CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

050814Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA:	50-814
Submission Date(s):	August 13, 2009
Brand Name	CAYSTON™
Generic Name	Aztreonam for inhalation solution (AZLI)
Primary Reviewer	Yongheng Zhang, Ph.D.
Team Leader	Charles Bonapace, Pharm.D.
OCP Division	DCP4
OND Division	DAIOP
Applicant	Gilead Science, Inc.
Relevant IND(s)	64,402
Submission Type; Code	Class 2 Resubmission
Formulation; Strength(s)	Lyophilized aztreonam for inhalation solution (75 mg aztreonam/vial)
Indication	To improve respiratory symptoms and pulmonary function in cystic fibrosis (CF) patients with *Pseudomonas aeruginosa*.

TABLE OF CONTENTS

1. EXECUTIVE SUMMARY

On November 16, 2007 Gilead Science Inc. submitted a New Drug Application (NDA 50-814) for aztreonam for inhalation solution (AZLI) indicated to improve respiratory symptoms and pulmonary function in cystic fibrosis (CF) patients with *Pseudomonas aeruginosa*. The proposed dosage regimen is AZLI 75 mg (one single use vial and one ampule of diluent) three times a day for 28 days. On September 16, 2008, the applicant received a Complete Response (CR) letter from the Division of Anti-Infective and Ophthalmology Products (DAIOP) stating that the application can not be approved in its present form because of the concerns on whether the applicant has demonstrated substantial evidence of effectiveness of AZLI in treating CF. On November 24, 2008, the applicant submitted a formal dispute resolution request (FDRR) to the Office of Antimicrobial Products, and the appeal was denied on February 18, 2009. On March 13, 2009 the applicant submitted another FDRR, which included a reanalysis of the data from the Phase 3 trial CP-AI-005, to the Office of New Drug, and the appeal was denied on June 17, 2009 with the two recommended actions: (1) to resubmit the NDA with new analyses of data, and (2) to present the application as part of the review to the Anti-Infective Drugs Advisory Committee (AC).

On August 13, 2009, the applicant submitted a Class 2 Resubmission of the NDA with new analyses of data. There is no new clinical pharmacology data in the Resubmission and a Clinical Pharmacology review on the original NDA has already been documented on June 27, 2008. In the current review, the reviewer focused on the applicant's responses to the CR letter, particularly to the response to deficiency #1 (Clinical/Clinical Pharmacology) stated below:

Before the application can be approved, it will be necessary for you to perform an additional adequate and well controlled Phase 3 study which demonstrates:

- **A delay in the Time to Need for IV or Inhaled Antibiotics due to Pre-defined Symptoms**
- **Changes in CFQ-R respiratory domain scores and changes in FEV_1 at Day 28 using a sequential testing procedure or other methodologies to control for the overall type-I error rate due to multiple testing.**

We strongly recommend the inclusion of a 150 mg BID arm in this study. Sparse sampling of sputum aztreonam concentrations should include additional collection times beyond 10 minutes post-dose (e.g. at 2, 4, and 6 hours post-dose).

As recommended in the response to the applicant's second formal dispute resolution request, the Anti-Infective Drugs Advisory Committee met on December 10, 2009 to discuss NDA 50-814. After reviewing the applicant's reanalysis of the data, the committee members dismissed the issue related to the regimen effect on the primary endpoint in Study CP-AI-005, which was one of the primary issues raised in the CR letter leading to the application not being approved, and focused on the secondary endpoint (i.e., FEV_1) and additional data from two other Phase 3 trials (CP-AI-006 and CP-AI-007). The committee members voted that the safety and efficacy of AZLI 75 mg TID for the requested indication of improvement of respiratory symptoms and pulmonary function in CF patients with *Pseudomonas aeruginosa* have been demonstrated (Yes = 15, No = 2). The committee members also ignored the agency's request in the CR letter for a new trial to evaluate a different dosing regimen and agreed that the applicant has identified a correct dose and regimen for AZLI for the requested indication (Yes = 17, No = 0).

1.1. RECOMMENDATIONS

The Applicant has adequately responded to deficiency #1 in the Complete Response letter dated September 16, 2008 and the Applicant's response constitutes a complete response.

An unresolved issue is whether an alternative dosing regimen (e.g. AZLI 150 mg BID) would be associated with a lower incidence of respiratory adverse events and greater efficacy than the proposed regimen of AZLI 75 mg TID. The reviewer recommends that an alternative dosing regimen be evaluated and compared to AZLI 75 mg TID in a well-controlled clinical trial should the NDA be approved. The applicant should submit the protocol to the division for comments prior to conduct of the study.

The reviewer's proposed label changes in Section 4.1 Proposed Package Insert should be forwarded to the sponsor.

1.2. PHASE 4 COMMITMENTS

Perform an adequate and well-controlled Phase 3 clinical trial(s) in cystic fibrosis patients with *Pseudomonas aeruginosa* to confirm the presence or absence of a regimen effect and determine the optimal AZLI dosing regimen. The objectives of this PMC are the following:

1. To evaluate the presence or absence of a regimen effect observed in study CP-AI-005 by comparing the efficacy of AZLI 75 mg BID to AZLI 75 mg TID;
2. To evaluate the safety and efficacy of an alternative AZLI dose (> 75 mg but ≤ 150 mg) either BID (presence of a regimen effect in #1 above) or TID (absence of a regimen effect in #1 above) compared to AZLI 75 mg TID for dose optimization.

Sparse sampling of sputum aztreonam concentrations should be obtained in the trial(s) at 10 minutes and approximately 4 hrs post-dose.

2. APPLICANT'S RESPONSES TO THE COMPLETE RESPONSE LETTER

On August 13, 2009 the applicant provided a Class 2 Resubmission and responded to the deficiencies outlined in the CR letter by the Division. The applicant's responses pertinent to Clinical Pharmacology are listed and commented below.

Deficiency & Recommendation
1. CLINICAL/STATISTICAL/CLINICAL PHARMACOLOGY
Before the application can be approved, it will be necessary for you to perform an additional adequate and well controlled Phase 3 study which demonstrates:

- A delay in the Time to Need for IV or Inhaled Antibiotics due to Pre-defined Symptoms
- Changes in CFQ-R respiratory domain scores and changes in FEV1 at Day 28 using a sequential testing procedure or other methodologies to control for the overall type-I error rate due to multiple testing.

We strongly recommend the inclusion of a 150 mg BID arm in this study. Sparse sampling of sputum aztreonam concentrations should include additional collection times beyond 10 minutes post-dose (e.g. at 2, 4, and 6 hours post-dose).

The sponsor's response: Several AZLI doses were studied in patients with CF during the AZLI clinical development program. A 75 mg AZLI TID dose was selected based on the need to minimize safety concerns, yet ensure effective treatment. This regimen was also the lowest effective dose tested in the Phase 1 through 3 studies. The higher doses under evaluation, 150 mg and 225 mg, were considered to be less safe and effective dosing regimens.

Reviewer's comments: Based on the data available, the findings from the Phase 1 and Phase 2 studies support that an alternative regimen such as 150 mg BID may result in improved efficacy and tolerability relative to the 75 mg BID and 75 mg TID regimens. Reviewer's comments are provided as below, immediately following each response from the sponsor.

The sponsor's response (cont'd): In a Phase 1 study (CP-AI-002), two cohorts of patients with CF were treated with single ascending doses of 75 mg, 150 mg, and 225 mg AZLI or 1 mL, 2 mL, and 3 mL placebo on consecutive days to determine the maximum tolerated dose (MTD). If a patient did not exceed their MTD, based on safety parameters, they were advanced to the next dose level. The first cohort included 18 adult patients (aged ≥ 18 years) and the second cohort included 17 adolescent patients (aged 13 to 17 years).

In the adult cohort, all 18 patients tolerated all three doses; none exceeded their MTD. In the adolescent cohort, 16/17 patients tolerated all three doses. One adolescent patient did not tolerate a dose of 150 mg AZLI (dosing was discontinued after the 150 mg dose due to an asymptomatic 20% decrease in FEV_1); therefore, this patient's MTD was 75 mg AZLI. Among adults, the percentage of AZLI-treated patients who experienced at least one adverse event (AE) increased with dose; no consistent dose-related trends were observed in the percentages of adolescents who experienced at least one AE during AZLI dosing. There was a dose-related trend in the incidence of respiratory AEs, notably cough, among adults. In addition, AEs of chest pain and chest tightness that were considered moderate in severity occurred in two adult patients (17%) treated with 150 mg AZLI. Taken together with the adolescent patient who was intolerant to the 150 mg dose, these events are of particular concern and indicate that the local airway tolerability of a 150 mg AZLI dose might preclude its use in patients with more reactive airways.

Reviewer's comments*: In Phase 1b Study CP-AI- 002 (single dose at 75mg, 150mg, and 225 mg), sputum concentrations of aztreonam following a single dose of AZLI 150 mg were approximately double those following AZLI 75 mg. As the dose was further increased from 150 mg to 225 mg, sputum concentrations were only minimally increased. Furthermore, the 225 mg dose was associated with a higher incidence of adverse events (AEs). The most common AEs were related to the respiratory system, for which the frequency was 2/12 (17%) of placebo patients, 5/23 (22%) of patients at the 75 mg dose level, 4/23 (17%) at 150 mg, and 6/22 (27%) at the 225 mg dose. The most common AE was aggravated cough, occurring in 0 of 12 patients in placebo, 1 of 23 (4%) at either 75 mg or 150 mg dose level, and 4 of 22 (18%) at the 225 mg dose level. Given the observed dose-related elevation in respiratory AEs, the sponsor's reason to exclude the intermediate dose of 150 mg but include the highest dose of 225 mg in subsequent trials is not valid.

The sponsor's response (cont'd): Based on the results of study CP-AI-002, a Phase 2 placebo-controlled study of AZLI in patients with CF was conducted (CP-AI-003). Patients were treated with AZLI (75 or 225 mg) or volume-matched placebo two times daily (BID) for 14 days. These doses were chosen based on the need to maximize tolerability (75 mg) and evaluate the maximum antibiotic effect (225 mg). One hundred five CF patients aged ≥ 13 years with *PA* and $FEV_1 \geq 40\%$ of predicted who had not used antipseudomonal or macrolide antibiotics within 56 days were included. The primary endpoint was change in pulmonary function (FEV_1) from baseline at Day 14. Although conducted in 21 centers, the enrollment criteria of 56 days off antibiotics made it impossible to fully recruit the study. The decision to limit the study to two active arms was based on an effort to balance the sample size required for comparison with the difficulty in recruiting CF patients meeting the entry criteria.

Reviewer's comments*: The sponsor's comment regarding that the 225 mg dose is associated with the maximum antibiotic effect may be true since a greater change in FEV_1 from day 0 to day 14, greater log_{10} reduction in sputum CFU/g from day 0 to day 14, and a lower proportion of patients requiring IV antibiotics was observed following administration of AZLI 225 mg BID compared to AZLI 75 mg BID in the Phase 2 Study CP-AI-003. However, the AZLI 150 mg BID regimen was not evaluated in this study and it is unknown how the efficacy of AZLI 150 mg BID would compare to AZLI 225 mg BID. In addition, only the BID dosing interval was selected for evaluation in Phase 2 (Study CP-AI-003) and the TID dosing interval was not explored until Phase 3. Thus, the tolerability of an AZLI BID regimen compared to a TID regimen has not been evaluated.

The sponsor's response (cont'd): Overall, *PA* colony forming units (CFU) decreased by 1.5 log10 for the 75 mg AZLI group and by 2.3 log_{10} for the 225 mg AZLI group relative to placebo after 14 days of treatment. FEV_1 increased among AZLI-treated patients in both dose groups at Day 7, but at the end of the treatment period (Day 14), the FEV_1 response among patients in the 225 mg AZLI group had decreased substantially, whereas FEV_1 among patients in the 75 mg AZLI group was maintained at approximately the same level as at Day 7. At Day 14, the FEV_1 treatment effects of 75 mg and 225 mg AZLI versus placebo were 3.4% and 0.4% predicted, respectively. An exploratory analysis was conducted in an effort to understand the reduced FEV_1 response in the 225 mg dose group at Day 14 and to evaluate the effect of bronchodilator use on clinical response. Bronchodilator use was associated with better peripheral drug delivery, increased antimicrobial effect, and sustained improvement in lung function in the 225 mg AZLI dose group.

Study CP-AI-003 revealed a trend toward decreased tolerance of the 225 mg AZLI dose. Overall, the number of patients with drug-related AEs was higher in the 225 mg AZLI group (38%) than in the 75 mg AZLI group (27%). There was a possible dose-related trend in the incidence and severity of drug-related cough, with three (10%) patients in the placebo group reporting mild cough, four (11%) patients and one (3%) patient in the 75 mg AZLI group reporting mild and moderate cough, respectively, and five (14%)

and two (5%) patients in the 225 mg AZLI group reporting mild and moderate cough, respectively. There was also a greater incidence of dysgeusia in the 225 mg AZLI group than in the placebo and 75 mg AZLI groups, with no patients in the placebo group, one patient (3%) in the 75 mg AZLI group, and four patients (11%) in the 225 mg AZLI group reporting dysgeusia. Bronchospasm is a risk associated with any drug administered by inhalation. Acute airway reactivity is assessed by spirometry before and after drug administration. Physicians become concerned with an acute drop of 15% to 20%, even though in most cases these changes do not lead to any symptoms. After the first AZLI dose, there appeared to be a dose response in airway reactivity. Four patients (one in the placebo group and three in the 225 mg AZLI group) had decreases of ≥ 15% between pre-dose and 30 minutes post-dose. Short-term airway obstruction could interfere with peripheral drug delivery. This may have occurred with the 225 mg AZLI dose, but appeared to have been prevented by bronchodilator use.

Reviewer's comments: In Phase 2 Study CP-AI- 003 (14 days of placebo BID, AZLI 75 mg BID or AZLI 225 mg BID), a dose-related increase in drug-related AEs was observed in which 6/31 (19.4%) patients receiving placebo, 10/37 (27.0%) of patients receiving AZLI 75 mg BID, and 14/37 (37.8%) patients receiving AZLI 225 mg BID reported drug-related AEs. This data confirmed (not "revealed" as the sponsor stated as above) the trend toward decreased tolerance of the 225 mg dose already observed in Study AP-AI-002. A dose-dependent reduction of P. aeruginosa CFUs/g sputum was also demonstrated; AZLI 225 mg BID resulted in a greater reduction in CFUs/g (-2.1 log_{10}) compared to AZLI 75 mg BID (-1.5 log_{10}), suggesting an intermediate dose (i.e. 150 mg BID) may also offer a greater reduction in CFUs/g than the 75 mg BID dose.

The sponsor's response (cont'd): The results of study CP-AI-003 provided sufficient safety and efficacy data to warrant continued evaluation of AZLI in patients with CF in Phase 3 studies. Based on these results, the 75 mg AZLI dose was selected for further study based on the increase in drug-related respiratory AEs and attenuated FEV_1 response in the 225 mg AZLI group at Day 14. A 28-day treatment course was proposed to potentially achieve a longer antibiotic effect. Further, both BID and TID dosing regimens were considered, as TID dosing would potentially achieve additional time above the MIC of aztreonam for *PA* compared to BID dosing, and thus have greater antimicrobial effect without exceeding total drug exposures that were previously shown to be well tolerated.

Reviewer's comments: If the sponsor had chosen to evaluate the 150mg BID dose instead of the 225 mg BID dose, which was associated with a high incidence of AEs and an attenuated FEV_1 response at Day 14, in the Phase 2 study, the 150 mg BID dose may have been selected for further evaluation in Phase 3 studies. In addition, the decision to include the TID regimen in phase 3 studies was not supported by any prior clinical data regarding this dosing interval because it was not evaluated until Phase 3, but on an assumption that a higher degree of T>MIC is expected for the TID regimen than the BID regimen.

The sponsor's response (cont'd): The pharmacokinetic and pharmacodynamic profiles of AZLI further support the 75 mg AZLI TID dose regimen. Aztreonam demonstrates time-dependent killing against sensitive gram negative bacteria; therefore, increasing the AZLI dose to above the minimum inhibitory concentration (MIC) of aztreonam does not substantially increase the speed or degree of bacterial killing. Sputum aztreonam concentrations following a single dose of 75 mg AZLI were well in excess of the MIC_{90} for all *PA* isolates. Further, due to the rapid airway clearance of aztreonam, it is unlikely that a 150 mg BID dose would result in more time above the MIC than a 75 mg TID dose.

Reviewer's comments: It may be true that the 150 mg BID dose would result in less time above the MIC than a 75 mg TID dose. However, without a direct comparison between these two dosing regimen in clinical study, it is not possible to conclude how the overall safety and efficacy profiles differ between the two treatments. The available clinical data from Phase 1 to 3 suggested that an alternative

dosing regimen (e.g., AZLI 150 mg BID) may be associated with a greater efficacy and lower incidence of respiratory AEs than AZLI 75 mg TID.

The sponsor's response (cont'd): The duration of drug administration time is an important consideration in determining patient compliance with an antibiotic regimen, which is crucial in preventing the development of antibiotic resistance. A 75 mg AZLI dose (1 mL total volume) delivered via the eFlow® Electronic Nebulizer takes approximately 2-3 minutes to complete. Therefore, a 75 mg AZLI TID treatment regimen (1 mL [1 vial] x 3 times daily) takes approximately 6-9 minutes per day to administer. A 150 mg AZLI BID treatment regimen (2 mL [2 vials] x 2 times daily) may therefore increase total drug administration time (approximately 8-12 minutes per day to administer) and thus, treatment burden.

Reviewer's comments: The sponsor's reasoning regarding the relationship between the duration of drug administration and patient burden is questionable. A 150 mg BID treatment may lead to a higher total drug administration time than a 75 mg TID treatment, but a less frequent daily dosing regimen (BID vs. TID) may actually outweigh this disadvantage. In addition, the primary goal is to identify the most appropriate dose for the patients and patient compliance is just one of the important issues to be accounted for.

The sponsor's response (cont'd): Overall, 274 CF patients have been treated with up to nine intermittent 28-day courses of AZLI (85 BID, 189 TID) in study CP-AI-006; 75 mg AZLI TID has proven to be more effective than 75 mg AZLI BID over multiple treatment cycles. Compliance data were collected over multiple courses of AZLI therapy in Study CP-AI-006 in order to make comparisons between the BID and TID dosing regimens. Overall, no consistent differences in compliance based on treatment regimen were observed. Mean relative compliance values within each course ranged from 93% to 96% in the AZLI BID group and from 92% to 95% in the AZLI TID group. The percentage of patients who used at least 80% of the vials expected within each course ranged from 84% to 93% in the AZLI BID group and from 86% to 93% in the AZLI TID group. Gilead has given careful consideration to selection of the 75 mg AZLI TID regimen and strongly believes this to be preferable based upon the mechanism of action of aztreonam (time above MIC) as well as efficacy, safety, tolerability, and treatment compliance data from both placebo-controlled and long-term studies. Given the observed increase in respiratory AEs associated with the 150 mg and 225 mg AZLI doses in the Phase 1 and 2 studies (CP-AI-002 and CP-AI-003), and the acceptable safety profile and robust FEV_1 responses demonstrated with the 75 mg AZLI TID dose in the Phase 3 studies (CP-AI-005 and CP-AI-007), Gilead believes it is unnecessary to evaluate an intermediate dose (150 mg BID), as a lower effective dose has been established.

Reviewer's comments: In Phase 3 Study CP-AI-005 (28 days of placebo BID, AZLI 75 mg BID, placebo TID or AZLI 75 mg TID), more frequent dosing of AZLI (i.e., 75 mg TID versus 75 mg BID) resulted in a greater reduction of P. aeruginosa CFUs/g sputum although the proportion of patients requiring inhaled or IV antibiotics, the primary efficacy endpoint, was lower for patients receiving AZLI 75 mg BID compared to AZLI 75 mg TID (p=0.0835). Respiratory AEs were reported more frequently with the AZLI 75 mg TID than AZLI 75 mg BID (productive cough [46% vs. 36%], wheezing [23% vs. 13%], dyspnea [17% vs. 6%] and decrease in pulmonary function tests [14% vs. 6%]) although there was no significant difference between the treatment groups with respect to total number of treatment-emergent AEs.

Although the safety and efficacy of AZLI 150 mg BID has not been compared to either AZLI 75 mg BID or AZLI 75 mg TID in CF patients, the results from the completed Phase 1, 2 and 3 studies suggest that an alternative regimen such as AZLI 150 mg BID may be an appropriate dosage regimen and should be further evaluated to address the following issues:

- ***Whether an alternative regimen would be associated with a lower incidence of respiratory AEs than AZLI 75 mg TID.***

- ***Whether an alternative regimen would result in a greater reduction of P. aeruginosa CFUs/g sputum than AZLI 75 mg TID.***
- ***Whether an alternative regimen would be associated with greater efficacy than AZLI 75 mg TID.***

- **Sparse sampling of sputum concentrations should be include additional collection times beyond 10 minutes post-dose (e.g. at 2, 4, and 6 hours post-dose)**

The sponsor's response : For additional studies, the Division suggests that sampling of sputum aztreonam concentrations should include additional collection times beyond 10 minutes post-dose (e.g., at 2, 4, and 6 hours post-dose).
Multiple timepoint sampling of aztreonam concentrations in expectorated sputum was conducted following single ascending doses of AZLI (75 mg, 150 mg and 225 mg) in study CP-AI-002. Sputum aztreonam concentrations were measured at the following post-dose timepoints: 10 minutes, 2 hours, and 4 hours. For both adults and adolescents participating in the study, sputum aztreonam concentrations exhibited considerable variability, but had dropped significantly at 2 and 4 hours relative to the maximum concentrations measured at10 minutes (as shown in **Table** below).

Table: **Sputum Aztreonam Concentrations (µg/g) by Treatment and Dose Group**

Time Post-Dose	75 mg AI Mean (SD)	150 mg AI Mean (SD)	225 mg AI Mean (SD)
Adults	**N = 12**	**N = 12**	**N = 12**
10 minutes	539 (571)	1129 (846)	1208 (935)
2 hours	60 (70)	104 (97)	125 (116)
4 hours	32 (32)	50 (44)	92 (144)
Adolescents	**N = 11**	**N = 11**	**N = 10**
10 minutes	329 (169)	587 (652)	479 (406)
2 hours	43 (87)	43 (63)	67 (90)
4 hours	16 (29)	209 (624)	15 (16)

Source: CP-AI-002 Final Statistical Report

These data indicate that AZLI is rapidly cleared from the airways, which is consistent with clearance rates of other inhaled therapies, including tobramycin. In addition, a saturation effect was observed, as sputum aztreonam concentrations were similar at each timepoint following administration of 150 mg and 225 mg AZLI, thereby suggesting that similar side effect profiles would be expected with these two dose levels. Based upon these data and the known pharmacokinetics of inhaled medications, additional sampling of sputum aztreonam concentrations beyond 10 minutes was considered uninformative and was therefore not undertaken in the Phase 2 and 3 studies.

Reviewer's comments: ***The data in the Table above suggest that the sputum concentrations are highly variable. Although AZLI appeared to be rapidly cleared from the airway, there are still substantial concentrations of aztreonam in the sputum up to 2 hours following administration of AZLI 75 mg and 4 hours following administration of 150 mg and 225 mg, when the sputum concentrations were either comparable to or slightly lower than the MIC_{90} value of 64 µg/mL. In addition, the sponsor's exposure-response analysis that the 150 mg and 225 mg doses would be expected to have a similar side effect profile based on similar sputum concentrations is contradictory to that observed in the Phase 1 Study CP-AI-002 in which a single 225 mg dose was associated with a higher incidence of respiratory AEs than a single 150 mg dose (27% vs. 17%, respectively).***

The sponsor's response (cont'd): In the Phase 3 placebo-controlled studies (CP-AI-005 and CP-AI-007), expectorated sputum was obtained from patients 10 minutes following in-clinic study drug dosing. Sputum aztreonam concentrations were evaluated in relation to aztreonam MIC values for *PA* isolates from sputum. Administration of a single dose of 75 mg AZLI to 195 CF patients resulted in mean sputum concentrations of 726 μg/g, 711μg/g, and 715 μg/g on Days 0, 14, and 28, respectively. These concentrations are more than 10 times the aztreonam MIC90 for all *PA* isolates obtained from AZLI-treated patients (64 μg/mL). No increased accumulation of aztreonam was observed following repeated dosing. Following dosing on Day 0, 93% of patients had sputum aztreonam concentrations ≥ 64 μg/mL, 87% of patients had sputum aztreonam concentrations ≥ 128 μg/mL and 95% of patients had sputum aztreonam concentrations at or above the MIC of their own least susceptible *PA* isolate. Thus, local aztreonam concentrations generally exceed aztreonam MIC values for *PA*, regardless of the level of *PA* susceptibility.
In summary, multiple timepoint sampling of sputum aztreonam concentrations during the Phase 1b study (10 minutes, 2 hours, and 4 hours) indicated that aztreonam is rapidly cleared from the airways. In the Phase 3 studies, no increased accumulation of aztreonam was observed following repeated dosing and sputum aztreonam concentrations generally exceeded the MIC value for each patient's least susceptible *PA* isolate. Therefore, Gilead believes that the pharmacokinetics of sputum aztreonam concentrations have been thoroughly examined in support of the proposed 75 mg AZLI TID dosing regimen and that further evaluation at 2, 4, and 6 hours post-dose would not provide additional pertinent information.

Reviewer's comments: ***Based on the available data, sparse sampling should be collected beyond the 10 min time point (i.e., at 2, 4, or 6 hour post-dose) for the 150 mg BID arm. This information will be informative and would enable a PK/PD analysis (i.e., T>MIC), which could not be done for the Phase 3 studies conducted so far due to insufficient sputum sample collection.***

3. LABELING RECOMMENDATIONS

See Appendix 4.1 for the proposed product label with reviewer annotations.

4. APPENDIX

4.1. PROPOSED PACKAGE INSERT WITH REVIEWER ANNOTATION

27 Pages of Draft Labeling 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/

YONGHENG ZHANG
01/22/2010

CHARLES R BONAPACE
01/22/2010
I concur with the reviewer's conclusions.

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA	50-814
Submission Date	November 16, 2007
Brand Name	Cayston™
Generic Name	Aztreonam lysine for inhalation
Primary Reviewer	Sarah Robertson, Pharm.D.
Team Leader (Acting)	Charles R. Bonapace, Pharm.D.
OCP Division	DCP4
OND Division	DAIOP
Applicant	Gilead Sciences Inc.
Relevant IND(s)	64,402
Submission Type; Code	NDA; 505(b)(2)
Formulation; Strength	Lyophilized aztreonam lysine for inhalation, 75 mg
Indication(s)	To improve respiratory symptoms and pulmonary function in cystic fibrosis (CF) patients with *Pseudomonas aeruginosa*.

TABLE OF CONTENTS

1. EXECUTIVE SUMMARY

Aztreonam for injection (Azactam®) has been approved in the U.S. since 1986 (NDA 50-580), and is indicated for the treatment of urinary tract infections, lower respiratory tract infections, septicemia, skin and skin structure infections, intra-abdominal infections and gynecological infections due to susceptible Gram-negative organisms. The recommended dose of IV aztreonam for adults with moderate to severe infection is 1-2 g every 8 to 12 hours.

The Sponsor submitted this 505(b)(2) NDA for a novel formulation of aztreonam, aztreonam lysine for inhalation (AI), to improve respiratory symptoms and pulmonary function in Cystic Fibrosis (CF) patients infected with *Pseudomonas aeruginosa* (*P. aeruginosa*). AI is administered with the PARI eFlow® Electronic Handheld Nebulizer. The proposed dose is 75 mg three times daily (TID) for 28 days in adult and pediatric CF patients age ≥ 6 years.

In support of the NDA, the Sponsor submitted six clinical studies of AI (Table 1). Two escalating single-dose PK studies were conducted – one in healthy adult subjects (CP-AI-001) and one in CF patients > 12 years of age (CP-AI-002). Single doses were escalated up to 225 mg in CF patient in the Phase 1 study with no safety issues. One Phase 2 study was conducted (CP-AI-003) comparing AI 75 mg BID and 225 mg BID with placebo for 14 days in adult and adolescent (>12 years) CF patients with *P. aeruginosa*. Two double-blind, placebo-controlled Phase 3 clinical trials (CP-AI-005 and -007) were conducted in adult and pediatric CF patients age ≥ 6 years. CP-AI-005 compared two dosing regimens of AI, 75 mg BID and 75 mg TID, to placebo, while CP-AI-007 compared one dosing regimen, 75 mg TID, to placebo. An open-label follow-on study (CP-AI-006) for patients enrolled in the two Phase 3 clinical trials is ongoing.

Table 1. Clinical Studies of AI Submitted by the Sponsor

Type of Study	Study Identifier	Objective(s) of the Study	Study Design	Dosage Regimen	N[a]	Duration of Treatment
Phase 1a	CP-AI-001	Assess safety, tolerability and PK	Double-blind, placebo-controlled in healthy subjects	AI: 3 escalating cohorts of 95 mg, 190 mg, 285 mg	24	Single Dose
Phase 1b	CP-AI-002	Assess safety, tolerability and PK	Double-blind, placebo-controlled in CF patients (> 12 years)	AI: 3 consecutive single doses of 75 mg, 150 mg, and 225 mg	35	3 days of AI (one dose/day); 3 days of follow-up
Phase 2	CP-AI-003	Assess safety, efficacy and PK Primary endpoint – % change in FEV_1 (Day 0 to Day 14)	Double-blind, placebo-controlled in CF patients (> 12 years)	AI: 75 mg BID or 225 mg BID	105	14 days of AI; 14 days of follow-up

Type of Study	Study Identifier	Objective(s) of the Study	Study Design	Dosage Regimen	N[a]	Duration of Treatment
Phase 3	CP-AI-005	Assess safety and efficacy of AI. Primary endpoint – time to need for inhaled or IV antibiotics	Double-blind, placebo-controlled; 28 days of inhaled tobramycin (open-label) followed by AI or placebo x 28 days in CF patients (> 6 years)	AI: 75 mg BID or TID	211	28-day run-in of inhaled tobramycin, then 28 days of AI and 56 days of follow-up
Phase 3	CP-AI-007	Assess safety and efficacy of AI. Primary endpoint – change in CFQ-R respiratory symptoms (Day 0 to Day 28)	Double-blind, placebo-controlled in CF patients > 6 years	AI: 75 mg TID	164	28 days of AI; 14 days of follow-up
Phase 3	CP-AI-006	Assess long-term safety of AI. Primary endpoint – AEs, airway reactivity, vital signs	Open-label, follow-on study from CP-AI-005 and -007); Patients receiving AI according to same regimen (BID or TID)	AI: 75 mg BID or TID	207[b]	Up to nine 28-day courses of AI, with 28 days off between each course

[a] Safety population (subjects received at least 1 dose of study drug)
[b] As of 3/2007 cutoff

In Phase 3 Study CP-AI-005, the 75 mg BID regimen performed better than the 75 mg TID regimen for the primary efficacy outcome, time to need for antibiotics. However, there was a regimen effect observed in this study, with BID placebo also performing better than TID placebo with respect to the primarily efficacy outcome. This suggests that TID administration of inhalation therapy results in poorer outcomes than BID, regardless of the treatment group (AI or placebo). Although there was no significant difference between the treatment groups with respect to total number of treatment-emergent adverse events (AEs), the following AEs with an incidence rate of $\geq$ 10% were reported more frequently in the AI TID arm than the AI BID arm: productive cough (46% vs. 36%), wheezing (23% vs. 13%), dyspnea (17% vs. 6%), pyrexia (15% vs. 9%) and decrease in pulmonary function tests (14% vs. 6%).

Despite the findings from CP-AI-005, the Sponsor selected the 75 mg TID regimen for further clinical development based on the assumption that the TID regimen would result in a longer time above MIC (T>MIC) in the lungs, and thus, improved antimicrobial activity. In the second Phase 3 study (CP-AI-007), AI 75 mg TID resulted in greater improvement in CFQ-R respiratory domain score, the primary efficacy outcome, relative to placebo. Thus, the Sponsor has completed only one successful clinical trial supporting the proposed dosage regimen of 75 mg TID. Without a second confirmatory study comparing the two regimens, it remains unclear whether 75 mg BID or 75 mg TID is the more appropriate regimen.

Alternatively, a higher AI dose of 150 mg BID may result in improved outcomes relative to the 75 mg BID or 75 mg TID regimens. In Study CP-AI-002, sputum concentrations of aztreonam following

150 mg AI were approximately double those of 75 mg, with no further increase observed at a dose of 225 mg. Further, a dose-dependent effect on lung *P. aeruginosa* CFUs was demonstrated in Phase 2 Study CP-AI-003, in which 225 mg BID resulted in a greater reduction in lung CFUs (-2.1 $\log_{10}$) relative to 75 mg BID (-1.5 $\log_{10}$). Given these observations, along with the efficacy results of Study CP-AI-005, and a higher rate of certain adverse events with TID dosing versus BID, a 150 mg BID dose may result in improved outcomes and improved tolerability relative to a 75 mg TID regimen.

1.1. Recommendation

The Clinical Pharmacology and Biopharmaceutics information provided by the Sponsor in the NDA submission is acceptable. However, the proposed dose of 75 mg TID may not be the most efficacious dose for the sought indication based on data from the Phase 2 and 3 studies. Therefore, it is recommended that if the Sponsor is to complete an additional Phase 3 clinical trial in CF patients, two dosing regimens of AI should be evaluated – 75 mg TID and 150 mg BID. Sparse sampling of sputum aztreonam concentrations should include additional collection times beyond 10 minutes post-dose (e.g. at 2, 4 and 6 hours post-dose).

Changes to the proposed label should be communicated to the Sponsor.

1.2. Phase IV Commitments

No Phase IV commitments are recommended.

1.3. Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

Following administration of AI to adult CF patients, mean plasma Tmax is achieved < 1 hour post-initiation of dose. The mean plasma Cmax value following an AI dose of 75 mg is 419 ng/mL, versus a Cmax of 90 µg/mL observed following a therapeutic 1 gram dose of IV aztreonam. Absorption of aztreonam following inhalation is low, with approximately 10% of the administered dose excreted unchanged in the urine, as compared to 60-65% following IV administration. The apparent terminal elimination half-life of aztreonam from plasma is approximately 2.1 hours following AI, similar to what is observed following intravenous administration.

In general, sputum levels of aztreonam are highly variable in CF patients, likely owing to the complex pathophysiology of the CF lung, as well as variation in expectorated sputum collection. In the Phase 1b study, CP-AI-002, the median sputum aztreonam concentration in adult CF patients was 383 µg/g (range 86 – 2170) 10 minutes after a single 75 mg dose of AI. Thereafter, sputum concentrations decreased rapidly, to a median value of 38 µg/g (range 7 – 267) by 2 hours post-dose. Sputum concentrations in adolescent CF patients were consistently lower than that of adults at all dose levels and at all time points in CP-AI-002. Sputum concentrations in adults increased approximately proportional to dose from 75 mg to 150 mg, but increased only minimally from 150 mg to 225 mg. Adolescent sputum concentrations generally did not increase with increasing dose from 75 mg to 150 mg or 225 mg, though considerable variability is noted. There was no accumulation of aztreonam in either the sputum or the plasma of CF patients following multiple dose administration of AI up to the highest dose studied, 225 mg BID.

Based on plasma concentration data, an unbound time above MIC (T>MIC) target of 50-60% of the dosing interval is considered to give near-maximal bactericidal activity for aztreonam. Although it is

unclear how this plasma PK/PD target relates to drug exposure at the site of infection, it is assumed that the longer concentrations in the lung remain above the MIC of *P. aeruginosa*, the greater the bactericidal activity of aztreonam in CF patients. In the Phase 2 and 3 efficacy studies sputum samples were collected from patients only at 10 minutes post-dose. Thus, T>MIC in the sputum cannot be determined for any of the patients enrolled in the efficacy studies, and therefore, cannot be assessed with regards to efficacy outcomes. However, serial sputum concentrations collected from CF patients in Phase 1 Study CP-AI-002 (up to 4 hours post-dose) can be used to compare sputum exposure with MIC_{90} values for *P. aeruginosa* isolated in the Phase 3 clinical trials. Sputum concentrations of adult CF patients (from Study CP-AI-002) remain above the MIC_{90} values obtained from Phase 3 clinical trials (64 and 128 μg/mL) for approximately 1 to 1.5 hours. By 2 hours post-dose, sputum concentrations in CP-AI-002 are below the MIC_{90} values in nearly all adult CF patients. In adolescent CF patients, sputum concentrations remain above MIC_{90} values for approximately 0.5 to 1 hour. However, it is noted that sputum exposure is highly variable over time in this cohort.

These observations would suggest a 75 mg TID dose of AI achieves a T>MIC of approximately 12% of the dosing interval in the lungs of adult and adolescent CF patients. However, given the significant variability in sputum concentrations observed in CF patients, it is difficult to make conclusions regarding the PK/PD parameter of interest, T>MIC in the lung.

In Phase 3 Study CP-AI-005, BID and TID regimens of AI 75 mg were compared with BID or TID placebo. One would have predicted that the TID regimen would produce a longer T>MIC in the lung, resulting in better outcomes relative to the BID regimen. However, for the primary efficacy outcome, time to need for antibiotics, a significant difference from the pooled placebo group was observed for the AI BID group, but not the AI TID group. When AI BID and AI TID groups were compared, the proportion of patients requiring inhaled or IV antibiotics was lower for the BID regimen than the TID regimen, though the difference did not reach statistical significance (p=0.0835). Despite the findings from CP-AI-005, the Sponsor selected the 75 mg TID regimen for further clinical development; the dose was selected based on the assumption that 75 mg TID results in a longer T>MIC compared to BID, and therefore provides better antimicrobial activity. In Study CP-AI-007, the 75 mg TID regimen was the sole treatment regimen compared with placebo. Thus, a dose-response evaluation cannot be conducted with regard to efficacy in the second Phase 3 study.

2. QUESTION BASED REVIEW

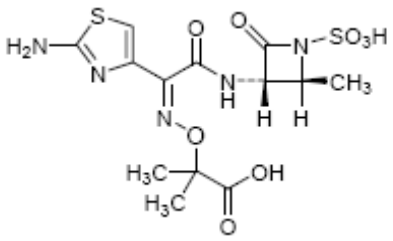
2.1. General Attributes of the Drug

2.1.1. What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product?

Aztreonam lysine for inhalation contains aztreonam as the active ingredient. L-lysine, a naturally occurring amino acid, is commercially available as L-lysine monohydrate. L-lysine monohydrate is used as a neutralizing agent for aztreonam in the inhaled formulation. The proposed commercial formulation contains (b) (4) mg lysine monohydrate per vial (46.7 mg of L-lysine), for an approximate (b) (4) of lysine monohydrate to aztreonam. The final formulation has a pH in the range of 4.5 to 6.0 upon reconstitution.

Chemical Structure:

Aztreonam



Chemical Name: (*Z*)-2-[[[(2-amino-4-thiazolyl)[[(2*S*,3*S*)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl]methylene]amino]oxy]-2-methylpropionic acid

Molecular Weight: 435.44

Solubility Profile:
Aztreonam has three ionizable moieties. In the pH range of 1.0 to 8.0 aztreonam exists either as a neutral zwitterion, monoanion or dianion. The aqueous solubility of aztreonam increases as the solution pH increases due to ionization of the sulfonyl and carboxyl groups. Solubility greater than 75 mg/mL is achieved at pH values above 4.2. Maximum stability of aztreonam in solution occurs from pH 4.5 to 6.

Drug Product:
Aztreonam lysine is provided as a sterile lyophilized powder in a single-use 2 mL glass vial containing 75 mg aztreonam. The powder is reconstituted with a sterile NaCl solution, which is co-packaged with the aztreonam lysine vial, to produce aztreonam lysine for inhalation. The reconstituted drug is nebulized using the eFlow® Electronic Nebulizer.

2.1.2. What is the proposed mechanism of drug action and therapeutic indication?

Aztreonam is a synthetic monobactam antibiotic, with activity against Gram-negative aerobic organisms, including *P. aeruginosa*. Aztreonam inhibits the synthesis of bacterial cell walls. It binds to penicillin-binding protein 3 of Gram-negative bacteria and does not bind to essential penicillin-binding proteins of Gram-positive or anaerobic bacteria. Thus, its activity is limited to that of Gram-negative pathogens.

Aztreonam for inhalation is proposed to [redacted] (b) (4)

2.1.3. What is the proposed dosage and route of administration?

The proposed dose of aztreonam for inhalation is 75 mg three times daily for 28 days. Doses of aztreonam are to be administered with the PARI eFlow® Electronic Nebulizer.

2.2. General Clinical Pharmacology

2.2.1. What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

Phase 1

The Sponsor conducted two clinical studies to evaluate the PK characteristics of single escalating doses of AI. Study CP-AI-001 (001) was conducted in healthy adult subjects and Study CP-AI-002 (002) was conducted in adult and adolescent (> 12 years) CF patients. Study 001 was a randomized, double-blind, placebo-controlled study evaluating single AI doses of 95 mg, 190 mg and 285 mg. Serial blood and urine samples were collected following inhalation of each dose for evaluation of aztreonam PK. Study 002 was a randomized, double-blind, placebo-controlled study evaluating three single escalating AI doses of 75 mg, 150 mg and 225 mg. Serial blood samples for evaluation of aztreonam PK in plasma were collected up to 8 hours post-dose in adult patients following administration of the 75 mg dose only. Sputum concentrations were collected from adult and adolescent patients in each of the three dose cohorts at the following time points: pre-dose, 10 minutes, 2 hours and 4 hours post-dose.

Phase 2

The Sponsor conducted one Phase 2 study, CP-AI-003 (003), to evaluate the safety and efficacy of 14-day treatment with AI at two dose levels in CF patients 13 years of age or older with pulmonary *P. aeruginosa* infection and mild-to-moderate disease (an FEV_1 of at least 40% predicted). A total of 105 CF patients were randomized 1:1:1 to receive placebo, 75 mg or 225 mg AI twice daily on Days 0 – 13 on an out-patient basis. Patients on short-acting bronchodilators (BD) were instructed to administer the BD prior to each dose of AI or placebo. Patients made visits on Days 0, 7, 14 and 28 for evaluation of sputum microbiology, spirometry assessments, monitoring of adverse events, laboratory evaluation, and analysis of plasma and sputum aztreonam concentrations. Blood for plasma aztreonam levels were obtained pre-dose on Day 0, one hour post-dose on Days 0 and 7, and on Day 14. Sputum samples were collected for determination of aztreonam concentration pre-dose on Day 0, 10 minutes post-dose on Days 0 and 7, and on Day 14. The primary efficacy outcome was percent change in FEV_1 from Day 0 to Day 14. Several secondary efficacy variables were analyzed, including absolute change in FEV_1 and change in *P. aeruginosa* CFUs in sputum.

Phase 3

Two pivotal Phase 3 efficacy trials were conducted in CF patients > 6 years of age with pulmonary *P. aeruginosa* infection (CP-AI-005 and CP-AI-007). The objective of CP-AI-005 (005) was to assess the safety and efficacy of AI 75 mg BID or TID for 28 days, immediately following a 28-day course of tobramycin inhalation therapy. Enrolled patients had an FEV_1 of 25 – 75% predicted (inclusive) at baseline. Patients were randomized to BID or TID dosing of AI 75 mg or BID or TID placebo (sterile lactose reconstituted with 0.17% saline). All patients were instructed to administer a BD prior to administering their dose of AI/placebo to improve drug disposition. Patients were also administered a BD in clinic 15 minutes prior to spirometry. There was a 56-day follow-up period following completion of the 28-day treatment period. The primary efficacy endpoint was time to need for IV or inhaled antipseudomonal antibiotics following the start of blinded AI treatment. Secondary endpoints included change in symptoms as measured by the CF Questionnaire-Revised (CFQ-R), change in pulmonary function, change in $\log_{10}$ *P. aeruginosa* CFU density, hospitalization and use of other antipseudomonal antibiotics. Sparse plasma and sputum samples were collected from all patients for evaluation of aztreonam concentrations. Plasma samples were collected on Day 0 (pre-treatment and 1-hour post-dose),

Day 14 (1-hour post-dose) and Day 28. Sputum samples were collected 10 minutes post-dose on Days 0 and 14.

Study CP-AI-007 (007) evaluated the safety and efficacy of AI 75 mg TID or placebo for 28 days. Unlike Study 005, there was no pre-treatment period with inhaled tobramycin. Any use of antipseudomonal or macrolide antibiotics was prohibited within 28 days of study treatment. As in Study 005, enrolled patients had an FEV_1 of 25 – 75% predicted (inclusive) at baseline. All patients were instructed to administer a BD prior to administering their dose of AI/placebo to improve drug disposition. There was a 14-day follow-up period following completion of the 28 day treatment period. The primary efficacy endpoint was change in respiratory symptoms as measured by the CFQ-R. Secondary endpoints included change in pulmonary function, change in $\log_{10}$ *P. aeruginosa* CFU density, hospitalization, use of other antipseudomonal antibiotics and change in weight or body mass index (BMI). Sparse plasma and sputum samples were collected from all patients for evaluation of aztreonam concentrations. Plasma samples were collected on Day 0 (pre-treatment and 1-hour post-dose), Day 14 (1-hour post-dose) and Day 28 (pre-dose and 1-hour post-dose). Sputum samples were collected 10 minutes post-dose on Days 0, 14 and 28.

2.2.2. What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics [PD]) and how are they measured in clinical pharmacology and clinical studies?

The primary efficacy endpoint was time to need for IV or inhaled antipseudomonal antibiotics in Study 005 and change in respiratory symptoms as measured by the CFQ-R in Study 007. Secondary endpoints in 005 included change in symptoms as measured by the CFQ-R, change in pulmonary function, change in $\log_{10}$ *P. aeruginosa* CFU density, hospitalization and use of other antipseudomonal antibiotics. Secondary endpoints in 007 included change in pulmonary function, change in $\log_{10}$ *P. aeruginosa* CFU density, hospitalization, use of other antipseudomonal antibiotics and change in weight or BMI.

2.2.3. Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

Aztreonam concentrations in human plasma and sputum were determined using validated liquid chromatography atmospheric pressure ionization tandem mass spectrometry (LC-API/MS/MS) methods, with demonstration of acceptable accuracy, selectivity and precision.

2.2.4. Exposure-Response

2.2.4.1. What are the characteristics of exposure-response relationships (dose-response, concentration-response) for efficacy?

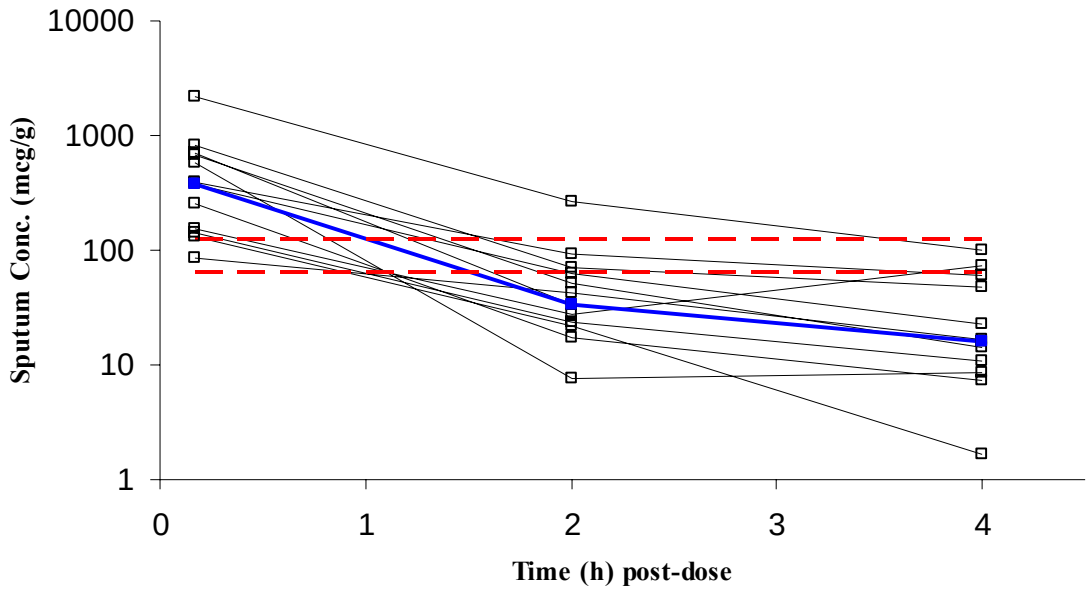
Based on plasma concentration data, an unbound T>MIC target of 50-60% of the dosing interval is considered to give near-maximal bactericidal activity for aztreonam. Although it is unclear how this plasma PK/PD target relates to exposure at the site of infection, it is assumed that the longer concentrations in the lung remain above the MIC of *P. aeruginosa*, the greater the bactericidal activity of aztreonam in CF patients.

In the Phase 2 and 3 efficacy studies, sputum samples were collected from patients at only one timepoint, 10 minutes post-dose. Thus, the PK/PD parameter of interest, T>MIC in the sputum, cannot be assessed with regards to efficacy outcomes. However, sputum samples were collected in 23 adult and adolescent CF patients up to 4 hours post-dose following a single-dose of AI 75 mg in Study 002. As would be expected, there was considerable inter-patient variability in sputum concentrations at all timepoints. Median (range) aztreonam concentrations at 4 hours post-dose were 16 µg/g (2 – 100) in adults and 2 µg/g (0 – 90) in adolescents. As shown below in Figure 2.2.4.1-1, sputum concentrations in adults (from Study CP-AI-002) remain above the MIC_{90} values obtained from Phase 3 clinical trials (64 and 128 µg/mL) for approximately 1 to 1.5 hours. By 2 hours post-dose, sputum concentrations are below the MIC_{90} values in nearly all adult CF patients. In adolescent CF patients, sputum concentrations remain above MIC_{90} values for approximately 0.5 to 1 hour. However, it is noted that sputum exposure over time is highly variable in this cohort.

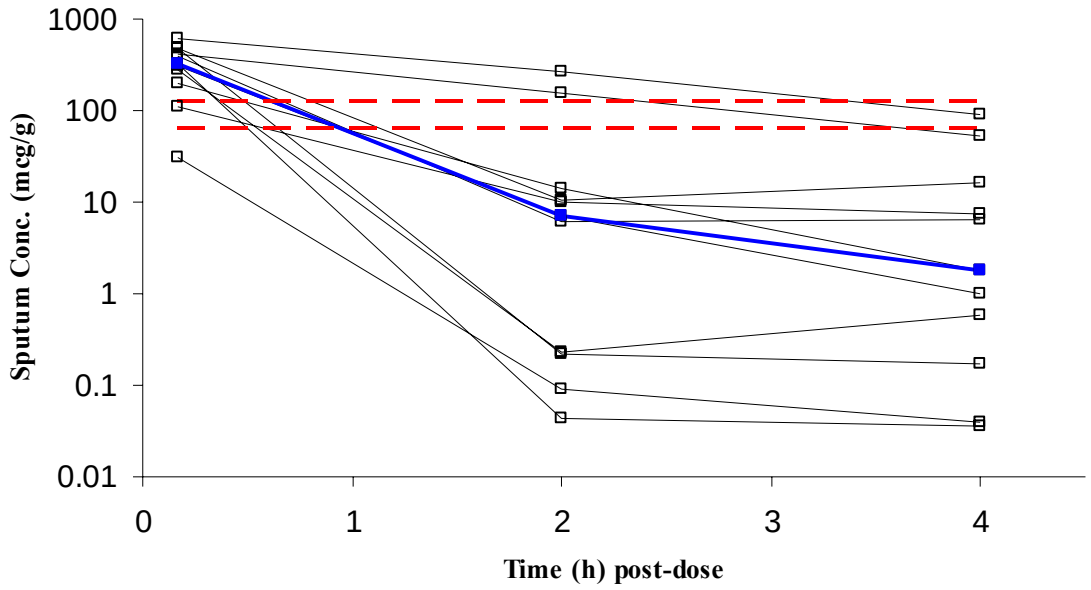
These observations would suggest a 75 mg TID dose of AI achieves a T>MIC of approximately 12% of the dosing interval in the lungs of adult and adolescent CF patients. However, the extreme variability in sputum concentrations, as well as the limited number of serially-collected samples, makes conclusions about the true exposure of aztreonam in the lungs of CF patients difficult.

Figure 2.2.4.1-1 Individual (—□—) and Median (—■—) Sputum Concentrations in Adult and Adolescent CF Patients Following AI 75 mg (Study 002) vs. MIC_{90} values (— — —) From the Phase 3 Clinical Trials

Adults

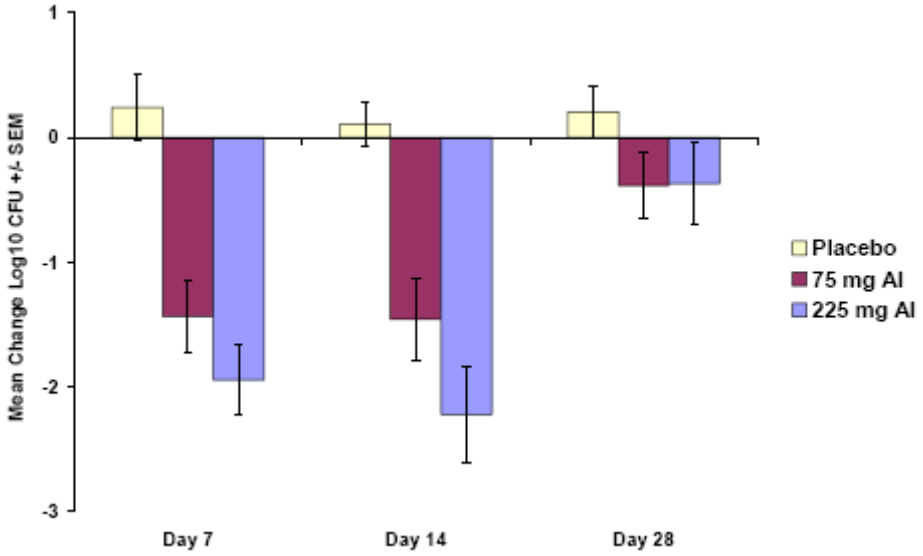


Adolescents



In the Phase 2 study, two dosing regimens of AI were compared with placebo in adult and adolescent CF patients. Doses of 75 mg BID, 225 mg BID or placebo were administered for 14 days to CF patients ≥ 13 years of age with *P. aeruginosa* infection and mild-to-moderate disease. In this study, sputum concentrations at 10 minutes post-dose were slightly greater than dose proportional from 75 mg to 225 mg. The results showed no significant difference between any of the three groups for the primary efficacy outcome, percent change in FEV_1 from Day 0 to Day 14. However, there was a difference in the secondary outcome measure, mean reduction in $\log_{10}$ *P. aeruginosa* CFUs in sputum. Mean change in $\log_{10}$ CFUs at Day 14 were 0.025, -1.506 and -2.109 for the placebo, 75 mg BID and 225 mg BID groups, respectively, suggesting a dose response relationship for reduction in *P. aeruginosa* CFUs (Figure 2.2.4.1-2). The MIC_{90} of aztreonam for *P. aeruginosa* isolates in this study was 64 µg/mL in the 75 mg treatment group and 128 µg/mL in the 225 mg treatment group. The proportion of patients requiring antipseudomonal antibiotics during the trial ("non-responders") was greatest in the 75 mg AI group (18.4% versus 9.4% and 5.7% in the placebo and 225 mg AI groups, respectively).

Figure 2.2.4.1-2 Change in Log_{10} CFUs by Visit and Treatment (Per Protocol Population)

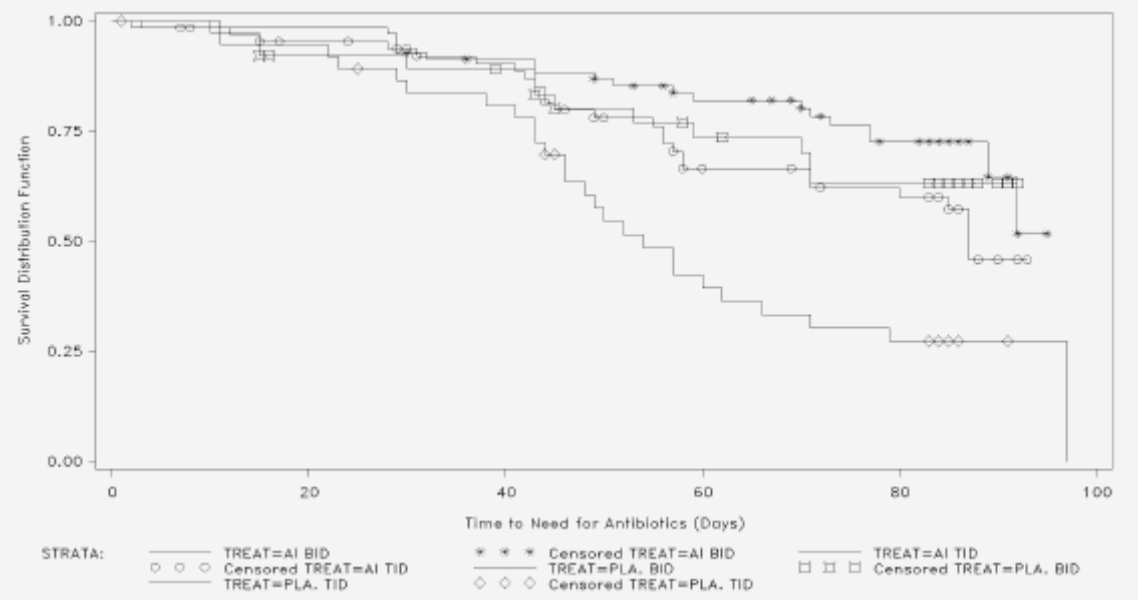


Source: CP-AI-003 Study Report

In Phase 3 Study 005, BID and TID regimens of AI 75 mg were compared with BID and TID administered placebo (sterile lactose). One would have predicted that the AI TID regimen would produce a longer T>MIC in the lung, resulting in better outcomes relative to the AI BID regimen. However, for the primary efficacy outcome, time to need for IV or inhaled antibiotics, a significant difference from the pooled placebo group was observed for the AI BID group, but not the AI TID group. When AI BID and AI TID groups were compared, the proportion of patients requiring inhaled or IV antibiotics was lower for the BID regimen than the TID regimen, though the difference did not reach statistical significance (p=0.0835). This finding is inconsistent with what would be expected for an antibiotic with time-dependent bactericidal activity. The time to need for antibiotics for all four treatment groups is presented below in Figure 2.2.4.1-3. An analysis of regimen effect revealed that the BID and TID regimens performed differently for both placebo and AI treatment. The time to need for antibiotics was significantly longer in the BID placebo group than in the TID placebo group (p=0.0043), similar to what was observed for AI. The Sponsor did not provide a hypothesis for the cause of the observed regimen effect.

All of the patients enrolled in Study 005 had a 28-day run-in period in which they received inhaled tobramycin (BID), prior to beginning study treatment. One potential explanation for the observed results is that patients who went from a BID inhaled drug (tobramycin) immediately to an inhalation therapy administered TID may have perceived worsening of their symptoms based on a more frequent dosing regimen, relative to those patients who were randomized to BID AI or placebo.

Figure 2.2.4.1-3 Post Hoc Analysis of Time to Need for Inhaled or IV Antibiotics for Placebo and AI Treated CF Patients



Source: CP-AI-005 Study Report

The AI BID regimen was also more efficacious than the AI TID regimen for the secondary efficacy outcome, change in CFQ-R respiratory domain score. Adjusted mean change in score was -0.66, 5.10 and 3.5 for the pooled placebo, BID AI and TID AI groups, respectively. The difference in score change was significant for the AI BID group versus the pooled placebo, but not for the AI TID group. At Day 14, the decline in *P. aeruginosa* CFU density was greater in the AI TID group than the AI BID group. However, by Day 28, the levels of CFU decline were similar for the two AI treatment groups.

In a subgroup analysis, the proportion of patients requiring inhaled or IV antibiotics was higher in patients with more severe disease ($\leq$ 50% of predicted FEV_1) than in those with less severe disease (> 50% of predicted FEV_1). When patients were stratified by their highest aztreonam MIC at baseline, the proportion of patients requiring inhaled or IV antibiotics was higher for patients with a baseline MIC value for *P. aeruginosa* above the parenteral breakpoint of 8 μg/mL versus those with a baseline MIC value $\leq$ 8 μg/mL

In Phase 3 study 007, only one AI regimen (75 mg TID) was compared to placebo. Thus, a dose-response evaluation cannot be conducted with regard to efficacy. The results demonstrated an adjusted mean change in CFQ-R respiratory domain score (Day 28 - baseline) of 7.08 for AI-treated patients and -2.63 for placebo-treated patients. There were a higher percentage of patients with improved symptom scores at Day 28 in the AI group (56%) than in the placebo group (37%) (p=0.0055). Patients in the AI treatment group had a 1.5 $\log_{10}$ drop in *P. aeruginosa* CFUs at Day 28, versus no change in the placebo group. However, CFUs returned to their baseline by Day 42 in the AI treatment group. There was no notable affect of baseline aztreonam MIC on mean CFQ-R score change.

2.2.4.2. What are the characteristics of exposure-response relationships (dose-response, concentration-response) for safety?

The majority of patients in placebo and AI treatment groups in the Phase 2 and 3 studies experienced at least one AE (70 – 92%). The most common AEs reported in the Phase 2 and 3 studies were pulmonary-related effects, including cough, chest discomfort and decrease in exercise tolerance. Non-pulmonary AEs occurring in ≥ 10% of patients were decreased appetite, dysgeusia, fatigue, headache, sinus/nasal congestion, pharyngolaryngeal pain, rhinorrhea and pyrexia. There was no statistically significant difference in drug-related AEs or AEs resulting in treatment withdrawal between treatment groups in any of the individual studies. However, there was a dose-related trend in the incidence of drug-related AEs (mostly pulmonary-related) in the Phase 2 study, with an occurrence rate of 19.4%, 27.0% and 37.8% in the placebo, 75 mg BID and 225 mg BID groups, respectively.

Of the individual AEs, only pyrexia was reported by significantly more AI-treated than placebo-treated patients in any study. In addition, the occurrence of pyrexia increased with dose in Study 005, from 3% in placebo to 9% in AI 75 mg BID and 15% in AI 75 mg TID. There was an overall trend toward more wheezing among AI-treated patients in Study 005 (p=0.0842). Although there was no significant difference between the treatment groups with respect to total number of treatment-emergent AEs in 005, the following AEs with an incidence rate of ≥ 10% were reported more frequently in the AI TID arm than the AI BID arm: productive cough (46% vs. 36%), wheezing (23% vs. 13%), dyspnea (17% vs. 6%), pyrexia (15% vs. 9%) and decrease in pulmonary function tests (14% vs. 6%).

The Sponsor did not conduct a concentration-response analysis for safety in any of the Phase 2 or 3 studies.

2.2.4.3. Does aztreonam prolong QT or QTc interval?

Intravenous aztreonam has not been associated with prolongation of the QT or QTc interval. Therefore, it is not expected that AI would be associated with this toxicity.

2.2.4.4. Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

As outlined in 2.2.4.1 above, in Study 005 the 75 mg BID regimen performed better than the 75 mg TID regimen for the primary efficacy outcome, time to need for antibiotics. However, there was a regimen effect observed in this study, with BID placebo also performing better than TID placebo with respect to the primarily efficacy outcome. This suggests that TID administration results in poorer outcomes than BID, regardless of the treatment group (AI or placebo). Despite this finding, the Sponsor selected the 75 mg TID regimen for further clinical development based on the assumption that the TID regimen would result in a longer T>MIC in the lungs, and thus, improved antimicrobial activity. Without a second confirmatory study comparing the two regimens, it remains unclear whether 75 mg BID or 75 mg TID is the more appropriate regimen.

Alternatively, a higher dose of 150 mg BID may result in improved outcomes relative to the 75 mg BID or 75 mg TID regimens. In Study 002, sputum concentrations of aztreonam following 150 mg AI were approximately double those of 75 mg, with no observable difference in safety or tolerability. As the dose was further increased from 150 mg to 225 mg, sputum concentrations were only

minimally increased. A dose-dependent effect on lung *P. aeruginosa* CFUs was demonstrated in Study 003, in which 225 mg BID resulted in a greater reduction in lung CFUs (-2.1 $\log_{10}$) relative to 75 mg BID (-1.5 $\log_{10}$). A 150 mg BID dose might be expected to reduce lung CFUs to a greater extent than 75 mg BID, and potentially with a lower AE rate than a TID AI regimen.

2.2.5. What are the PK characteristics of aztreonam for inhalation?

2.2.5.1. What are the single and multiple dose PK parameters?

Following administration of AI 75 mg to adult CF patients, the mean plasma Tmax occurs within 1 hour post-initiation of dose (Table 2.2.5.1-1). The mean plasma Cmax value following an AI dose of 75 mg is 419 ng/mL, versus a Cmax of 90 µg/mL observed following a 1 gram dose of IV aztreonam. The apparent terminal elimination half-life of aztreonam from plasma was approximately 2.1 hours following single-dose AI, similar to what has been reported for IV aztreonam.

Table 2.2.5.1-1 Plasma PK Parameter Values in Adult CF Patients Following a Single 75 mg Dose of AI

PK Parameter	**Mean**	**SD**
Cmax (ng/mL)	419	155.32
tmax (h)	0.99	0.348
t ½ (h)	2.1	0.32
AUC_{0-t} (ng·h/mL)	1496	494.86
$AUC_{0-\infty}$ (ng·h/mL)	1629	521.73
Apparent CL (mL/h)	51173	18693.15

Sputum aztreonam concentrations in adult and adolescent CF patients administered single ascending doses of AI are summarized below in Table 2.2.5.1-2. Concentrations were greatest at 10 minutes post-treatment, decreasing rapidly thereafter, to ≤ 10% by 2 hours post-dose. In general, sputum levels were highly variable in both dose cohorts, with %CVs ranging from 75% to 300%. This high inter-patient variability in sputum concentrations was observed in all clinical studies. Sputum concentrations in adolescents were consistently lower than that of adults at all dose levels and at all time points. Sputum concentrations in adults appear to increase approximately proportional to dose from 75 mg to 150 mg at all time points. However, from 150 mg to 225 mg, there is only a minimal increase in sputum concentrations. Adolescent sputum concentrations do not appear to increase with increasing dose, though considerable variability is noted. In general, clearance of aztreonam from the sputum appears to occur more rapidly in adolescents. There is no accumulation of aztreonam in either the sputum or the plasma of CF patients following multiple dose administration of AI up to the highest dose studied, 225 mg BID.

Table 2.2.5.1-2 Sputum Aztreonam Concentrations (μg/g) in Adult and Adolescent CF Patients Following Single Ascending Doses of AI (Study 002)

	Adults (n=12)			Adolescents (n=11[b])		
	Mean (SD)	**Median**	**Range**	**Mean (SD)**	**Median**	**Range**
Day 0 (75 mg)						
10 min[a]	539 (571)	383	86 – 2170	329 (169)	324	31 – 601
2 hr	60 (70)	38	7 -267	43 (87)	7	0 – 267
4 hr	32 (32)	16	2 -100	16 (29)	2	0 – 90
Day 1 (150 mg)						
10 min	1129 (846)	879	376 -3090	587 (652)	387	17 – 1830
2 hr	104 (97)	83	17 – 361	43 (63)	19	0 – 182
4 hr	50 (44)	36	4 – 139	209 (624)	7	0 – 2090
Day 2 (225 mg)						
10 min	1208 (935)	985	303 – 3440	479 (406)	260	79 – 1140
2 hr	125 (116)	78	9 – 396	67 (90)	26	0 – 272
4 hr	92 (144)	52	2 – 531	15 (16)	8	0 – 50

[a] Time post-dose collection
[b] n=10 for the 225 mg dose

2.2.5.2. How does the PK of aztreonam in healthy volunteers compare to that in patients?

The PK properties of aztreonam in sputum cannot be compared between healthy subjects and CF patients, as sputum concentrations were not assessed in healthy volunteers. Plasma $AUC_{0-\infty}$ following single-dose AI was similar between healthy subjects and adult CF patients after correcting for differences in dose. Plasma Cmax, however, was higher in CF patients. Tmax and the elimination half-life from plasma were similar for both patients and healthy subjects.

2.2.5.3. What are the characteristics of drug absorption?

The systemic bioavailability of aztreonam following administration of AI has not been assessed. However, absorption of aztreonam following inhalation appears low, with approximately 10% of the administered dose excreted unchanged in the urine, as compared to 60-65% excretion of unchanged drug following IV administration.

2.2.5.4. What are the characteristics of drug distribution?

Not applicable.

2.2.5.5. What are the characteristics of drug metabolism?

Approximately 7% of intravenously administered aztreonam is hydrolyzed in the liver to the beta-lactam ring-opened metabolite. The Sponsor investigated the oxidative metabolism of aztreonam in pulmonary and hepatic microsomal fractions prepared from preclinical species and humans

(Study 22175). Aztreonam was relatively resistant to oxidative metabolism in human pulmonary and hepatic microsomes, with very little to no disappearance at concentrations of 15 – 100 μM. Based on these in vitro findings, it can be assumed that aztreonam does not undergo any appreciable metabolism in the lungs.

2.2.5.6. What are the characteristics of drug excretion?

Systemically available aztreonam is excreted by glomerular filtration and tubular secretion. Approximately 60-65% of IV aztreonam is eliminated by urinary excretion as unchanged drug. In healthy volunteers, approximately 10% of the total AI dose was recovered in the urine in a 24-hour collection period.

2.2.5.7. Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

In healthy volunteers administered single ascending AI doses of 95 mg, 190 mg, and 285 mg, plasma Cmax and AUC increased greater than dose proportionally from 95 mg to 190 mg (Table 2.2.5.7-1). However, as the dose increased from 190 to 285 mg, no further increases in exposure were apparent.

Table 2.2.5.7-1 Plasma Cmax and AUC Values Following Single Ascending AI Doses in Healthy Subjects

Dose of Aztreonam (mg)	Cmax (ng/mL)			$AUC_{0-\infty}$ (ng·h/mL)		
	N	Geometric Mean (CV%)	Dose Proportionality	N	Geometric Mean (CV%)	Dose Proportionality
95	6	284 (275.5)	1	5	2591 (119.7)	1
190	5	1330 (116.1)	4.68	5	6792 (90.5)	2.62
285	5	1250 (316.0)	4.40	4	5191 (285.2)	2.00

In Study 002, sputum concentrations in adult CF patients appeared to increase approximately proportional to dose from 75 mg to 150 mg at all timepoints assessed. However, from 150 mg to 225 mg, there is only a minimal increase in sputum concentrations (~ 12%). In general, adolescent sputum concentrations do not appear to increase with increasing dose, though considerable variability in concentrations is noted for this cohort.

Taken together, the sputum data from CF patients and the plasma data from healthy subjects suggest that local pulmonary deposition and, possibly, absorption from the lungs, are saturated at AI doses greater than 150-190 mg. In contrast, the 10-minute post-dose sputum concentrations in Study 003 were slightly greater than dose-proportional from 75 mg to 225 mg. This would imply that lung deposition is not limited up to AI doses of at least 225 mg.

2.2.5.8. How do PK parameters change with time following chronic dosing?

As noted above, there is no accumulation of aztreonam in either the sputum or the plasma of CF patients following multiple dose administration of AI up to the highest dose studied, 225 mg BID.

Peak sputum (10 minutes post-dose) and plasma (1 hour post-dose) concentrations of aztreonam were similar on Day 0 and Days 7 and/or 14, across all of the doses studied.

2.2.5.9. What is the inter- and intra-subject variability in volunteers and patients, and what are the major causes of variability?

The inter-subject variability in plasma aztreonam exposure (Cmax, AUC) was greater in healthy subjects (90 – 316%) than in adult CF patients (33 – 37%) following single-dose AI. There is no clear explanation for the observed difference in variability between the two populations.

Sputum concentrations of aztreonam were highly variable between CF patients in all clinical studies (Phase 1, 2 and 3), with %CV values of up to 126% for the 10 minute post-dose samples. The variability was even greater (up to 300%) at subsequent sampling timepoints (2 hours and 4 hours post-dose). This finding is not unexpected given the complexity of lung pathophysiology in CF patients, the range of disease severity studied, and the method of sputum collection employed (expectorated sputum).

2.3. Intrinsic Factors

2.3.1. What intrinsic factors influence exposure and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

Age

In Study 002, sputum concentrations of aztreonam were compared between adult and adolescent CF patients at 10 minutes, 2 hours and 4 hours post-dose. In general, sputum concentrations in adolescents were consistently lower than that of adults at all dose levels and at all time points. While sputum concentrations appear to increase approximately proportional to dose from 75 mg to 150 mg in adults, adolescent sputum concentrations increase minimally with increasing dose from 75 mg up to 225 mg. In addition, the clearance of aztreonam from the sputum appears to occur more rapidly in adolescents than adults. Similar to adults, sputum concentrations were considerably variable in adolescents at all time points assessed (%CV 51 – 300%).

It is not known what effect, if any, these observed differences in sputum exposure in adolescents have on the efficacy or safety of AI. In both of the Phase 3 studies patients < 18 years of age had better outcomes for the primary efficacy measurement relative to patients aged 18 years or older. However, the number of children and adolescents enrolled in the Phase 3 studies was relatively small ($\geq$ 6 to $\leq$ 12 years, n=25 total; > 12 to < 18 years, n=58 total).

Renal Impairment

Systemically available aztreonam is excreted by combined mechanisms of glomerular filtration and tubular secretion. Approximately 60-65% of IV aztreonam is eliminated in the urine as unchanged drug. It is recommended the dose of IV aztreonam be reduced by 50% in patients with a CrCl between 10 and 30 mL/min/1.73m^2, after an initial 1 or 2 gram loading dose. In patients with a CrCl < 10 mL/min/1.73m^2, the usual loading dose should be administered followed by a reduced maintenance dose of 25% of the loading dose. Maintenance doses are to be given at the regular dosing interval (every 6-12 hours) in renally impaired patients.

The systemic exposure of aztreonam following administration of AI to patients with renal impairment has not been assessed. The Phase 3 clinical studies conducted with AI excluded

patients with a baseline serum creatinine > 2x ULN. Given the low systemic exposure of aztreonam following administration of AI 75 mg (Cmax 0.4 to 0.7 µg/mL), it is unlikely that systemic accumulation of aztreonam in renally impaired patients would approach the concentrations observed following therapeutic doses of IV aztreonam (Cmax 58 to 242 µg/mL). Assuming, conservatively, that 20% of the 75 mg AI dose is absorbed from the lungs, the systemically available dose of 15 mg is well below the recommended IV dose of 125-250 mg for anephric patients. This estimate is also based on 100% delivery of AI to the lungs, though the true respirable dose is likely less than the full 75 mg dose. Further, systemic aztreonam is generally regarded as having a wide margin of safety. Thus, no dosage adjustment is necessary for CF patients with renal impairment.

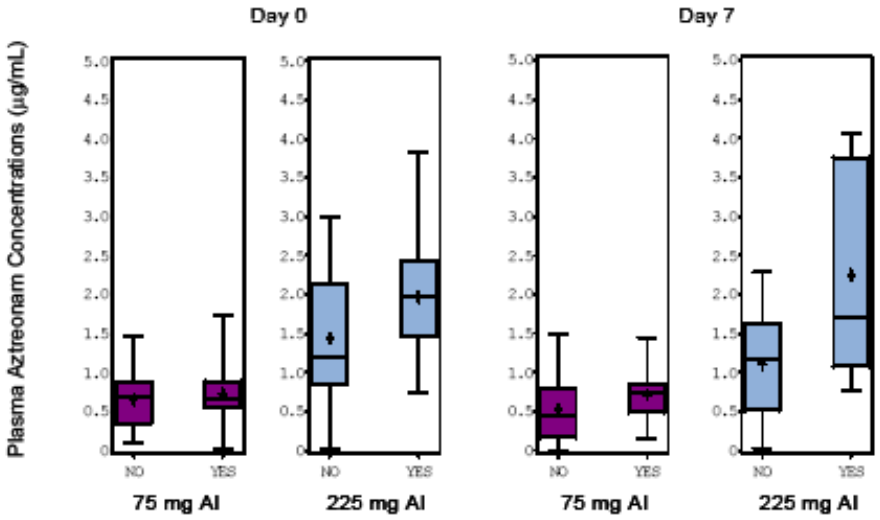
2.4. Extrinsic Factors

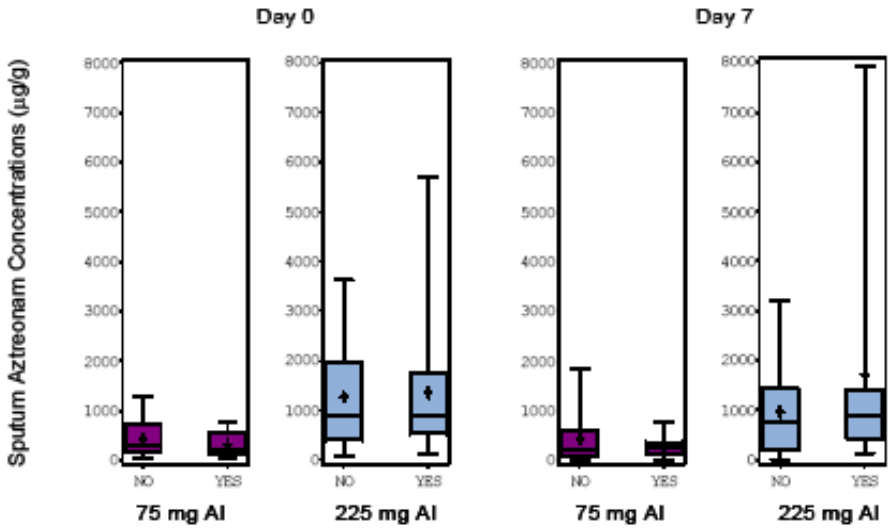
2.4.1. What extrinsic factors influence dose-exposure and/or –response, and what is the impact of any differences in exposure on response?

Bronchodilator Use

Study 003 assessed the effect of bronchodilator use prior to AI administration in CF patients. There were no notable differences in mean plasma aztreonam concentrations between bronchodilator users and non-users in the 75 mg AI group one hour after dosing on Days 0 and 7 (Figure 2.4.1-1). In the 225 mg AI group, bronchodilator users achieved higher mean plasma aztreonam concentrations than non-users at both timepoints. There were no notable differences in mean sputum aztreonam concentrations between bronchodilator users and non-users in the 75 mg or 225 mg treatment groups.

Figure 2.4.1-1 Plasma and Sputum Aztreonam Concentrations by Bronchodilator Use (Yes vs. No)





Source: CP-AI-003 Study Report

In both of the Phase 3 efficacy studies, a short-acting BD was to be administered by patients before administration of all AI doses to improve drug disposition in the lung.

Nebulizer

The first handheld nebulizer used to deliver AI was the eFlow IMP model, a prototype used in the Phase 1a study. The control unit and nebulizer handset for the eFlow IMP were different in appearance than later eFlow models, but the aerosol head and vibrating mesh were comparable and the aerosol characteristics were the same. The Phase 1b study used the Pilot Series 2 version of the eFlow, while the Phase 2 study used the Pilot Series 3 version. The Phase 3 clinical studies used a 510(k) cleared version of the eFlow, model 78G1004.

The manufacturer of the nebulizer, PARI, has modified the base unit of the eFlow to incorporate an electronic display. A 510(k) has been submitted for the modified configuration. The final proposed commercial model, 678G1002, was compared to model 78G1004 used in the Phase 3 studies to evaluate their performance standards. Tests were conducted on the two models to evaluate consistency in emitted dose, particle size and size distribution, drug product aerosolization and drug product/nebulizer leachable interaction. The two models were comparable with regard to the four characterization tests.

2.4.2. What issues related to dose, dosing regimens or administration are unresolved and represent significant omissions?

In Study 005 the 75 mg BID regimen of AI performed better than the 75 mg TID regimen for the primary efficacy outcome, time to need for antibiotics. However, there was a regimen effect observed in this study, with BID placebo also performing better than TID placebo with respect to the primarily efficacy outcome. This suggests that TID administration of inhalation treatment to CF patients results in poorer outcomes than BID, regardless of whether the treatment is active drug or placebo. Although there was no significant difference between the treatment groups with respect to total number of treatment-emergent AEs, the following AEs with an incidence rate of $\geq$ 10% were reported more frequently in the AI TID arm than the AI BID arm: productive cough (46% vs. 36%), wheezing (23%

vs. 13%), dyspnea (17% vs. 6%), pyrexia (15% vs. 9%) and decrease in pulmonary function tests (14% vs. 6%).

Despite the findings of Study 005, the Sponsor selected the 75 mg TID regimen for further clinical development based on the assumption that the TID regimen would result in a longer T>MIC in the lungs, and thus, improved antimicrobial activity. Without a second confirmatory study to compare the two regimens, it remains unclear whether 75 mg BID or 75 mg TID is the more appropriate regimen for the proposed indication. Alternatively, a higher AI dose of 150 mg BID may result in improved outcomes relative to the 75 mg BID or 75 mg TID regimens. In Study 002, sputum concentrations of aztreonam following 150 mg AI were approximately double those of 75 mg, with no further increase observed at a dose of 225 mg. Further, a dose-dependent effect on lung *P. aeruginosa* CFUs was demonstrated in Phase 2 Study 003, in which 225 mg BID resulted in a greater reduction in lung CFUs (-2.1 $\log_{10}$) relative to 75 mg BID (-1.5 $\log_{10}$). Given these observations, along with the efficacy results of Study 005, and a higher rate of certain adverse events with TID dosing versus BID, a 150 mg BID dose may result in improved outcomes and improved tolerability relative to a 75 mg TID regimen.

2.5. General Biopharmaceutics

2.5.1. Based on the biopharmaceutics classification system (BCS) principles, in what class is this drug and formulation? What solubility, permeability, and dissolution data support this classification?

Not applicable for AI.

2.6. Analytical Section

2.6.1. How are the active moieties identified and measured in the clinical pharmacology and biopharmaceutics studies?

The active moiety, aztreonam, was measured in plasma, urine and sputum samples in the clinical studies using validated analytical methods.

2.6.2. Which metabolites have been selected for analysis and why?

No metabolites were assessed in any of the clinical studies.

2.6.3. For all moieties measured, is free, bound, or total measured? What is the basis for that decision, if any, and is it appropriate?

Total aztreonam was assessed in plasma and sputum studies in all studies. Assessment of plasma protein binding is not relevant for the proposed route of administration since the protein binding of aztreonam is expected to be substantially lower in pulmonary fluid as compared to plasma.

2.6.4. What bioanalytical methods are used to assess concentrations?

Aztreonam concentrations in human plasma and sputum were determined using validated liquid chromatography atmospheric pressure ionization tandem mass spectrometry (LC-API/MS/MS) methods.

2.6.4.1. What is the range of the standard curve? How does it relate to the requirements for clinical studies? What curve fitting techniques are used?

For the determination of aztreonam in plasma, a set of 8 calibration standards were extracted to produce a calibration curve ranging from 2.50 – 1,000 ng/mL. The quadratic regression of the curves for peak area ratios versus concentration was weighted $1/x^2$. For determination of aztreonam in sputum, processed 10% mucin was used as a surrogate matrix for preparation of the calibration standards. A set of 8 points were used to prepare a curve ranging from 0.500 to 500 ng/g. The calibration curve ranges were appropriate for the clinical studies conducted.

2.6.4.2. What are the lower and upper limits of quantification (LLOQ/ULOQ)?

The respective lower and upper limits of quantitation were 2.50 ng/mL and 1,000 ng/mL in plasma and 0.500 and 500 ng/g in sputum.

2.6.4.3. What are the accuracy, precision, and selectivity at these limits?

	Plasma		10% Mucin[a]		Sputum	
	LLOQ	ULOQ	LLOQ	ULOQ	LLOQ	ULOQ
Accuracy (%bias)	≤ 13.3%	≤ 5.9 %	≤ 5.0%	≤ 5.3%	11%	-
Precision (%CV)	≤ 5.36%	≤ 2.5 %	4.26%	2.6%	15.2%	-
Selectivity	No interference		No interference		No significant interference[b]	

[a] Diluted and processed 10% mucin extracts were used as a surrogate during validation until a supply of human sputum could be obtained. Upon obtaining human sputum samples, QC samples were prepared at 3 concentrations (1.50, 200 and 400 ng/g) to confirm the process validation.

[b] A small, later eluting endogenous peak was present in 2 of the 3 individual sputum samples evaluated; the peak could slightly impact quantitation at the LLOQ

2.6.4.4. What is the sample stability under the conditions used in the study (long-term, freeze-thaw, sample-handling, sample transport, autosampler)?

Test Conditions	**Plasma**	**10% Mucin***	**Sputum**
Long-term freezer (-20 ºC ± 10ºC)	8 days	79 days	440 days
Long-term freezer (-70 ºC ± 10ºC)	188 days	Not assessed	Not assessed
Benchtop stability	4 hours	23 hours	16 hours

Freeze/thaw stability	3 cycles	3 cycles	3 cycles
Refrigerated (4ºC ± 4ºC) extract stability	73 hours	25 hours	-
Autosampler stability (room temperature)	63 hours	24 hours	-

*Diluted and processed 10% mucin extracts were used as a surrogate during validation until a supply of human sputum could be obtained

Long-term storage stability for aztreonam in human plasma was demonstrated at -70ºC (±10ºC) for up to 188 days. The stability criterion defined a priori was QC mean concentrations within 15% of expected. The mean concentrations of the stored QCs were within 20% of nominal for the low and mid QCs and 15% of nominal for the high QCs at 365 days of storage at -70ºC (±10ºC). After 429 days of storage at -70ºC (±10ºC), the low, mid and high QCs were 79.4%, 84.9% and 91.6% of nominal concentration. For Phase 3 Study 005, plasma samples were stored at -70ºC for up to 482 days. The reported values for 465 plasma samples analyzed outside of the 188 days of established stability may be underestimated due to degradation during storage. For Phase 3 Study 007, plasma samples were stored for up to 567 days. The reported values for 206 plasma samples analyzed outside of the 188 days of established stability may be underestimated due to degradation during storage.

Sputum samples were stored at -20ºC (±10ºC) for up to 477 days in Study 005 and for 437 days in Study 007. The stability of processed human sputum samples has been demonstrated for 440 days at -20ºC (±10ºC).

Plasma samples from the Study 003 were originally stored at -20ºC (± 10ºC), until long-term storage stability results established aztreonam stability in plasma for only 8 days at -20ºC (± 10ºC). Samples were subsequently moved to -70ºC storage. However, many of the samples were at -20ºC for longer than 8 days before being transferred to the colder freezer conditions; some samples were in -20ºC for as long as 119 days. It is expected that plasma samples stored at -20ºC for > 8 days may have been impacted by stability; the total number of affected samples was not reported. Plasma samples were stored at -70ºC for a maximum of 239 days. Plasma samples analyzed outside of the 188 days of established stability may be underestimated due to degradation during storage; the total number of affected samples was not reported. Sputum samples and QC samples were also originally stored at -20ºC. However, like the plasma samples, they were moved to -70ºC due to suspected instability at -20ºC. Sputum samples were stored at -20ºC (± 10ºC) for up to 141 days and at -70ºC (± 10ºC) for up to 247 days.

2.6.4.5. What is the QC sample plan?

For determination of aztreonam in plasma, three QC samples containing 7.50, 400 and 800 ng/mL of the analyte were prepared in control plasma. Intra-assay precision and accuracy were evaluated using replicates (N=6) from each of the three concentrations. QC samples at three concentrations (1.50, 200 and 400 ng/g) were prepared in diluted and processed 10% mucin (as a surrogate for sputum) and analyzed to determine intra- and inter-assay accuracy and precision. They were also used to assess analyte stability. A diluted "Above Quantitation Limit" sample (2000 ng/g) was prepared to evaluate the accuracy and precision at a concentration above the calibration curve range. Upon obtaining human sputum samples, QC samples were prepared at 3 concentrations (1.50, 200 and 400 ng/g) to confirm the process validation and stability testing.

3. LABELING RECOMMENDATIONS

See Appendix 4.1 for the proposed product label with reviewer annotations.

4. APPENDICES

4.1. Proposed Package Insert with Reviewer Annotation

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

4.2. Individual Study Reviews

4.2.1 In Vitro Studies

22175 (b) (4)

The Comparative Metabolism of Aztreonam in Pulmonary and Hepatic Microsomal Fractions From Rat, Dog and Human

Dates: March – May 2002

Study Site: (b) (4)

Objective:
To investigate the oxidative metabolism of aztreonam in pulmonary and hepatic microsomal fractions prepared from preclinical species and humans.

Methods:

Preparation of Microsomal Fractions
Weighed lung or liver samples were washed in 0.067 M sodium potassium phosphate buffer, pH 7.4, containing 1 .I 5% KC1 before the samples were quickly blotted semi-dry and transferred to a tared beaker containing the same buffer. The liver and lung were finely chopped with scissors and then homogenized. The homogenized material was centrifuged at ~12500 g for ~15 min to pellet whole cells and cellular debris. The supernatant was recovered and centrifuged at ~100000 g for ~60 min, the pellet recovered, re-suspended (washing step) in the same buffer and re-centrifuged at ~100000 g for ~60 min. The microsomal pellet was then finally re-suspended in 0.067 M sodium potassium phosphate buffer, pH 7.4. Microsomes were stored in 0.5 ml aliquots at -80°C. All procedures used in the preparation of sub-cellular fractions were carried out on ice, where possible.

Use of Stored Samples
To ensure the viability of the microsomal preparations, samples were thawed only once immediately before commencing each incubation.

Tissue Viability
Hepatic and pulmonary microsomal preparations used for this study were stored at -80°C. The viability of the tissue preparations were assessed prior to commencing the study by measuring alkoxyresorufin 0-dealkylation activities of the pooled sample by the continuous fluorimetric method (Biochem. Pharmacol., *34,* 3337-345, 1985). Protein content of microsomes was determined by following the Lowry protein method (Biol Chem., 1951,193, 265-1 75). Microsomal Cytochrome P450 content was estimated according to the method of Omura and Sato (J. Biol. Chem., *239,* 2370-2378, 1964).

Metabolism of Aztreonam in Microsomes From Each Species
The effect of varying incubation time (3 timepoints, *eg* 15, 30 and 60 min) and substrate concentration (3 concentrations, *eg* 15, 30 and 100 μM) on the rate of metabolism of aztreonam was investigated. Reaction mixtures contained 0.5 mg microsomal protein. Each reaction contained an NADPH-generating system comprised of 0.6 units isocitric dehydrogenase, 2.5 mM DL-isocitrate, 250 μM NADP and 5 mM $MgCI_2$. Each reaction mixture was made up to 0.25 ml with suitable reaction buffer. All reaction mixtures were pre-incubated for ~2 min at ~37°C in a

water bath prior to initiating the reaction. Each incubation was performed in duplicate. Analysis of microsomal incubates was performed using HPLC as described below. Blank reactions contained all the components of the reaction minus the NADPH-generating system which was replaced by addition of reaction buffer. At the end of incubation the reactions were terminated by addition of cold methanol, centrifuged and the supernatant containing parent compound and metabolites transferred to a clean vial and stored at - 20°C until analysis by HPLC.

Chromatography
Post incubation supernatants were analyzed by HPLC with UV detection using a mobile phase of phosphate buffer in methanol and a reverse phase CI8 HPLC column.

Results:

The cytochrome P450 content and metabolic competence of the liver microsomal preparations were within acceptable limits. The values observed in lung microsomes were lower, or in some cases, too low for detection, consistent with previously reported low CYP isozyme activity in the lung.

Following incubation of aztreonam with hepatic and pulmonary microsomes from the rat there appeared to be little or no loss of the parent compound (Table 1). Disappearance of aztreonam did not appear to show any dependency upon starting concentration, incubation time or presence of co-factor. Furthermore, visual assessment of chromatograms did not provide any evidence for the presence of any additional components other than aztreonam.

Similar to the rat, aztreonam was relatively resistant to oxidative metabolism in microsomes from dog liver and lung (Table 2). Starting concentration, incubation time or presence of co-factor did not have any effect on the rate of metabolism. Inspection of the chromatograms did not reveal any additional components.

The results observed following incubation of aztreonam with human hepatic and pulmonary microsomes was identical to that observed for both rat and dog. Disappearance of aztreonam was minimal and did not show any dependency upon starting concentration, incubation time or presence of co-factor (Table 3). The HPLC chromatograms did not provide any supporting evidence for the formation of metabolic products

Table 1. Rate of Aztreonam Metabolism Following Incubation with Hepatic and Pulmonary Microsomes from Rats

Tissue	Time (min)	Concentration (μM)	Rate of Disapperance of Aztreonam ($nmol.min^{-1}.mg^{-1}$)	
			+NADPH	-NADPH
Liver	15	15	0.02	0.02
	30		0.02	0.01
	60		0.01	0.01
	15	30	0.15	0.04
	30		0.03	0.05
	60		0.03	0.01
	15	100	0.43	0.24
	30		0.22	0.11
	60		0.10	0.06
Lung	15	15	0.01	0.03
	30		0.02	0.06
	60		0.02	0.02
	15	30	0.02	0.09
	30		0.03	0.11
	60		0.04	0.09
	15	100	0.76	0.71
	30		0.16	0.18
	60		0.12	0.19

Table 2. Rate of Aztreonam Metabolism Following Incubation with Hepatic and Pulmonary Microsomes from Dogs

Tissue	Time (min)	Concentration (μM)	Rate of Disappearance of Aztreonam ($nmol.min^{-1}.mg^{-1}$)	
			+NADPH	-NADPH
Liver	15	15	0.00	0.00
	30		0.00	0.00
	60		0.00	0.00
	15	30	0.00	0.00
	30		0.09	0.00
	60		0.00	0.00
	15	100	0.13	0.00
	30		0.00	0.00
	60		0.00	0.00
Lung	15	15	0.09	0.06
	30		0.03	0.00
	60		0.00	0.00
	15	30	0.26	0.16
	30		0.09	0.01
	60		0.01	0.00
	15	100	0.35	0.62
	30		0.48	0.08
	60		0.01	0.08

Table 3. Rate of Aztreonam Metabolism Following Incubation with Hepatic and Pulmonary Microsomes from Humans

Tissue	Time (min)	Concentration (μM)	Rate of Disappearance of Aztreonam ($nmol.min^{-1}.mg^{-1}$)	
			+NADPH	-NADPH
Liver	15	15	0.01	0.02
	30		0.03	0.03
	60		0.01	0.02
	15	30	0.00	0.00
	30		0.05	0.05
	60		0.02	0.03
	15	100	0.00	0.00
	30		0.06	0.25
	60		0.12	0.12
Lung	15	15	0.03	0.08
	30		0.03	0.24
	60		0.00	0.03
	15	30	0.15	0.24
	30		0.05	0.05
	60		0.03	0.05
	15	100	0.72	0.61
	30		0.24	0.23
	60		0.15	0.15

Conclusions:

Following incubation with rat, dog and human hepatic and pulmonary microsomes, aztreonam was found to be relatively resistant to oxidative metabolism in microsomes from both tissues. This finding is consistent with previous study findings in which it was reported that only 7% of a dose is metabolized (via hydrolytic opening of the beta-lactam ring).

4.2.2 Clinical Studies

Study CP-AI-001

A Randomized, Double-Blind, Placebo Controlled Trial to Assess the Safety and Tolerability of Inhaled Aztreonam in Healthy Male and Female Volunteers

Dates: November – December 2002

Study Sites: Salus Pharma Inc., 2025 1st Avenue, Suite 800, Seattle WA 98121

Objective:
To evaluate the safety, tolerability and PK of 3 escalating doses of aztreonam for inhalation when administered as a single dose to healthy volunteers.

Methods:

Study Design
This was a randomized double-blind, placebo-controlled, single escalating dose study of inhalational aztreonam. Within each of the 3 dose levels, subjects were randomized to either active treatment (n=6) or placebo (n=2). The doses of aztreonam evaluated were 95 mg, 190 mg and 285 mg by inhalation via the eFlow™IMP nebulizer. Progression to the 190 mg and 285 mg dose levels occurred only after the blinded safety data from the 95 mg and 190 mg groups, respectively, were evaluated. Subjects self-administered the single dose of aztreonam in clinic 30 minutes after a light breakfast. Serial blood and urine samples were collect prior to and following dose administration for determination of aztreonam concentrations.

Test Product
Inhaled aztreonam at a dose of 95 mg (1 ml), 190 mg (2 ml) or 285 mg (3 ml) (Batch Number G002-TOX-004) was self-administered by the subject using an eFlow™IMP nebulizer (PARI).

Reference Product
Sterile 0.9% saline, manufactured by (b) (4) (Batch #139006)

Inclusion criteria
Subjects were male or female non-smokers, aged 18 to 55 years, weighing between 50 and 100 kg with a body mass index of 18 to 28 kg/m^2, with a negative Coombs' test result and a forced expiratory volume in one second (FEV1) of at least 80% of the predicted normal.

Pharmacokinetic assessment
Blood samples were collected at the following time points for determination of aztreonam concentrations in plasma: pre-dose, immediately post-dose, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 hours post-initiation of the dose. Urine samples were collected for evaluation of drug concentrations at the following time intervals: predose, 0-6 hours, 6-12 hours, 12-18 hours and 18-24 hours post-dose. Following collection, plasma and urine samples were stored at -80ºC until the time of analysis.

Analytical Methods

Plasma and urine aztreonam concentrations were analyzed by (b) (4) using a validated assay. Plasma samples were spiked with internal standard ((b) (4) Following protein precipitation with acetonitrile the extracts were analyzed by reverse-phase LC-MS. The validated assay range is 12.5 to 625 ng/mL in plasma. Urine samples were spiked with internal standard, serially diluted with de-ionized water, and then analyzed by reverse-phase LC-MS/MS. The validated assay range is 100 to 20,000 ng/mL in human urine.

Plasma and urine samples were stored at -80ºC for a maximum of approximately 210 days. Long-term storage stability of plasma samples has been demonstrated at -70 ºC for up to 188 days. Mean concentrations of QCs stored at -70 ºC for 365 days were within 20% of nominal for the low and mid QCs and within 15% of nominal for the high QCs. Long-term storage stability at -80 ºC has not been evaluated.

Pharmacokinetic Methods

The PK parameters of aztreonam were estimated by non-compartmental analysis using WinNonlin®.

Results:

Study Population

All 24 subjects dosed were included in the safety dataset, while all 18 subjects who received an active dose were included in the PK dataset. Plasma concentrations for one subject who received 190 mg were below the LOQ and therefore this subject was not included in the final statistical analysis. Two subjects had "unreliable" estimates of $AUC_{0-\infty}$ and therefore these values were not included in the summary statistics.

The mean (SD) age of the subjects was 34.8 (12.5) years. The mean (SD) weight of the subjects was 69.95 (10.39) kg. The mean (SD) body mass index was 24.5 (2.8) kg/m^2. Fifteen subjects (62.5%) were female and 9 (37.5%) were male. Twenty-two subjects were white, one was black, and one was Asian.

Analytical Performance

The analysis of aztreonam in plasma and urine was conducted between December 9, 2002 and June 14, 2003.

Plasma Analysis:

Calibration standards ranged in concentrations of 12.5 to 625 ng/mL. QC samples were prepared at concentrations of 25, 60 and 500 ng/mL. Inter-batch accuracy for aztreonam in plasma ranged from 96.5 to 97.8%. Precision (%CV) was 10.3 to 10.5%. Coefficients of Determination for the calibration curves were ≥ 0.9975.

Urine Analysis:

Calibration standards ranged from 100 to 20,000 ng/mL. QC samples were prepared in concentrations of 200, 1,600 and 16,000 ng/mL. Inter-batch accuracy for aztreonam in urine ranged from 98.2 to 100.8%. Precision (%CV) was 5.2 to 6.8%. Coefficients of Determination for the calibration curves were ≥ 0.9952.

Pharmacokinetic Analysis

Mean (range) duration of dosing of inhaled aztreonam was 3.5 (2.3 – 3.9) minutes for 95 mg, 6.6 (2.5 – 12.6) minutes for 190 mg, and 8.4 (6.3 – 10.9) minutes for 285 mg.

Plasma Data
Plasma concentration profiles were generally consistent with inhalation dosing. Peak plasma aztreonam concentrations were attained between 0.5 and 2.0 hours post-dose; Tmax was unchanged with increasing dose. Following Tmax, plasma concentrations declined in a monophasic manner up to 8 hours after dosing. Two subjects had quantifiable plasma concentrations up to 24 hours after dosing, one each in the 190 mg and 285 mg dose groups. Clearance (CL/F) was highly variable, ranging from 12.8 to 428 L/h. In general, CL/F decreased as the dose increased from 95 to 190 mg, and then increased from 190 to 285 mg. Mean (SD) CL/F estimates were 50.3 (38.9), 37.5 (38.2) and 131.7 (198) L/h for the 95 mg, 190 mg and 285 mg dose groups, respectively. Apparent terminal elimination half-life estimates were 2.7, 2.9 and 2.7 hours for the three ascending dose groups, respectively.

Systemic exposure to aztreonam (Cmax and AUC) increased as the dose increased from 95 to 190 mg. As the dose increased from 190 to 285 mg, no further increases in exposure were apparent (Table 1).

Table 1. Plasma Cmax and AUC values Following a Single Dose of Aztreonam by Inhalation

Dose of Aztreonam (mg)	Cmax (ng/mL)			$AUC_{0-\infty}$ (ng*h/mL)		
	n	Geometric Mean (CV%)	Dose Proportionality*	n	Geometric Mean (CV%)	Dose Proportionality*
95	6	284 (275.5)	1	5	2591 (119.7)	1
190	5	1330 (116.1)	4.68	5	6792 (90.5)	2.62
285	5	1250 (316.0)	4.40	4	5191 (285.2)	2.00

* Relative to lowest dose

Urinary Excretion
Urine concentrations of aztreonam were quantifiable up to at least 6 hours after dosing in all subjects. The mean amount of unchanged aztreonam excreted (Ae) over the 24h collection period was 8.6 mg, 19.8 mg and 18.6 mg in the 3 ascending dose groups, respectively. These values represent 9.1%, 10.4% and 6.5% of the total dose administered in the three respective groups. As the dose increased in a ratio of 1:2:3, the total amount of unchanged aztreonam in the urine increased in a ratio of 1:2.3:2.2.

Safety
There were no serious adverse events (AEs) and no subject was withdrawn due to an AE. A total of 7 AEs were reported in 7 subjects after dosing. Six of the 7 events were Grade 1 in severity and 1 event was Grade 2. The AEs included GI complaints, a decrease in FEV, dizziness, dysgeusia, headache and cough. There was no apparent difference in the incidence of AEs among the different dose groups.

Conclusions
Inhaled aztreonam was generally safe and tolerated well when administered at doses of 95 mg, 190 mg and 285 mg. There were no clinically significant changes in FEV_1 (defined as a decrease from baseline of 15% or more) in any subject treated with aztreonam. The relatively short Tmax (ranging from 0.50 to 2.00 h) and terminal elimination half-life (ranging from 1.86 to 4.34 h) suggest aztreonam was both rapidly absorbed and eliminated following inhalation dosing. The

plasma elimination half-life is similar to what is observed following IV administration of aztreonam (1.6 to 2.9 hours). Aztreonam exposure (Cmax and AUC) increased greater than dose proportionally from 95 to 190 mg. However, as the dose increased to 285 mg, Cmax and AUC values did not increase further, indicating saturation of absorption from the lung and/or limitations in drug disposition at doses > 190 mg. Similarly, the amount of drug excreted in urine over 24 hours increased with dose from 95 to 190 mg; thereafter, no further increases were apparent. Aztreonam excretion in the urine was approximately 10% of the total dose administered up to 190 mg.

Study CP-AI-002
Safety and Tolerability of Ascending Single Doses of Aztreonam for Inhalation in Patients with Cystic Fibrosis (CF)

Dates: May – October 2003

Study Sites: The study was conducted at 8 U.S. Cystic Fibrosis Centers

Objective:
To evaluate the safety, tolerability and PK of single escalating doses of aztreonam for inhalation in patients with CF.

Methods:

Study Design
This was a multi-center, randomized, double-blind, placebo-controlled, single escalating dose study of aztreonam for inhalation. Two cohorts of patients with CF were treated with up to 3 single ascending doses of aztreonam or placebo on consecutive days. Patients were randomized within each cohort in a 2:1 fashion to treatment with aztreonam or placebo. The two cohorts consisted of adults ≥ 18 years and adolescents > 12 to < 18 years. Dosing in the adult cohort was completed and the findings reviewed before commencing with dose administration in the adolescent cohort. The ascending aztreonam doses administered to both cohorts were 75 mg, 150 mg and 225 mg, delivered by the eFlow Electronic Nebulizer (PARI). The maximum tolerated dose (MTD) for each patient was determined based on a pre-specified decline in forced expiratory volume in one second (FEV_1) or oxygen saturation. Serial plasma concentrations were evaluated in adults after the first (75 mg) dose only, while sputum concentrations were evaluated in adults and adolescents after each of the 3 doses.

Test Product
Aztreonam doses of 75 mg (1 ml), 150 mg (2 ml) or 225 mg (3 ml) (Batch number D103001) were dissolved in sterile 0.17% saline for inhalation by nebulizer.

Reference Product
Placebo doses contained 5 mg lactose and 7.3 mg NaCl reconstituted with 1 mL of 0.17% saline (Batch #P403001).

Inclusion criteria
Enrolled patients were diagnosed with CF, but clinically stable with no evidence of acute upper or lower respiratory tract infection or current pulmonary exacerbation, with an FEV_1 of at least 40% of the predicted normal and an oxygen saturation of at least 90%. Patients were excluded if they received any IV or aerosolized antibiotics or any bronchodilator, anti-inflammatory or corticosteroid medication within 7 days prior to Screening. In addition, patients were excluded if they had abnormal renal (SCr > 1.5x ULN) or hepatic (AST or ALT > 2.5x ULN) function.

Pharmacokinetic assessment
Blood samples were collected from adult patients after the administration of the 75 mg dose only. Samples were collected at the following time points for evaluation of plasma aztreonam concentrations: pre-dose, immediately post-dose, 0.5, 1, 2, 4, 5, 6 and 8 hours post-dose. Following collection, samples were stored at -70ºC until the time of analysis.

Sputum samples were collected from adult and adolescent patients after each of the 3 ascending doses for evaluation of aztreonam concentrations. Samples were collected at the following time points: screening, 10 minutes, 2 hours and 4 hours post-treatment. Patients gargled and rinsed with saline three times prior to the first sputum collection post-dose. A minimum of 1 mL of sputum was collected and frozen at -70 ºC until the time of analysis.

Analytical Methods

Plasma and sputum samples were analyzed by (b) (4) using validated assay methods. Plasma samples were extracted using acetonitrile:methanol precipitation and spiked with internal standard ((b) (4) prior to analysis by LC-API/MS/MS. The validated assay range in plasma was 2.50 to 1,000 ng/mL. Aztreonam and the IS (carumonam) were extracted from processed and diluted sputum samples using acetonitrile precipitation. The sputum extracts were analyzed by LC-API/MS/MS. The validated assay range in sputum was 0.500 to 500 ng. Diluted and processed 10% mucin was used as a matrix for preparing calibration standards and QC samples for analysis of sputum samples.

Pharmacokinetic Methods

Aztreonam plasma PK parameters were estimated using a one-compartment model with first-order input and first-order elimination using S-PLUS 6.1.

Results:

Study Population

A total of 18 adult CF patients and 17 adolescent CF patients were randomized. All randomized patients received at least one dose of study drug. Demographic and clinical characteristics are shown below in Table 1.

Table 1. Demographic and Clinical Characteristics of Patients

		Placebo		Aztreonam	
		Adults (N=6)	**Adolescents (N=6)**	**Adults (N=12)**	**Adolescents (N=11)**
Gender:	**Male**	3 (50%)[a]	3 (50%)	7 (58%)	5 (45%)
	Female	3 (50%)	3 (50%)	5 (42%)	6 (55%)
Race:	**Caucasian**	6 (100%)	6 (100%)	12 (100%)	9 (82%)
	African American	–	–	–	2 (18%)
Age (yr):	**Mean (SD)**	33.4 (13.8)	15.8 (1.29)	32.5 (8.65)	15.3 (1.14)
	Median	33.7	16.2	30.9	15.0
	Range	18.6 – 54.2	13.4 – 16.9	19.6 – 47.5	13.6 – 17.0
FEV1 (L):	**Mean (SD)**	2.31 (0.71)	2.85 (1.11)	2.36 (0.59)	2.58 (0.72)
	Median	2.21	2.66	2.27	2.76
	Range	1.53 – 3.60	1.50 – 4.27	1.28 – 3.20	1.31 – 3.68
FEV1 % Predicted:	**Mean (SD)**	67.7 (12.6)	79.5 (20.5)	66.9 (17.3)	88.4 (16.5)
	Median	63.5	75.7	65.0	94.4
	Range	54 – 91	51 – 111	42 – 98	49 – 111

[a] Percentage of patients based on the number of treated patients in each treatment group

Patients in the adult cohort tolerated all doses of aztreonam and were considered to have MTDs of at least 225 mg. Sixteen of the 17 adolescent patients tolerated all of the doses up to 225 mg. One adolescent had an MTD of 75 mg (dosing was discontinued after the 150 mg dose due to an asymptomatic 20% decrease in FEV_1).

Analytical Performance

Plasma Analysis:

The calibration curve contained 8 standards ranging from 2.5 to 1,000 ng/mL. Calibration curve R^2 values were ≥0.9949. Analytical QC samples were 1.50, 200 and 400 ng. The accuracy of calibration standards ranged from 90.1 – 104%, while precision (%CV) was 3.18 – 6.09%. The accuracy of QC samples ranged from 85.3 – 105%, while precision (%CV) was 2.13 – 7.61%.

Sputum Analysis:

The calibration curve contained 8 standards ranging from 0.500 to 500 ng. Calibration curve R^2 values were ≥0.9955. Analytical QC samples were 7.50, 400 and 800 ng/mL. The accuracy of calibration standards ranged from 90.4 to 107%, while precision (%CV) was 2.67 – 5.25%. The accuracy of QC samples ranged from 92.3 to 108%, while the precision (%CV) was 10.3%.

Pharmacokinetic Analysis

Plasma Concentrations
Following administration of aztreonam 75 mg by inhalation, plasma concentrations of aztreonam were quantifiable up to 8 hours post-dose in all adults. Peak plasma concentrations were achieved rapidly, with a mean Tmax of < 1 hour post-initiation of dose.

Table 2. Summary Plasma PK Parameters in Adults Following a Single 75 mg Dose of Aztreonam by Inhalation

PK Parameter	Mean	SD
Cmax (ng/mL)	419	155.32
tmax (h)	0.99	0.348
t ½ (h)	2.1	0.32
AUC_{0-t} (ng·h/mL)	1496	494.86
$AUC_{0-\infty}$ (ng·h/mL)	1629	521.73
Apparent CL (mL/h)	51173	18693.15

Sputum Concentrations
Sputum aztreonam levels in adults and adolescents are summarized below by time and dose group in Table 3. Concentrations were greatest at 10 minutes post-treatment and decreased at subsequent time points. In general, sputum levels were highly variable in both dose cohorts, with %CVs ranging from 75% to 300%. Sputum concentrations in adolescents were consistently lower than that of adults at all dose levels and at all time points. Sputum concentrations in adults appear to increase approximately proportional to dose from 75 mg to 150 mg at all time points. However, from 150 mg to 225 mg, there is only a minimal increase in sputum concentrations. In general, adolescent sputum concentrations do not appear to increase with increasing dose. An increase in mean concentrations occurred only at 10 minutes post-dose when increasing from 75

mg to 150 mg, though considerable variability is noted. In general, clearance of aztreonam from the sputum appears to occur more rapidly in adolescents.

Table 3. Summary of Sputum Aztreonam Levels (µg/g) in Adults and Adolescent CF Patients

	Adults (n=12)			Adolescents (n=11[b])		
	Mean (SD)	**Median**	**Range**	**Mean (SD)**	**Median**	**Range**
Day 0 (75 mg)						
10 min[a]	539 (571)	383	86 – 2170	329 (169)	324	31 – 601
2 hr	60 (70)	38	7 -267	43 (87)	7	0 – 267
4 hr	32 (32)	16	2 -100	16 (29)	2	0 – 90
Day 1 (150 mg)						
10 min	1129 (846)	879	376 -3090	587 (652)	387	17 – 1830
2 hr	104 (97)	83	17 – 361	43 (63)	19	0 – 182
4 hr	50 (44)	36	4 – 139	209 (624)	7	0 – 2090
Day 2 (225 mg)						
10 min	1208 (935)	985	303 – 3440	479 (406)	260	79 – 1140
2 hr	125 (116)	78	9 – 396	67 (90)	26	0 – 272
4 hr	92 (144)	52	2 – 531	15 (16)	8	0 – 50

[a] Time post-dose collection
[b] N = 10 for 225 mg dose due to 1 subject reaching MTD early

Safety
There were no serious adverse events (AEs) reported. A total of 41 AEs were reported by 16 patients. In the adult cohort, there was a trend for increased number of AEs with increasing dose. However, this finding was not observed in the adolescents. The most common AEs were related to respiratory symptoms, such as cough, congestion, wheezing and increased sputum. The most commonly reported AE was aggravation of cough, reported in 4 of 22 (18%) patients at the 225 mg dose level. Two adult patients receiving 150 mg aztreonam reported moderate AEs, including chest pain and tightness. One adolescent receiving 75 mg aztreonam experienced moderate cough and increased sputum.

No clinically relevant changes in pulmonary function tests occurred during treatment or follow-up in either cohort, with the exception of one adolescent patient who experienced an asymptomatic decrease in FEV_1 pf greater than 20% following the 150 mg dose. No clinically relevant changes in oxygen saturation were observed in either cohort.

Conclusions:

In general, inhalation aztreonam was safe and well-tolerated in adults and adolescents with CF up to a dose of 225 mg. Peak plasma aztreonam levels occurred around 1 hour post-dose in adult CF patients, similar to what was observed in healthy subjects administered aztreonam by inhalation in Study CP-AI-001. Cmax following the 75 mg dose in adults with CF was approximately 1.5-fold that observed in healthy adults administered a single 95 mg dose. This would suggest greater systemic absorption from the lungs of CF patients as compared to healthy subjects. However, mean $AUC_{0-\infty}$ was only 63% of that seen in healthy adults following a 95 mg dose – nearly

proportional to the difference in dose (75 mg vs. 95 mg). Systemic clearance was approximately 50 L/hr in both adult CF patients administered a 75 mg dose and healthy adults administered 95 mg.

Sputum levels of aztreonam were highly variable in both adults and adolescents (%CV 75 - 300%). Sputum concentrations in adolescents were consistently lower than that of adults. Sputum concentrations in adults appear to increase approximately proportional to dose from 75 mg to 150 mg at all time points. However, from 150 mg to 225 mg, there is only a minimal increase in sputum concentrations. In general, adolescent sputum concentrations do not appear to increase with increasing dose, though there is considerable variability.

Study CP-AI-003

A blinded, multicenter, randomized, placebo-controlled trial with aztreonam for inhalation in CF patients with lung disease due to *P. aeruginosa* infection.

Dates: November 25, 2003 – August 4, 2004

Study Sites: The study was conducted at 21 U.S. Cystic Fibrosis Centers

Objective:
To assess the safety and efficacy of 14-day treatment with aztreonam for inhalation at two dosage levels compared to placebo in CF patients with pulmonary *P. aeruginosa* infections.

Methods:

Study Design
This was a multi-center, randomized, double-blinded, placebo-controlled study of 14 days of aztreonam for inhalation in adult and adolescent CF patients. Up to 138 CF patients with pulmonary infection due to *P. aeruginosa* were randomized 1:1:1 to receive placebo, 75 mg or 225 mg aztreonam for inhalation (AI) twice daily for 14 days. With the exception of the first dose and a dose on Day 7, all doses were self-administered at home. The twice daily doses were to be separated by at least 8 hours. No instructions were given regarding administration with regard to mealtime. Treatment was administered on Days 0 – 13. Patients made visits on Days 0, 7, 14 and 28 for evaluation of sputum microbiology, spirometry assessment, monitoring of adverse events (AEs), laboratory evaluation, and analysis of plasma and sputum aztreonam concentrations.

CF patients 13 years of age or older with *P. aeruginosa* infection and mild-to-moderate disease (an FEV_1 of at least 40% predicted) were enrolled. Exclusion criteria included the presence of lung infection due to *B. cepacia* in the previous 2 years, administration with any antipseudomonal antibiotic via any route of administration in the previous 56 days, oral corticosteroid use in doses exceeding 10 mg/day and abnormal renal or hepatic function. Patients on short-acting bronchodilators were instructed to administer the bronchodilator before their inhalation treatment.

Test Product
Aztreonam was supplied as a lyophilized, sterile powder (Batch number A103006). Diluent (sterile, pyrogen-free 0.17% saline) was added to the powder to produce 75 mg/mL solution for inhalation.

Reference Product
Placebo doses contained 5 mg lactose and 7.3 mg NaCl per ampule for reconstitution with 1 mL of 0.17% saline (Batch #P403001). One half of patients randomized to placebo received one vial of lactose powder, while the other half received three vials of lactose powder to reconstitute for administration.

Reviewer Comment: The study report states that the study was blinded to patients and investigators. However, the 75 mg dose of aztreonam was reconstituted by the patient using one ampule of diluent and one vial of powder, while the 225 mg dose was reconstituted by the patient using three ampules of diluent and three vials of powder. Thus, patients were not blinded with regard to dose, but only to treatment vs. placebo.

Efficacy Measurements

The primary efficacy outcome was percent change in FEV_1 from Day 0 to Day 14. Several secondary efficacy variables were analyzed, including absolute change in FEV_1 and change in *P. aeruginosa* CFUs in sputum. Other microbiologic variables that were assessed included the disappearance or appearance or other pathogens from Day 0 to Day 7 and Day 14, and change in the susceptibility of *P. aeruginosa*.

Pharmacokinetic assessment

Blood for plasma aztreonam levels were obtained pre-dose on Day 0, one hour post-dose on Days 0 (first day of treatment) and 7, and on Day 14. Sputum samples for determination of aztreonam levels were obtained pre-dose on Day 0, 10 minutes post-dose on Days 0 and 7, and on Day 14. Patients gargled and rinsed with normal saline three times before producing the post-treatment sputum samples. A minimum of 1 mL of sputum was collected for determination of aztreonam concentrations.

Analytical Methods

Aztreonam and the internal standard (b) (4) were extracted from human plasma by protein precipitation using 3:1 acetonitrile:methanol. After evaporation and reconstitution with 0.1% formic acid, samples were analyzed by LC-API/MS/MS.

Aztreonam and the internal standard (b) (4)) were extracted from processed and diluted human sputum using acetonitrile precipitation. After evaporation and reconstitution, the extracts were analyzed by LC-API/MS/MS. Diluted and processed 10% mucin was used as a surrogate matrix for the calibration standards, QCs, and study sample dilutions.

Results:

Study Population

A total of 105 patients were randomized to one of the three treatment groups, of which 98 completed study participation. Seven patients discontinued participation due to AEs. Demographic characteristics are shown below in Table 1.

Table 1. Demographic and Clinical Characteristics of Patients

Variable	Treatments as Randomized			
	Placebo (N = 32)	**75 mg AI (N = 38)**	**225 mg AI (N = 35)**	**Total (N = 105)**
Gender; n (%)				
Male	16 (50.0%)	22 (57.9%)	16 (45.7%)	54 (51.4%)
Female	16 (50.0%)	16 (42.1%)	19 (54.3%)	51 (48.6%)
Race; n (%)				
African American	0	0	1 (2.9%)	1 (1.0%)
Caucasian	31 (96.9%)	36 (94.7%)	33 (94.3%)	100 (95.2%)
Hispanic	1 (3.1%)	2 (5.3%)	1 (2.9%)	4 (3.8%)
Genotype; n (%)				
Homozygous for ΔF508	15 (46.9%)	19 (50.0%)	16 (45.7%)	50 (47.6%)
Heterozygous for ΔF508	10 (31.3%)	10 (26.3%)	5 (14.3%)	25 (23.8%)
Unidentified	3 (9.4%)	7 (18.4%)	5 (14.3%)	15 (14.3%)

Variable	Treatments as Randomized			
	Placebo (N = 32)	**75 mg AI (N = 38)**	**225 mg AI (N = 35)**	**Total (N = 105)**
Other	4 (12.5%)	0	2 (5.7%)	6 (5.7%)
Missing	0	2 (5.3%)	7 (20.0%)	9 (8.6%)
Sweat chloride test (mEQ/L)[a]				
Mean (SD)	102.4 (16.3)	110.9 (27.0)	105.9 (25.8)	106.8 (24.0)
n	28	37	34	99
Age group (years); n (%)				
> 12 to < 18	6 (18.8%)	9 (23.7%)	12 (34.3%)	27 (25.7%)
≥ 18	26 (81.3%)	29 (76.3%)	23 (65.7%)	78 (74.3%)
Age (years)				
Mean (SD)	26.4 (9.8)	28.1 (11.7)	23.3 (8.4)	26.0 (10.2)
Disease severity; n (%)				
FEV_1 % pred. < 60%	6 (18.8%)	14 (36.8%)	4 (11.4%)	24 (22.9%)
FEV_1 % pred. ≥ 60%	26 (81.3%)	24 (63.2%)	31 (88.6%)	81 (77.1%)
BD use; n (%)				
Yes	8 (25.0%)	16 (42.1%)	12 (34.3%)	36 (34.3%)
No	24 (75.0%)	22 (57.9%)	23 (65.7%)	69 (65.7%)
Aztreonam MIC for PA in sputum; n (%)[b]				
≤ 8 µg/mL	24 (75.0%)	20 (52.6%)	22 (62.9%)	66 (62.9%)
> 8 µg/mL	5 (15.6%)	15 (39.5%)	8 (22.9%)	28 (26.7%)
Missing	3 (9.4%)	3 (7.9%)	5 (14.3%)	11 (10.5%)
MIC of aztreonam for all PA isolates (µg/mL)				
MIC50	≤ 1	2	2	≤ 1
MIC90	16	64	128	64
Minimum MIC	≤ 1	≤ 1	≤ 1	≤ 1
Maximum MIC	128	1024	1024	1024

[a] From medical history
[b] The highest MIC isolate from each patient; PA = *P. aeruginosa*

Efficacy Analysis

There was no statistically significant difference in the mean percent change in FEV_1 from Day 0 to Day 14 between the 225 mg AI and placebo treatment groups in the intent-to-treat (ITT) dataset (Table 2). There were no statistically significant differences between any of the three dose levels (placebo, 75 mg AI, and 225 mg AI). T he relationship between the dose and the percent change in FEV_1 was not found to be linear. These results were confirmed in the per-protocol (PP) dataset for treatment received as well. There was a possible placebo effect on percent change in FEV1 at Day 14, with an adjusted mean percent change from Day 0 to Day 14 of 4.08%. In the overall analysis, the term for treatment in the percent change in FEV_1 analysis

was not statistically significant (p = 0.3746); however, the term for disease severity was statistically significant (p = 0.0002).

There were no statistically significant differences in the mean percent change in FEV_1 from Day 0 to Day 14 between the 225 mg AI and placebo treatment groups for any of the subsets analyzed (age group, gender, and MIC category). These results were confirmed by the PP dataset for treatment received. None of the results for the remaining spirometry tests (FEV_1, FVC, and FEF_{25-75}) was statistically significant at any timepoint.

Table 2. Percent Change in FEV_1 at Day 14 (ITT Dataset)

% Change from Day 0 to Day 14:	**Treatment as Randomized**		
	Placebo (N = 32)	**75 mg AI (N = 38)**	**225 mg AI (N = 35)**
Mean (SD)	1.02 (8.14)	4.53 (13.23)	3.88 (10.94)
n	31	36	34
Adjusted mean	4.08	6.20	7.71
Treatment difference: 225 mg AI – placebo	3.62		
95% CI (p-value)	-1.50, 8.75 (0.1638)		
Treatment difference: 75 mg AI – placebo	2.12		
95% CI (p-value)	-2.97, 7.21 (0.4112)		
Treatment difference: 225 – 75 mg AI	1.51		
95% CI (p-value)	-3.54, 6.55 (0.5548)		
p-value for trend test	0.1784		

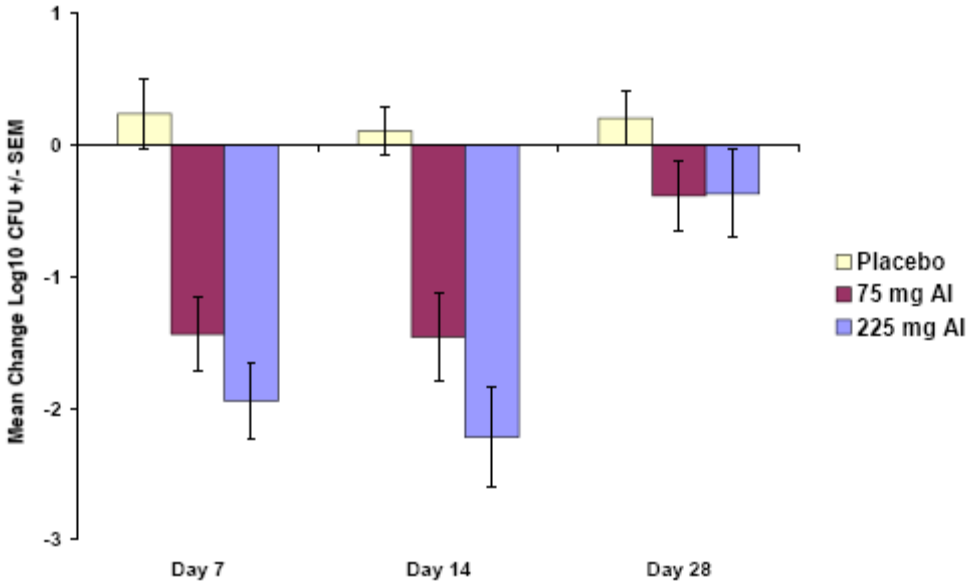
There was a mean reduction in log_{10} *P. aeruginosa* CFUs in the two AI treatment groups at both Day 7 and Day 14, and a small mean increase in the placebo group at both timepoints (Table 3). The mean reduction in log_{10} CFUs observed after treatment with AI was greater in the 225 mg AI group than in the 75 mg AI group. The treatment ratio (95% CI) was 0.01 (0.00, 0.05), indicating that, on average, the reduction in log_{10} CFUs for placebo was only 1% of the reduction for 225 mg AI. These results were confirmed by the PP dataset for treatments as received. As shown in Figure 1, by Day 28 *P. aeruginosa* CFUs had almost returned to their baseline in both treatment groups.

Table 3. Changes in Log_{10} *P. aeruginosa* (PA) CFUs in Sputum (CFUs/g Sputum): ITT Dataset

Change in log10 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 log10 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 14			
Mean (SD)	0.025 (1.313)	-1.453 (1.575)	-2.109 (1.990)
n	28	30	27
Adjusted mean	-0.103	-1.506	-2.249
Treatment difference: 225 mg AI-placebo	-2.146		
95% CI (p-value)	-3.029, -1.263 (< 0.0001)		
Treatment ratio 95% CI	0.01		
	0.00, 0.05		

Figure 1. Log_{10} Change in CFUs by Treatment Group and Visit



Pharmacokinetic Analysis

A summary of plasma and sputum concentrations are shown below in Table 4. There was considerable variability in plasma and sputum concentrations among patients in both treatment cohorts. Plasma concentrations were generally low, with the highest concentration observed at 1-hour post-dose on Day 0 in the 225 mg cohort (1.6 µg/mL). Sputum concentrations were slightly greater than dose proportional from the 75 mg to 225 mg dose cohorts. Day 14 sputum concentrations were 6.0 and 9.3 µg/g in the 75 mg and 225 mg treatment groups, respectively. However, the time interval between sputum collection and the last dose administration (on Day 13) was variable depending on the Day 14 clinic visit. The time intervals from administration of the last dose were not reported.

Table 4. Mean (SD) Aztreonam Concentrations in Plasma and Sputum Samples (Safety Dataset)

Extent of Exposure	Treatments as Received				
	Placebo (N = 31)	75 mg AI (N = 37)		225 mg AI (N = 37)	
Treatment days per patient	14	14		14	
Doses per day	2	2		2	
Mean (SD)Plasma Concentrations (ng/mL)		n		n	
Day 0, pretreatment	-	35	15.11 (86.17)	36	28.55 (158.43)
Day 0, + 1 hour	-	37	685.99 (364.96)	37	1613.31 (832.40)
Day 7, + 1 hour	-	35	607.53 (357.49)	36	1488.17 (1079.21)
Day 14	-	36	45.61 (78.33)	34	81.66 (94.26)
Mean (SD) Sputum Concentrations (μg/g)					
Day 0, pretreatment	-	36	0.0 (0.0)	35	0.0 (0.0)
Day 0, + 10 minutes	-	37	384.2 (313.5)	35	1,297.4 (1,241.9)
Day 7, + 10 minutes	-	37	366.7 (401.0)	35	1,202.0 (1,520.6)
Day 14	-	33	6.0 (7.0)	32	9.3 (17.1)

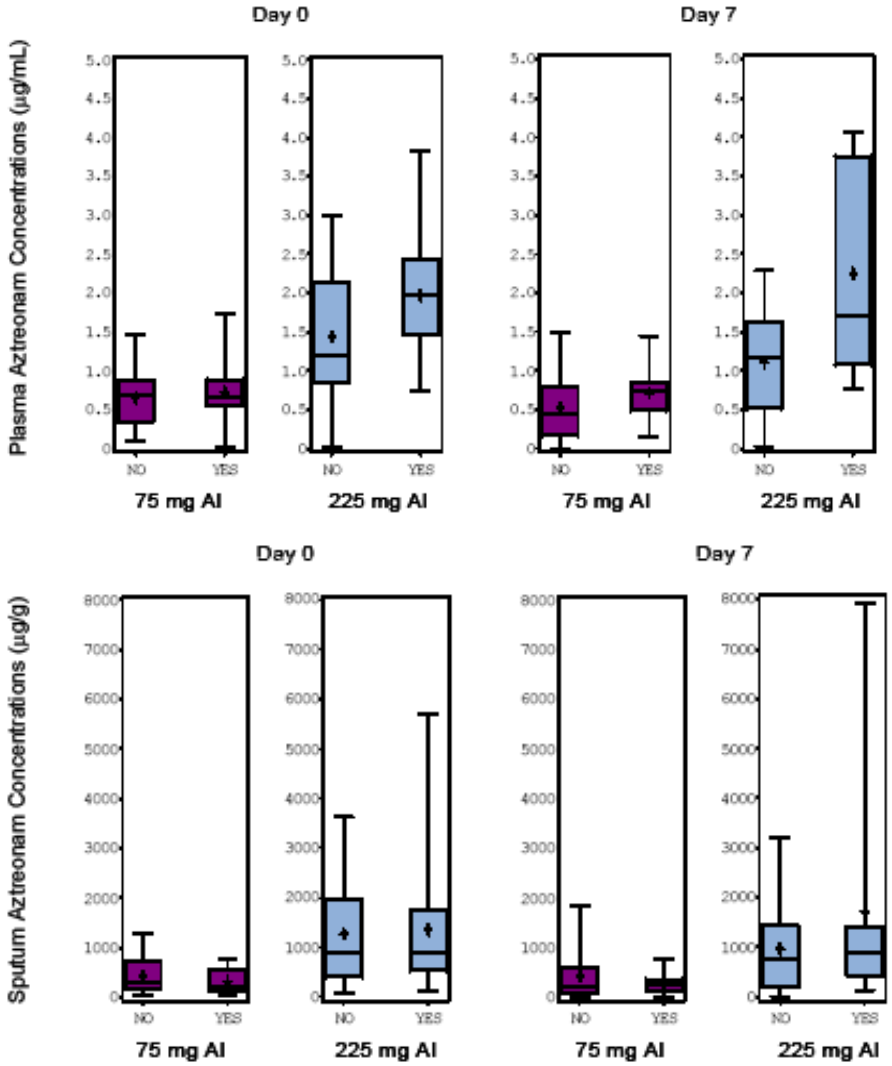
Reviewer Comment:
It appears that several errors were made during the separation and transfer of plasma samples. It was noted that seven patients in the 75 mg (2 patients) and 225 mg (5 patients) AI groups had measurable plasma aztreonam levels before dosing. Three patients in the placebo group also had measurable aztreonam levels. As samples were processed at the same time at the trial site, it is postulated that in the case of the 7 AI-treated patients, possible cross-contamination of the pre-dose and post-dose samples may have occurred during plasma separation and transfer to clean storage tubes. Five of the 7 patients had pre-dose levels ranging from 4.66 to 75.2 ng/mL. Two patients had pre-dose levels of 951 ng/mL and 510 ng/mL, while their post-dose levels were 11.3 and 22.5 ng/mL, respectively. The Sponsor suggests the possibility that these patients may have had their pre-dose and post-dose tubes mislabeled, in addition to possible cross-contamination. All questionable plasma samples were re-run a[redacted] (b) (4) to verify the results. None of these potentially mislabeled and/or contaminated concentrations were omitted from the final results presented above in Table 4.

Placebo-treated Patient 09808 (pre-dose plasma level 38.6 ng/mL) was seen at the trial site on the same day as Patient 09809, who received 225 mg AI; thus, possible cross-contamination may have occurred during sample processing. However, there was no concurrent processing of samples to explain the values for placebo-treated Patients 03007 (27.9 ng/mL) and 31003 (149 ng/mL at Day 0 and 19.3 ng/mL at Day 7). In addition, one set of sputum samples from a patient

who received placebo (Patient 01409) had measurable aztreonam levels at Days 0, 7, and 14. These sputum samples were not re-analyzed by (b) (4) *to verify the results.*

There were no notable differences in mean plasma aztreonam concentrations between bronchodilator users and non-users in the 75 mg AI group one hour after dosing on Days 0 and 7 (Figure 2). In the 225 mg AI group, bronchodilator users achieved higher mean plasma aztreonam concentrations than non-users at both timepoints. No statistical analyses on the differences between bronchodilator users and non-users were performed within either AI treatment group. There were no notable differences in mean sputum aztreonam concentrations between bronchodilator users and non-users in the 75 mg or 225 mg treatment groups. There appears to be greater variability in individual sputum concentrations in the 225 mg AI group compared with the 75 mg AI group.

Figure 2. Plasma and Sputum Aztreonam Concentrations by Bronchodilator Use (Yes vs. No) (Safety Dataset)



Analytical Performance

Plasma Analysis:

The calibration curve ranged from 2.50 to 1,000 ng/mL. The calibration curve R^2 values were ≥ 0.9898. The accuracy (%bias) of calibration standards ranged from 86.4 – 113%, while precision (%CV) was ≤ 6.80%. QC samples were prepared at concentrations of 7.50, 400 and 800 ng/mL. The accuracy (%bias) of QC samples ranged from 86.5 – 113%, while precision (%CV) was ≤ 12.9%. Six samples were re-analyzed due to a positive result in a pre-dose sample, while seven samples were re-analyzed to confirm a positive result found in a placebo-treated patient sample.

Plasma samples from the study were originally stored at -20ºC (± 10ºC), until long-term storage stability results established aztreonam stability in plasma for only 8 days at -20ºC (± 10ºC). Samples were subsequently moved to -70ºC storage. However, many of the samples were at -20ºC for longer than 8 days before being transferred to the colder freezer conditions; some samples were in -20ºC for as long as 119 days. Long-term storage of plasma samples at -20ºC has been validated for a period of 8 days only. Thus, samples stored at -20ºC for > 8 days may have been impacted by stability. Plasma samples were stored at -70ºC for a maximum of 239 days. Long-term stability have confirmed the stability of plasma stored at -70ºC for up to 188 days.

Sputum Analysis:

The calibration curve for aztreonam in human sputum ranged from 0.500 to 500 ng. The LLOQ for sputum samples was 0.500 ng. R^2 values were ≥ 0.9921. The accuracy (% bias) of calibration standards was 86.8 – 111%, while precision (%CV) was ≤ 7.03%. Analytical QC samples were prepared at concentrations of 1.50, 200 and 400 ng. Dilution QC samples were analyzed at 400 and 2,000 ng. Accuracy (% bias) of the QC standards was 85.3 – 114%, while precision (%CV) was ≤ 9.20%.

All study sputum samples and QC samples were originally stored at -20ºC. However, like the plasma samples, they were move to -70ºC due to suspected instability at -20ºC. Sputum samples were stored at -20ºC (± 10ºC) for up to 141 days and at -70ºC (± 10ºC) for up to 247 days. Long term stability of sputum samples at -20ºC has been demonstrated up to 440 days. Stability studies of sputum at -70ºC have not been conducted.

Safety

There were no notable differences between treatment groups in the percentages of patients reporting AEs, with 22 (71.0%), 26 (70.3%), and 27 (73.0%) patients reporting AEs in the placebo, 75 mg and 225 mg AI groups, respectively. There appeared to be a dose-related trend in the percentages of patients reporting drug-related AEs, with 19.4%, 27.0%, and 37.8% of patients in the placebo, 75 mg and 225 mg AI groups, respectively, reporting drug-related AEs. All drug-related AEs were mild or moderate in severity. Three patients had AEs that led to discontinuation of trial drug (one patient in each treatment group). None of the AEs leading to discontinuation was considered to be related to the trial drug. The most commonly reported AE and drug-related AE was cough, reported in 12 (38.7%), 12 (32.4%), and 13 (35.1%) patients in the placebo, 75 mg and 225 mg AI groups, respectively. There were no clinically relevant changes in any of the laboratory parameters evaluated.

Conclusions:
The study failed to demonstrate a significant finding for the primary efficacy outcome, percent change in FEV_1 at Day 14 versus Day 0 for either the 75 mg or 225 mg AI dose groups. There did appear to be an effect for patients with greater disease severity (FEV_1 < 75% of predicted at baseline); however, only the difference between the 225 mg AI and placebo groups at Day 7 met statistical significance for % change in FEV_1 (approximately 9.0%). The Sponsor cites the large percentage of patients with normal baseline lung function as the likely reason for the lack of findings.

During the trial, reductions in $\log_{10}$ CFUs were observed for both AI treatment groups, despite 27% of isolates having MICs at Day 0 that exceeded the parenteral breakpoint of 8 μg/mL. At the end of the trial (Day 28), CFU densities had returned to near Day 0 levels.

There was considerable variability in plasma and sputum concentrations among patients in both treatment cohorts. Plasma concentrations were generally low, with the highest concentration observed at 1-hour post-dose on Day 0 in the 225 mg cohort (1.6 μg/mL). Sputum concentrations at 10 minutes post-dose were slightly greater than dose proportional from 75 mg to 225 mg. A PK/PD relationship for response cannot be evaluated, since T>MIC is the PK/PD parameter of interest for aztreonam and only 10 minute post-dose sputum concentrations were collected.

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

/s/

Sarah M. Robertson
6/27/2008 12:34:32 PM
BIOPHARMACEUTICS

Charles Bonapace
6/27/2008 12:51:13 PM
BIOPHARMACEUTICS