepacia* from any patient.

The MIC₅₀ and MIC₉₀ of aztreonam for all PA isolates, and for the isolate with the highest MIC from each patient generally remained unchanged for both treatment groups. The only notable effects were reported for MIC₉₀ after dosing in the AI group; for all PA isolates, there was a transient increase in MIC₉₀ from Day 0 to Day 14 (from 128 to 512 µg/mL), which returned to unchanged from Day 0 at the subsequent time point (Day 28). At Days 28 and 42 the percentage of patients whose highest MICs increased >4-fold was higher in the AI group than in the placebo group. At Day 28, 16 (28%) patients in the AI group had an increase in their highest MICs, compared with 8 (12%) patients in the placebo group; this difference approached statistical significance based on a CMH test ($p = 0.0804$). The difference between the AI and placebo groups was not statistically significant at Day 42 and the percentage of patients with increases in their highest MICs had not increased in the AI group relative to Day 28. However, the percentage of patients with increases in their highest MICs remained higher in the AI group than in the placebo group (14 [25%] patients treated with AI vs. 7 [13%] patients treated with placebo). There were no notable changes from Day 0 in either treatment group in percentages of patients with MICs of aztreonam above the parenteral breakpoint (≥ 8 µg/mL) for the PA isolate with the highest MIC. There was no difference between the AI and placebo treatment groups in the change in MIC of tobramycin from Day 0 for the PA isolate with the highest MIC from each patient.

There was no difference between the AI and placebo treatment groups in the percentage of patients with MIC of tobramycin ≥ 8 µg/mL for the PA isolate with the highest MIC. There were no notable increases in MIC₅₀ or MIC₉₀ of other antibiotics tested against PA after treatment with AI for 28 days.

No correlations were found between highest baseline aztreonam MIC for PA and three key efficacy parameters (CFQ-R respiratory domain score, FEV₁, and log₁₀ PA CFU density).

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Study AI-006 was an open-label, multicenter follow-on study of multiple courses of AI designed to assess safety and disease-related outcomes following repeated exposure of up to nine courses of AI in CF patients who participated in either **AI-005** or **AI-007**. Patients enrolled into the **AI-006** study receive 75 mg AI in the same treatment regimen, BID or TID, to which they were randomized in **AI-005** or **AI-007** for 28-day AI courses with 25 days between courses. The original open-label follow-on study protocol was designed to have patients receive two AI treatment courses with an optional third course. A protocol amendment extended the treatment period to allow up to nine courses of AI. At the time of interim analysis (01 March 2007), 207 patients had been treated with AI and had at least one post-baseline evaluation; of these patients, 162 had completed at least three AI courses.

In **Study AI-006**, *P. aeruginosa* CFU/g sputum were decreased from baseline at each subsequent visit among patients in the BID and TID AI treatment groups. In the TID treatment group, a consistent decrease in *P. aeruginosa* CFU/g sputum was observed following each of the first three treatment courses. For BID- treated patients, decreases in *P. aeruginosa* CFU/g did not directly cycle with treatment. Increases in *P. aeruginosa* CFU/g were generally observed during the off-treatment periods for AI BID and TID groups. Overall, the decreases in *P. aeruginosa* CFU/g in sputum were consistently larger in the TID group than the BID group. There were no notable differences between treatment groups in the appearance of other pathogens.

No changes were noted in the MIC50 of aztreonam for all *P. aeruginosa* isolates or for the *P. aeruginosa* isolate with the highest MIC from each patient. Transient increases in aztreonam MIC90 values occurred, but did not directly correlate with the on- and off-treatment courses. The MICs of aztreonam for the *P. aeruginosa* isolate with the highest MIC from each patient were largely unchanged in both the BID and TID AI groups during the first three courses of AI treatment. There were no notable increases in the MIC50 or MIC90 of other antibiotics for *P. aeruginosa* following AI treatment.

Microbiology results from the individual clinical studies of AI in the treatment of CF patients with pulmonary *P. aeruginosa* are summarized in Table I.

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	CP-AI-003 (N = 105)				CP-AI-005 (N = 211)				CP-AI-007 (N = 164)		CP-AI-006 (N = 207)	
	Placebo (N = 32)		AI (N = 38)		Placebo (N = 76)		AI (N = 69)		Placebo (N = 84)	AI (N = 80)	75 mg BID (N = 82)	75 mg TID (N = 125)
	75 mg BID	225 mg BID										
Change from baseline in log₁₀ <i>P. aeruginosa</i> CFU/g, Mean (SD)												
Mid-Treatment (1 course)*	0.014 (1.595)	-1.509 (1.453)	-2.080 (1.542)		0.239 (1.505)	-0.055 (1.407)	-0.479 (1.999)		0.032 (1.135)	-0.813 (2.104)	-	-
End-Treatment (1 course)*	0.025 (1.313)	-1.453 (1.575)	-2.109 (1.990)		0.284 (1.167)	-0.387 (1.333)	-0.222 (1.691)		-0.025 (0.786)	-1.476 (2.540)	-0.250 (1.675)	-0.617 (1.793)
End-Treatment (3 courses)	-	-	-		-	-	-		-	-	-0.390 (1.382)	-0.601 (2.253)
Treatment-emergent persistent isolation of pathogens, n (%)												
<i>S. aureus</i>	-	-	-		6 (8.5)	4 (6.1)	8 (12.3)		5 (6.2)	2 (2.7)	9 (11.3)	7 (5.7)
<i>S. maltophilia</i>	-	-	-		1 (1.4)	2 (3.0)	3 (4.6)		0	2 (2.7)	2 (2.5)	7 (5.7)
<i>A. xylosoxidans</i>	-	-	-		2 (2.8)	1 (1.5)	0		2 (2.5)	1 (1.4)	0	0
<i>B. cepacia</i>	-	-	-		0	0	0		0	0	0	0
MIC₅₀ and MIC₉₀ (µg/mL) of aztreonam for all <i>P. aeruginosa</i> isolates												
Baseline	≤ 1, 16	2, 64	≤ 1, 128		≤ 1, 64	2, 64	2, 32		2, 64	4, 128	4, 128	4, 64
End-Treatment (1 course)	≤ 1, 16	≤ 1, 64	4, 128		≤ 1, 64	4, 128	4, 64		2, 64	8, 128	8, 256	8, 128
End-Treatment (3 courses)	-	-	-		-	-	-		-	-	8, 256	8, 512
MIC₅₀ and MIC₉₀ (µg/mL) of aztreonam for <i>P. aeruginosa</i> isolate with the highest MIC from each patient												
Baseline	4, 64	8, 128	4, 256		4, 128	4, 128	4, 32		8, 128	8, 256	8, 128	8, 256
End-Treatment (1 course)	4, 128	4, 128	4, 256		4, 128	8, 256	8, 128		4, 64	8, 256	8, 512	8, 512
End-Treatment (3 courses)	-	-	-		-	-	-		-	-	8, 256	16, 512

Source: CP-AI-003 CSR Tables 14.2.1.3.3, 14.2.2.2, 14.2.2.4; CP-AI-005 CSR Tables 14.2.12.3, 14.6.3.2, 14.6.3.5; CP-AI-007 Tables 14.2.10.3, 14.6.3, 14.6.4.1, 14.6.4.3; CP-AI-006 CSR Tables 14.6.2.1, 14.7.3, 14.7.7, and SCM Table 3.5.2.3.

* Mid-treatment (1 course) data represent Day 7 for CP-AI-003 and Day 14 for CP-AI-005 and CP-AI-007. End-treatment (1 course) data represent Day 14 for CP-AI-003 and Day 28 for CP-AI-005, -006, and -007. End-treatment (3 courses) data represent Day 140 (end of treatment course 3) for CP-AI-006.

- Data not available.

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In an **integrated analysis** of the two Phase 3 placebo-controlled CF studies, 160/215 (74.4%) patients who received a 28-day treatment course of 75 mg AZLI were aged ≥ 18 years. Adult patients treated with AZLI demonstrated statistically significant improvements in respiratory symptoms, pulmonary function, and sputum PA density in comparison with placebo-treated patients following a 28-day treatment course. The treatment difference at Day 28 for change in CFQ-R Respiratory Symptoms score was 5.25 points ($p = 0.0078$). The treatment difference at Day 28 for percent change in FEV₁ was 7.0% ($p < 0.0001$). This improvement persisted through Day 42 (treatment difference: 5.3%; $p < 0.0001$). The treatment difference at Day 28 for change in log₁₀ PA CFUs in sputum was -0.80 ($p < 0.0001$). Among patients treated with AZLI in [Study CP-AI-007](#), 59/80 (73.8%) patients were aged ≥ 18 years. Robust improvements were observed in this adult patient population for CFQ-R Respiratory Symptoms score (treatment difference: 6.35 points; $p = 0.0495$) and FEV₁ (treatment difference: 10.5%; $p < 0.0001$). Improvement in FEV₁ persisted through Day 42 (treatment difference: 5.9%; $p = 0.0018$). Since patients who enrolled in [Study CP-AI-007](#) were required to have been off all antibiotics for 28 days prior to AZLI administration, of the three Phase 3 studies in the AZLI program for CF, they most closely match patients with non-CF bronchiectasis, who in general are exposed to fewer courses of antibiotics per year than similarly infected patients with CF. Therefore, patients who enroll in the proposed Phase 3 clinical study of AZLI for bronchiectasis may respond to therapy in a similar fashion to patients with CF who were treated with AZLI in [Study CP-AI-007](#).

Microbiology data were collected in the CF studies to track the potential development of antibiotic resistance and emergence of other pathogens. Overall, no changes in the susceptibility of PA to aztreonam or to other antibiotics, including aminoglycosides, quinolones, and beta-lactams, were observed following one 28-day course of 75 mg AZLI in the Phase 3 placebo-controlled studies and up to six 28-day courses (with 28 days between courses) of 75 mg AZLI in the open-label follow-on study. Further, a trend toward increased tobramycin susceptibility was observed, which may reflect the activity of AZLI against tobramycin-resistant PA isolates.

No concerning trends in the treatment-emergent isolation of other bacterial respiratory pathogens, including *B. cepacia*, *S. maltophilia*, *A. xylosoxidans*, or *S. aureus*, were observed following one 28-day course of 75 mg AZLI in the Phase 3 placebo-controlled studies and up to six 28-day courses of 75 mg AZLI in the open-label follow-on study.

The Applicant seeks approval of AI for the improvement of respiratory symptoms and pulmonary function in CF patients with *Pseudomonas aeruginosa*. AI is intended as a suppressive therapy and is not expected to eradicate *P. aeruginosa* in CF patients with established airway infections. Consequently, in the Phase 2 and three Phase 3 clinical trials, there are no definitive endpoints for microbiological eradication or clinical cure. Under these circumstances, the objective of the review team is to determine **what constitutes microbiological or clinical success?** While it is not the place for this Reviewer to define clinical success, he shall attempt to define microbiological success.

There are two ways to evaluate the data from this submission. First, the microbiological efficacy endpoints were compared to the most common clinical

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efficacy endpoint, improvement in FEV₁. Second, the results of the microbiological and primary clinical efficacy endpoint data were compared to the corresponding data derived from the package insert for TOBI Solution for Inhalation (TSI), a drug for which AI is intended to be an alternative treatment for CF. TOBI is the only other Agency-approved drug for the management of cystic fibrosis and also is administered in a similar manner.

There are three measures of microbiological outcome:

1. Change in log₁₀ *P. aeruginosa* CFUs/g sputum specimens from patients;
2. Appearance of other pathogens, specifically *S. aureus*, *B. cepacia*, *S. maltophilia* or *A. xylosoxidans* in CF patients; and
3. Changes in aztreonam MIC50s and MIC90s of *P. aeruginosa* isolates from CF patients.

Change in *P. aeruginosa* CFUs in Sputum

AI is intended as a suppressive therapy and is not expected to eradicate *P. aeruginosa* in CF patients with established airway infections. While reductions in *P. aeruginosa* CFU/g in sputum were observed following up to three 28-day treatment courses of 75 mg AI, the majority of patients continued to have positive sputum culture results for *P. aeruginosa*.

Overall, the reductions in log₁₀ *P. aeruginosa* CFU/g in sputum observed among patients treated with AI for 28 days in the integrated Phase 3 placebo-controlled studies achieved significance over those observed for placebo-treated patients at Days 14 and 28 of treatment. *P. aeruginosa* CFU/g declined from Day 0 to Day 14, and further reduction in *P. aeruginosa* CFU/g was observed from Day 14 to Day 28, supporting a 28-day AI treatment regimen for extended antibiotic effect.

Patients treated with AI administered TID demonstrated greater reductions in *P. aeruginosa* CFU/g at Days 14 and 28 than did patients treated with AI administered BID. *P. aeruginosa* CFU/g in sputum returned to near baseline values during the 14-day period following treatment (Day 42). The greater reductions in *P. aeruginosa* CFU/g observed among patients in the AI TID group may be due to better efficacy with the TID dosing regimen or to the contribution of patients treated in [CP-AI-007](#), who would be expected to demonstrate greater antibiotic effect after having been off anti-pseudomonal antibiotics for at least 28 days prior to AI treatment.

In contrast, all patients in the BID group were treated with a 28-day course of TSI immediately prior to AI therapy, which may have suppressed the CFU response to AI. When AI-treated patients were analyzed by subgroup, reductions in *P. aeruginosa* CFU/g were greater at Days 14 and 28 for patients < 18 years old, males, patients with less severe disease (FEV₁ > 50% predicted), and patients with more susceptible *P. aeruginosa* isolates (MIC ≤ 8 µg/mL).

Among all patients completing at least three AI courses in the open-label follow-on study, consistent reductions in log₁₀ *P. aeruginosa* CFU/g were observed at the end of each AI treatment course. The TID dosing regimen resulted in larger reductions in *P. aeruginosa* CFU/g after each treatment course than did BID dosing, and no attenuation in *P. aeruginosa* CFU killing was observed following three AI treatment courses.

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When AI-treated patients in the open-label follow-on study were analyzed by subgroup, the results were largely consistent with those observed in the Phase 3 placebo-controlled studies. Reductions in *P. aeruginosa* CFU/g were greater for patients < 18 years old, males, and patients with more susceptible *P. aeruginosa* isolates ($\text{MIC} \leq 8 \mu\text{g/mL}$). However, patients with more severe disease ($\text{FEV}_1 \leq 50\%$ predicted) demonstrated greater reductions in *P. aeruginosa* CFU/g than patients with less severe disease ($\text{FEV}_1 > 50\%$ predicted).

Presence or Absence of CF Pathogens

In the integrated Phase 3 placebo-controlled studies, slight increases in the percentages of patients with positive cultures for *S. aureus* and *S. maltophilia* were observed for the pooled placebo and AI groups. The percentage of patients from which *A. xylosoxidans* was isolated increased among placebo-treated patients and decreased among AI-treated patients; *B. cepacia* was isolated from a total of three AI-treated patients during the two studies.

In the open-label follow-on study, there were no notable changes in the appearance of *S. maltophilia* or *A. xylosoxidans*. Three AI TID patients had a positive culture for *B. cepacia* during the open-label follow-on study; two of these patients also had positive isolations in their previous AI study. Slight increases in the percentages of patients with positive cultures for *S. aureus* were observed following AI treatment administered BID or TID; however, the percentage of AI-treated patients with appearance of *S. aureus* after three AI treatment courses in the open-label follow-on study (12.4%) was similar to the percentage of patients with appearance of *S. aureus* after 28 days of placebo treatment (8.0%) in the integrated Phase 3 placebo-controlled studies. Data from patients completing additional treatment courses will be required to determine whether long-term AI treatment is associated with increased isolation of *S. aureus*.

In the integrated Phase 3 placebo-controlled studies, there were no differences between the pooled placebo and AI groups in the treatment-emergent intermittent isolation of other pathogens. Treatment-emergent persistent isolation of *S. maltophilia* occurred among more AI-treated than placebo-treated patients (3.4% AI vs. 0.7% placebo), while treatment-emergent persistent isolation of *A. xylosoxidans* occurred among more placebo-treated than AI-treated patients (2.6% placebo vs. 1.0% AI).

In the open-label follow-on study, there were no differences between the AI BID and TID groups in the treatment-emergent intermittent isolation of other pathogens. A higher percentage of AI BID patients experienced treatment-emergent persistent isolation of *S. aureus* (11.3% AI BID patients vs. 5.7% AI TID patients), *Aspergillus* spp. (10.0% AI BID patients vs. 4.9% AI TID patients), and *Candida* spp. (20.0% AI BID patients vs. 12.2% AI TID patients), while a higher percentage of AI TID patients experienced treatment-emergent persistent isolation of *S. maltophilia* (5.7% AI TID patients vs. 2.5% AI BID patients). These results should be interpreted with caution, since there was no placebo group for comparison with AI over multiple treatment courses in the open-label follow-on study. Overall, a higher percentage of patients with treatment-emergent persistent isolation of *Aspergillus* or *Candida* spp. used concomitant oral/inhaled corticosteroids (93% and 74%, respectively), compared to 64% of total patients in the integrated Phase 3 placebo-controlled

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studies and 66% of patients receiving at least one dose of AI in the combined Phase 2/3 placebo-controlled and open-label follow-on studies.

Among patients enrolled in the Phase 3 placebo-controlled studies who used concomitant medications for mycobacterial or fungal infections, 4/5 (80%) patients in the CP-AI-007 study and 12/14 (86%) patients in the CP-AI-005 study were receiving treatment for a pre-existing condition. Among the patients without a reported pre-existing condition necessitating concomitant medication usage, one placebo patient in CP-AI-007 received concomitant nystatin on Day 12 for oral thrush, one placebo patient in CP-AI-005 received concomitant rifampin on Day 52 for history of MRSA and increased cough, and one AI BID patient in CP-AI-005 received concomitant rifampin on Day 28 for sinus pressure headache. These indications suggest that the majority of these concomitant medications were likely prescribed empirically or for pathogens common to CF disease other than mycobacterial or fungal pathogens.

Among patients enrolled in the open-label follow-on study who were prescribed concomitant medications for mycobacterial or fungal infections, 6/21 (29%) patients were receiving AI BID treatment and 15/21 (71%) patients were receiving AI TID treatment. The majority of patients prescribed concomitant medication more than one time during the first three treatment courses were receiving treatment for a pre-existing condition.

MICs for CF Pathogens

For patients treated with AI in the integrated Phase 3 placebo-controlled studies, the aztreonam MIC50 and MIC90 values for all *P. aeruginosa* isolates and for the *P. aeruginosa* isolate with the highest MIC remained unchanged from baseline for the BID and TID treatment groups.

Among all patients in the open-label follow-on study, the MIC50 of aztreonam for all *P. aeruginosa* isolates remained unchanged while the MIC90 increased transiently from baseline for the AI BID and TID treatment groups through three AI treatment courses. The increases in MIC50 observed for TID-treated patients originating in CP-AI-005 and in MIC90 observed for TID-treated patients completing at least three AI courses were largely a reflection of low baseline values for both subsets in comparison with all patients enrolled in the study.

For the *P. aeruginosa* isolate with the highest MIC from each patient, the MIC50 of aztreonam remained unchanged from baseline for AI BID- and TID-treated patients. Among patients originating in CP-AI-005 or completing at least three AI courses, the MIC90 of aztreonam for the *P. aeruginosa* isolate with the highest MIC increased by 2 step dilutions from baseline at several subsequent visits. These increases in MIC90 of aztreonam were largely attributable to patients in the BID treatment group.

For the Phase 3 placebo-controlled studies, there were no notable changes after dosing in the percentages of placebo-treated patients with highest aztreonam MIC for *P. aeruginosa* above the parenteral breakpoint ($\geq 8 \mu\text{g/mL}$). For AI-treated patients, these percentages increased from Day 0 to Days 14 and 28 in the AI BID and AI TID groups. For patients treated with AI TID (CP-AI-007 and CP-AI-005), but not AI BID (CP-AI-005 only), these percentages returned to baseline following

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cessation of AI treatment. In general, the MICs of aztreonam for the *P. aeruginosa* isolate with the highest MIC from each patient were largely unchanged (± 1 dilution step change) from baseline at each subsequent visit for the pooled placebo and AI groups. Larger percentages of AI- than placebo-treated patients demonstrated increased MICs (≥ 2 dilution step change) at each time point. These data should be interpreted with caution, as changes in MIC may reflect sampling variability rather than decreased susceptibility to aztreonam.

When the AI and placebo treatment groups were divided by age group (< 18 years old, ≥ 18 years old), similar percentages of patients in each age subgroup demonstrated increased aztreonam MIC at Day 28. By sex, a higher percentage of males than females demonstrated increased aztreonam MIC at Day 28. By baseline disease severity ($FEV_1 \leq 50\%$ predicted, $FEV_1 > 50\%$ predicted), similar percentages of patients in each subgroup demonstrated increased aztreonam MIC at Day 28. By baseline *P. aeruginosa* susceptibility to aztreonam ($MIC \leq 8 \mu\text{g/mL}$, $MIC > 8 \mu\text{g/mL}$), a higher percentage of patients with more susceptible *P. aeruginosa* isolates ($MIC \leq 8 \mu\text{g/mL}$) demonstrated increased aztreonam MIC at Day 28 while a higher percentage of patients with less susceptible *P. aeruginosa* isolates ($MIC > 8 \mu\text{g/mL}$) demonstrated decreased aztreonam MIC at Day 28.

For the open-label follow-on study, the percentages of patients whose *P. aeruginosa* isolate with highest aztreonam MIC was above the parenteral breakpoint ($> 8 \mu\text{g/mL}$) increased from Day 0 to the end of each AI treatment course (Days 28, 84, and 140). In general, these increases were observed during both on- and off-treatment courses for patients in the AI BID group, but only during the on-treatment courses for patients in the AI TID group. In general, the MICs of aztreonam for the *P. aeruginosa* isolate with the highest MIC from each patient were largely unchanged (± 1 dilution step change) from baseline at the end of each treatment course for the AI BID and TID groups. A larger percentage of patients treated with AI TID than BID demonstrated increased MICs (2 dilution step change) following each of the first three treatment courses, both on and off treatment. These data should be interpreted with caution, as changes in MIC may reflect sampling variability rather than decreased susceptibility to aztreonam.

When patients completing at least three AI courses were divided by age group (< 18 years old, ≥ 18 years old), a higher percentage of patients < 18 years old had decreased MICs following three AI treatment courses. By sex, a higher percentage of males had increased MICs following three AI treatment courses. By baseline disease severity ($FEV_1 \leq 50\%$ predicted, $FEV_1 > 50\%$ predicted), a higher percentage of patients with more severe disease ($FEV_1 \leq 50\%$ predicted) demonstrated either increased or decreased MICs following three AI treatment courses. By baseline *P. aeruginosa* susceptibility to aztreonam ($MIC \geq 8 \mu\text{g/mL}$, $MIC > 8 \mu\text{g/mL}$), a higher percentage of patients with more susceptible *P. aeruginosa* isolates ($MIC \leq 8 \mu\text{g/mL}$) demonstrated increased MICs following treatment courses 1 and 2; no differences between susceptibility subgroups were observed following treatment course 3.

For the integrated Phase 3 placebo-controlled studies, there were no notable increases in the MIC₅₀ or MIC₉₀ of any of the other antibiotics tested (tobramycin, gentamicin, amikacin, piperacillin, cefepime, meropenem, ceftazidime, ciprofloxacin, and ticarcillin/clavulanate) for all *P. aeruginosa* isolates or for the *P. aeruginosa*

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isolate with the highest aztreonam MIC following AI or placebo treatment. In the AI TID treatment group, the MIC90 values of tobramycin for *P. aeruginosa* were lower than baseline by Day 28. This apparent increase in tobramycin susceptibility may reflect the activity of aztreonam against tobramycin-resistant *P. aeruginosa* isolates.²⁴ No changes in tobramycin MIC were observed during the 28-day TSI run-in period of the CP-AI-005 study.

For the open-label follow-on study, there were generally no notable increases in the MIC50 or MIC90 of any of the other antibiotics tested for all *P. aeruginosa* isolates or for the *P. aeruginosa* isolate with the highest aztreonam MIC following AI treatment. The MIC90 of tobramycin decreased from baseline for all *P. aeruginosa* isolates following the first treatment course for AI BID patients originating in CP-AI-005, for all *P. aeruginosa* isolates following the third treatment course for AI TID patients completing at least three AI courses, and for the *P. aeruginosa* isolate with the highest MIC following the first treatment course for AI TID patients originating in CP-AI-005.

B. cepacia was isolated from two patients in the CP-AI-007 study and one patient in the CP-AI-005 study. Three patients had positive cultures for *B. cepacia* in the CP-AI-006 study; two of these patients also had positive isolations in their previous AI study. All *B. cepacia* isolates were generally susceptible to aztreonam and resistant to tobramycin, with varying susceptibilities to other antibiotics.

However, none of the three microbiology responses correlated with clinical responses.

To investigate the possible correlation between microbiological and clinical endpoints, scatterplots were produced. Scatterplots examining these endpoints showed the following:

- Changes in numbers of PA in sputum were not associated with changes in FEV₁ or aztreonam concentrations in study CP-AI-003, CP-AI-005 and slightly negatively associated in study CP-AI-007.
- Changes in numbers of PA in sputum were not associated with changes in aztreonam concentrations in study CP-AI-003.
- Changes in aztreonam MIC for the PA isolate with the highest MIC from each patient were slightly positively associated with changes in FEV₁ in study CP-AI-003 but slightly negatively associated in study CP-AI-007.
- Changes in numbers of PA in sputum were not associated with changes in aztreonam MIC for the PA isolate with the highest MIC in study CP-AI-005 and CP-AI-007.
- Changes in numbers of PA in sputum were not associated with changes in CFQ-R respiratory domain score in study CP-AI-007.
- The Applicant did not present scattergrams to explain relationships between microbiological to clinical outcomes.

The lack of correlation of microbiology outcomes with clinical outcomes is not unprecedented. In the Microbiology subsection of the package insert for TOBI for the management of cystic fibrosis patients with *P. aeruginosa*, the Agency states "The relationship between in vitro susceptibility test results and clinical outcome with TOBI therapy is not clear".

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As none of the examined microbiology endpoints correlate with clinical outcomes, this Reviewer believes the clinical endpoints should be the deciding factors in the determination of clinical efficacy of AI. Therefore, ***this Reviewer defers to the opinion of the Medical Officer, Dr. Menfo Imiosili.***

**MICROBIOLOGY SUBSECTION OF THE PACKAGE
INSERT**

The approval of this application will be dependent upon the successful completion of another pivotal clinical trial, as deemed by the Medical Officer. Consequently, the microbiology subsection of the package insert was not reviewed pending the completion of this successful clinical trial.

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INTRODUCTION

Cystic fibrosis (CF) is the most common, life-shortening genetic disorder in Caucasians, with approximately 60,000 affected persons worldwide. CF patients produce thickened airway secretions that predispose them to recurrent pulmonary bacterial infections. Unlike acute pulmonary infections in non-CF individuals, bacterial infections in the lower respiratory tracts of CF patients begin as transient infections that are reversible with antimicrobial therapy and progress to chronic infections that cannot be eradicated with treatment.

Pseudomonas aeruginosa is the most commonly isolated pathogen in CF. Initial infection typically occurs with a non-mucoid strain, but mucoid variants that produce alginate can emerge within three months of initial (*P. aeruginosa*) infection and predominate as infections become chronic. Chronic infection with mucoid *P. aeruginosa* has been clearly associated with decreased survival. Despite the availability of several CF mouse models, an appropriate model of chronic lung infection in CF mice has not been developed.

Among CF patients, isolation of a particular bacterial species tends to vary according to age. *Staphylococcus aureus* infections predominate in infants less than one year of age, while *Haemophilus influenzae* (typically unencapsulated) is more frequently cultured after age 1, often in the presence of other pathogens such as *S. aureus*. In the US, approximately 50% of patients are chronically infected with *P. aeruginosa* by the age of 10; this number increases to 80% by adulthood. *Burkholderia cepacia* complex is also an important pathogen in CF and can cause a rapidly fulminating fatal systemic infection or result in persistent lung infection that is associated with increased loss of lung function.

Other commonly isolated organisms include *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans*, although the clinical significance of these organisms is not well understood. Patients are frequently co-infected with multiple organisms, multiple colony phenotypes of the same species or both. The vast majority of CF patients ultimately succumb to respiratory failure due to damage resulting from chronic pulmonary infection.

Antibiotics are used in several ways to treat infections in CF patients:

- Prophylactic treatment to delay or avoid chronic pulmonary infection (primarily for *S. aureus*),
- Early treatment following bacterial colonization to eradicate pulmonary infection,
- Treatment of acute pulmonary exacerbations to stabilize patients and reduce sputum bacterial loads, or
- Regularly scheduled treatment of chronically infected patients (regardless of symptomatology) to reduce inflammation and slow lung function decline.

Beta-lactams comprise a chemically diverse group of antibiotics that are broadly categorized as penems (penicillins), penems (carbapenems), or cepems (cephalosporins). All beta-lactams have a four-member, nitrogen-containing beta-lactam nucleus. The nucleus is open to side chain substitutions; the nature of the substitution influences the spectrum of activity.

Aztreonam lysine for inhalation (AI) was investigated to improve respiratory symptoms and pulmonary function in CF patients with pulmonary *P. aeruginosa*. Azactam has been approved for parenteral (IV or IM) administration in the US since 1986 and is sometimes used to treat acute pulmonary exacerbations in CF. The parenteral formulation contains approximately 780 mg arginine per gram of aztreonam, as a buffer. Aerosolized arginine has been tested as a mucolytic in CF patients and was shown to cause airway inflammation. AI incorporates a lysine salt, which eliminates the inflammatory component of the IV formulation. Additionally, the pH and osmolality

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of AI are adjusted to be safe and tolerable in the airway. The change in buffer from arginine to lysine did not impact MIC values; the two aztreonam formulations demonstrated identical activity against a panel of CF clinical isolates.

PRECLINICAL EFFICACY—*IN VITRO*

MECHANISM OF ACTION

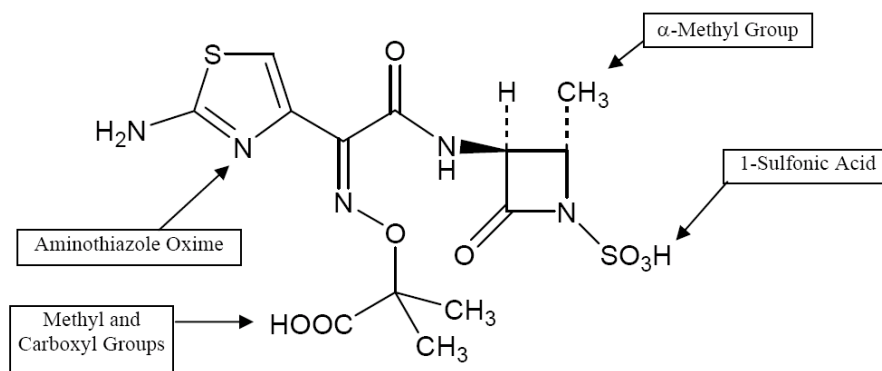
Aztreonam is a synthetic bactericidal monobactam antibiotic that was originally isolated from *Chromobacterium violaceum*. The monobactams are structurally different from the beta-lactam antibiotics due to a unique monocyclic nucleus; this nucleus contains a number of side chains that confer various activities (Table 1, Figure 1).

Table 1. Structure-activity Relationships in the Aztreonam Molecule

Structure	Activity
1-sulfonic acid group attached to nitrogen of the monocyclic nucleus	Activation of the monocyclic nucleus
α -methyl group at position 4	Enhanced stability to beta-lactamases, increased antibacterial activity
Aminothiazole oxime moiety on acyl side chain	Activity against aerobic gram-negative bacteria
Two methyl groups and carboxyl acid on oxime side chain	Enhanced activity against <i>Pseudomonas</i>

Source: Table 1, this submission.

Figure 1. Structure of Aztreonam (Z)-2-[[[(2-amino-4-thiazolyl)[[(2S,3S)-2methyl-4-oxo-1-sulfo-3-azetidiny]carbonyl]methylene]amino]oxy]-2-methylpropionic acid.



Source: Figure 1, this submission.

Aztreonam (Azactam®) is the only monobactam approved for clinical use in the US. It is also approved in 31 other countries, including 15 European Union member states. A second monobactam, carumonam sodium (AMASULIN®), has been approved for human use in Japan.

Aztreonam, like beta-lactam antibiotics, inhibits bacterial cell wall biosynthesis, resulting in cell lysis and death through irreversible inhibition of penicillin-binding proteins (PBPs), enzymes that catalyze the elongation and crosslinking of the peptidoglycan chains. In general, most bacteria possess at least four different PBPs with various functions in peptidoglycan production. In aerobic

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Gram-negative bacteria, PBP3 is responsible for cross-linking peptidoglycan in the division septum.

Aztreonam binds preferentially and with high affinity to PBP3 of aerobic Gram-negative bacteria, accounting for its potent antibacterial activity against *P. aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter cloacae* and *Klebsiella pneumoniae*. In contrast, aztreonam has poor affinity for the PBP3 of Gram-positive and anaerobic bacteria and consequently is inactive against these bacteria. Binding of aztreonam to PBP3 results in filamentation and cell lysis, an effect similar to that observed with cephalosporins. Electron microscopy studies demonstrate that within two hours of exposure to aztreonam ($= 4 \mu\text{g/mL}$), *P. aeruginosa* cells become filamentous and lack cross walls or septa. After further incubation, the filaments collapse and cells lyse.

Aztreonam, like beta-lactam antibiotics, demonstrates time-dependent killing against sensitive Gram-negative bacteria; increasing the concentration of aztreonam above the minimum inhibitory concentration (MIC) of the test organism does not substantially increase the speed or degree of bacterial killing.

SPECTRUM OF ACTIVITY

Aztreonam is active against several aerobic Gram-negative pathogens, but is relatively inactive against Gram-positive and anaerobic organisms.

Aztreonam is active against most isolates of the following Gram-negative microorganisms, both *in vitro* and following parenteral administration in the clinic:

- *Citrobacter* species, including *C. freundii*
- *Enterobacter* species, including *E. cloacae*
- *E. coli*
- *H. influenzae* (including ampicillin-resistant and other penicillinase-producing strains)
- *Klebsiella oxytoca*
- *K. pneumoniae*
- *P. mirabilis*
- *P. aeruginosa*
- *Serratia* species, including *S. marcescens*

Other aerobic Gram-negative bacteria show *in vitro* susceptibility to aztreonam, although the clinical significance of these organisms in CF is unknown, and data regarding clinical efficacy is not available. Susceptible bacteria are typically killed at concentrations one to four times their MICs. However, in some cases, the concentration of aztreonam required to achieve bactericidal killing of *P. aeruginosa* has been reported to be 4 to 16 times the MIC. Aztreonam exhibits MICs of $\leq 8 \mu\text{g/mL}$ against at least 90% of the following aerobic Gram-negative bacteria: *Aeromonas hydrophila*, *Morganella morganii*, *Neisseria gonorrhoeae* (including penicillinase-producing strains), *Pasturella multocida*, *P. vulgaris*, *Providencia stuartii*, *Providencia rettgeri*, and *Yersinia enterocolitica*. The safety and efficacy of aztreonam in treating clinical infections due to these microorganisms, however, have not been established in clinical trials.

Established susceptibility breakpoints for parenteral administration of aztreonam using agar or broth dilution are $\leq 8 \mu\text{g/mL}$ (susceptible), $16 \mu\text{g/mL}$ (intermediate), and $\geq 32 \mu\text{g/mL}$ (resistant). The MIC values that were determined in the early 1980s at the time of the Azactam submission are provided in [Table 2](#).

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Table 2. MIC values (µg/mL) of Aztreonam for Aerobic Bacteria

Organism	No. of strains	MIC Range	MIC ₅₀	MIC ₉₀
Gram-negative aerobes:				
<i>Acinetobacter calcoaceticus</i>	25	1.6–100	20.1	58.3
<i>Citrobacter freundii</i>	25	< 0.1–50	0.2	0.7
<i>Enterobacter aerogenes</i>	13	0.1–50	0.1	33.3
<i>Enterobacter cloacae</i>	29	< 0.1–50	0.2	12.5
<i>E. coli</i>	79	< 0.1–0.8	0.1	0.2
<i>H. influenzae</i>	36 ^a	< 0.1–0.2	< 0.1	0.2
<i>K. pneumoniae</i>	68	< 0.1–> 100	< 0.1	0.3
<i>M. (Proteus) morganii</i>	19	< 0.1–1.6	< 0.1	0.6
<i>N. gonorrhoeae</i>	20	< 0.1–0.4	< 0.1	0.2
<i>P. (Providencia) rettgeri</i>	6	< 0.1	< 0.1	< 0.1
<i>P. mirabilis</i>	25	< 0.1–0.1	< 0.1	< 0.1
<i>P. vulgaris</i>	11	< 0.1–0.1	< 0.1	< 0.1
<i>P. stuartii</i>	15	< 0.1–0.1	< 0.1	< 0.1
<i>P. aeruginosa</i>	61	0.2–50	4.3	12.0
<i>Salmonella spp.</i>	25	0.1–0.8	0.1	0.3
<i>Serratia marcescens</i>	113	0.1–6.3	0.3	1.6
<i>Shigella spp</i>	25	< 0.1–12.5	< 0.1	5.7
Gram-positive aerobes:				
<i>S. aureus</i>	12	> 100	> 100	> 100
<i>Streptococcus faecalis</i>	12	> 100	> 100	> 100
<i>Streptococcus pneumoniae</i>	11	50–> 100	> 100	> 100

^a 18 Amp^S and 18 Amp^R

Source: Table 2, this submission.

SURVEILLANCE STUDIES (Non-CF Isolates)

Repeated administration of any antibiotic exerts selective pressure favoring microbial isolates resistant to the agent used. Parenterally administered Azactam (aztreonam arginine) has been used since 1986 to treat a wide variety of infections caused by aerobic gram-negative bacilli. Although there is little longitudinal data specific to aztreonam use, surveillance type data collected following approval suggest some selective pressure related to beta-lactam antibiotic usage.

North American Trends in Susceptibility

In North America, aztreonam susceptibility of non-extended-spectrum beta-lactamases (ESBL) producing *E. coli* and *Klebsiella* has remained stable for the last 20 years: the MIC₉₀ values remain ≤ 0.5 µg/mL and more than 94% of isolates are susceptible at the parenteral breakpoint of 8 µg/mL. Changes in susceptibility to aztreonam for *Enterobacter* species are more difficult to ascertain due to the range of dilutions tested; minimum inhibitory concentration for 50% of isolates (MIC₅₀) values remain low at ≤ 0.12 µg/mL; however, MIC₉₀ values exceeded the range tested between 1997 and 2003. The proportion of susceptible *Enterobacter* isolates actually increased from 70.7 % (1997) to 81.1% (1998–2003). In contrast, susceptibility of *P. aeruginosa* to aztreonam has declined over time. The proportion of isolates susceptible at the parenteral

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breakpoint of 8 µg/mL decreased from 77.4% (1997) to 63.7% (1998-2003) (Table 3). Similar data have been observed in other antimicrobial surveillance studies.

Table 3. Changes in Susceptibility to Aztreonam in North America

Organism	1997 ⁵⁶				1998-2003 ⁵⁷			
	N	MIC ₅₀	MIC ₉₀	%S	N	MIC ₅₀	MIC ₉₀	%S
<i>P. aeruginosa</i>	189	8	> 16	77.4	5517	8	> 16	63.7
<i>E. coli</i>	769	≤ 0.12	≤ 0.12	97.6	10,361	≤ 0.12	0.25	98.9
<i>E. coli (ESBL)</i>	NR				386	4	> 16	71.2
<i>Enterobacter</i>	151	≤ 0.12	> 16	70.7	2836	≤ 0.12	> 16	81.1
<i>Klebsiella</i>	311	≤ 0.12	0.25	96.1	5166	≤ 0.12	0.5	94.5
<i>Klebsiella (ESBL)</i>	NR				442	> 16	> 16	35.7

Data from 1997 are from the US only. Data from 1998-2003 are from the SENTRY program and include isolates from the US and Canada.

Source: Table 3, this submission.

Global Trends in Aztreonam Susceptibility

The SENTRY program also monitors the prevalence and antibiotic susceptibility profiles of bacterial pathogens collected from participating medical centers in four international regions: Asia-Pacific, Europe, Latin America, and North America. Changes in aztreonam susceptibility in these regions mirror the North American data.

During the first five years of the program (1997-2001), 48,440 *Enterobacteriaceae* isolates were collected and analyzed. The most frequently isolated pathogens were *E. coli* (46%), *Klebsiella* spp. (21%), and *Enterobacter* spp. (12%); no other pathogen comprised more than 5% of the collection. For these *Enterobacteriaceae*, 75.6% of isolates were inhibited at the lowest aztreonam concentration tested (0.12 µg/mL), and the susceptibility rate at the parenteral breakpoint (8 µg/mL) was 91.2%.

From 1997 to 1999, 6,631 *P. aeruginosa* isolates from respiratory tract, urinary tract, skin/soft tissue, and bloodstream infections (one isolate per patient) were analyzed. The respiratory tract was the most common source of *P. aeruginosa*. While the proportion of *P. aeruginosa* isolates susceptible to aztreonam varied by geographic region (Table 4), the overall proportion of *P. aeruginosa* isolates susceptible to aztreonam decreased slightly over time with the largest decline in susceptibility noted for Europe.

Table 4. Regional Trends in the Susceptibility of *P. aeruginosa* to Aztreonam

	Percentage of Isolates Susceptible to Aztreonam				
	Asia-Pacific (11.4%) ^a	Canada (8.7%) ^a	Europe (25.0%) ^a	Latin America (17.1%) ^a	United States (37.7%) ^a
1997	—	73.4	80.2	55.5	67.0
1998	66.5	67.1	73.2	45.0	64.7
1999	65.3	75.2	55.6	48.2	62.3

^a Distribution of isolates by region during the study period (N = 6,631 total).

Source: Table 4, this submission.

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P. aeruginosa Susceptibility to Aztreonam Compared with Other Antibiotics

Recent data regarding the activity of aztreonam compared with other antibiotics commonly used to treat Gram-negative infections is shown in [Table 5](#). Overall, aztreonam is less active than other commonly used antibiotics in all geographic regions.

Table 5. Susceptibility and Resistance of *P. aeruginosa* to Aztreonam and Selected Antibiotics

Antimicrobial Agent	Percentage of Isolates Susceptible/Resistant to Aztreonam			
	Asia-Pacific (N = 894)	Europe (N = 1702)	Latin America (N = 1103)	North America (N = 3270)
Aztreonam	66.9/19.1	72.8/14.9	48.7/32.9	66.1/19.9
Beta-lactams				
Ceftazidime	78.7/14.1	79.7/15.0	65.5/27.6	80.1/13.6
Meropenem	90.3/5.5	85.1/9.8	79.5/15.0	91.4/4.8
Piperacillin	85.6/14.4	79.6/20.4	68.2/31.8	87.1/12.9
Quinolones				
Ciprofloxacin	84.6/11.6	72.4/24.7	62.7/33.3	77.8/15.8
Levofloxacin	83.6/12.3	71.4/24.7	60.4/33.5	74.0/17.5
Aminoglycosides				
Gentamicin	84.2/15.8	71.7/28.3	61.8/38.2	84.2/15.8
Tobramycin	89.6/10.4	75.8/24.2	65.5/34.5	92.3/7.8

Source: Table 5, this submission.

Susceptibility of CF *P. aeruginosa* to Aztreonam

Antibiotic susceptibility trends in bacterial isolates collected from CF patients may differ from the general patient population due to the chronic nature of antibiotic therapy in CF patients. In the US, susceptibility of *P. aeruginosa* to aztreonam has gradually decreased within both the CF and non-CF populations over the last 10 years with 80% of *P. aeruginosa* isolates susceptible (MIC $\leq$ 8 μ g/mL) to aztreonam in 1995, 78.9 % susceptible in 2004, and 68.6% susceptible in 2006 ([Table 6](#)). In Europe, the aztreonam susceptibility of *P. aeruginosa* isolated from CF patients is lower than in the US (56%).

Table 6. Aztreonam Susceptibility Trends in CF Isolates of *P. aeruginosa*

Reference	Region	No. of Isolates (Patients)	MIC ₅₀	MIC ₉₀	Range	%S	%R
1995-1996 ⁶³	US	1240 (508 pts)	≤ 2	32	$\leq 2 \rightarrow 512$	80.0	11.9
2004-2005	US	242 (105 pts)	≤ 1	64	$\leq 1 \rightarrow 2048$	78.9	10.3
Phase 2 AI baseline data ⁶⁴							
1993-1997 ⁶⁷	Italy	1267 (224 pts)	NR	NR		NR	19.7
2004-2005 ⁶⁶	Europe	150	8	256	0.125 $\rightarrow$ 512	56	30
2006 ⁶⁵	US	1826	8	≥ 32		68.6	23.1

NR = Not reported

Source: Table 6, this submission.

Aztreonam has been shown to have *in vitro* activity against tobramycin-resistant and multidrug-resistant *P. aeruginosa* isolates from CF patients. In a study of 1,240 *P. aeruginosa* isolates

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collected from 1995 to 1996 from 508 CF patients, 67 isolates were shown to be resistant to tobramycin ($\text{MIC} \geq 16 \mu\text{g/mL}$). Of these 67 tobramycin-resistant isolates, 44 (65.7%) were susceptible to aztreonam ($\text{MIC} \leq 8 \mu\text{g/mL}$). One investigator examined the susceptibility of multidrug resistant *P. aeruginosa* isolated in 2001-2003 from CF patients in the US (one isolate per patient) (Table 7) and reported that 15 to 20% of multidrug resistant isolates remained susceptible to aztreonam.

Table 7. Activity of Aztreonam against Multidrug-Resistant Strains of *P. aeruginosa*

Organism	No. of Strains	MIC ($\mu\text{g/mL}$)			%S	%R
		Range	MIC ₅₀	MIC ₉₀		
<i>P. aeruginosa</i> , mucoid	200	0.25-> 512	64	> 512	20	71
<i>P. aeruginosa</i> , nonmucoid ^a	200	0.25-> 512	128	> 512	15	80

^a US Cystic Fibrosis Consensus definition of resistance to all agents tested in 2 of the following 3 classes of antibiotics: aminoglycosides, beta-lactams, and quinolones.

Source: Table 7, this submission.

MECHANISMS OF RESISTANCE

Multiple mechanisms of bacterial resistance to aztreonam and beta-lactam antibiotics have been identified. These mechanisms include the derepression of chromosomal beta-lactamases, the acquisition of beta-lactamase encoding plasmids, overexpression of efflux pump systems, impermeability of the outer membrane, and alteration of the drug target (PBPs).

In Gram-negative bacteria the prevalence of various resistance mechanisms varies by organism and two or more mechanisms may contribute to the overall resistance profile.

For *P. aeruginosa*, the relative frequency of each resistance mechanism has typically not been reported due to the interplay among multiple resistance mechanisms. The first study to systematically evaluate the contributions of six potential mechanisms of carbapenem resistance was published in May 2006; the authors concluded that for some isolates it was still not possible to fully explain the basis for their beta-lactam resistance.

In general, researchers have reported only on the presence or absence of specific beta-lactamases. In 1998, investigators evaluated 735 consecutively isolated *P. aeruginosa* isolates from 13 teaching hospitals in France. They reported that in non-CF *P. aeruginosa*, the most frequent mechanisms of resistance included:

- non-enzymatic resistance (14.3%),
- overproduction of a constitutive cephalosporinase (13.8%),
- production of a transferable beta-lactamase (8.6%) and
- a combination of these mechanisms (4.2%).

In contrast, for *P. aeruginosa* isolated from CF patients, resistance to beta-lactam antibiotics was primarily due to overproduction of a constitutive cephalosporinase (18.6%), and rarely due to production of a transferable beta-lactamase (1.4%). However, these researchers also reported that susceptibility to aminoglycosides and fluoroquinolones was lower in isolates producing beta-lactamases, thereby suggesting involvement of other mechanisms. Other researchers have noted that in the polymicrobial chronic infections of CF patients, resistant isolates that appear during treatment are often not present after treatment due to a selective growth advantage of the susceptible isolates in the absence of antibiotic.

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Beta-lactamases

Beta-lactamases are enzymes that hydrolyze the four-member, nitrogen-containing ring of beta-lactam antibiotics. Genes encoding beta-lactamases may be located on the bacterial chromosome or on plasmids, and expression may be constitutive or inducible. In Gram-negative bacteria, inducible beta-lactamases are invariably encoded by chromosomal genes whereas constitutively expressed enzymes may be either chromosomal or plasmid encoded.

Beta-lactamases are concentrated in the periplasmic space located between the outer and inner membranes of Gram-negative bacteria. Bacteria become resistant when the concentration of active drug in the periplasm falls below the level needed to inhibit PBPs. The degree of antibiotic degradation by beta-lactamases depends on the enzyme's affinity for a specific antibiotic, the rate of hydrolysis, and the amount of beta-lactamase produced.

Aztreonam is a poor inducer of beta-lactamase production and, although not used as such, is an inhibitor of many beta-lactamases.

The beta-lactamases are classified using the Ambler system into four groups (A, B, C, and D) according to their amino acid sequences. Classes A, C, and D beta-lactamases use a catalytically active serine molecule for inactivation of the beta-lactam drug, while Class B enzymes are metallo-enzymes requiring zinc for activity.

Class A Beta-lactamases

Two class A beta-lactamase families commonly encountered among *Enterobacteriaceae* include the TEM and SHV types, which primarily hydrolyze penicillins and have little activity toward aztreonam or cephalosporins. Of more concern are the clavulanic acid-inhibited Ambler Class A ESBLs that are broadly active against most beta-lactams, as well as aztreonam. The TEM- and SHV-type ESBLs have been extensively reported in *Enterobacteriaceae* but have been only rarely reported from *P. aeruginosa*. Non-TEM, non-SHV ESBLs reported to hydrolyze aztreonam include the VEB type (primarily Southeast Asia origin), PER type (Turkey), and GES and IBC types (France, Greece, and S. Africa).

Class B Beta-lactamases

The class B metallo-beta-lactamases (MBLs) hydrolyze a wide variety of beta-lactams, including carbapenems, but do not hydrolyze aztreonam. These enzymes are not inhibited by the serine beta-lactamase inhibitors clavulanate, sulbactam, and tazobactam. Within the group there are four main types of transferable MBLs: IMP, VIM, SPM-1 and GIM. While there have been rare reports of organisms with MBLs that are also resistant to aztreonam [VIM-5 (*K. pneumoniae*) and *P. aeruginosa*-Turkey], VIM-6 (*P. putida*-Singapore) and VIM-7 (*P. aeruginosa*-United States)], it is possible that these organisms were expressing multiple resistance mechanisms.

Reviewer's comments: Recently, a strain of *P. aeruginosa* was found to express an ESBL, GES-9 which differs from GES-1 by a Gly243Ser substitution [1]. This ESBL hydrolyzes aztreonam but is inhibited by clavulanic acid and imipenem. The gene for GES-9 is located inside a class 1 integron structure containing two copies of an insertion sequence.

Class C Beta-lactamases

Class C beta-lactamases are produced by almost all Gram-negative bacteria and hydrolyze cephalosporins more effectively than penicillins. AmpC, the best characterized enzyme of this class, is normally produced at low levels in clinical isolates of *P. aeruginosa* and *Enterobacteriaceae* but production can be induced to high levels by the presence of beta-lactams, especially cefoxitin and imipenem. The chromosomal AmpC gene has moved onto plasmids recovered from clinical isolates of *E. coli* and *K. pneumoniae*. High-level production of

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both chromosomal- and plasmid-encoded AmpC is responsible for cross-resistance to aztreonam and to multiple beta-lactam antibiotics.

Class D Beta-lactamases

Class D beta-lactamases preferentially hydrolyze penems, including oxacillin. Most of the genes encoding for OXA enzymes are chromosomal, but several have been encoded by genes associated with mobile elements; generally gene cassettes inserted into integrons. Over 121 variants of OXA enzymes have been described, of which 45 exhibit carbapenem hydrolyzing activity. The OXA-type carbapenemases generally have little or no activity against aztreonam, but there have been rare reports of oxacillinases from clinical isolates of *P. aeruginosa* (OXA-11 [Turkey] and OXA-45 [United States]) that have hydrolyzed aztreonam.

Impermeability of the Outer Membrane

Gram-negative bacteria are surrounded by a low permeability layer called the outer membrane, which serves as a barrier to antibiotics. Water-filled channels in the outer membrane, termed porins, allow diffusion of some particles, including antibiotics, into the bacterial cell. Some porins provide entry for a wide variety of drugs and others are specific for a single drug. In *P. aeruginosa*, porins have extremely low permeability to most beta-lactams and thereby play a major role in intrinsic resistance. Modifications in outer membrane porins may further decrease the amount of antibiotic entering the cell. Typically changes in permeability are strongly synergistic with beta-lactamase activity. Due to variability in porin specificity, alterations may lead to resistance to one or more antibiotics. An example of specific drug resistance is seen in loss of the OprD porin, which can result in resistance of *P. aeruginosa* to carbapenems but does not confer cross-resistance to non-carbapenem beta-lactams or aztreonam.

Active Efflux

If a beta-lactam antibiotic reaches the inside of the cell, it becomes subject to extrusion by active efflux. Efflux pumps transport drug from the cytoplasm across the inner and outer membranes to the outside of the bacterial cell. Overexpression of an efflux system increases bacterial resistance to any antibiotic that is a pump substrate.

Several chromosomally-encoded efflux systems play an important role in *P. aeruginosa* antibiotic resistance. The MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM systems extrude a wide variety of antibiotics including beta-lactams. Most beta-lactams, (e.g., penicillins, cepheems, and meropenem) are pump substrates for all of these efflux systems. In contrast, aztreonam can only be extruded by the MexAB-OprM pump system.

Alteration of PBPs

Clinical resistance associated with alteration of PBPs is exceedingly rare in *P. aeruginosa*, but cannot be ruled out. In 1981 two beta-lactam-resistant *P. aeruginosa* serotypes emerged in sputum from a CF patient receiving high-dose tobramycin and piperacillin. One serotype, K, had modified PBP3, while the other, serotype M, had numerous PBP modifications and demonstrated resistance to several beta-lactam antibiotics. Although aztreonam was not evaluated in this study, it is likely that these isolates would have increased resistance to aztreonam because its target, PBP3, was altered.

Frequency of Mutation

In vitro resistance frequencies for *P. aeruginosa* ATCC 27853 and 10 clinical isolates of *P. aeruginosa* were determined for aztreonam and beta-lactam antibiotics. Colonies that grew on antibiotic-containing media were randomly selected for susceptibility testing. The resistance frequency for ATCC 27853 and the clinical isolates was less than 1×10^{-8} in the presence or absence of aztreonam (500 µg/mL). When agar plates containing aztreonam were inoculated with 10^8 CFUs, a thin film of bacteria was visible after 72 hours incubation; microscopic examination

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and subculture revealed highly filamentous but viable bacteria that had retained susceptibility to beta-lactam antibiotics.

A modified Szybalski method was used to demonstrate that aztreonam, at 4 times the MIC, selected resistant variants of 10 *P. aeruginosa* isolates (two lab isolates, eight isolated from blood of cancer patients) *in vitro*. Mutants resistant to aztreonam were generated at a frequency of approximately 1×10^{-6} to 1×10^{-7} . Some mutants produced large amounts of cephalosporinase; however, aztreonam was stable to hydrolysis by this enzyme.

POST ANTIBIOTIC EFFECT

Post antibiotic effect (PAE) refers to the suppression of bacterial growth that persists after short-term exposure to an antibiotic. Aztreonam, like beta-lactam antibiotics, has minimal or no persistent effects on most organisms. The duration of PAEs for aztreonam, ceftazidime, and piperacillin against *P. aeruginosa* ATCC 27853 is less than 0.5 hour when tested at concentrations equivalent to four times, 16 times, and four times the MIC, respectively. By comparison, gentamicin (4 times the MIC), tobramycin (two times the MIC), and ciprofloxacin (4 times the MIC) have PAEs of 2.2, 2.1, and 2.4 hours, respectively, against *P. aeruginosa* ATCC 27853. Similarly, the PAE for imipenem against *P. aeruginosa* ATCC 27853 is 3.7 hours when tested at a concentration equivalent to two times the MIC and 4.1 hours at 4 times the MIC. Aztreonam produces PAEs of one hour or less against *E. coli* ATCC 25922 at concentrations up to 512 times the MIC.

A long PAE suggests the possibility of increasing the time interval between antibiotic doses. Because aztreonam does not have a demonstrable PAE for *P. aeruginosa*, the dosing schedule was determined on the basis of the pharmacokinetic (PK) parameters.

MISCELLANEOUS STUDIES

Pharmacodynamic Model

A pharmacodynamic model was developed to facilitate evaluation of aztreonam activity when delivered to an *in vitro*, mucin-covered epithelial cell surface. CF human airway cells were grown at an air-liquid interface to promote differentiation. Well-differentiated cells, which demonstrated mucous production and ciliary beating, were infected with a clinical isolate of *P. aeruginosa*, then treated with aerosolized aztreonam lysine (2 mL of 75 mg/mL) or Tobramycin Solution for Inhalation (TSI, TOBI®) (2 mL of 150 mg/mL). Bacteria were harvested from the culture surface and quantified. Although the model's reproducibility limited its utility, a significant decrease in CFUs was observed by 6 to 12 hours for both drugs with regrowth at 24 hours.

Enhancement of Phagocytic Function

Exposure of bacteria to aztreonam can modify the interactions between Gram-negative bacteria and phagocytic cells, resulting in enhanced phagocytic killing due to aztreonam-induced alterations in the bacterial cells.

In a murine peritoneal macrophage model, aztreonam concentrations below the minimum bactericidal concentration (MBC) (64 µg/mL) did not affect extracellular growth of *E. coli* strain 775, but significantly reduced the number of bacteria within macrophages. Concentrations above the MBC (up to 16 times MBC) reduced extracellular *E. coli* by 20% to 30% and intracellular *E. coli* by > 90% within one hour.

Exposure of *P. aeruginosa* ATCC 9721 and 11 clinical isolates of *Enterobacteriaceae* to aztreonam (0.05 to 20 times MIC and 4 times MIC, respectively) resulted in enhanced phagocytic killing by human polymorphonuclear leukocytes (PMNs). The enhanced killing required the presence of serum opsonins and was associated with increased uptake of bacteria by the PMNs. The observed effects were not due to any direct bactericidal effects of serum or to the direct

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action of aztreonam on the PMNs; the enhanced phagocytic killing was attributed to modification of bacterial cell surface structures upon exposure to aztreonam.

Sub-MIC concentrations of aztreonam enhanced the phagocytosis and killing of *E. coli* S615, *S. marcescens* US5, *K. pneumoniae* 12 and *P. aeruginosa* K1 by a murine macrophage cell line. With *E. coli*, a synergistic effect was seen with aztreonam and lysozyme or macrophage lysate. Additionally, aztreonam increased the sensitivity of *E. coli* to the macrophage products hydrogen peroxide and superoxide anion. Results of this study suggested that alterations in the bacterial cells by aztreonam increased their susceptibility to macrophage bactericidal substances.

Effect on Gastrointestinal Flora

Aztreonam has the potential to alter the normal gastrointestinal flora, thereby decreasing patients' resistance to colonization with other pathogens such as *Candida* and *Clostridium* spp.

Overgrowth of such organisms, notably *Clostridium difficile*, can lead to pseudomembranous colitis. Although animal models failed to show evidence of *C. difficile* or its cytotoxin, *C. difficile*-associated diarrhea or colitis has been reported rarely (3 of 2,388 patients) following treatment with parenteral aztreonam. However, systemic exposure to aztreonam is very low and is unlikely to lead to pseudomembranous colitis.

Biofilm Studies

In chronic airway infections in CF patients, *P. aeruginosa* is likely to exist in both planktonic and biofilm phases. The presence of a biofilm in CF helps to explain why *P. aeruginosa* lung infections are rarely eradicated with antibiotic therapy, regardless of the organism's reported *in vitro* susceptibility. To date, *in vitro* antibiotic susceptibility tests used to support clinical practice have been performed on planktonically growing bacteria. Recent reports have suggested that these tests often are not good predictors of clinical response in CF, with patients having resistant isolates responding to treatment and the converse, patients with susceptible isolates not responding to treatment. Recently a methodology to test antibiotic susceptibility within a biofilm was developed with the hope that it might be more predictive.

As with beta-lactams, biofilm inhibitory concentrations (BICs) for aztreonam are much higher than conventionally determined MICs. Against 85 clinical isolates of *P. aeruginosa* from the US, the aztreonam MIC₅₀ and MIC₉₀ were 4 and 32 µg/mL, respectively, whereas the BIC₅₀ and BIC₉₀ were both > 128 µg/mL. The clinical utility of this methodology to predict response to therapy has not yet been shown. Despite their low activity in biofilms, beta-lactam antibiotics given as single agents have been shown to reduce symptoms of pulmonary exacerbation and inflammatory markers associated with lung damage in CF patients.

Effect of Nebulization on Drug Product Potency

To determine whether nebulization impacts the potency of aztreonam, the *in vitro* activity of aztreonam lysine was tested before and after nebulization against 161 clinical isolates of *B. cepacia* complex, *S. maltophilia*, *A. xylosoxidans*, *S. aureus*, and *P. aeruginosa* from CF patients. Aztreonam lysine was nebulized using the investigational eFlow, which uses a vibrating perforated membrane to produce a fine mist of antibiotic solution; the solution was trapped following nebulization and subsequently incorporated into broth microdilution MIC testing trays. Nebulization of aztreonam did not significantly affect its activity against CF pathogens. Of the 161 isolates tested, 82% had little or no change in MIC values after nebulization; 63 (39%) had identical MICs and 69 (43%) had MICs within one dilution (plus or minus) between pre and post nebulization. The MICs for the remaining isolates (N = 29, 18%) were more variable, with a slight tendency toward decreased activity of aztreonam after nebulization. In aggregate, these results support the physical finding that the antimicrobial activity of aztreonam is not significantly reduced during nebulization by the eFlow device.

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Reviewer's comments: A recent report by Gasink et al. concluded that curbing the use of fluoroquinolones and antimicrobials with anti-anaerobic activity may be an effective strategy to limit the emergence of aztreonam-resistant *P. aeruginosa* [2]. These investigators identified risk factors for infection or colonization with aztreonam-resistant *P. aeruginosa*. Nosocomial mortality was greater among patients infected or colonized with aztreonam-resistant *P. aeruginosa* compared with those infected with aztreonam-susceptible *P. aeruginosa*.

ANTIMICROBIAL INTERACTIONS

The *in vitro* activity of aztreonam combined with other antibiotics has been studied for a variety of Gram-negative bacteria. The frequency of synergistic effects varies depending on the specific antibiotic combinations and bacterial isolates studied.

Aztreonam is not antagonistic with the antibiotics commonly used on- and off-label to treat respiratory infections in CF patients, suggesting that clinical use of aztreonam lysine in conjunction with another antibiotic to treat CF respiratory infections will not reduce the antibacterial activity of either drug due to antagonistic interactions.

Beta-lactams

When *P. aeruginosa* or *Enterobacteriaceae* are treated with aztreonam in combination with beta-lactam antibiotics, the interactions are typically indifferent or synergistic.

In a study of 14 multidrug-resistant isolates of *Enterobacteriaceae* (N = 10) and *P. aeruginosa* (N = 4 [ATCC 27853 and three clinical isolates]), synergy was noted with 42% (6 of 14) of the organisms when aztreonam was combined with any of the beta-lactam antibiotics tested (piperacillin, moxalactam, cefotaxime, or ceftazidime); indifferent effects were observed with all other organisms. When determinations were based on MBC results, antagonism was observed for one isolate each of *P. aeruginosa* (aztreonam in combination with piperacillin or with moxalactam) and *K. pneumoniae* (aztreonam in combination with moxalactam).

The synergistic activity of aztreonam with ceftazidime was studied in 96 isolates of *P. aeruginosa* and 20 isolates of *B. cepacia* from CF patients. Synergy was observed with 29% of *P. aeruginosa* isolates but not in any *B. cepacia* isolate. No antagonism was observed for any combination.

Investigators have demonstrated that combinations of aztreonam plus cefepime or piperacillin resulted in indifference against 98 and 99 of 100 *P. aeruginosa* isolates, 22 of which were resistant to piperacillin monotherapy. Synergy was observed with the remaining *P. aeruginosa* isolates.

It has been reported that interactions between aztreonam and imipenem were generally indifferent for *P. aeruginosa* (N = 40), whereas interactions with cephalosporins were predominantly partially synergistic (Table 8). No antagonism was observed.

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Table 8. Interactions of Aztreonam with Beta-lactam Antibiotics

Organism (No. of Isolates)	Co-drug (% of observations) ^a		
	Ceftazidime	Cefepime	Imipenem
<i>P. aeruginosa</i> (N = 40)			
Synergy ^b	7.5	2.5	0.0
Partial synergy ^c	77.5	72.5	17.5
Additive ^d	12.5	22.5	17.5
Indifferent ^e	2.5	2.5	65.0
<i>Enterobacteriaceae</i> (N = 40)			
Synergy ^b	5.0	12.5	32.5
Partial synergy ^c	80.0	52.5	55.0
Additive ^d	7.5	17.5	10.0
Indifferent ^e	7.5	17.5	2.5

a No indeterminate results were recorded. b Synergy = decrease in MIC of each agent $\geq$ 4-fold. c Partial synergy = decrease in MIC $\geq$ 4-fold for one agent and 2-fold for the other. d Additive = 2-fold reduction in MIC for both agents. e Indifference = any interaction that met none of the preceding criteria.

Source: Table 8, this submission.

Quinolones

Combinations of aztreonam with quinolones sometimes result in synergy, but indifferent or additive effects are more commonly observed.

The synergistic activity of aztreonam with ciprofloxacin was studied using 96 isolates of *P. aeruginosa* and 20 isolates of *B. cepacia* from CF patients. Synergy was observed against 30% of *P. aeruginosa* isolates and 15% of *B. cepacia*, but no synergy was observed for the aztreonam-ceftazidime combination against *B. cepacia*. No antagonism was observed.

Investigators have demonstrated that combinations of aztreonam plus ciprofloxacin resulted in indifference against 100 of 100 *P. aeruginosa* isolates.

Other workers found interactions between aztreonam and three quinolones to be additive or partially synergistic against a majority of recently isolated *P. aeruginosa* and *Enterobacteriaceae* strains (Table 9). No antagonism was observed.

Table 9. Interactions of Aztreonam with Quinolones

Organism (No. of Isolates)	Co-drug (% of Observations) ^a		
	Ciprofloxacin	Gatifloxacin	Levofloxacin
<i>P. aeruginosa</i> (N = 40)			
Synergy ^b	12.5	10.0	12.5
Partial synergy ^c	45.0	57.5	52.5
Additive ^d	37.5	25.0	27.5
Indifferent ^e	5.0	7.5	7.5
<i>Enterobacteriaceae</i> (N = 40)			
Synergy ^b	2.5	12.5	10.0
Partial synergy ^c	52.5	37.5	42.5
Additive ^d	17.5	25.0	22.5
Indifferent ^e	27.5	25.0	25.0

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a No indeterminate results were recorded. b Synergy = decrease in MIC of each agent $\geq$ 4-fold. c Partial synergy = decrease in MIC $\geq$ 4-fold for one agent and 2-fold for the other. d Additive = 2-fold reduction in MIC for both agents. e Indifference = any interaction that met none of the preceding criteria.

Source: Table 9, this submission.

One investigator examined interactions between aztreonam and ciprofloxacin, levofloxacin, gatifloxacin or moxifloxacin using the checkerboard method. Interactions were evaluated against four isolates each of *P. aeruginosa*, *B. cepacia*, *E. cloacae*, *K. pneumoniae*, *E. coli* and *P. mirabilis*. All combinations demonstrated uniformly additive activity against *P. aeruginosa*. Synergy was isolate dependent and occurred less frequently than additive activity against non-*P. aeruginosa* isolates.

Aminoglycosides

In vitro interactions between aztreonam and aminoglycosides have been shown to be synergistic against *P. aeruginosa*, *Enterobacteriaceae*, and other Gram-negative aerobic bacilli.

Synergy of aztreonam and amikacin was demonstrated against 45% (10 of 22) and 41% (9 of 22), respectively, of amikacin-resistant (i.e., MICs > 64 μ g/mL) strains of *P. aeruginosa* and *B. cepacia* isolated from CF patients.

In a study of 14 multidrug-resistant isolates of *Enterobacteriaceae* (N = 10) and *P. aeruginosa* (N = 4), synergy was noted with 71% (10 of 14) of the organisms when aztreonam was combined with amikacin; indifferent effects were observed with the other four organisms. Regardless of whether determinations were based on MIC or MBC results, no antagonism was observed.

Investigators reported that aztreonam combined with tobramycin, gentamicin, amikacin or netilmicin was synergistic for 50% to 70% and additive for 30% to 50% of *P. aeruginosa* isolates (N = 10). No antagonism was observed.

Investigators demonstrated that the combination of aztreonam and tobramycin was synergistic with 10 of 16 (62%) *P. aeruginosa* isolates. No antagonism was noted.

Aztreonam and amikacin were synergistic against 28 of 30 (93%) multidrug-resistant *P. aeruginosa* isolates from urine, blood, sputum and wound cultures from patients in Greece. Combinations of aztreonam with netilmicin, gentamicin, or tobramycin were also synergistic with 83% to 97% of the *P. aeruginosa* isolates tested.

Aztreonam and amikacin were synergistic against 16 of 100 clinical isolates of *P. aeruginosa* isolates from southern Taiwan. The remaining 84 interactions were indifferent. Among the 22 isolates in this study that were resistant to piperacillin, synergy was observed for four isolates (18%).

PRECLINICAL EFFICACY—IN VIVO

ANIMAL MODELS OF EFFICACY

As there are no reliable animal models for cystic fibrosis, no animal efficacy studies were presented.

PHARMACOKINETICS

AI is delivered using the PARI eFlow electronic nebulizer (eFlow). The eFlow produces an aerosol with a relatively monodispersed particle spectrum (average mass median diameter 2.5 μ m, geometric standard deviation approximately 1.6), which is well-suited for lower airway drug deposition.

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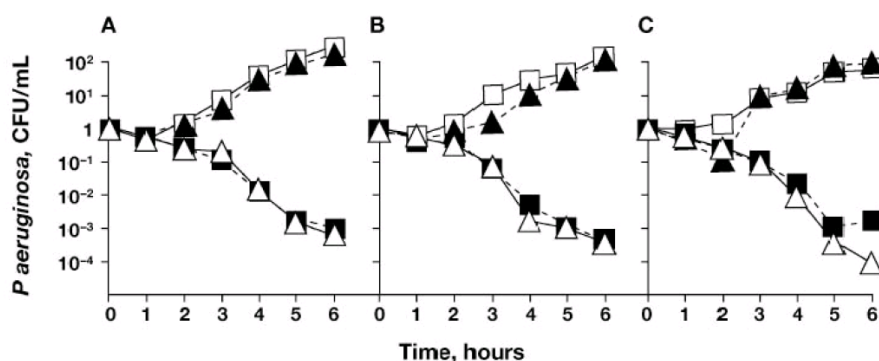
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Reviewer's Note: Aztreonam for Inhalation is delivered via an aerosol to the targeted organs. Thus, this drug is essentially a topical antibiotic and does not have the same pharmacokinetic properties of a paternally administered antibiotic.

Effect of CF Sputum on *In Vitro* Activity of Aztreonam

The airways of CF patients contain copious amounts of sputum, which contains mucins, glycoproteins, DNA, lipid and cellular debris. Sputum provides a growth environment for bacteria and can significantly inhibit the activity of antibiotics. Gibson et al. evaluated aztreonam lysine time-kill kinetics for *P. aeruginosa* American Type Culture Collection (ATCC) 27853 using aztreonam concentrations equivalent to 0.1 times, 1 time, and 10 times the MIC (Figure 2).

Figure 2. Lack of Inhibition of Aztreonam Lysine Activity in the Presence or Absence of CF Sputum or Porcine Mucin



Source: Figure 2, this submission.

Aztreonam lysine was added to bacterial cultures (A) without sputum or porcine mucin (control), (B) with 1% CF sputum, or (C) with 10% weight/volume mucin to yield a final antibiotic concentration of 0 (open squares), 0.1 (closed triangles), 1.0 (closed squares), and 10 (open triangles) times the MIC. Cultures were exposed to aztreonam lysine for the indicated time period, and then plated for quantitation of bacterial colony-forming units (CFUs).

No killing was observed at 0.1 times the aztreonam MIC. Bactericidal levels of killing ($= 3 \log_{10}$) were reached by five hours, and the speed and degree of killing were comparable at aztreonam concentrations of 1 time and 10 times the MIC. The killing kinetics of aztreonam against *P. aeruginosa* ATCC 27853 in the presence of 1% CF sputum and 10% porcine mucin were nearly identical to those conducted in media alone. By comparison, the activity of aminoglycosides is known to be antagonized by sputum.

Similar time-kill studies were conducted with 20 CF *P. aeruginosa* isolates using 10% porcine gastric mucin as a model for the protein-binding component of sputum. Aztreonam MIC values for the 20 isolates ranged from 0.25 to 64 $\mu\text{g/mL}$. Nine of the 20 isolates used in the study had a mucoid morphotype although no differences in killing were observed between mucoid and non-mucoid isolates. CFU reduction was similar at 1 time and 10 times the MIC in either the presence or absence of mucin. Most clinical isolates (18/20 at 10 times the MIC, and 14/20 at 1 time the MIC) showed at least a 1- to 2- $\log_{10}$ CFU reduction at six hours of exposure. In these experiments, aztreonam was bactericidal at 10 times the MIC for *P. aeruginosa* ATCC 27853 and one of the 20 clinical isolates.

The sputum and mucin kill-curve data suggest that aztreonam administered as an aerosol to the CF lungs will not be significantly inhibited by sputum.

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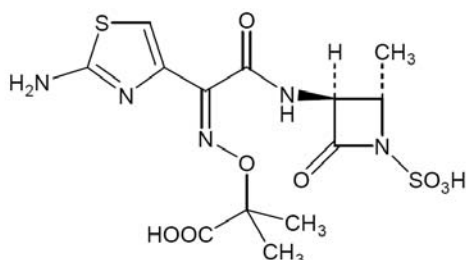
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Pharmacokinetics of Aztreonam Following Parenteral Administration

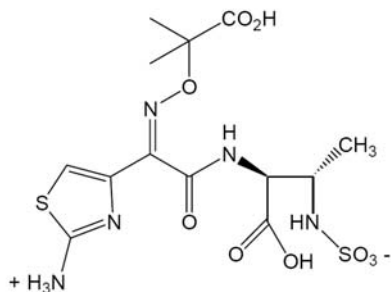
Nonclinical studies of ^{14}C -aztreonam disposition following IV and subcutaneous doses to dogs or IV and IM doses to monkeys and rats showed that the predominant route of excretion of radioactivity in rats and dogs was via the urine, while approximately equal amounts were excreted in the urine and feces of monkeys. Across species, 77% to 86% of urinary radioactivity was excreted as unchanged aztreonam, while SQ 26,992, a microbiologically inactive metabolite formed by hydrolysis of the monobactam ring, accounted for 10% to 15% of the urinary radioactivity (Figures 3 and 4). Biliary excretion of parent and metabolite-associated radioactivity was demonstrated in rats and, presumably, also occurred in monkeys. Aztreonam was metabolized most extensively in the monkey, and aztreonam and SQ 26,992 accounted for most of the radioactivity present in the sera of dogs and monkeys.

Figure 3. Structure of Aztreonam



Source: Figure 2.7.2.1-1, this submission.

Figure 4. Structure of SQ26,992



Source: Figure 2.7.2.1-2, this submission

Aztreonam was reported to be bound to serum proteins only to the extent of 28% to 35% in dogs and 49% to 59% in monkeys.

Following administration of an intramuscular dose of ^{14}C -aztreonam to rats, radioactivity was rapidly distributed to most tissues, including the lung. Tissue concentrations of total radioactivity and unchanged aztreonam declined more slowly in the lung than did serum levels, suggesting that the duration of activity of aztreonam in some tissues may be longer than predicted from serum concentration-time curves.

Aztreonam treatment decreased hepatic microsomal P-450 content and associated metabolic activities in male rats which were attributed to a decrease in P-450 2C11. It did not have the same effect in females. In monkeys, repeated treatment with aztreonam resulted in a decrease in hepatic microsomal content of P-450 3A.

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HUMAN PHARMACOLOGY

About 66% of a 500 mg IV or IM dose of ^{14}C aztreonam administered to healthy volunteers was excreted as unchanged drug in urine, with 1% as unchanged aztreonam in the feces over 144 hours after dosing. Total radioactive dose recovery approximated 90%, and excretion of unchanged aztreonam in urine was essentially complete by six hours. The monobactam ring-opened, inactive metabolite SQ 26,992 accounting for 7% of the dose in urine and 3% in feces, was excreted in urine more slowly than aztreonam. Aztreonam accounted for all of the antimicrobial activity present in serum. Aztreonam was reported to be bound to human serum proteins to the extent of 56% to 71%.

There are numerous literature reports on the pharmacokinetics of aztreonam in adults and children after IV or IM injection, and information is also presented in the package insert for aztreonam for injection (Azactam). Pharmacokinetics in healthy subjects have been described following single, 500 mg, IV and IM doses, and following multiple 500 mg and 1 g doses given IV and IM every eight hours for seven days. Following single doses, the average elimination half-life ($t_{1/2}$) was 1.6 to 1.7 hours, and this did not change on multiple dosing. In the multiple-dose study, mean peak serum concentrations (C_{max}) were 39 and 99 $\mu\text{g/mL}$ after the initial 2-minute IV infusions of 500 mg or 1 g, respectively. Following IV multiple-dose administration, the mean steady-state volume of distribution (V_{ss}) was 0.21 L/kg, serum clearance (CL) was 1.7 mL/min/kg, and urinary recovery of unchanged aztreonam was 60% of the dose. An average of about 6% of a 1 g IM dose was excreted in the eight-hour urine collection on Day 8 as metabolite SQ 26,992. Investigators have demonstrated that oral bioavailability of aztreonam, administered as a 500 mg dose in solution or capsule formulation, was less than 1%.

A study in healthy elderly volunteers (aged 65 to 75 years) reported on pharmacokinetics in the elderly with normal renal function [$t_{1/2}$ 2.06 hours, area under the concentration time curve from zero to 24 hours (AUC_{0-24}) 232 $\mu\text{g}\cdot\text{hr/mL}$, CL 0.94 mL/min/kg]. Other investigators reported a somewhat longer half-life (mean 2.67 hours) in nine elderly patients aged 64 to 82 years, given a 1 g IV dose of aztreonam. In another study, Investigators demonstrated differences in some pharmacokinetic parameters between elderly (half-life 3.1 hours, area under the concentration versus time curve extrapolated to infinite time ($\text{AUC}_{0-\infty}$) 469 $\mu\text{g}\cdot\text{hr/mL}$) and young ($t_{1/2}$ 1.8 hours, AUC_{0-8} 294 $\mu\text{g}\cdot\text{hr/mL}$) healthy volunteers following a 2-g IV dose of aztreonam. Similar observations were made after a dose of 1 g. These changes were probably due to decreased renal function in the elderly group, as indicated by decreased creatinine clearance.

Total clearance of aztreonam was correlated with corrected creatinine clearance (CLCR) by investigators in a study of pharmacokinetics in patients with CL_{CR} ranging from < 10 to > 80 mL/min. They reported mean elimination half-lives ranging from two hours in normal patients to six hours in anephric patients, with total clearance of aztreonam varying from 107 mL/min to 29 mL/min in these patient groups.

The pharmacokinetics of aztreonam in children aged from newborn to approximately 12 years old were evaluated after a 30-mg/kg single IV dose. Aztreonam pharmacokinetics in children from 2 years to 12 years old was comparable to those in adults. Clearance decreased and half-life increased with decreasing age to values of 0.94 mL/min/kg and 5.71 hours, respectively, for children less than seven days old and weighing less than 2,500 g. In a separate study, pharmacokinetics in ten male CF patients, aged 10 to 18 years, following a 30-mg/kg IV dose, appeared to be generally similar to those in 2 year to 12 year old non-CF patients.

Taken together, these data indicate that following parenteral administration to humans, aztreonam is eliminated from the body rapidly and predominantly unchanged in the urine. The metabolite SQ 26,992 circulates in plasma and is excreted in relatively small amounts; however, this metabolite is microbiologically inactive. Clearance of aztreonam and SQ 26,992 decreases,

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and apparent elimination half-lives increase with decreasing renal function in the elderly and in patients with compromised renal function. This consistency in pharmacokinetics of aztreonam when given parenterally is expected to apply to pharmacokinetics of the much reduced fraction of aztreonam which is systemically available following administration by inhalation. There should also be little difference in aztreonam pharmacokinetics in children and CF patients compared to adults, with the exception of very young children. Given the poor oral bioavailability of aztreonam, the fraction of the inhaled dose that is swallowed will lead to no meaningful additional systemic exposure.

Furthermore, an *in vitro* study (Inveresk Report 22175) using hepatic and lung microsomes from rat, dog, and humans showed no oxidative metabolism in either matrix in any species, suggesting that no unexpected biotransformation products would be formed following pulmonary administration of aztreonam. These data are in accord with *in vivo* data in animals and humans, demonstrating that no significant oxidative metabolites are formed via cytochrome P-450 involvement and that the major biotransformation product of aztreonam is SQ 26,992, an inactive analog formed by hydrolysis.

Pharmacokinetics of Aztreonam following Inhalation Administration of AI

The pharmacokinetics of aztreonam following inhalation of AI was assessed in a total of five clinical studies. There were two Phase 1 ascending-dose studies: one in healthy volunteers (CP-AI-001) and one in CF patients (CP-AI-002). There was one Phase 2 multicenter study where CF patients with pulmonary PA received one of two twice daily (BID) dose levels (75 mg or 225 mg, CP-AI-003), one multicenter Phase 3 study of BID or TID AI (75 mg) in CF patients with pulmonary PA (CP-AI-005), and one multicenter Phase 3 study with TID dosing of 75 mg AI in CF patients with pulmonary PA (CP-AI-007). Sputum aztreonam concentrations were determined in all of these studies except the Phase 1 volunteer trial CP-AI-001. Individual study data are summarized in Section 2.7.2.2, this submission. The characterization of sputum concentrations and plasma pharmacokinetics of inhaled aztreonam are summarized in Section 2.7.2.3 this submission.

CLINICAL LABORATORY SUSCEPTIBILITY TEST METHODS

A single sputum sample from a CF patient may contain multiple morphotypes of *P. aeruginosa* and each morphotype may have a different level of *in vitro* susceptibility. The approved antimicrobial susceptibility test methods and quality control parameters used for parenteral aztreonam therapy were used to monitor the activity of AI against *P. aeruginosa* isolates from CF patients. It should be noted that susceptibility testing of *P. aeruginosa* isolates from CF patients may require extended incubation up to 24 hours for accurate determination of MIC values.

CLINICAL MICROBIOLOGY METHODS

Microbiology data were collected in four studies of AI in the treatment of CF patients with pulmonary *P. aeruginosa*: one Phase 2 placebo-controlled study (CP-AI-003), two Phase 3 placebo-controlled studies (CP-AI-005 and CP-AI-007), and one open-label follow-on study (CP-AI-006). Extensive microbiology methodology is found in the study reports included in this submission.

Sputa were collected at all patient visits (1—7) of each study for analysis of microbiology endpoints. Sputum samples were processed for qualitative and quantitative culture of *P. aeruginosa* (each morphotype), *S. aureus*, *S. maltophilia*, *A. xylosoxidans* and *B. cepacia* complex. In addition, qualitative and quantitative culture data for *Candida* spp. and *Aspergillus* spp. were collected in the CP-AI-006 study. The *in vitro* susceptibility of all *P. aeruginosa* and *B. cepacia* isolates to aztreonam and other selected antibiotics was determined using standard susceptibility testing methods. Established susceptibility breakpoints for parenteral administration

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of aztreonam using agar or broth dilution are ≤ 8 $\mu\text{g/mL}$ (susceptible), 16 $\mu\text{g/mL}$ (intermediate), and ≥ 32 $\mu\text{g/mL}$ (resistant) [3].

Sputum Collection

Sputum samples (minimum of 0.5 mL) were collected at each patient visit in sterile 50 mL tubes; if the patient was unable to produce sputum, an oropharyngeal swab was collected instead. Since collection of a swab rather than sputum significantly limits microbiology testing, every effort was made to collect expectorated (not induced) sputum.

Sputum for microbiology testing was collected at least four hours after the most recent dose of AI. The date and time of sputum collection were recorded. After collection, a sterile swab was inserted into the most purulent section of sputum and a small amount of the sputum was spread onto a glass slide. This procedure was repeated twice in order to prepare two slides.

Sputum Storage and Transport

Sputum was generally shipped for microbiology testing on the day of collection. Sputum was stored refrigerated ($2 - 8^{\circ}\text{C}$) and swabs were stored at room temperature prior to shipment to the central laboratory. Sputum samples were sent on frozen gel packs and swabs on ambient gel packs by overnight mail. Upon receipt, the shipping time and conditions were recorded. Sputum samples were rejected if they met any one of the following criteria: sample was more than 48 hours old upon arrival, sample arrived with no gel pack or a warm gel pack, sample was leaking or frozen, or quantity of sample was not sufficient for microbiology testing.

Pathogen Isolation and Susceptibility Testing

Gram stains were performed on the two sputum slides to assess the quality of the sample. The macroscopic appearance of the sputum was recorded as mucoid or non-mucoid and purulent (containing pus) or nonpurulent (no pus present).

Biochemical identification and quantitation of *P. aeruginosa* (each morphotype), *S. aureus*, *S. maltophilia*, *A. xylosoxidans* and *B. cepacia* were performed in accordance with current clinical laboratory standards [4].

In vitro susceptibility testing was performed on all *P. aeruginosa* and *B. cepacia* isolates using custom minimum inhibitory concentration (MIC) panels containing the following antibiotics: aztreonam, tobramycin, amikacin, cefepime, ceftazidime, ciprofloxacin, gentamicin, meropenem, piperacillin, and ticarcillin/clavulanate [5]. Quality control (QC) organisms (*Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212) were also tested against appropriate concentrations of the selected antibiotics according to standard procedures for testing patient isolates [3]. Susceptibility of each pathogen to the antibiotics tested was assessed using established breakpoints [4].

QUALITY CONTROL

Aztreonam (as AZACTAM) has been approved by the Agency for treatment of the following indications caused by *Pseudomonas aeruginosa*: urinary tract infection, lower respiratory tract infection, septicemia, skin and soft structure infection and intra-abdominal infection. In addition, since AI is a topically administered antibiotic, interpretive criteria will not be determined. Therefore, quality control parameters were not determined.

PROVISIONAL INTERPRETIVE CRITERIA

Aztreonam has been approved by the Agency for other indications as noted above. Therefore, provisional interpretive criteria were not determined.

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CLINICAL EFFICACY

Reviewer's Note: The Applicant presents the protocols and results from four clinical trials: one phase 2 trial, [Study AI-003](#), and three phase 3 trials. Among the three phase 3 trials were two pivotal studies, [Study AI-005](#) and [Study AI-007](#), as well as a follow-on study, [AI-006](#), which will not be discussed in detail.

Study CP-AI-003: A Blinded, Multicenter, Randomized, Placebo-Controlled Trial With Aztreonam for Inhalation (AI) In Cystic Fibrosis Patients with Lung Disease Due to *P. aeruginosa* Infection

OBJECTIVES

To assess the safety and efficacy of 14-day treatment with AI at two dosage levels compared to placebo in CF patients with pulmonary *P. aeruginosa* (PA) infections

METHODOLOGY

Up to 138 CF patients were to be randomized into three cohorts (75 mg AI, 225 mg AI, and placebo). Patients were screened for inclusion in the trial within seven days of the start of the treatment period. After randomization, each patient was instructed on the use of the eFlow® Electronic Nebulizer (eFlow). Patients were instructed to self-administer one dose twice-daily for 14 days, with the two daily doses being separated by at least eight hours. Patients self-administered the first dose and one of the Day 7 doses at the clinic (Visits 2 and 3), and the remaining doses were self-administered at home. Patients returned to the clinic at the end of the treatment period (Day 14, Visit 4) and for a follow-up visit 14 days after the end of the treatment period (Day 28, Visit 5).

NUMBER OF PATIENTS PLANNED, ENROLLED, AND ANALYZED

	Treatment			Total
	Placebo	75 mg AI	225 mg AI	
Planned	46	46	46	138
Safety Dataset ^a	31	37	37	105
ITT Dataset ^a	32	38	35	105
PP Dataset ^a	29	34	31	94

^a Some patients did not receive their treatments as randomized. Data are presented for patients by treatment as received in the Safety and PP datasets, and for patients by treatment as randomized in the ITT dataset. Diagnosis and Main Criteria for Inclusion: CF patients chronically infected with PA, with FEV₁ values = 40% of predicted, 13 years of age and above.

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION

AI (75 mg/mL sterile pyrogen-free aztreonam lysinate, pH 4.2 to 7.0 and osmolality 300 to 550 mOsmol/kg) dosed at 75 or 225 mg (in 1 and 3 mL, respectively), self-administered to the lungs using the eFlow. Placebo was reference therapy with volume-matched to active trial drug (1 and 3 mL), self-administered to the lungs using the eFlow.

DURATION OF TREATMENT

Up to 37 days, including a variable screening period of up to 7 days, 14-day treatment period, and a follow-up visit 14 days after the last dose of trial drug was administered.

CRITERIA FOR EVALUATION OF EFFICACY

The primary efficacy variable was the percent change from pretreatment Day 0 to Day 14 in FEV₁.

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Secondary efficacy variables were as follows:

- Percent change from pretreatment Day 0 to pretreatment Day 7 in FEV₁ (L)
- Absolute change from pretreatment Day 0 to pretreatment Day 7 in FEV₁ (L)
- Absolute change from pretreatment Day 0 to Day 14 in FEV₁ (L)
- Absolute change from pretreatment Day 0 to Day 14 in FEV₁ percent of predicted
- Absolute change from pretreatment Day 0 to pretreatment Day 7 in FEV₁ percent of predicted
- Percent change from pretreatment Day 0 to Day 14 in FVC (L)
- Absolute change from pretreatment Day 0 to Day 14 in FVC (L)
- Percent change from pretreatment Day 0 to pretreatment Day 7 in FVC (L)
- Absolute change from pretreatment Day 0 to pretreatment Day 7 in FVC (L)
- Percent change from pretreatment Day 0 to Day 14 in FEF₂₅₋₇₅ (L/sec)
- Absolute change from pretreatment Day 0 to Day 14 in FEF₂₅₋₇₅ (L/sec)
- Percent change from pretreatment Day 0 to pretreatment Day 7 in FEF₂₅₋₇₅ (L/sec)
- Absolute change from pretreatment Day 0 to pretreatment Day 7 in FEF₂₅₋₇₅ (L/sec)
- Change in log₁₀ PA colony-forming units (CFUs) in sputum (CFUs/g sputum) from Day 0 to Day 7 and Day 14.
- Number and percent of patients unable to expectorate sputum at Day 14 (Visit 4)
- Hospitalizations and emergency treatment
- Antibiotic use

CRITERIA FOR OTHER EVALUATIONS

The following microbiology variables were assessed:

- Disappearance or appearance of other pathogens (*Staphylococcus aureus*, *Burkholderia cepacia* [appearance only], *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*) from Day 0 to Day 7 and to Day 14
- Change in MIC₅₀ and MIC₉₀ of aztreonam for PA from Day 0 to Day 28
- Post hoc exploratory analyses were performed by the sponsor to assess data by disease severity, with the disease severity categories separated at 75% rather than at 60%, and by BD use (BD users and non-users). By-subgroup analyses were performed on baseline and demographic data, change in log₁₀ CFU data, and spirometry change data. Spirometry change data were also analyzed by placebo subgroup (placebo-75 and placebo-225). Extent of exposure data were re-analyzed by BD use. Duration of nebulization data were summarized.

CLINICAL EFFICACY RESULTS

There was an increase in adjusted mean percent change in FEV₁ (L) from Day 0 to Day 14 in all treatment groups (4.08% in the placebo group, 6.20% in the 75 mg AI group, and 7.71% in the 225 mg AI group). There was no significant difference in the mean percent change in FEV₁ from Day 0 to Day 14 between the 225 mg AI and placebo treatment groups, either overall (treatment difference: 3.62%; CI: -1.50, 8.75) or for any of the subsets tested (disease severity [$\leq 60\%$ FEV₁ percent predicted], age, gender, and MIC category [≥ 8 $\mu\text{g/mL}$]) for the ITT or PP datasets. There was an increase in adjusted mean percent change in FEV₁ from Day 0 to Day 7 in all treatment groups (5.03% in the placebo group, 7.59% in the 75 mg AI group, and 9.74% in the 225 mg AI group). The difference of 4.71% between the 225 mg AI and placebo groups was statistically significant (CI: 0.06, 9.35), however, it was not confirmed by the PP dataset.

There was a mean reduction in log₁₀ PA CFUs at Days 7 and 14 in both AI treatment groups, and the mean reductions in the 225 mg group were slightly larger than those in the 75 mg AI group. The difference (CI) between the 225 mg AI and placebo groups in change in log₁₀ PA CFUs from Days 0 to 14 of -2.146 (-3.029, -1.263) was highly statistically significant (p-value < 0.0001).

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There was a potential loss of power in the trial because there were only 105 evaluable patients in the ITT dataset, which was less than the planned sample size of 138, and this may have contributed to the lack of statistical significance shown in the results.

MICROBIOLOGY RESULTS

The majority of patients in each treatment group showed no changes in the presence or absence of the pathogens tested. After dosing, PA infection disappeared from two patients in the 75 mg AI group (Day 28), and three and seven patients in the 225 mg AI group (Days 7 and 14, respectively). PA infection did not disappear at any postdosing day for any patients in the placebo group. There were no notable differences between treatments in the numbers of patients becoming positive after dosing for any of the pathogens tested (*S. aureus*, *B. cepacia*, *S. maltophilia*, or *A. xylosoxidans*). There were no notable differences between treatment groups in the numbers of patients who were positive at Day 0 and became negative for any of the other pathogens tested.

There were no notable increases from Day 0 in MIC50 or MIC90 (using all PA isolates and the PA isolate with the highest MIC from each patient) in any treatment group, indicating there was no evidence of reduced susceptibility of PA to aztreonam. There were no notable increases from Day 0 in the numbers of patients whose PA isolate with the highest MIC was above the parenteral susceptibility breakpoint for aztreonam (≥ 8 µg/mL) in either AI treatment group.

MICROBIOLOGY

Variables

- Disappearance or appearance of pathogens (PA, *S. aureus*, *B. cepacia* [appearance only], *S. maltophilia*, *A. xylosoxidans*) from Day 0 to Day 7 and Day 14. CFU counts in sputum for each pathogen of interest were measured at Days 0 (Visit 2), 7 (Visit 3) and 14 (Visit 4), and a binary indicator was created, where 0 = presence and 1 = absence of each pathogen, at each visit.
- Change in MIC50 and MIC90 of aztreonam (µg/mL) for PA from Day 0 to Day 28.

Methods of Analysis

- Presence or absence of PA, *S. aureus*, *B. cepacia*, *S. maltophilia* and *A. xylosoxidans* separately was summarized (n and percent) at Day 0, Day 7, Day 14, and Day 28 by disease severity (FEV₁ percent predicted $\leq 60\%$) and overall and by treatment, including shifts from Day 0.
- MIC50 and MIC90 of aztreonam for all PA isolates (µg/mL) at Day 0, Day 14, and Day 28 were both presented by treatment, visit, disease severity, and overall, with the range of MICs, including changes from Day 0 (Visit 2).
- MIC of aztreonam for all PA isolates (µg/mL) was summarized (n and percent) by visit and treatment, with percentages displayed cumulatively.
- MIC50 and MIC90 of aztreonam for the PA isolate with the highest MIC from each patient (µg/mL) at Day 0, Day 14, and Day 28 were presented by treatment, with the range of MICs for the PA isolates with the highest MIC from each patient.
- MIC of aztreonam for the PA isolate with the highest MIC from each patient (µg/mL) at Day 0, Day 14, and Day 28 was summarized (n and percent) by treatment, with percentages displayed cumulatively.
- Change in MIC of aztreonam (increased, unchanged, or decreased) from Day 0 to Days 14 and 28 for the PA isolate with the highest MIC from each patient was analyzed using a Chi-squared test (or a Fisher's Exact test if [20 = N < 40 and expected frequency of one of the cells was < 5] or N < 20) for overall treatment effect, where increased included patients with a = 4-fold increase in MIC, unchanged included patients with MIC values $\pm$ a 2-fold change, and decreased included patients with a = 4-fold decrease in MIC. Missing values were included in the summary statistics and excluded from the statistical analysis.

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Change in MIC of aztreonam (increased, unchanged, or decreased) from Day 0 to Days 14 and 28 for the PA isolate with the highest MIC from each patient and change in MIC of aztreonam (increased, unchanged, or decreased) from Day 14 to Day 28 for the PA isolate with the highest MIC from each patient were summarized (n and percent) by treatment.

- Patients with MIC of aztreonam ≥ 8 $\mu\text{g/mL}$ for the PA isolate with the highest MIC at Day 0, Day 14, and Day 28 were summarized (n and percent) by treatment.
- MIC50 and MIC90 for other antibiotics against the PA isolate with the highest MIC from each patient ($\mu\text{g/mL}$) at Day 0 and Day 14 were presented by treatment.

Changes in CFUs of PA

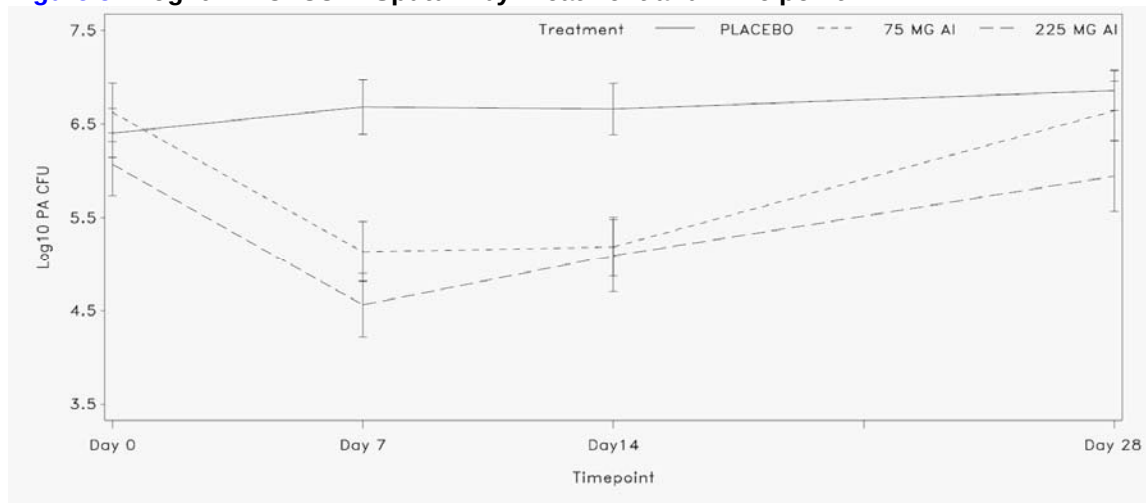
Changes in log₁₀ PA CFUs in sputum are summarized in Table 10. Plots of log₁₀ PA CFUs in sputum vs. time point are presented in Figure 5. While the table presents the exact numerical changes in CFU counts, the figure presents a more obvious and visual representation of these same changes.

Table 10. Changes in Log₁₀ PA CFUs in Sputum (CFUs/g Sputum): ITT Dataset

Change in log ₁₀ PA CFUs (CFUs/g sputum)	Treatment as Randomized		
	Placebo (N = 32)	75 mg AI (N = 38)	225 mg AI (N = 35)
Change in log ₁₀ PA CFUs from Day 0 to Day 7			
Mean (SD)	0.014 (1.595)	-1.509 (1.453)	-2.080 (1.542)
n	27	32	26
Change in log ₁₀ PA CFUs from Day 0 to Day 14			
Mean (SD)	0.025 (1.313)	-1.453 (1.575)	-2.109 (1.990)
n	28	30	27

Source: Table 14.2.1.3.3, Study Report AI-003, this submission.

Figure 5. Log₁₀ PA CFUs in Sputum by Treatment and Time point



Source: Figure 14.6.4, Study Report AI-003, this submission.

There was a mean reduction in log₁₀ PA CFUs in the two AI treatment groups at both Day 7 and Day 14, and a small mean increase in the placebo group at both time points. The mean reduction in log₁₀ PA CFUs observed after treatment with AI (75 mg) was 1.51 at Day 7 and 1.45 at Day

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14. The mean reduction in log₁₀ PA CFUs observed after treatment with AI (225 mg) was 2.08 at Day 7 and 2.11 at Day 14. The mean reduction in log₁₀ PA CFUs observed after treatment with AI was greater in the 225 mg AI group than in the 75 mg AI group.

There was a large degree of variation in individual CFU counts in all treatment groups and at all time points, as indicated by the large SDs. The mean values were higher than the median values in all treatment groups and at all time points, indicating that the data were skewed with some individual patients having high values.

Plots of log₁₀ PA CFUs vs. time point illustrate that, between Day 0 and Day 7, a similar rate of decline in log₁₀ PA CFUs occurred in both AI treatment groups, compared with virtually no change in the placebo group. There were slight increases in log₁₀ PA CFUs between Day 7 and Day 14 both AI treatment groups and noticeable differences in the log₁₀ PA CFUs between Day 14 and Day 28. The values for log₁₀ PA CFUs at Day 14 were low compared with Day 0 for both AI treatment groups. At Day 28, values for log₁₀ PA CFUs were similar to Day 0 for both AI treatment groups.

Appearance or Disappearance of Pathogens

The numbers of patients with an appearance or disappearance of pathogens are summarized in [Table 11](#).

Table 11. Numbers of Patients with an Appearance or Disappearance of Pathogens: Safety Dataset

Pathogen	Type of Change	Day n ^a	Treatment as Received											
			Placebo (N = 31)				75 mg AI (N = 37)				225 mg AI (N = 37)			
			Change from Day 0				Change from Day 0				Change from Day 0			
			0 ^b	7	14	28	0 ^b	7	14	28	0 ^b	7	14	28
			29	27	29	28	34	34	32	33	32	33	31	31
<i>P. A</i>	Appearance		29	0	0	0	33	1	1	0	32	0	0	0
	No change			25	27	26		30	28	29		27	22	29
	Disappearance			0	0	0		0	0	2		3	7	0
<i>S. aureus</i>	Appearance		19	0	2	2	15	1	1	4	21	2	1	2
	No change			24	25	23		29	28	27		25	27	25
	Disappearance			1	0	1		1	0	0		3	1	2
<i>B. cepacia</i>	Appearance		1	0	0	0	0	0	0	0	0	0	1	0
	No change			25	27	25		31	29	31		30	28	29
<i>S. maltophilia</i>	Appearance		3	1	1	0	3	2	2	1	2	1	1	3
	No change			22	24	25		29	27	29		28	27	26
	Disappearance			2	2	1		0	0	1		1	1	0
<i>A. xylosoxidans</i>	Appearance		1	0	0	0	1	1	1	1	1	1	1	2
	No change			25	27	26		30	28	30		29	27	26
	Disappearance			0	0	0		0	0	0		0	1	1

Source: Tables 14.2.2.1.1 to 14.2.2.1.5.

a n = Number of patients with available data at the specified trial day. Note that patients were only counted in the change table if they had data at both Day 0 and the specified trial day. b 0 = Number of patients positive for the specified infection at Day 0.

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P. aeruginosa: In the placebo group, PA was cultured in all patients' samples at each time point. In the 75 mg AI group, PA disappeared from two patients at Day 28. In the 225 mg AI group, PA disappeared from three and seven patients at Days 7 and 14, respectively.

S. aureus: The 75 mg AI group had a smaller proportion of patients with positive *S. aureus* cultures at Day 0 compared with the other two groups. There were no notable differences between treatment groups in the number of patients with appearance or disappearance of *S. aureus* at any of the other trial time points.

B. cepacia: Infection appeared for one patient in the trial (Patient 18403, 225 mg AI) at Day 14. This patient had been positive for *B. cepacia* at Screening, but was negative at Day 0. Another patient (Patient 31001, placebo) was positive for *B. cepacia* at Screening, and Days 0, 7, and 14, but negative at Day 28. There was no evidence of *B. cepacia* infection appearing in any patient who did not already have it at Screening.

S. maltophilia: Similar numbers of patients were positive for *S. maltophilia* at Day 0. There were no notable differences between treatment groups in the numbers of patients who became positive or negative for *S. maltophilia* at each time point after dosing.

A. xylosoxidans: One patient in each treatment group was positive for *A. xylosoxidans* at Day 0. There were no notable differences between treatment groups in the numbers of patients who became positive or negative for *A. xylosoxidans* at each time point after dosing.

Changes in the Aztreonam MIC for PA

Details of MIC for aztreonam are summarized in Tables 14.2.2.2 to 14.2.2.9. Individual patient data are presented in Data Listing 16.2.6.3. Changes in aztreonam MIC50 and MIC90 values are summarized in [Table 12](#).

Table 12. Aztreonam MICs for All PA Isolates (µg/ml) by Visit and Treatment as Received

MIC	PLACEBO (N=31)			75 MG AI (N=37)			225 MG AI (N=37)		
	Day 0 (n*=79)	Day 14 (n*=79)	Day 28 (n*=82)	Day 0 (n*=85)	Day 14 (n*=71)	Day 28 (n*=80)	Day 0 (n*=76)	Day 14 (n*=52)	Day 28 (n*=68)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
<=1	46 (58.2%)	45 (57.0%)	45 (54.9%)	38 (44.7%)	36 (50.7%)	32 (40.0%)	38 (50.0%)	17 (32.7%)	34 (50.0%)
2	6 (65.8%)	6 (64.6%)	4 (59.8%)	5 (50.6%)	4 (56.3%)	2 (42.5%)	6 (57.9%)	7 (46.2%)	7 (60.3%)
4	11 (79.7%)	12 (79.7%)	9 (70.7%)	12 (64.7%)	16 (78.9%)	20 (67.5%)	8 (68.4%)	6 (57.7%)	6 (69.1%)
8	7 (88.6%)	6 (87.3%)	7 (79.3%)	7 (72.9%)	5 (85.9%)	2 (70.0%)	5 (75.0%)	4 (65.4%)	5 (76.5%)
16	4 (93.7%)	5 (93.7%)	8 (89.0%)	8 (82.4%)	1 (87.3%)	3 (73.8%)	4 (80.3%)	4 (73.1%)	2 (79.4%)
32	1 (94.9%)	1 (94.9%)	4 (93.9%)	6 (89.4%)	1 (88.7%)	2 (76.3%)	3 (84.2%)	2 (76.9%)	5 (86.8%)
64	2 (97.5%)	0 (94.9%)	0 (93.9%)	3 (92.9%)	3 (93.0%)	6 (83.8%)	2 (86.8%)	3 (82.7%)	0 (86.8%)
128	2 (100%)	3 (98.7%)	2 (96.3%)	4 (97.6%)	2 (95.8%)	5 (90.0%)	3 (90.8%)	4 (90.4%)	4 (92.6%)
256	0 (100%)	1 (100%)	2 (98.8%)	1 (98.8%)	3 (100%)	4 (95.0%)	3 (94.7%)	4 (98.1%)	1 (94.1%)
512	0 (100%)	0 (100%)	1 (100%)	0 (98.8%)	0 (100%)	3 (98.8%)	3 (98.7%)	0 (98.1%)	3 (98.5%)
1024	0 (100%)	0 (100%)	0 (100%)	1 (100%)	0 (100%)	1 (100%)	1 (100%)	0 (98.1%)	0 (98.5%)
2048	0 (100%)	0 (100%)	0 (100%)	0 (100%)	0 (100%)	0 (100%)	0 (100%)	0 (98.1%)	0 (98.5%)
>2048.00	0 (100%)	0 (100%)	0 (100%)	0 (100%)	0 (100%)	0 (100%)	0 (100%)	1 (100%)	1 (100%)

Source: Table 14.2.2.3, Study Report AI-003.

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Table 13. Aztreonam MICs for PA Isolates with the Highest MIC from each Patient (µg/ml) by Visit and Treatment as Received

MIC	PLACEBO (N=31)			75 MG AI (N=37)			225 MG AI (N=37)		
	Day 0 (n*=29) n (%)	Day 14 (n*=29) n (%)	Day 28 (n*=28) n (%)	Day 0 (n*=33) n (%)	Day 14 (n*=31) n (%)	Day 28 (n*=30) n (%)	Day 0 (n*=32) n (%)	Day 14 (n*=24) n (%)	Day 28 (n*=31) n (%)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
<=1	9 (31.0%)	12 (41.4%)	10 (35.7%)	7 (21.2%)	7 (22.6%)	6 (20.0%)	12 (37.5%)	5 (20.8%)	11 (35.5%)
2	3 (41.4%)	2 (48.3%)	0 (35.7%)	2 (27.3%)	2 (29.0%)	1 (23.3%)	3 (46.9%)	3 (33.3%)	4 (48.4%)
4	8 (69.0%)	4 (62.1%)	3 (46.4%)	5 (42.4%)	11 (64.5%)	8 (50.0%)	4 (59.4%)	4 (50.0%)	3 (58.1%)
8	4 (82.8%)	3 (72.4%)	2 (53.6%)	5 (57.6%)	4 (77.4%)	2 (56.7%)	4 (71.9%)	2 (58.3%)	2 (64.5%)
16	1 (86.2%)	4 (86.2%)	6 (75.0%)	3 (66.7%)	1 (80.6%)	0 (56.7%)	1 (75.0%)	2 (66.7%)	2 (71.0%)
32	1 (89.7%)	1 (89.7%)	3 (85.7%)	4 (78.8%)	0 (80.6%)	2 (63.3%)	0 (75.0%)	2 (75.0%)	4 (83.9%)
64	2 (96.6%)	0 (89.7%)	0 (85.7%)	2 (84.8%)	2 (87.1%)	3 (73.3%)	1 (78.1%)	1 (79.2%)	0 (83.9%)
128	1 (100%)	2 (96.6%)	1 (89.3%)	3 (93.9%)	1 (90.3%)	4 (86.7%)	1 (81.3%)	1 (83.3%)	2 (90.3%)
256	0 (100%)	1 (100%)	2 (96.4%)	1 (97.0%)	3 (100%)	1 (90.0%)	3 (90.6%)	3 (95.8%)	0 (90.3%)
512	0 (100%)	0 (100%)	1 (100%)	0 (97.0%)	0 (100%)	2 (96.7%)	2 (96.9%)	0 (95.8%)	2 (96.8%)
1024	0 (100%)	0 (100%)	0 (100%)	1 (100%)	0 (100%)	1 (100%)	1 (100%)	0 (95.8%)	0 (96.8%)
2048	0 (100%)	0 (100%)	0 (100%)	0 (100%)	0 (100%)	0 (100%)	0 (100%)	0 (95.8%)	0 (96.8%)
>2048..00	0 (100%)	0 (100%)	0 (100%)	0 (100%)	0 (100%)	0 (100%)	0 (100%)	1 (100%)	1 (100%)

Source: Table 14.2.2.5, Study Report AI-003.

Table 14. MIC50 and MIC90 of Aztreonam against PA, Using All Isolates and the Isolate with the Highest MIC from Each Patient (µg/mL): Safety Dataset

Day:	Treatment as Received								
	Placebo (N = 31)			75 mg AI (N = 37)			225 mg AI (N = 37)		
	0	14	28	0	14	28	0	14	28
All isolates from each patient									
n	79	79	82	85	71	80	76	52	68
MIC ₅₀ (µg/mL)	≤ 1	≤ 1	≤ 1	2	≤ 1	4	≤ 1	4	≤ 1
MIC ₉₀ (µg/mL)	16	16	32	64	64	128	128	128	128
Highest MIC isolate from each patient									
n	29	29	28	33	31	30	32	24	31
MIC ₅₀ (µg/mL)	4	4	8	8	4	4	4	4	4
MIC ₉₀ (µg/mL)	64	128	256	128	128	256	256	256	128
Highest MIC (µg/mL)	128	256	512	1024	256	1024	1024	>2048	>2048
Patients with isolates whose highest MIC > 8 µg/mL, n (%)	5 (17.2)	8 (27.6)	13 (46.4)	14 (42.4)	7 (22.6)	13 (43.3)	9 (28.1)	10 (41.7)	11 (35.5)

Source: Tables 14.2.2.2 to 14.2.2.5 and 14.2.2.8, Study Report AI-003.

MIC50 values were within one dilution step (for all PA isolates from each patient) for each treatment group at Day 0. There were differences between treatment groups in MIC90 at Day 0, with the lowest MIC90 recorded in the placebo group (16 µg/mL) and the highest MIC90 recorded in the 225 mg AI group (128 µg/mL). There was an increase in MIC50 of at least two dilutions (from 1 to 4 µg/mL) at Day 14 in the 225 mg AI group, however, the MIC50 dropped back to 1 µg/mL by Day 28. There were no notable increases from Day 0 in MIC50 or MIC90 for the placebo and 75 mg AI groups, and there was no notable increase from Day 0 in MIC90 for the 225 mg AI treatment group. Overall, the increase in MIC50 and MIC90 values was a 1-step dilution or less and thus, a *change in aztreonam susceptibility during therapy was not discernable*.

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Similar results were found in the Using the isolate with the highest MIC from each patient. The numbers of patients with increases and decreases in the MIC (using the highest MIC isolate from each patient) of aztreonam against PA are summarized in [Table 15](#).

Table 15. MIC50 and MIC90 of Aztreonam for the *P. aeruginosa* Isolate with the Highest MIC from Each Patient: Integrated Phase 3 Placebo-Controlled Studies

	Result (µg/mL)					Change from Day 0	
	MIC ₅₀	MIC ₉₀	n*	Min	Max	MIC ₅₀	MIC ₉₀
Pooled Placebo (N = 160)							
Day 0	8	128	147	≤1	1024	-	-
Day 14	4	128	146	≤1	512	unchanged	unchanged
Day 28	4	128	132	≤1	512	unchanged	unchanged
Day 42	4	64	113	≤1	> 2048	unchanged	unchanged
AI BID (N = 69)							
Day 0	4	128	59	≤1	> 2048	-	-
Day 14	8	128	64	≤1	> 2048	unchanged	unchanged
Day 28	8	256	55	≤1	> 2048	unchanged	unchanged
Day 42	8	256	57	≤1	512	unchanged	unchanged
AI TID (N = 146)							
Day 0	8	128	132	≤1	> 2048	-	-
Day 14	8	256	131	≤1	> 2048	unchanged	unchanged
Day 28	8	256	119	≤1	> 2048	unchanged	unchanged
Day 42	8	128	116	≤1	2048	unchanged	unchanged
Pooled AI (N = 215)							
Day 0	4	128	191	≤1	> 2048	-	-
Day 14	8	128	195	≤1	> 2048	unchanged	unchanged
Day 28	8	256	174	≤1	> 2048	unchanged	unchanged
Day 42	8	128	173	≤1	2048	unchanged	unchanged

n* = number of patients with at least one isolate. Increased: = 4-fold increase in MIC, Unchanged: ± 2-fold change in MIC, Decreased: = 4-fold decrease in MIC.

Source: Table 28, Microbiology section, this submission.

Using the isolate with the highest MIC from each patient, MIC50 values were within one dilution step for each treatment group at Day 0. The lowest MIC90 at Day 0 was recorded in the placebo group (64 µg/mL) and the highest MIC90 was recorded in the 225 mg AI group (256 µg/mL). There was an increase from Day 0 to Day 28 in MIC90 in the placebo group (from 64 to 256 µg/mL). There were no notable increases from Day 0 in MIC50 or MIC90 (using the PA isolate with the highest MIC from each patient) in either of the active treatment groups during the trial.

There were increases in the highest MIC in the placebo and 225 mg AI groups between Days 0 and 28 (from 128 to 512 µg/mL and from 1024 to > 2048 µg/mL for the placebo and 225 mg AI groups, respectively), but there were no increases in the 75 mg AI group. There were no notable increases from Day 0 in either AI treatment group in the numbers of patients whose highest MIC isolate was above the breakpoint (> 8 µg/mL). Again, overall, the increase in MIC50 and MIC90 values was a 1-step dilution or less and thus, a *change in aztreonam susceptibility during therapy was not discernable*.

CORRELATION OF *IN VITRO* SUSCEPTIBILITY AND CLINICAL OUTCOME

The following were examined to determine if there was a relationship between microbiological and clinical response in this study:

- Change in FEV₁ (L) vs. change in log10 PA CFUs in sputum (CFUs/g sputum) from Day 0 (Visit 2) to Day 14 (Visit 4) was plotted by treatment.
- Change in log10 PA CFUs in sputum (CFUs/g sputum) from Day 0 (Visit 2) to Day 7 (Visit 3) vs. sputum aztreonam concentrations at Day 0 (Visit 2) and change in log10 PA CFUs

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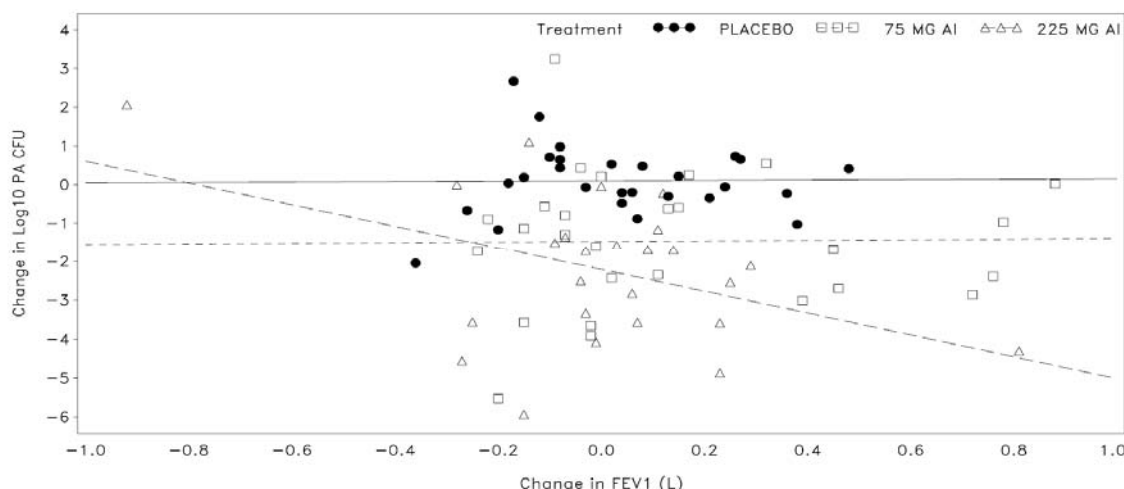
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in sputum (CFUs/g sputum) from Day 7 (Visit 3) to Day 14 (Visit 4) vs. sputum aztreonam concentrations at Day 7 (Visit 3) were plotted by treatment.

- MIC of aztreonam for the PA isolate with the highest MIC from each patient ($\mu\text{g/mL}$) vs. FEV_1 (L) was plotted for Day 7 (Visit 3) and Day 14 (Visit 4) by treatment.

Plots of change in FEV_1 vs. change in $\log_{10}$ PA CFUs in sputum from Day 0 to Day 14 for the PP dataset are presented in [Figure 6](#).

Figure 6. Change in FEV_1 vs. Change in $\log_{10}$ PA CFUs in Sputum from Day 0 to Day 14 by Treatment as Received (Individual Values: PP Dataset).



Source: Figure 14.6.5.1, Final Report AI-003.

Changes in $\log_{10}$ PA CFUs appeared not to be associated with changes in FEV_1 for the placebo and 75 mg AI groups at the end of the treatment period (Day 14). However, in the 225 mg AI group, a decline in $\log_{10}$ PA CFUs was associated with a small increase in FEV_1 .

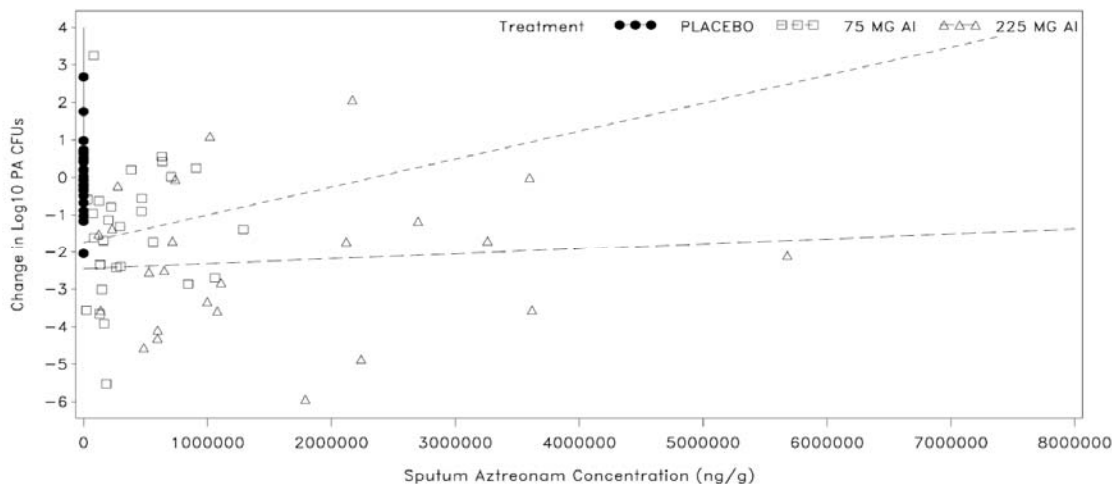
Plots of change in $\log_{10}$ PA CFUs in sputum from Day 0 to Day 7 vs. sputum aztreonam concentrations at Day 0, and change in $\log_{10}$ PA CFUs in sputum from Day 7 to Day 14 vs. sputum aztreonam concentrations at Day 7 are presented in [Figures 7](#) and [8](#), respectively.

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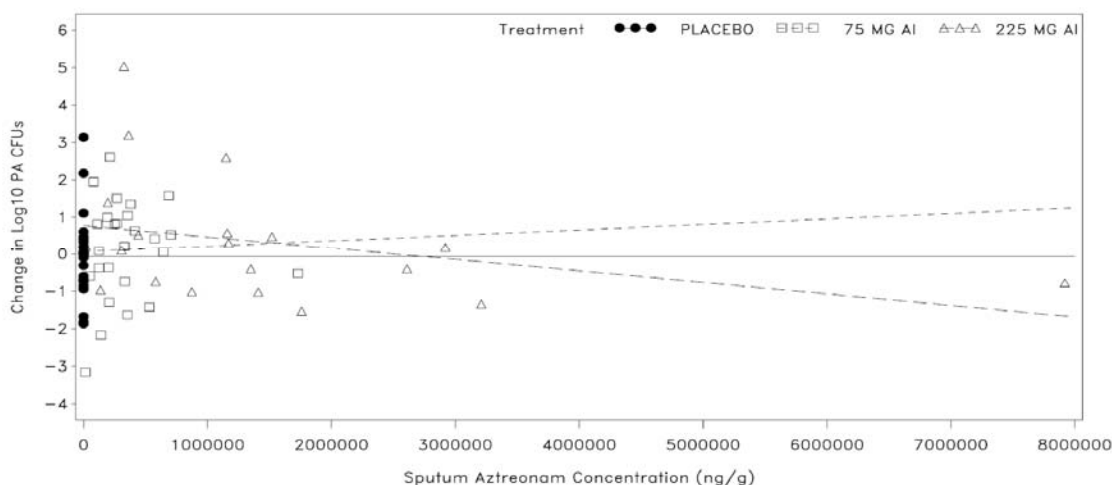
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Figure 7. Change in Log10 PA CFUs in Sputum from Day 0 to Day 7 vs. Sputum Aztreonam Concentration at Day 0 by Treatment as Received. Individual Values: PP Dataset.



Source: Figure 14.6.5.2.1, Final Report AI-003.

Figure 8. Change in Log10 PA CFUs in Sputum from Day 7 to Day 14 vs. Sputum Aztreonam Concentration at Day 7 by Treatment as Received. Individual Values: PP Dataset.



Source: Figure 14.6.5.2.1, Final Report AI-003.

Concentrations of aztreonam in sputum were highly variable. Correlations between the aztreonam sputum concentrations and changes in PA CFUs were not distinguishable.

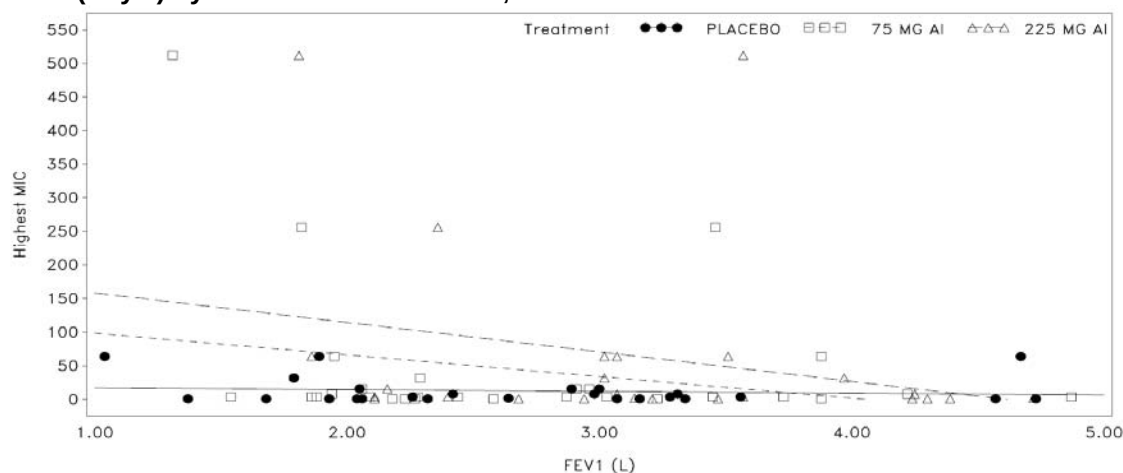
Plots of MIC of aztreonam for the PA isolate with the highest MIC from each patient vs. FEV₁ at Days 7 and 14 are presented in [Figures 9 and 10](#), respectively.

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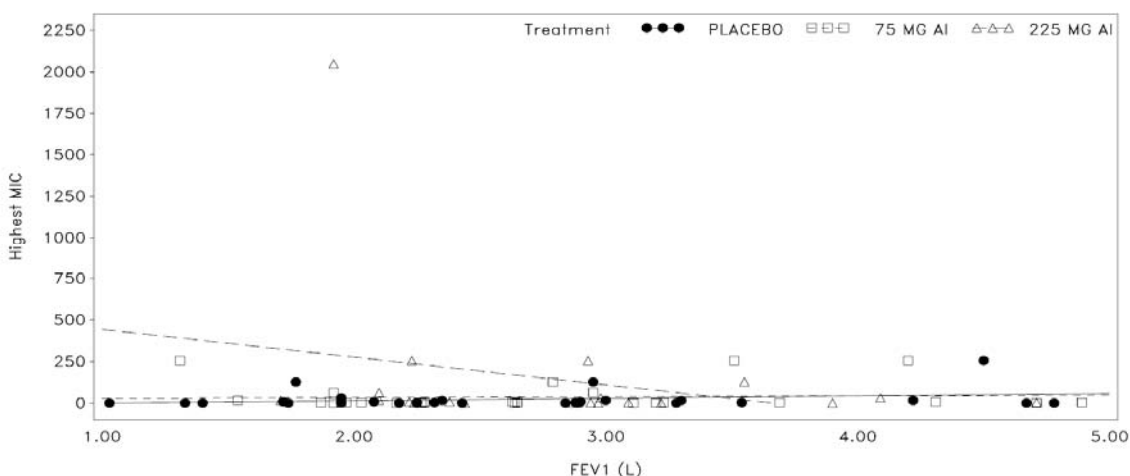
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Figure 9. MIC of Aztreonam for PA Isolate with the Highest MIC from Each Patient vs. FEV₁ Visit 3 (Day 7) by Treatment as Received; Individual Values: PP Dataset



Source: Figure 14.6.5.3.1, Final Report AI-003.

Figure 10. MIC of Aztreonam for PA Isolate with the Highest MIC from Each Patient vs. FEV₁ Visit 4 (Day 14) by Treatment as Received; Individual Values: PP Dataset



Source: Figure 14.6.5.3.2, Final Report AI-003.

In both the 75 and 225 mg AI groups, lower MICs were associated with larger FEV₁ values at Day 7. At Day 14, lower MICs were associated with larger FEV₁ values for the 225 mg AI group, however, there was no apparent correlation between the value of the highest MIC and the FEV₁ values in the 75 mg AI group. In the placebo group, there was no apparent correlation between the value of the highest MIC isolate and the FEV₁ values at Day 7 or at Day 14.

Reviewer's comments: From the microbiological viewpoint, the results of **Study AI-003** were encouraging since treatment arms showed statistically significant log₁₀ reductions of *P. aeruginosa*, there were few changes in the appearance of new pathogens and there were no notable increases in MIC₉₀ values from baseline to TOC, thus no reduced susceptibility of the drug on therapy.

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Study CP-AI-005: Trial with Aztreonam Lysinate for Inhalation in Cystic Fibrosis Patients with Pulmonary *P. aeruginosa* requiring Frequent Antibiotics (AIR-CF2)

OBJECTIVES

This trial was designed to assess the safety and efficacy of a 28-day treatment with Aztreonam Lysine for Inhalation (AI) and the ability of AI to maintain or improve clinical status following a 28-day course of Tobramycin Inhalation Solution (TSI, TOBI®) therapy in cystic fibrosis (CF) patients with pulmonary *Pseudomonas aeruginosa* (PA).

METHODOLOGY

Patients were screened for eligibility at Visit 1 (Day -42) and returned to the center for Visit 2 after a 14-day evaluation period. At Visit 2 (Day -28), patients were randomized and began a 28-day course of TSI. At Visit 3 (Day 0), following completion of the 28-day course of TSI, patients began treatment at the clinic with their randomized therapy, either AI (twice daily [BID] or three times daily [TID]) or volume-matched placebo (BID or TID), and continued treatment at home for a total of 28 days, with a clinic visit (including one of the daily doses) after 14 days of treatment (Visit 4 [Day 14]) and at the end of treatment (Visit 5 [Day 28]). Patients returned for visits every two weeks for eight weeks after the end of the AI/placebo treatment (Visits 6 to 9 [Days 42 to 84]).

TSI was self-administered using the PARI LC PLUS® Jet nebulizer, and AI was self-administered using the PARI eFlow® Electronic Nebulizer (eFlow). All doses of AI/placebo were administered after using a short-acting bronchodilator.

Patients in this trial were eligible to enroll in an open-label follow-on trial, [CP-AI-006](#).

NUMBER OF SUBJECTS

Population	Treatment				Total
	Placebo		75 mg AI		
	BID	TID	BID	TID	
Planned	-	-	-	-	Approx 250
Enrolled	41	41	82	82	247 ^a
Safety	38	38	69	66	211
ITT	38	38	69	66	211
PP	37	35	62	59	193

Patients were randomized in a 1:1:2:2 ratio to Placebo BID: Placebo TID: AI BID: AI TID. The Safety and ITT populations were identical. ^a One patient received TSI but was not randomized and is included in the Enrolled total only.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION

The main criterion for inclusion was that patients were eligible if aged ≥ 6 years with CF and pulmonary PA having received ≥ 3 courses of TSI within the last 12 months and having lung function within the range $FEV_1 \geq 25\%$ and $\leq 75\%$ predicted.

TEST PRODUCT

AI (75 mg/mL aztreonam lysine when reconstituted in diluent [0.17% saline]; sterile, pH 4.2 to 7.0, and osmolality 300 to 550 mOsmol/kg). AI was self-administered by inhalation using the eFlow BID or TID. AI was self-administered by inhalation using the PARI eFlow nebulizer. The eFlow is 510(k) approved in the US and is CE-marked in Europe. It is approved in Canada and is considered investigational in Australia and New Zealand.

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REFERENCE THERAPY

Placebo (5 mg/mL lactose when reconstituted in diluent [0.17% saline]; sterile, pH 4.2 to 7.5, and osmolality 200 to 400 mOsmol/kg). Placebo was self-administered by inhalation using the eFlow BID or TID.

DURATION OF TREATMENT

28 days of AI/placebo. The study period lasted 18 weeks (126 days), including a 14-day Screening period, followed by a 28-day course of TSI (TSI treatment period), and a 28-day course of AI/placebo (AI/placebo treatment period) with a 56-day follow-up.

CRITERIA FOR EVALUATION OF EFFICACY

The primary efficacy endpoint was the time to need for a course of inhaled or IV anti-pseudomonal antibiotics other than trial drug with documented symptom(s) predictive of pulmonary exacerbation.

The secondary efficacy endpoints were as follows:

- Clinical symptoms as assessed by the Cystic Fibrosis Questionnaire – Revised (CFQ-R) respiratory domain
- Change in pulmonary function (forced expiratory volume in one second [FEV₁], FEV₁ percent of predicted, forced vital capacity [FVC] and forced expiratory flow from 25% to 75% [FEF₂₅₋₇₅])
- Hospitalization: time to hospitalization, number of days hospitalized, proportion of patients hospitalized, and percent of days hospitalized
- School, work missed or both: number of missed school or work days, proportion of patients missing school or work, and percent of school or work days missed
- Change in CFQ-R nonrespiratory domains
- Physician's and patient's assessment of change in symptoms using the Global Rating of Change Questionnaire (GRCQ)
- Percent change in weight
- Change in body mass index (BMI)
- Change in CF symptoms and severity
- Change in patient's ability to produce sputum
- Change in log₁₀ PA colony-forming units (CFUs) in sputum

OTHER EVALUATIONS

Clinical Pharmacology:

Aztreonam concentrations in plasma and sputum

Clinical Microbiology:

- Disappearance or appearance of other pathogens (*Staphylococcus aureus*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*)
- Minimum Inhibitory Concentration (MIC) of aztreonam for PA

CLINICAL MICROBIOLOGY METHODOLOGY

Sputum for microbiology was collected for qualitative and quantitative culture for PA, *Staphylococcus aureus*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, and to determine MICs for PA and *B. cepacia*.

Samples were collected at all visits up to Day 56, and were collected before treatment at Days -28, 0, and 14. At Days 0, 14, and 28, sputum was collected at least 4 hours after the most recent dose of TSI or AI/placebo taken. A minimum of 0.5 mL of sputum was to be collected. If the patient could not expectorate, a throat swab was to be collected from which two slides were to be

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prepared. Every effort was made to obtain an expectorated sputum sample because a throat swab significantly limited microbiology testing.

Sputum for microbiology was stored refrigerated until shipment was made to the central laboratory. Sputum samples were shipped on cold gel packs.

CLINICAL EFFICACY RESULTS

The median time to need for inhaled or IV anti-pseudomonal antibiotics due to pre-defined symptoms was 21 days longer in the pooled AI group (92 days) than in the pooled placebo group (71 days) ($p = 0.0070$). The median time to antibiotic need for the AI BID group was significantly different than that in the pooled placebo group ($p = 0.0019$; median time not estimable), and the median time to antibiotic need was 87 days for the AI TID group ($p = 0.1816$). There was a strong suggestion of a regimen effect (BID vs. TID), which was confirmed by a post hoc analysis showing that the time to need for inhaled or IV antibiotics was different between the pooled BID and pooled TID regimens ($p = 0.0012$). The regimen effect was not seen for other efficacy endpoints.

Results of the microbiologically pertinent secondary endpoints are presented in the table below:

Table 16. Results of Microbiologically Pertinent Secondary Endpoints

Secondary Endpoint Key Test or Evaluation	Result			
	Pooled Placebo (N = 76)	Pooled AI (N = 135)	Treat. Diff.	p-value
Clinical symptoms as assessed by CFQ-R respiratory domain				
Mean change in CFQ-R respiratory domain score at Day 28	-0.66	4.34	5.01	0.0196
Categorical result: % of patients who improved at Day 28 ^a	37.0	51.5	-	0.0289
% of patients who worsened at Day 28 ^a	38.4	28.0	-	
Change in pulmonary function				
Mean percent change in FEV ₁ at Day 28 ^b	-2.4	3.9	6.3	0.0012
Change in log ₁₀ PA CFUs in sputum				
Mean change in sputum log ₁₀ PA CFUs at Day 28 ^d	0.225	-0.434	-0.659	0.0059

Notes: - not applicable; NS = not significant; Treat. Diff. = Treatment Difference. ^{aA} greater % of patients improved (had an increase in score of >5) in the AI BID group than in the AI TID group (55% vs. 48%, Day 28). ^b There was no apparent dose response for FEV₁. ^c Number of hospitalizations was low. There were no treatment differences in time to first hospitalization. Respiratory symptoms were the main cause of hospitalization: 11/14 patients (AI) vs. 3/3 patients (placebo). Of the 11 AI-treated patients with respiratory hospitalizations, 7 occurred after Day 28. ^d The mean reduction in log₁₀ CFU at Day 14 was lower in the AI BID group than in the AI TID group (-0.184 vs. -0.753), indicating CFU reduction occurred more quickly in the AI TID group than in the AI BID group. However, the decrease from Day 0 in mean log₁₀ PA CFUs was similar for the two AI groups at Day 28 (-0.489 vs. -0.373 for the AI BID and TID groups, respectively).

For the remaining secondary endpoints, there were no statistically significant differences between the pooled AI and pooled placebo groups in school and/or work missed, CFQ-R nonrespiratory domain scores, and patient's ability to produce sputum at Days 14 or 28, and there were no notable differences between pooled groups in CF nonrespiratory symptom severity or in patients' or physicians' GRCQ results.

MICROBIOLOGICAL EFFICACY RESULTS

Log₁₀ PA Colony-forming Units in Sputum

Details of log₁₀ PA CFUs in sputum are summarized in [Table 17](#) and are illustrated graphically in [Figure 11](#).

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The difference between the pooled AI and pooled placebo groups for change in log₁₀ PA CFUs in sputum approached statistical significance at Day 14 (-0.490, $p = 0.0586$) and achieved significance at Day 28 (-0.66, $p = 0.0059$). From Day 0 to Day 28, log₁₀ PA CFU sputum density decreased (improved) by 0.43 in the pooled AI group, and increased (deteriorated) by 0.23 in the pooled placebo group. The change in log₁₀ PA CFU density was also significantly different for the AI BID (-0.72, $p = 0.011$) and TID (-0.60, $p = 0.031$) groups compared with the pooled placebo group.

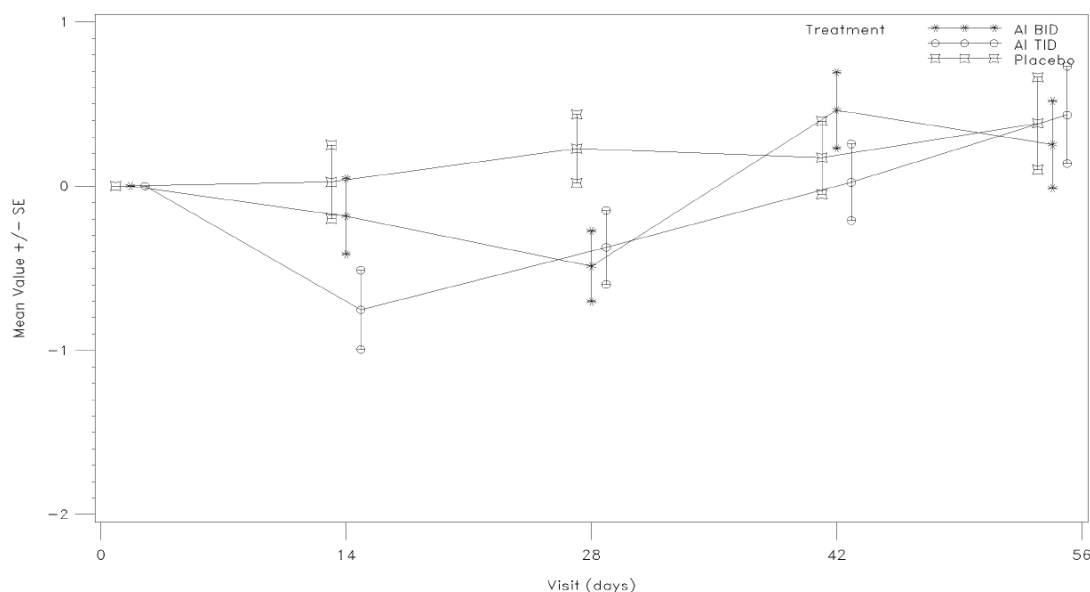
After cessation of therapy (Day 28), log₁₀ PA CFU density among AI-treated patients was greater than baseline at Days 42 and 56 (Table 14.2.12.3). A summary of the log₁₀ PA CFUs in sputum is presented in Table 17.

Table 17. Mean (SD) Log₁₀ PA CFUs in Sputum: ITT Population

Timepoint	Treatment			
	Placebo	AI		
	Pooled (N = 76)	BID (N = 69)	TID (N = 66)	Pooled (N = 135)
Day 0 (TSI)				
Mean (SD)	5.978 (1.776)	5.037 (2.346)	5.966 (1.783)	5.485 (2.135)
n	67	58	54	112
Day 14 (AI/placebo)				
Mean (SD)	6.361 (1.188)	5.246 (2.058)	5.252 (2.058)	5.249 (2.049)
n	63	60	54	114
Day 28 (AI/placebo)				
Mean (SD)	6.342 (1.532)	4.979 (2.271)	5.472 (1.921)	5.209 (2.119)
n	59	56	49	105

Source: Table 33, Microbiology section, this submission.

Figure 11. Mean Change in Log₁₀ PA CFUs in Sputum from Day 0



Source: Figure 14.2.12.3, Study Report AI-005 (also Figure 12, Microbiology section, this submission).

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The reduction in log₁₀ CFUs at Day 14 was greater in the AI TID group than in the AI BID group (-0.753 vs -0.184), indicating that CFU reduction occurred more quickly in the AI TID group than in the AI BID group. However, the decrease from Day 0 in mean log₁₀ PA CFUs was similar for the two AI groups at Day 28 (-0.373 vs. -0.489 for the AI TID and BID groups, respectively).

Results for the PP population were similar to those in the ITT population.

Presence or Absence of Pathogens

Presence or absence of pathogens is summarized for the safety population in [Table 18](#).

Table 18. Presence or Absence of *Pseudomonas aeruginosa* for AI/Placebo Period: Safety Population

Treatment	Visit	n ^a	Result		Change		
			Presence	Absence	App	No	Dis
			n (%)	n (%)	n	n	n
Pooled Placebo (N = 76)	Day 0	73	71 (97.3)	2 (2.7)	–	–	–
	Day 14	73	72 (98.6)	1 (1.4)	1	69	0
	Day 28	64	62 (96.9)	2 (3.1)	0	63	0
	Day 42	57	56 (98.2)	1 (1.8)	0	54	0
	Day 56	45	45 (100)	0	0	42	0
AI BID (N = 69)	Day 0	66	59 (89.4)	7 (10.6)	–	–	–
	Day 14	69	64 (92.8)	5 (7.2)	3	62	1
	Day 28	62	55 (88.7)	7 (11.3)	3	53	4
	Day 42	60	58 (96.7)	2 (3.3)	4	54	0
	Day 56	55	52 (94.5)	3 (5.5)	5	46	2
AI TID (N = 66)	Day 0	66	64 (97.0)	2 (3.0)	–	–	–
	Day 14	64	59 (92.2)	5 (7.8)	1	59	4
	Day 28	57	53 (93.0)	4 (7.0)	1	52	4
	Day 42	52	51 (98.1)	1 (1.9)	1	50	1
	Day 56	41	40 (97.6)	1 (2.4)	1	39	1
Pooled AI (N = 135)	Day 0	132	123 (93.2)	9 (6.8)	–	–	–
	Day 14	133	123 (92.5)	10 (7.5)	4	121	5
	Day 28	119	108 (90.8)	11 (9.2)	4	105	8
	Day 42	112	109 (97.3)	3 (2.7)	5	104	1
	Day 56	96	92 (95.8)	4 (4.2)	6	85	3

Source: Table 14.6.2.6.

App, No, and Dis indicate the number of patients with appearance/disappearance of pathogen, or no change from Day 0. na The number of patients with available data. – Not applicable.

***P. aeruginosa*:** Presence of PA during screening was an inclusion criterion for the trial. After dosing, PA was isolated in almost all patients in the pooled placebo group between Days 14 and 56 (97% to 100% of patients, pooled placebo). PA was isolated in 91% to 97% of patients (pooled AI) over the course of the trial. Eradication of established PA infection is not expected for suppressive therapy, and the presence or absence of PA is more likely to be attributable to variations in sampling technique, or to the patients' ability to produce sputum, than to the disappearance of PA in individual patients.

***S. aureus*:** The percentages of patients with *S. aureus* were slightly higher in the pooled AI group than in the pooled placebo group at all time points in the AI/placebo period, including Day 0 (44% to 55% vs. 32% to 41%).

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A. xylosoxidans: The percentages of patients with *A. xylosoxidans* were generally slightly lower in the pooled AI group than in the pooled placebo group at all time points in the AI/placebo period, including Day 0 (4 to 8% vs. 8 to 12%).

There were no other notable differences between the AI and placebo groups in the percentage of patients with positive results, or in the numbers of patients with an appearance or disappearance of pathogens for any of the other pathogens tested (*B. cepacia* and *S. maltophilia*).

Treatment-emergent isolation of other organisms for the AI/placebo period is summarized in [Table 19](#).

Table 19. Treatment-emergent Isolation of Other Organisms for AI/Placebo Period: Safety Population

Nature of Isolation	Organism	Treatment			
		Placebo	AI		
		Pooled (N = 76)	BID (N = 69)	TID (N = 66)	Pooled (N = 135)
		n (%)	n (%)	n (%)	n (%)
Treatment-emergent intermittent isolation ^a	<i>S. aureus</i>	2 (2.8)	4 (6.1)	4 (6.2)	8 (6.1)
	<i>B. cepacia</i>	0	0	0	0
	<i>S. maltophilia</i>	6 (8.5)	5 (7.6)	6 (9.2)	11 (8.4)
	<i>A. xylosoxidans</i>	0	1 (1.5)	2 (3.1)	3 (2.3)
Treatment-emergent persistent isolation ^b	<i>S. aureus</i>	6 (8.5)	4 (6.1)	8 (12.3)	12 (9.2)
	<i>B. cepacia</i>	0	0	0	0
	<i>S. maltophilia</i>	1 (1.4)	2 (3.0)	3 (4.6)	5 (3.8)
	<i>A. xylosoxidans</i>	2 (2.8)	1 (1.5)	0	1 (0.8)

Source: Table 14.6.2.11.

Number of patients with available data (i.e., with valid culture at Day 0 and between Day 0 and Day 42): Pooled Placebo = 71, AI BID = 66, AI TID = 65, and Pooled AI = 131. a Intermittent Isolation: Organism not isolated at Day 0 but isolated once between Day 0 and Day 42, and not a persistent isolation. b Persistent Isolation: Organism not isolated at Day 0 but isolated at least twice or at an Early Termination Visit between Day 0 and Day 42.

S. aureus: In the pooled AI group, eight (6%) patients had *S. aureus* isolated intermittently compared with two (2.8%) patients in the pooled placebo group. There were no differences between the placebo and AI groups in the percentages of patients with persistent *S. aureus* isolation. The percentage of patients with persistent isolation of *S. aureus* was four (6%) patients in the AI BID group vs. eight (12%) patients in the AI TID group.

B. cepacia: This organism was not isolated from any patient at any time point during the AI/placebo period.

S. maltophilia: There were no notable differences between the placebo and AI treatment groups in the percentages of patients with intermittent or persistent isolation of *S. maltophilia*.

A. xylosoxidans: There were no differences between treatment groups in the percentage of patients with intermittent or persistent isolation of *A. xylosoxidans*. Over the 28-day treatment period in this study, there was no evidence of a persistent increase in positive test cultures for any of the organisms evaluated.

Minimum Inhibitory Concentrations for PA

Aztreonam MIC values for all *P. aeruginosa* isolates in each treatment arm treated BID are presented in [Tables 20](#) and [21](#). MIC90 values are indicated by the shaded cells.

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Table 20. Aztreonam MICs for all PA Isolates: Placebo--BID

MIC (µg/ml)	Day 0	N=61	Day14	N=70	Day 28	N=49	Day 56	N=50
	n	%	n	%	n	%	n	%
<1	28	45.9%	35	50.0%	22	44.9%	20	40.0%
2	2	3.3%	5	7.1%	4	8.2%	4	8.0%
4	3	4.9%	5	7.1%	4	8.2%	5	10.0%
8	6	9.8%	3	4.3%	3	6.1%	9	18.0%
16	4	6.6%	11	15.7%	5	10.2%	5	10.0%
32	6	9.8%	2	2.9%	2	4.1%	1	2.0%
64	7	11.5%	2	2.9%	3	6.1%	2	4.0%
128	4	6.6%	4	5.7%	4	8.2%	1	2.0%
256	0	0.0%	1	1.4%	0	0.0%	1	2.0%
512	1	1.6%	2	2.9%	2	4.1%	1	2.0%
1024	0	0.0%	0	0.0%	0	0.0%	2	4.0%
2048	0	0.0%	0	0.0%	0	0.0%	0	0.0%
>2048	0	0.0%	0	0.0%	0	0.0%	0	0.0%

Source: Table 14.6.3.3, Study Report AI-005.

Table 21. Aztreonam MICs for all PA Isolates: AI (75 mg)--BID

MIC (µg/ml)	Day 0	N=105	Day14	N=101	Day 28	N=93	Day 56	N=90
	n	%	n	%	n	%	n	%
<1	47	44.8%	30	29.7%	30	32.3%	40	44.4%
2	8	7.6%	14	13.9%	9	9.7%	9	10.0%
4	15	14.3%	8	7.9%	9	9.7%	7	7.8%
8	15	14.3%	20	19.8%	15	16.1%	10	11.1%
16	5	4.8%	11	10.9%	11	11.8%	7	7.8%
32	4	3.8%	2	2.0%	6	6.5%	4	4.4%
64	3	2.9%	4	4.0%	2	2.2%	7	7.8%
128	3	2.9%	8	7.9%	3	3.2%	2	2.2%
256	2	1.9%	2	2.0%	5	5.4%	2	2.2%
512	1	1.0%	0	0.0%	1	1.1%	1	1.1%
1024	1	1.0%	1	1.0%	1	1.1%	1	1.1%
2048	0	0.0%	0	0.0%	0	0.0%	0	0.0%
>2048	1	1.0%	1	1.0%	1	1.1%	0	0.0%

Source: Table 14.6.3.3, Study Report AI-005.

During the treatment period, MIC90s of aztreonam for all PA isolates generally remained unchanged. There were no increases of two dilution steps or more during therapy. In the placebo arm, MIC90 values were 64 µg/ml at Day 0, Day 14 and Day 56 and 128 µg/ml at Day 28. In the AI treatment arm, MIC90 values were 64 µg/ml at Day 0 and 128 µg/ml at Days 14, 28 and 56.

Aztreonam MIC values for all *P. aeruginosa* isolates in each treatment arm treated TID are presented in [Tables 22](#) and [23](#). MIC90 values are indicated by the shaded cells.

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Table 22. Aztreonam MICs for all PA Isolates: Placebo--TID

MIC	Day 0 N=64		Day 14 N=70		Day 28 N=64		Day 56 N=33	
	n	%	n	%	n	%	n	%
<1	35	54.7%	38	54.3%	40	62.5%	19	57.6%
2	3	4.7%	7	10.0%	3	4.7%	1	3.0%
4	4	6.3%	5	7.1%	4	6.3%	4	12.1%
8	9	14.1%	6	8.6%	6	9.4%	3	9.1%
16	3	4.7%	6	8.6%	3	4.7%	3	9.1%
32	3	4.7%	4	5.7%	1	1.6%	1	3.0%
64	2	3.1%	1	1.4%	2	3.1%	0	0.0%
128	0	0.0%	0	0.0%	5	7.8%	1	3.0%
256	2	3.1%	2	2.9%	0	0.0%	1	3.0%
512	2	3.1%	1	1.4%	0	0.0%	0	0.0%
1024	1	1.6%	0	0.0%	0	0.0%	0	0.0%
2048	0	0.0%	0	0.0%	0	0.0%	0	0.0%
>2048	0	0.0%	0	0.0%	0	0.0%	0	0.0%

Source: Table 14.6.3.3, Study Report AI-005.

Table 23. Aztreonam MICs for all PA Isolates: AI (75 mg)--TID

MIC	Day 0 N=111		Day 14 N=92		Day 28 N=95		Day 56 N=70	
	n	%	n	%	n	%	n	%
<1	53	47.7%	34	37.0%	29	30.5%	39	55.7%
2	5	4.5%	4	4.3%	12	12.6%	4	5.7%
4	12	10.8%	7	7.6%	9	9.5%	4	5.7%
8	11	9.9%	14	15.2%	17	17.9%	7	10.0%
16	8	7.2%	12	13.0%	9	9.5%	1	1.4%
32	12	10.8%	9	9.8%	7	7.4%	4	5.7%
64	3	2.7%	1	1.1%	5	5.3%	5	7.1%
128	2	1.8%	6	6.5%	2	2.1%	1	1.4%
256	3	2.7%	1	1.1%	3	3.2%	1	1.4%
512	1	0.9%	3	3.3%	1	1.1%	0	0.0%
1024	1	0.9%	1	1.1%	1	1.1%	3	4.3%
2048	0	0.0%	0	0.0%	0	0.0%	1	1.4%
>2048	0	0.0%	0	0.0%	0	0.0%	0	0.0%

Source: Table 14.6.3.3, Study Report AI-005.

During the treatment period, MIC90s of aztreonam for all PA isolates generally remained stable. There were no lasting increases of two dilution steps or more during therapy. In the placebo arm, MIC90 values were 64 μ g/ml at Day 0 and Day 28 and 32 μ g/ml at Day 14. Interestingly, the MIC90 was only 16 μ g/ml at Day 56, the last treatment visit. In the AI treatment arm, MIC90 values were 32 μ g/ml at Day 0, 64 μ g/ml at Days 28 and 56 and 128 μ g/ml at Day 14.

MIC50 and MIC90 of aztreonam for the PA isolate with the highest MIC from each patient are summarized in [Table 24](#). MIC90 values are indicated by the shaded cells.

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Table 24. MIC50 and MIC90 of Aztreonam for PA Isolate With the Highest MIC from Each Patient for the TSI and AI/Placebo Periods: Safety Population

Treatment	Visit	Result (µg/mL)				
		MIC ₅₀	MIC ₉₀	n	Min	Max
Pooled placebo (N = 76)	Day -28	4	128	72	≤1	>2048
	Day 0	4	128	71	≤1	1024
	Day 14	4	128	71	≤1	512
	Day 28	4	128	62	≤1	512
	Day 42	2	64	56	≤1	512
	Day 56	8	256	45	≤1	1024
AI BID (N = 69)	Day -28	4	32	58	≤1	1024
	Day 0	4	128	59	≤1	>2048
	Day 14	8	128	64	≤1	>2048
	Day 28	8	256	55	≤1	>2048
	Day 42	8	256	57	≤1	512
	Day 56	8	64	52	≤1	1024
AI TID (N = 66)	Day -28	4	128	57	≤1	1024
	Day 0	4	32	63	≤1	1024
	Day 14	8	128	59	≤1	1024
	Day 28	8	128	53	≤1	1024
	Day 42	4	64	51	≤1	1024
	Day 56	4	256	39	≤1	2048
Pooled AI (N = 135)	Day -28	4	64	115	≤1	1024
	Day 0	4	64	122	≤1	>2048
	Day 14	8	128	123	≤1	>2048
	Day 28	8	256	108	≤1	>2048
	Day 42	8	128	108	≤1	1024
	Day 56	4	128	91	≤1	2048

n = number of patients with data.

Source: Table 51, Microbiology section, this submission.

During the TSI period, MIC50 and MIC90 values of aztreonam for the PA isolate with the highest MIC from each patient generally remained unchanged in the pooled AI and pooled placebo groups. However, the variability observed in the aztreonam MIC90 between Day -28 and Day 0 (prior to treatment with AI) in the AI BID and TID groups was in the range of two 2-fold dilutions. This was evident as an increase in MIC90 from Day -28 to Day 0 for the AI BID group (32 to 128 µg/mL), and a decrease in MIC90 from Day -28 to Day 0 for the AI TID group (128 to 32 µg/mL). There were no meaningful differences in MIC50 and MIC90 between treatment groups at Day 0.

Throughout the AI/placebo period, MIC50 and MIC90 (using the PA isolate with the highest MIC from each patient) generally remained unchanged for all treatment groups. From Day -28 to Day 56, the MIC50 for PA isolates with the highest MIC from each patient in the pooled AI group varied from 4 to 8 µg/mL compared to 2 to 8 µg/mL in the pooled placebo group. Over the same time period the MIC90 varied from 64 to 256 µg/mL for both of these pooled groups.

The only increase of concern occurred in the AI TID treatment arm. The MIC90 in this group rose from 32 to 256 µg/mL from Day 0 to Day 56, an increase of three dilution steps. However, the aztreonam MIC increase was only one dilution step (128 to 256 µg/mL) from Day -28 to Day 56.

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MICs of Tobramycin for PA

Overall, from Day -28 to Day 0, the MIC of tobramycin remained unchanged for 123 (70%) patients. There were no differences between isolates from randomized treatment groups at Day 0 in the percentages of patients with an increase in MIC of tobramycin.

During the AI/placebo period, the percentages of patients with increased highest MICs of tobramycin from Day 0 were generally much lower than those with decreased highest MICs of tobramycin in all treatment groups. There were no notable or statistically significant differences between pooled treatment groups in change in MIC of tobramycin from Day 0 for the PA isolate with the highest MIC from each patient.

The pooled AI group had a lower percentage of patients with MIC of tobramycin ≥ 8 $\mu\text{g/mL}$ for the PA isolate with the highest MIC than the pooled placebo group, at all time points.

MICs of other Antibiotics for PA

Apart from transient changes, there were no notable increases in the MIC50 or MIC90 of any of the other antibiotics tested after treatment with AI (tobramycin, gentamicin, amikacin, piperacillin, cefepime, meropenem, ceftazidime, ciprofloxacin, and ticarcillin/clavulanate).

Aztreonam Concentrations in Plasma and Sputum

Plasma and sputum aztreonam concentrations are summarized in [Table 25](#).

Table 25. Plasma and Sputum Aztreonam Concentrations: Safety Population

Treatment	Visit	Timepoint	n	Mean	Median	SD	Min	Max
Aztreonam concentrations in plasma (ng/mL)								
Placebo BID (N = 38)	Day 0	Pre-treatment	36	0.13	0.00	0.81	0.00	4.8
		+1h	35	0.00	0.00	0.00	0.00	0.00
	Day 14	+1h	33	0.00	0.00	0.00	0.00	0.00
	Day 28 ^a		31	0.00	0.00	0.00	0.00	0.00
Placebo TID (N = 38)	Day 0	Pre-treatment	32	0.00	0.00	0.00	0.00	0.00
		+1h	35	0.18	0.00	1.09	0.00	6.4
	Day 14	+1h	33	0.00	0.00	0.00	0.00	0.00
	Day 28 ^a		27	0.00	0.00	0.00	0.00	0.00
AI BID (N = 69)	Day 0	Pre-treatment	68	28.93	0.00	134.74	0.0	971.0
		+1h	66	536.90	533.00	322.46	0.0	1390.0
	Day 14	+1h	65	617.38	581.00	307.75	45.1	1540.0
	Day 28 ^a		59	48.79	29.30	74.63	0.0	353.0
AI TID (N = 66)	Day 0	Pre-treatment	60	31.21	0.00	139.73	0.0	776.0
		+1h	63	685.42	585.00	456.89	0.0	2920.0
	Day 14	+1h	59	706.33	622.00	385.56	30.7	1710.0
	Day 28 ^a		54	75.56	43.05	92.44	0.0	411.0
Aztreonam concentrations in sputum (ng/g)								
Placebo BID (N = 38)	Day 0	+10 min	37	0.54	0.00	3.27	0.0	19.9
	Day 14	+10 min	32	0.58	0.00	3.27	0.0	18.5
Placebo TID (N = 38)	Day 0	+10 min	35	68.93	0.00	369.63	0.0	2,190
	Day 14	+10 min	33	7680	0.00	43,860	0.0	252,000 ^b
AI BID (N = 69)	Day 0	+10 min	63	552,931	422,000	494,740	0.0	2,160,000
	Day 14	+10 min	61	681,731	429,000	708,117	273.0	3,430,000
AI TID (N = 66)	Day 0	+10 min	58	585,376	536,500	507,590	182.0	3,010,000
	Day 14	+10 min	51	638,714	406,000	616,068	67,600	3,240,000

a At Day 28, trial drug was not administered in the clinic. Patients were instructed not to dose with trial drug within four hours before the visit. b Patient 18101: sputum sample mislabeled at the central laboratory.

Source: Table 47, Microbiology section, this submission.

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Concentrations of aztreonam in both plasma and sputum varied substantially between patients in both the AI BID and TID groups, as indicated by the high standard deviations.

Mean and median plasma aztreonam concentrations one hour posttreatment at Day 0 were similar to those one hour after dosing at Day 14, and mean and median values in the AI BID group were generally similar to those in the AI TID group at all time points. Mean and median plasma aztreonam concentrations were much lower at Day 28 (when no dose was given in the clinic and samples were obtained at least four hours after dosing) than at Days 0 and 14 in both AI groups.

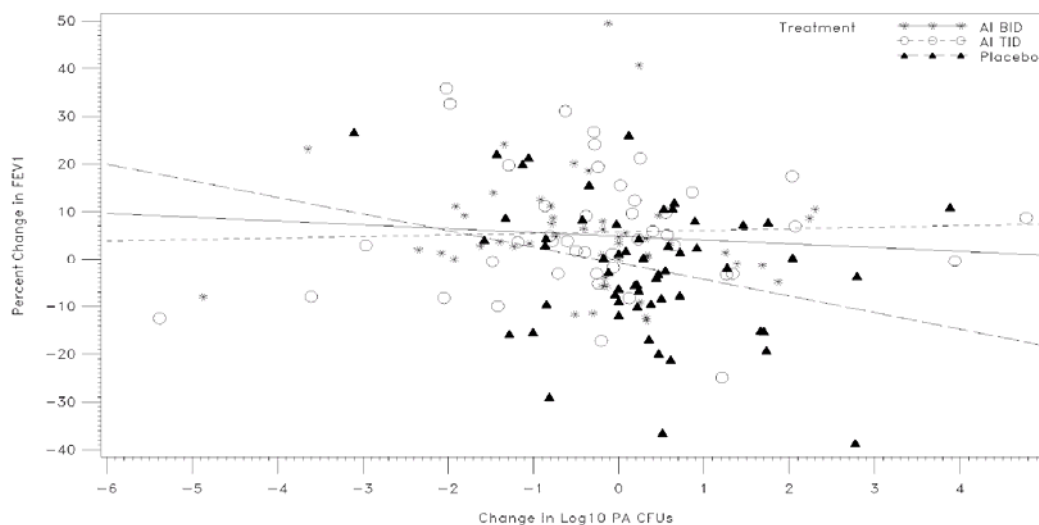
Mean aztreonam concentrations in sputum were approximately 1000-fold higher than those in plasma. Mean and median aztreonam concentrations in sputum 10 min after treatment at Day 0 were similar to those after dosing at Day 14, and mean and median values in the AI BID group were similar to those in the AI TID group at all time points.

CORRELATION OF *IN VITRO* SUSCEPTIBILITY AND CLINICAL OUTCOME

Correlation Between Log₁₀ PA CFUs and FEV₁ (L)

Log₁₀ PA CFU and FEV₁ (L) correlation data are plotted in [Figure 12](#).

Figure 12. Day 28 Percent Change in FEV₁ vs. Day 28 Change in Log₁₀ PA CFUs for Pooled Placebo, AI BID and AI TID Individual Values



Source: Figure 14.6.26, Study report AI-005.

There was little correlation between change in log₁₀ PA CFUs and percent change in FEV₁ at Day 28 for the AI BID ($r = -0.09$) and AI TID ($r = 0.04$) groups. There was an inverse correlation for these variables in the pooled placebo group ($r = -0.29$).

Correlation between Log₁₀ PA CFUs and Aztreonam MIC

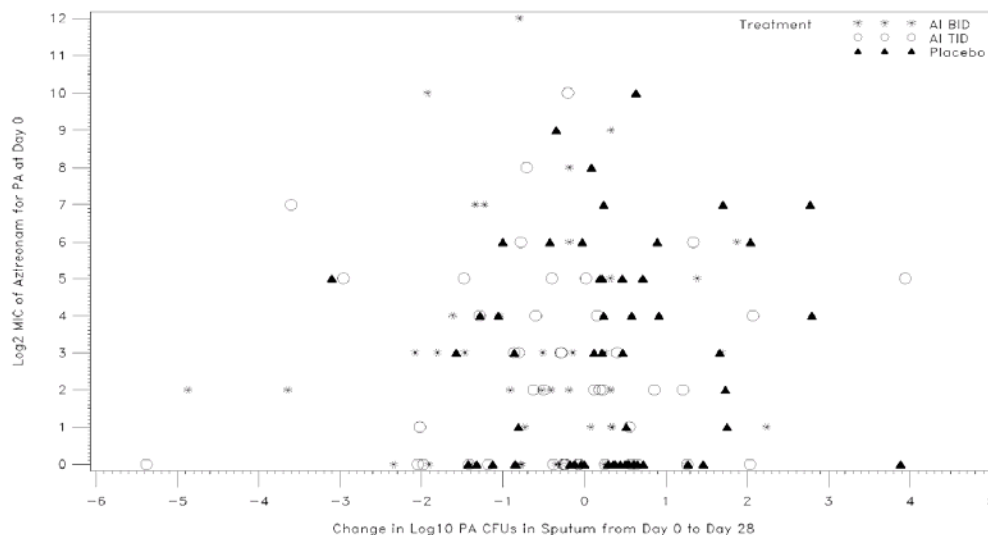
The change in aztreonam MIC for the PA isolate with the highest MIC was plotted against log₁₀ PA CFUs in sputum in [Figure 13](#).

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Figure 13. Aztreonam Log2 MIC for PA Isolate with the Highest MIC at Day 0 vs. Day 28 Actual Change in Log10 PA CFUs in Sputum for Pooled Placebo, AI BID and AI TID Individual Values



Source: Figure 14.6.23, Study report AI-005.

Figure demonstrates there was no correlation between the MIC for the PA isolate with the highest MIC from a patient and the change in log10 PA CFUs in sputum samples.

Relationship between Aztreonam MIC and Clinical Outcomes

MIC50 and MIC90 of aztreonam at Day 0 for all PA isolates for subsets of patients (those who, at Day 28, had:

- ≥ 0.75 decrease in log10 PA CFUs in sputum;
- < 0.75 decrease in log10 PA CFUs in sputum;
- 8% increase in FEV₁;
- $< 8\%$ increase in FEV₁;
- ≥ 5 increase in CFQ-R respiratory domain score; and
- < 5 increase in CRQ-R respiratory domain score are summarized in [Table 26](#).

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Table 26. MIC50 and MIC90 of Aztreonam at Day 0 for all PA Isolates (µg/mL) for Subsets of Patients

Statistics	Treatment			
	Placebo	AI		
	Pooled (N = 76)	BID (N = 69)	TID (N = 66)	Pooled (N = 135)
Patients with ≥ 0.75 decrease in $\log_{10}$ PA CFUs at Day 28				
n ^a	12	17	13	30
n ^b	26	33	22	55
MIC ₅₀	≤ 1	4	≤ 1	4
MIC ₉₀	16	128	32	64
Patients with < 0.75 decrease in $\log_{10}$ PA CFUs at Day 28				
n ^a	43	29	31	60
n ^b	77	47	56	103
MIC ₅₀	4	2	2	2
MIC ₉₀	64	64	128	64
Patients with $\geq 8\%$ increase in FEV ₁ at Day 28				
n ^a	15	16	21	37
n ^b	25	29	36	65
MIC ₅₀	≤ 1	2	≤ 1	≤ 1
MIC ₉₀	64	8	16	16
Patients with $< 8\%$ increase in FEV ₁ at Day 28				
n ^a	47	39	35	74
n ^b	85	70	62	132
MIC ₅₀	≤ 1	2	2	2
MIC ₉₀	64	64	128	64
Patients with ≥ 5 increase in CFQ-R respiratory domain score at Day 28				
n ^a	22	31	27	58
n ^b	34	50	46	96
MIC ₅₀	≤ 1	≤ 1	≤ 1	≤ 1
MIC ₉₀	64	32	32	32
Patients with < 5 increase in CFQ-R respiratory domain score at Day 28				
n ^a	36	24	27	51
n ^b	67	47	49	96
MIC ₅₀	2	4	≤ 1	4
MIC ₉₀	64	64	256	128

a n = the number of patients with available data.

b n = the number of isolates.

Source: Table 14.6.4.1, Study report AI-005.

PA CFU response was categorized according to the magnitude of change (< 0.75 decrease in $\log_{10}$ PA CFUs and ≥ 0.75 decrease in $\log_{10}$ PA CFUs). In the pooled placebo group, the baseline MIC90 was at two dilution steps higher among patients who had a $\log_{10}$ decrease in PA CFU density of < 0.75 . In the BID AI group, the MIC90 was one dilution step higher among patients with ≥ 0.75 decrease in $\log_{10}$ PA CFUs. In the TID AI group, the MIC90 was two dilution steps lower among patients with ≥ 0.75 decrease in $\log_{10}$ PA CFUs.

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FEV₁(L) response was categorized according to the magnitude of change (< 8% increase and ≥ 8% increase). In the BID AI group and TID AI group, the baseline MIC90 was three-dilution steps higher among patients with an increase of < 8% compared with patients with an increase of ≥ 8%. The baseline MIC90 were not clearly associated with FEV₁ response in the placebo groups.

CFQ-R respiratory domain response was categorized according to the magnitude of change (< 5 point increase and ≥ 5 point increase). In the BID AI group, the MIC90 was a one-dilution step higher among patients with an increase in CFQ-R score of < 5. In the TID AI group, the MIC90 was a three-dilution step higher among patients with an increase in CFQ-R score of < 5. In the pooled placebo group, baseline MIC90 was comparable among patients in the two CFQ-R response subgroups.

To summarize, by the categorical analyses, *there was insufficient evidence to establish a relationship between MICs and three measures of efficacy.*

Study CP-AI-007: A Phase 3, Double-blind, Multicenter, Multinational, Randomized, Placebo-controlled Trial Evaluating Aztreonam Lysinate for Inhalation in Cystic Fibrosis Patients with Pulmonary *P. aeruginosa* (AIR-CF1)

OBJECTIVES

This trial was designed to assess the safety and efficacy of a 28-day treatment with aztreonam lysine for inhalation (AI) (75 mg three times daily [TID]) compared to placebo in cystic fibrosis (CF) patients with lung disease due to *Pseudomonas aeruginosa* (PA).

METHODOLOGY

Patient eligibility was initially assessed at a Screening Visit that occurred 7 to 14 days prior to the baseline visit (Day 0). Those patients who continued to meet eligibility criteria at Day 0 were randomized and began a 28-day course of AI TID or placebo TID. Patients returned for clinic visits at Day 14, an end of treatment visit at Day 28, and a follow-up visit 14 days after the last dose of the trial drug (Day 42).

AI/placebo was self-administered using the PARI eFlow® Electronic Nebulizer (eFlow). All doses of AI/placebo were administered after using a short- or long-acting bronchodilator.

Patients in this trial were eligible to enroll in an open-label follow-on trial, [CP-AI-006](#).

NUMBER OF PATIENTS

The planned trial size was approximately 140 patients randomized in a 1:1 ratio to AI TID or placebo TID. There were 166 patients randomized to a treatment group (83 AI, 83 placebo). Two patients randomized to AI withdrew before receiving drug. In addition, one patient randomized to receive AI received placebo in error. Thus, 80 patients received AI and 84 patients received placebo. There were 164 patients included in both the Safety population and the Intent-to-treat (ITT) population.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION

Patients aged ≥ 6 years with CF and pulmonary PA and having forced expiratory volume in one second FEV₁ ≥ 25% and ≤ 75% predicted. Patients were not to have received anti-pseudomonal antibiotics for at least 28 days before receiving AI or placebo.

TEST PRODUCT

AI (75 mg/mL aztreonam lysine when reconstituted in diluent [0.17% saline]; sterile, pH 4.2 to 7.0, and osmolality 300 to 550 mOsmol/kg). AI was self-administered by inhalation using the eFlow BID or TID. AI was self-administered by inhalation using the PARI eFlow nebulizer. The eFlow is

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510(k) approved in the US and is CE-marked in Europe. It is approved in Canada and is considered investigational in Australia and New Zealand.

REFERENCE THERAPY

Placebo (5 mg/mL lactose when reconstituted in diluent [0.17% saline]; sterile, pH 4.2 to 7.5, and osmolality 200 to 400 mOsmol/kg). Placebo was self-administered by inhalation using the eFlow TID.

DURATION OF TREATMENT

28 days of AI/placebo. The study period lasted 56 days including a 14-day Screening period, a 28-day course of AI/placebo (AI/placebo treatment period) and a follow-up visit 14 days after the last dose of the trial.

CRITERIA FOR EVALUATION OF EFFICACY

The primary efficacy endpoint was change from Day 0 (baseline) to Day 28 in clinical symptoms as assessed by the respiratory domain of the Cystic Fibrosis Questionnaire-Revised (CFQ-R).

The key secondary efficacy endpoints were as follows:

- Percent change in FEV₁ from Day 0 to Day 28
- Change in log₁₀ PA colony-forming units (CFUs) in sputum from Day 0 to Day 28
- Proportion of patients receiving IV or inhaled anti-pseudomonal antibiotics other than trial drug through nominal Day 42
- Proportion of patients hospitalized through nominal Day 42

Other secondary efficacy endpoints were as follows:

- Change from baseline (Day 0) to Day 14 and Day 42 in clinical symptoms as assessed by the CFQ-R respiratory domain
- Change in pulmonary function (FEV₁ percent of predicted, AUC analysis of change in FEV₁ [L] from Days 0 to 42, change in forced vital capacity [FVC], and change in forced expiratory flow from 25% to 75% [FEF₂₅₋₇₅])
- Change in the CFQ-R nonrespiratory domains
- Percent of days and number of days hospitalized
- Use of nontrial drug anti-pseudomonal antibiotics (oral and overall)
- Change in CF symptoms and severity
- Missed school and/or work days due to CF
- Change in patient's ability to produce sputum
- Percent change in weight
- Change in Body Mass Index (BMI)

OTHER EVALUATIONS

- Aztreonam concentrations in plasma and sputum
- Disappearance or appearance of other pathogens (*Staphylococcus aureus*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*)
- Minimum Inhibitory Concentration (MIC) of aztreonam for PA

CLINICAL MICROBIOLOGY METHODOLOGY

Sputum for microbiology was collected for qualitative and quantitative culture for PA, *B. cepacia*, *S. maltophilia*, *Achromobacter xylosoxidans*, and *Staphylococcus aureus*, and to determine MICs for PA and *B. cepacia*.

Samples were collected at all visits, and were collected before trial drug treatment at Days 0, 14, and 28. At Days 14 and 28, sputum was collected at least four hours after the most recent dose of AI/placebo taken. A minimum of 0.5 mL of sputum was to be collected. If the patient could not

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expectorate, a throat swab was to be collected from which two slides were to be prepared. Every effort was made to obtain an expectorated sputum sample because a throat swab significantly limited microbiology testing.

Sputum for microbiology was stored refrigerated until shipment was made to the central laboratory. Sputum samples were shipped on frozen gel packs.

CLINICAL EFFICACY RESULTS

Patients in the AI group had a clinically significant improvement in respiratory symptoms as measured by the CFQ-R respiratory domain score for Child/Teen/Adult combined. The adjusted mean change from Day 0 at Day 28 was 7.08 compared with -2.63 in the placebo group. The adjusted mean difference (9.71) between AI and placebo at this time point was statistically significant ($p = 0.0005$). Significant differences were also observed between the treatments at Day 14 (7.98, $p = 0.0006$) and Day 42 (6.33, $p = 0.0154$). A greater proportion of patients in the AI group showed clinically meaningful improvement in CFQ-R respiratory domain score at Day 28 (56%) compared with patients in the placebo group (37%), $p = 0.0055$. Results of the microbiologically significant clinical endpoints are as follows:

Table 27. Results of Microbiologically Significant Clinical Endpoints in AI-007

Efficacy Endpoint Key Test or Evaluation	Result			
	Placebo (N = 84)	75 mg AI (N = 80)	Treat. Diff.	p-value
Clinical symptoms as assessed by CFQ-R respiratory domain				
Mean (adj) change in CFQ-R respiratory domain score at Day 14	-0.98	7.01	7.98	0.0006
Mean (adj) change in CFQ-R respiratory domain score at Day 28	-2.63	7.08	9.71	0.0005
Categorical result: % of patients who improved at Day 28	37.3	56.3	-	0.0055
% of patients who worsened at Day 28	44.6	25.0	-	
Mean (adj) change in CFQ-R respiratory domain score at Day 42	-5.71	0.62	6.33	0.0154
Change in pulmonary function				
Mean (adj) percent change in FEV ₁ at Day 28	-2.408	7.886	10.294	< 0.0001
Mean (adj) percent change in FEV ₁ at Day 42	-2.591	3.140	5.731	0.0024
Change in log ₁₀ PA CFUs in sputum				
Mean (adj) change in sputum log ₁₀ PA CFUs at Day 28	0.069	-1.384	-1.453	< 0.0001
Mean (adj) change in sputum log ₁₀ PA CFUs at Day 42	-0.010	-0.078	-0.069	NS

Notes: NS = not significant; Treat. Diff. = Treatment Difference; adj = adjusted. a Pulmonary exacerbation was the predominant cause of hospitalization between Days 0 and 42 in both groups: 2/4 patients in the AI group and 8/12 patients in the placebo group.

MICROBIOLOGICAL EFFICACY RESULTS

Log₁₀ PA Colony-forming Units in Sputum

Mean log₁₀ PA CFUs in sputum is presented in [Table 28](#).

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Table 28. Mean ($\pm$ SD) Log₁₀ PA CFUs in Sputum: ITT Population

Timepoint	Treatment	
	Placebo (N = 84)	75 mg AI (N = 80)
Day 0		
Mean ($\pm$ SD)	6.273 (2.081)	6.574 (2.000)
n	74	62
Day 14		
Mean ($\pm$ SD)	6.409 (1.873)	5.485 (2.236)
n	70	68
Day 28		
Mean ($\pm$ SD)	6.096 (2.234)	5.027 (2.564)
n	66	64
Day 42		
Mean ($\pm$ SD)	6.171 (1.763)	6.352 (2.057)
n	50	59

Source: Table 14.2.10.1, Study report AI-007.

At baseline (Day 0), mean log₁₀ PA CFU sputum density values were nearly equivalent in the AI and placebo groups. In the AI group, log₁₀ PA CFU sputum density had decreased by more than 1 log₁₀ CFU at Day 14 and by more than 1.5 log₁₀ CFUs at Day 28. Log₁₀ PA CFU sputum density in the placebo group remained near baseline values at Days 14 and 28.

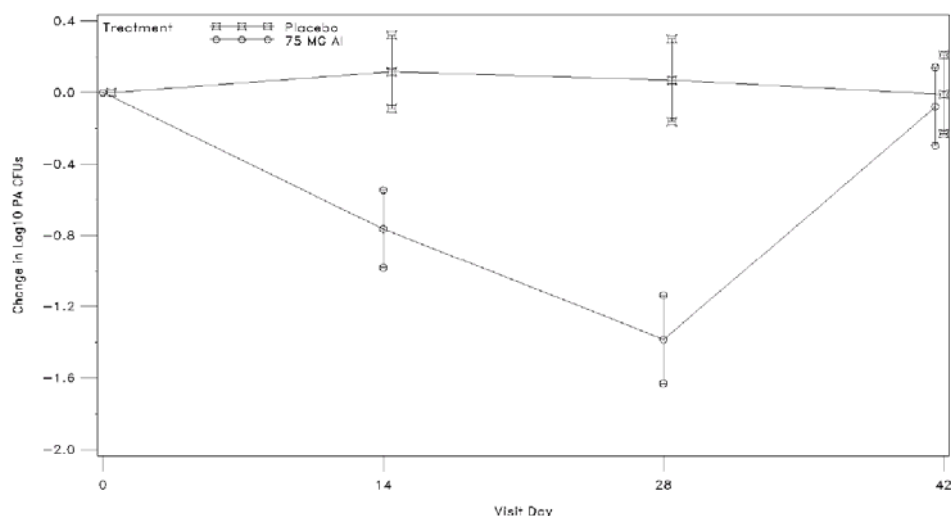
At Day 42, two weeks after cessation of therapy, log₁₀ PA CFU sputum density had returned to near baseline values in both the AI and placebo groups. The change in log₁₀ PA CFUs in sputum from Day 0 is presented in [Figure 14](#) and [Table 29](#).

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Figure 14. Mean Change in Log10 PA CFUs in Sputum from Day 0: ITT population



Source: Table 14.2.10.3, Study report AI-007.

Table 29. Change in Sputum Log10 PA CFUs from Day 0: ITT Population

Timepoint	Treatment	
	Placebo (N = 84)	75 mg AI (N = 80)
Day 14		
Mean (± SD)	0.032 (1.135)	-0.813 (2.104)
Median (range)	-0.045 (-2.22, 5.16)	-0.907 (-6.78, 8.19)
n	64	55
Adjusted mean	0.116	-0.764
Treatment difference: 75 mg AI – placebo	-0.880	
95% CI (p-value)	-1.469, -0.290 (0.0038)	
Day 28		
Mean (± SD)	-0.025 (0.786)	-1.476 (2.540)
Median (range)	-0.108 (-1.48, 2.50)	-1.114 (-8.12, 4.76)
n	61	53
Adjusted mean	0.069	-1.384
Treatment difference: 75 mg AI – placebo	-1.453	
95% CI (p-value)	-2.115, -0.791 (< 0.0001)	
Day 42		
Mean (± SD)	-0.118 (1.724)	-0.193 (1.375)
Median (range)	-0.227 (-5.29, 7.06)	-0.026 (-6.78, 3.60)
n	49	50
Adjusted mean	-0.010	-0.078
Treatment difference: 75 mg AI – placebo	-0.069	
95% CI (p-value)	-0.673, 0.535 (0.8218)	

Source: Table 14.2.10.3.

As the results of the analysis of percent change in FEV₁ at Day 28 achieved statistical

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significance, the change in log₁₀ PA CFUs in sputum at Day 28 was tested at a significance level of 0.025.

The difference between the AI and placebo groups for change in log₁₀ PA CFUs in sputum was statistically significant at Day 14 (-0.880, $p = 0.0038$) and at Day 28 (-1.453, $p < 0.0001$). From Day 0 to Day 28, adjusted mean log₁₀ PA CFU sputum density decreased (improved) by 1.384 in the AI group and increased (worsened) by 0.069 in the placebo group. Log₁₀ PA CFU density continued to decline in the AI group from Day 14 to Day 28. At Day 42 there was no statistically significant difference between the AI and placebo groups for change in log₁₀ PA CFUs in sputum (-0.069, $p = 0.8218$).

Presence or Absence of Pathogens

Presence or absence of PA is summarized in [Table 30](#).

Table 30. Presence or Absence of *Pseudomonas aeruginosa*: Safety Population

Treatment	Visit	n ^a	Result		Change		
			Presence	Absence	App	No	Dis
			n (%)	n (%)	n	n	n
Placebo (N = 84)	Day 0	81	76 (93.8)	5 (6.2)	–	–	–
	Day 14	80	76 (95.0)	4 (5.0)	3	73	1
	Day 28	75	70 (93.3)	5 (6.7)	1	71	2
	Day 42	59	58 (98.3)	1 (1.7)	3	55	1
75 mg AI (N = 80)	Day 0	74	70 (94.6)	4 (5.4)	–	–	–
	Day 14	78	72 (92.3)	6 (7.7)	3	66	3
	Day 28	76	66 (86.8)	10 (13.2)	3	59	8
	Day 42	69	65 (94.2)	4 (5.8)	2	59	2

App, No, and Dis indicate the number of patients with appearance/disappearance of pathogen, or no change from Day 0. na The number of patients with available data. – Not applicable.

Source: Table 14.6.2, Study report AI-007.

***Pseudomonas aeruginosa*:** Presence of PA during screening was an inclusion criterion for this trial for patients enrolled under protocol versions 1, 2, and 3. For patients enrolled under protocol version 4 the eligibility criterion was changed as follows: PA present in expectorated sputum or throat swab culture at Visit 1 or documented PA in 2 expectorated sputum or throat swab cultures within the 12 months prior to Visit 1 (one of the previous PA positive cultures had to be no more than 3 months prior to Visit 1).

After dosing, PA was isolated in the majority of patients between Days 14 and 42 (87% to 94% of patients in the AI group, 93% to 98% of patients in the placebo group). Eradication of established PA infection is not expected for suppressive therapy, and the presence or absence of PA is more likely to be attributable to variations in sampling technique, or to the patients' ability to produce sputum, than to the disappearance of PA in individual patients.

Other Pathogens: The percentages of patients testing positive for *S. aureus* were slightly higher in the AI group than in the placebo group at all time points, including Day 0 (46% to 50% in AI-treated patients vs. 37% to 42% in placebo-treated patients) (Table 14.6.2, not shown).

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The percentages of patients testing positive for *A. xylosoxidans* were generally slightly lower in the AI group than in the placebo group at all time points, including Day 0 (1.3% to 2.6% in AI-treated patients vs. 5.0% to 9% in placebo-treated patients).

There were no other notable differences between the AI and placebo groups in the percentages of patients with positive culture results, or in the numbers of patients with appearance or disappearance of pathogens for the other pathogens tested (*B. cepacia* and *S. maltophilia*).

Treatment-emergent isolation of other organisms is summarized in [Table 31](#).

Table 31. Treatment-emergent Isolation of Other Organisms: Safety Population

Nature of Isolation	Organism	Treatment	
		Placebo (N = 84) n (%)	75 mg AI (N = 80) n (%)
Treatment-emergent intermittent isolation ^a	<i>S. aureus</i>	7 (8.6)	4 (5.4)
	<i>B. cepacia</i>	0	0
	<i>S. maltophilia</i>	3 (3.7)	3 (4.1)
	<i>A. xylosoxidans</i>	2 (2.5)	0
Treatment-emergent persistent isolation ^b	<i>S. aureus</i>	5 (6.2)	2 (2.7)
	<i>B. cepacia</i>	0	0
	<i>S. maltophilia</i>	0	2 (2.7)
	<i>A. xylosoxidans</i>	2 (2.5)	1 (1.4)

Number of patients with available data (i.e. with valid culture at Day 0 and between Day 0 and Day 42): Placebo = 81, 75 mg AI = 74. a Intermittent Isolation: Organism not isolated at Day 0 but isolated once between Day 0 and Day 42, and not a persistent isolation. b Persistent Isolation: Organism not isolated at Day 0 but isolated in at least two subsequent specimens or at the Early Termination Visit.

Source: Table 14.6.3, Study report AI-007.

***Staphylococcus aureus*:** A smaller percentage of patients in the AI group had intermittent isolation of *S. aureus* (four [5%] patients) compared with the placebo group (seven [9%] patients), and a smaller percentage of patients in the AI group had persistent isolation of *S. aureus* (two [2.7%] patients) compared with the placebo group (five [6%] patients).

***Burkholderia cepacia*:** Patient 14756 tested positive for *B. cepacia* at screening and at Day 0, but not at Day 14. This patient discontinued the study and was lost to follow-up after Day 14. Patient 16066 tested positive at screening but not at Day 0 or thereafter. This patient completed the study. There was no intermittent or persistent isolation of *B. cepacia* from any patient.

***Stenotrophomonas maltophilia*:** A slightly higher percentage of patients in the AI group had persistent isolation of *S. maltophilia* (two [2.7%] patients) compared with the placebo group (no patients). There was no difference between the AI and placebo groups in the percentages of patients with intermittent isolation of *S. maltophilia*.

***Achromobacter xylosoxidans*:** A smaller percentage of patients in the AI group had intermittent isolation of *A. xylosoxidans* (no patients) compared with the placebo group (two [2.5%] patients). There was no difference between the AI and placebo groups in the percentage of patients with persistent isolation of *A. xylosoxidans*.

Over the 28-day treatment and 2-week follow-up periods in this trial, there was no evidence of a persistent increase in positive test cultures for any of the organisms evaluated.

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Minimum Inhibitory Concentrations for PA

MICs of aztreonam for PA isolates in each treatment arm are presented in [Tables 32](#) and [33](#).
MIC90 values are indicated by the shaded cells.

Table 32. Aztreonam MICs for all PA Isolates: Placebo--TID

MIC (µg/ml)	Day 0	N=140	Day 14	N=128	Day 28	N=116	Day 56	N=94
	n	%	n	%	n	%	n	%
<1	64	45.7%	53	41.4%	49	42.2%	44	46.8%
2	9	6.4%	12	9.4%	9	7.8%	7	7.4%
4	10	7.1%	4	3.1%	11	9.5%	9	9.6%
8	25	17.9%	23	18.0%	18	15.5%	12	12.8%
16	10	7.1%	6	4.7%	6	5.2%	7	7.4%
32	7	5.0%	7	5.5%	6	5.2%	9	9.6%
64	4	2.9%	11	8.6%	10	8.6%	1	1.1%
128	10	7.1%	8	6.3%	6	5.2%	0	0.0%
256	1	0.7%	3	2.3%	1	0.9%	2	2.1%
512	0	0.0%	1	0.8%	0	0.0%	2	2.1%
1024	0	0.0%	0	0.0%	0	0.0%	0	0.0%
2048	0	0.0%	0	0.0%	0	0.0%	0	0.0%
>2048	0	0.0%	0	0.0%	0	0.0%	1	1.1%

Source: 14.6.4.2, Study report AI-007.

Table 33. Aztreonam MICs for all PA Isolates: AI—TID

MIC (µg/ml)	Day 0	N=128	Day 14	N=118	Day 28	N=102	Day 56	N=113
	n	%	n	%	n	%	n	%
<1	51	39.8%	39	33.1%	31	30.4%	43	38.1%
2	8	6.3%	11	9.3%	8	7.8%	9	8.0%
4	12	9.4%	9	7.6%	8	7.8%	15	13.3%
8	16	12.5%	19	16.1%	16	15.7%	15	13.3%
16	13	10.2%	8	6.8%	5	4.9%	9	8.0%
32	4	3.1%	6	5.1%	11	10.8%	5	4.4%
64	7	5.5%	9	7.6%	5	4.9%	4	3.5%
128	8	6.3%	3	2.5%	9	8.8%	6	5.3%
256	4	3.1%	2	1.7%	3	2.9%	3	2.7%
512	3	2.3%	5	4.2%	4	3.9%	1	0.9%
1024	0	0.0%	4	3.4%	1	1.0%	2	1.8%
2048	0	0.0%	0	0.0%	0	0.0%	1	0.9%
>2048	2	1.6%	3	2.5%	1	1.0%	0	0.0%

Source: 14.6.4.2, Study report AI-007.

During the treatment period, MIC90s of aztreonam for all PA isolates generally remained unchanged. There were no lasting increases of two dilution steps or more during therapy. In the placebo arm, MIC 90 values were 64 µg/ml at Days 0, 14 and 28 and decreased to 32 µg/ml by Day 56. In the AI treatment arm, MIC90 values were 128 at Days 0, 28 and 56. The MIC90 value on Day 14 was 512 µg/ml.

Aztreonam MIC50 and MIC90 values for the PA isolate with the highest MIC from each patient are summarized in [Table 34](#).

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Table 34. Aztreonam MIC50 and MIC90 Values for PA Isolate with the Highest MIC from Each Patient: Safety Population

Treatment	Visit	Result (µg/mL)				
		MIC ₅₀	MIC ₉₀	n	Min	Max
Placebo (N = 84)	Day 0	8	128	76	≤ 1	256
	Day 14	8	128	75	≤ 1	512
	Day 28	4	64	70	≤ 1	256
	Day 42	4	64	57	≤ 1	> 2048
75 mg AI (N = 80)	Day 0	8	256	69	≤ 1	> 2048
	Day 14	8	512	72	≤ 1	> 2048
	Day 28	8	256	66	≤ 1	> 2048
	Day 42	8	256	65	≤ 1	2048

Source: Table 14.6.4.3, Study report AI-007.

MIC50 and MIC90 for the PA isolate with the highest MIC from each patient generally remained unchanged for both the AI and placebo treatment groups. For the placebo arm, the MIC90 decreased by one dilution step (128 to 64 µg/ml) between Day 0 and Day 42. In the AI treatment group, the MIC90 remained the same (256 µg/ml) at Days 0 and 42.

MICs of Tobramycin for PA

For the AI group, at all time points, the percentages of patients with increased highest MICs of tobramycin from Day 0 for the PA isolate with the highest MIC from each patient (9% to 12%) were generally much lower than the percentages of patients with decreased highest MICs of tobramycin (20% to 30%). There were no notable differences within the placebo group for the percentages of patients with increased (10% to 14%) vs. decreased (12% to 20%) highest MIC of tobramycin from Day 0 for the PA isolate with the highest MIC. There were a slightly higher percentage of AI-treated patients with a decrease in MIC of tobramycin at all time points (20% to 30%) compared with the placebo group (12% to 20%) for the PA isolate with the highest MIC; none of these differences were statistically significant (Table 14.6.4.6, not shown).

There were no differences between the AI and placebo treatment groups in the percentage of patients with MIC of tobramycin = 8 µg/mL for the PA isolate with the highest MIC. The percentages of patients who are highest MIC was = 8 µg/mL generally declined over the course of the trial in both treatment groups, particularly after the end of AI/placebo treatment (Table 14.6.4.8, not shown).

MICs of other Antibiotics for PA

Apart from transient changes, there were no notable increases in the MIC50 or MIC90 of any of the other antibiotics tested after treatment with AI (gentamicin, amikacin, piperacillin, cefepime, meropenem, ceftazidime, ciprofloxacin, and ticarcillin/clavulanate) (Tables 14.6.4.9 and 14.6.4.10, not shown).

Aztreonam Concentrations in Plasma and Sputum

Plasma and sputum aztreonam concentrations are summarized in [Table 35](#).

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Table 35. Plasma and Sputum Aztreonam Concentrations: Safety Population

Treatment	Visit	Timepoint	n	Mean	Median	SD	Min	Max
Aztreonam concentrations in plasma (ng/mL)								
75 mg AI (N = 80)	Day 0	Pretreatment	71	5.55	0.00	36.37	0.0	300.0 ^a
			72	550.35	495.00	308.51	0.0	1,620.0
	Day 14	+1H	76	666.83	595.00	356.90	12.1	1,660.0
			Day 28	Pretreatment	63	103.59	63.40	123.61
	+1H	68		652.90	602.50	394.28	0.0	1,740.0
		Aztreonam concentrations in sputum (ng/g)						
75 mg AI (N = 80)	Day 0	+10 min	74	984,162	530,500	1,106,805	8,170	6,010,000
	Day 14	+10 min	67	793,420	677,000	619,268	2,080	2,780,000
	Day 28	+10 min	63	715,026	451,000	689,609	614	2,800,000

^a The maximum aztreonam plasma concentration at Day 0 pretreatment for Patient 17151 was 300.00 ng/mL. However, at + 1 H this patient's plasma concentration was BQL; the pretreatment and + 1 H tubes were likely switched.

Source: Tables 14.6.1.1 and 14.6.1.2, Study report AI-007.

Concentrations of aztreonam in both plasma and sputum varied substantially as indicated by high SDs. Mean aztreonam concentrations in sputum were at least 1000-fold higher than those in plasma. There were no notable changes in mean or median aztreonam concentrations in sputum.

CORRELATION OF *IN VITRO* SUSCEPTIBILITY AND CLINICAL OUTCOME

To investigate possible correlation between endpoints, scatterplots were produced as changes from Day 0 to Day 28 on the ITT (observed case data) population (unless otherwise stated) with a regression line displayed and a correlation coefficient reported in the footnotes. These data are presented in Figures 14.7.9 (highest aztreonam log₂ MIC for PA Day 0 vs. change in log₁₀ PA CFUs in sputum); 14.7.10 (highest aztreonam log₂ MIC for PA Day 0 vs. percent change in FEV₁); 14.7.11 (percent change in FEV₁ vs. change in log₁₀ PA CFUs in sputum); 14.7.12 (percent change in FEV₁ vs. change in CFQ-R respiratory domain score [for the ITT (imputed data) population]); and 14.7.13 (change in CFQ-R respiratory domain score vs. change in log₁₀ PA CFUs in sputum).

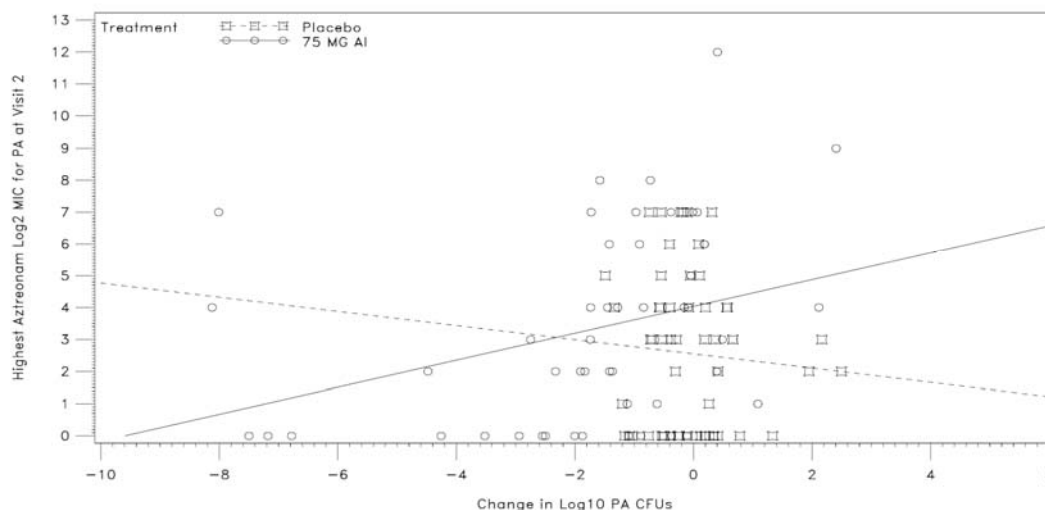
In the first analysis, the highest aztreonam log₂ MIC for PA Day 0 was plotted against the change in log₁₀ PA CFUs in sputum as seen in [Figure 15](#).

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Figure 15. Supplementary Analysis Highest Aztreonam Log2 MIC for PA at Visit 2 (Day 0) vs. Change in Log10 PA CFUs in Sputum from Visit 2 (Day 0) to Visit 4 (Day 28) on Observed Case Data Individual Values

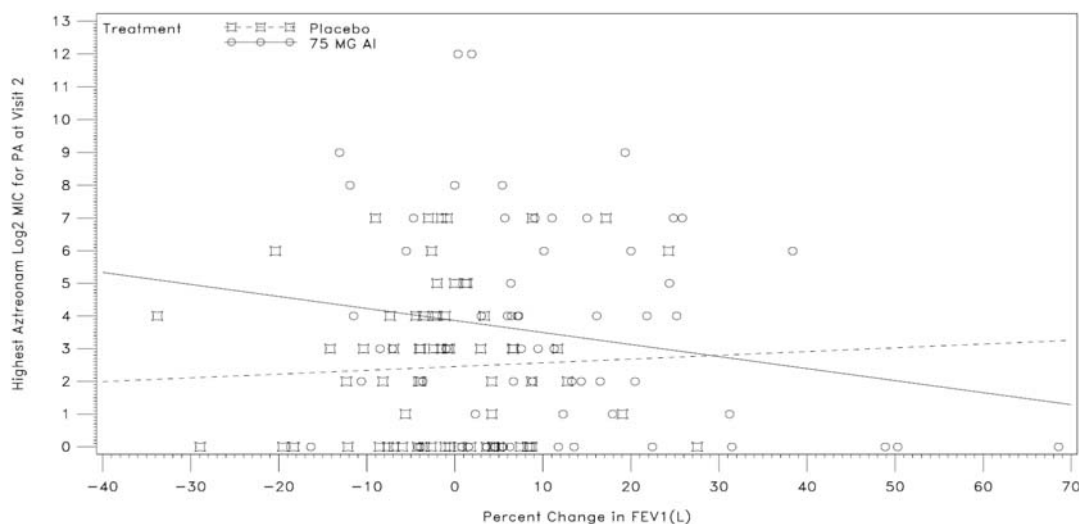


Source: Figure 14.7.9, Study report AI-007.

In the AI group, there was a positive correlation between highest aztreonam log2 MIC for PA at Day 0 and change in log10 PA CFUs in sputum (correlation coefficient = 0.33), but there was no correlation between highest aztreonam log2 MIC for PA at Day 0 and change in log10 PA CFUs in sputum for the placebo group (correlation coefficient = -0.07). This result indicated that change in log10 PA CFUs in sputum became more negative (i.e., greater bacterial killing) as highest aztreonam log2 MIC for PA at Day 0 decreased in the AI group but not in the placebo group.

The highest aztreonam log2 MIC for PA Day 0 was plotted against the percent change in FEV₁ as shown in Figure 16.

Figure 16. Supplementary Analysis Highest Aztreonam Log2 MIC for PA at Visit 2 (Day 0) vs. Percent Change in FEV₁ (L) from Visit 2 (Day 0) to Visit 4 (Day 28) on Observed Case Data Individual Values



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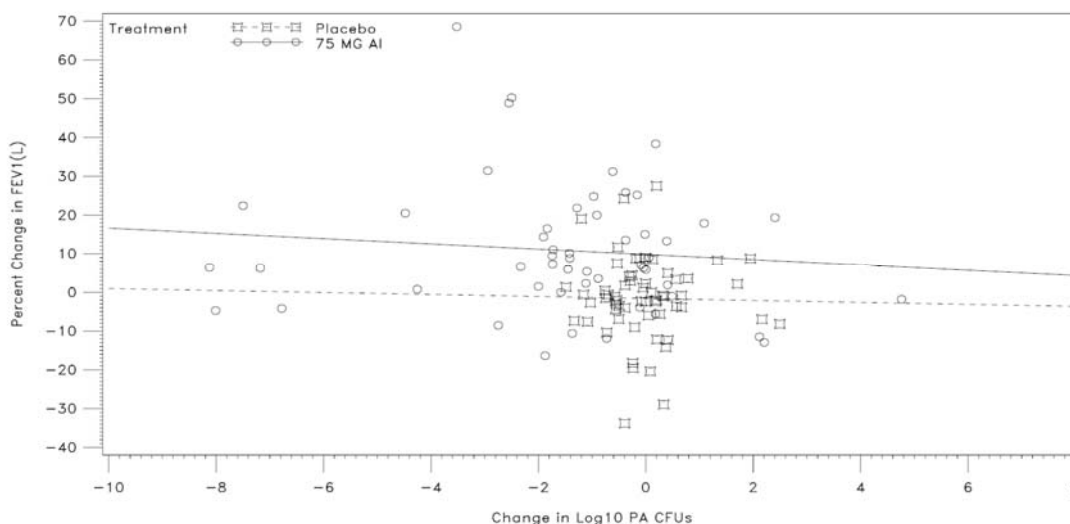
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Source: Figure 14.7.10, Study report AI-007.

The plot of highest aztreonam log₂ MIC for PA at Day 0 and percent change in FEV₁ indicate a slight negative correlation in the AI group (correlation coefficient = -0.18) and no correlation for the placebo group (correlation coefficient = 0.05).

The percent change in FEV₁ was plotted against the change in log₁₀ PA CFUs in sputum as shown in [Figure 17](#).

Figure 17. Supplementary Analysis Percent Change in FEV₁ (L) vs. Change in Log₁₀ PA CFUs in Sputum from Visit 2 (Day 0) to Visit 4 (Day 28) on Observed Case Data Individual Values



Source: Figure 14.7.11, Study report AI-007.

The plot of change in log₁₀ PA CFUs and percent change in FEV₁ indicate a very slight negative correlation in the AI group.

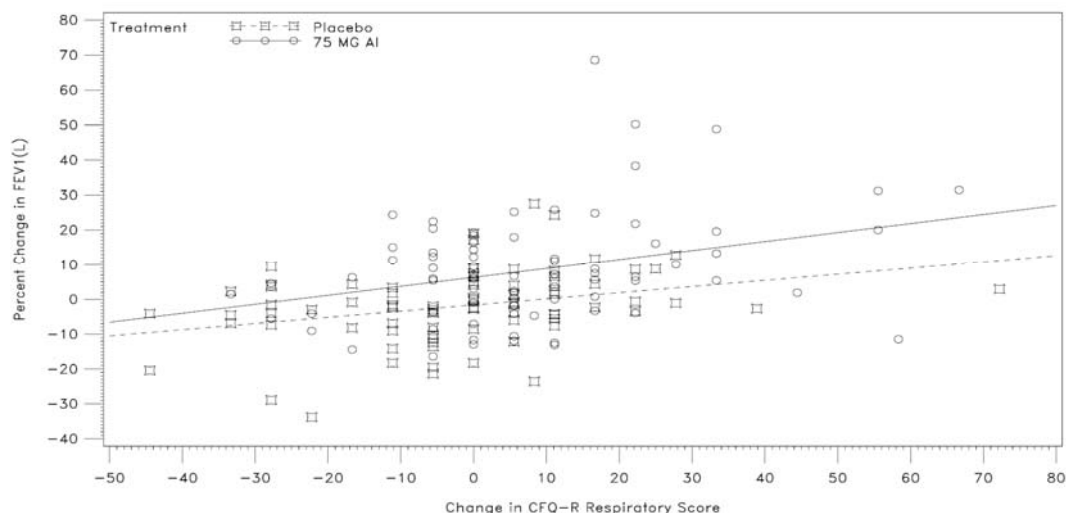
The percent change in FEV₁ was plotted against the change in CFQ-R respiratory domain score [for the ITT (imputed data) population] as shown in [Figure 18](#).

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Figure 18. Supplementary Analysis Percent Change in FEV₁ (L) vs. Change in CFQ – R Respiratory Domain Scores from Visit 2 (Day 0) to Visit 4 (Day 28) on Imputed Data Individual Values

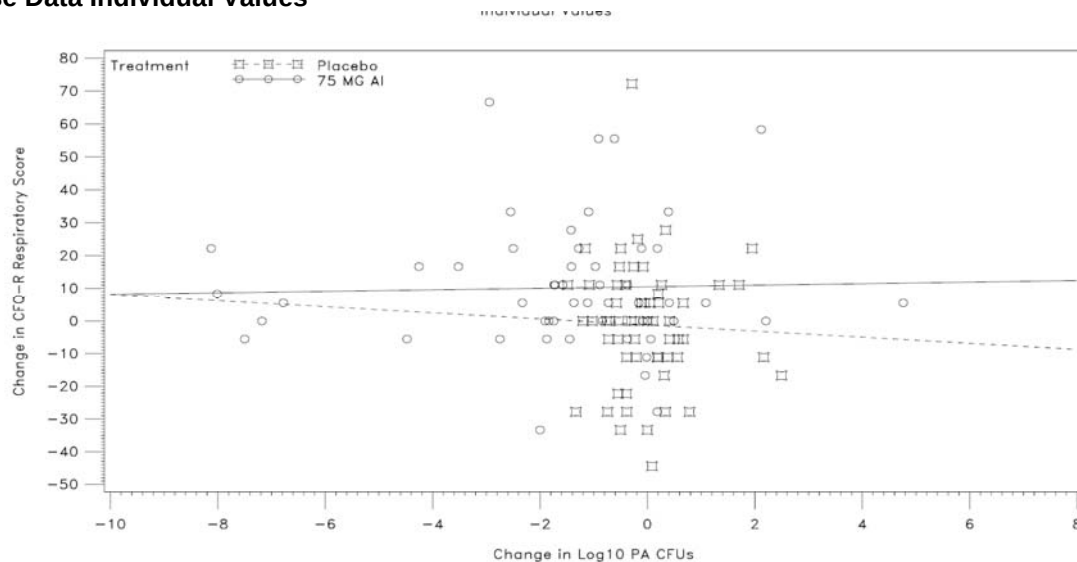


Source: Figure 14.7.12, Study report AI-007.

There was no correlation for percent change in FEV₁ or CFQ-R respiratory domain scores and change in log₁₀ PA CFUs in sputum for either treatment group (correlation coefficients were between -0.1 and 0.1).

The change in CFQ-R respiratory domain score was plotted against the change in log₁₀ PA CFUs in sputum as shown in Figure 19.

Figure 19. Supplementary Analysis Change in CFQ – R Respiratory Domain Scores vs. Change in Log₁₀ PA CFUs in Sputum from Visit 2 (Day 0) to Visit 4 (Day 28) on Observed Case Data Individual Values



Source: Figure 14.7.13, Study report AI-007.

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There was a moderate positive correlation between percent change in FEV₁ and change in CFQ-R respiratory domain score for the ITT (imputed data) population in both treatment groups (correlation coefficient = 0.32 for AI and placebo), indicating that change in CFQ-R respiratory domain score became more positive as percent change in FEV₁ became more positive in the both treatment groups.

Study CP-AI-006: A Phase 3, Open-label, Follow-on Study of Multiple Courses of Aztreonam Lysinate for Inhalation (AI) in Cystic Fibrosis Patients (AIR-CF3)

Reviewer's note: This study was not a pivotal trial but a follow-on study for AI-005 and AI-007. Consequently, this study will be summarized rather than reviewed in detail.

CP-AI-006 was an open-label, multicenter, follow-on study of multiple courses of AI designed to assess safety and disease-related outcomes following repeated exposure of up to nine courses of AI in CF patients who participated in either CP-AI-005 or CP-AI-007. Patients enrolled into the CP-AI-006 study receive 75 mg AI in the same treatment regimen, BID or TID, to which they were randomized in CP-AI-005 or CP-AI-007 for 28-day AI courses with 28 days between courses. The original open-label follow-on study protocol was designed to have patients receive two AI treatment courses with an optional third course. A protocol amendment extended the treatment period to allow up to nine courses of AI. At the time of interim analysis (01 March 2007), 207 patients had been treated with AI and had at least one post-baseline evaluation; of these patients, 162 had completed at least three AI courses.

In CP-AI-006, *P. aeruginosa* CFU/g in sputum were decreased from baseline at each subsequent visit among patients in the BID and TID AI treatment groups (see CP-AI-006 CSR Table 14.6.2.1, this submission, not shown). In the TID treatment group, a consistent decrease in *P. aeruginosa* CFU/g in sputum was observed following each of the first three treatment courses. For BID-treated patients, decreases in *P. aeruginosa* CFU/g did not directly cycle with treatment. Increases in *P. aeruginosa* CFU/g were generally observed during the off-treatment periods for AI BID and TID groups. Overall, the decreases in *P. aeruginosa* CFU/g in sputum were consistently larger in the TID group than the BID group. There were no notable differences between treatment groups in the appearance of other pathogens (see SCM Table 3.5.2.3, this submission, not shown). No changes were noted in the MIC50 of aztreonam for all *P. aeruginosa* isolates or for the *P. aeruginosa* isolate with the highest MIC from each patient (see CP-AI-006 CSR Tables 14.7.3 and 14.7.7). Transient increases in aztreonam MIC90 values occurred, but did not directly correlate with the on- and off-treatment courses. The MICs of aztreonam for the *P. aeruginosa* isolate with the highest MIC from each patient were largely unchanged in both the BID and TID AI groups during the first three courses of AI treatment (see CP-AI-006 CSR Table 14.7.8, this submission, not shown). There were no notable increases in the MIC50 or MIC90 of other antibiotics for *P. aeruginosa* following AI treatment (see CP-AI-006 CSR Table 14.7.4, this submission, not shown).

BREAKPOINT DISCUSSION

Aztreonam (as AZACTAM) has been approved by the Agency for treatment of the following indications caused by *Pseudomonas aeruginosa*: urinary tract infection, lower respiratory tract infection, septicemia, skin and soft structure infection and intra-abdominal infection. In addition, since AI is a topically administered antibiotic, interpretive criteria will not be determined.

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