CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

050814Orig1s000

PHARMACOLOGY REVIEW(S)

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION:
RESUBMISSION OF NDA 50-814, Cayston™ (Aztreonam for Inhalation, AZLI)

DATE: 11/20/09

TO: File, NDA 50-814

FROM: Amy L. Ellis, Ph.D.
Pharmacologist, DAIOP

THROUGH: Wendelyn Schmidt, Ph.D.
Pharmacology/Toxicology Supervisor, DAIOP

RE: NDA 50-814 resubmission: Pharm/Tox review of additional nonclinical studies

The sponsor of this NDA, Gilead Sciences, Inc. (Seattle, WA), resubmitted it to the DAIOP on 8/11/09. This resubmission is intended to address the issues preventing approval that were raised in the Division's complete response letter dated 9/16/08. The pharmacology/toxicology reviewer did not object to the approval of the NDA when it was submitted initially and this recommendation has not changed. The pharmacologist's review of the original NDA submission, including labeling recommendations, can be found in DARRTS and was filed on 7/18/08. The labeling recommendations have not changed, with the exception that data from the genotoxicity tests recently submitted by the sponsor should be included.

This NDA resubmission contains reports of a safety pharmacology study in dogs (SDN 46, 8/26) and several genotoxicity studies (SDN 48, 9/22) on both the drug substance, aztreonam, and the drug product, AZLI. The Multiple Study Data Analyses submitted on 8/11 (SDN 44) contains summaries of some *in vitro* PK/metabolism studies conducted with aztreonam. The results from these studies do not change the original recommendation of the pharmacologist regarding approval of this NDA or the reasoning behind it. The genotoxicity studies conducted on the drug product used degraded AZLI as well as non-degraded AZLI; results were negative for both. These data could be used for setting limits for degradation products in Cayston™.

PK/Metabolism:

The following table lists the PK/metabolism studies conducted by the sponsor since the original NDA was submitted. The sponsor has not submitted full reports of these studies to the IND or NDA for aztreonam for inhalation, but included summaries of each in the Multiple Module Information Amendments contained in the 8/11 NDA resubmission.

Study Title	Test System	Administration	Testing Facility	Study Number
Plasma Stability of Aztreonam	*In vitro*	*In vitro*	Gilead Sciences	AD-205-2001
In Vitro Metabolism of Aztreonam in Rat, Dog, Cynomolgus Monkey, and Human	*In vitro*	*In vitro*	Gilead Sciences	AD-205-2002
Induction of Metabolizing Enzymes by Aztreonam	*In vitro*	*In vitro*	Gilead Sciences	AD-205-2003
Cytochrome P450 Phenotyping of Aztreonam	*In vitro*	*In vitro*	Gilead Sciences	AD-205-2004
In Vitro Assessment of Human Liver Cytochrome P450 Inhibition Potential of Aztreonam	*In vitro*	*In vitro*	Gilead Sciences	AD-205-2005
In Vitro Protein Binding Determination of Aztreonam Lysine (GS-9268) in CD-1 Mouse, Sprague-Dawley Rat, Beagle Dog, and Human Plasma by Equilibrium Dialysis	*In vitro*	*In vitro*	(b) (4)	AD-205-2006

These studies confirmed that aztreonam is resistant to metabolism by plasma esterases and liver microsomal enzymes obtained from several species. *In vitro* studies demonstrated that aztreonam has a low potential for inducing or inhibiting human cytochrome P450 enzymes, and that it is not metabolized or is metabolized very slowly by these enzymes. These data suggest a low potential for drug interactions involving cytochrome P450. Protein binding of aztreonam was determined by dialysis of plasma collected from mice, rats, dogs, and humans. It was low to moderate, as can be seen in this table from the submission:

Nominal Concentration (μM)	Percent Unbound (Mean ± SD) [a]			
	Species			
	Mouse	Rat	Dog	Human
1	16.9 ± 1.9	14.5 ± 0.5	53.9 ± 9.8	17.2 ± 1.3
3	20.6 ± 1.1	14.3 ± 2.2	55.8 ± 7.5	28.3 ± 3.7
10	21.0 ± 1.0	14.7 ± 1.8	65.9 ± 8.8	25.6 ± 4.3
30	29.5 ± 2.2	31.2 ± 3.5	80.3 ± 2.0	56.5 ± 6.7
50	32.1 ± 2.6	40.3 ± 2.9	79.3 ± 5.4	84.5 ± 10.1

[a] Mean and SD represent N=3.

Safety Pharmacology:

Aztreonam: Evaluation by Inhalation Administration on Blood Pressure, Heart Rate, Lead II ECG and Respiratory Function in Conscious Telemetered Beagle Dogs (PC-205-2001)

Key study findings: Single estimated inhaled doses of aztreonam up to 163 mg/kg were not associated with treatment-related changes in cardiovascular or respiratory parameters or the core body temperature of conscious male beagle dogs.

Study no.: 670220 (Gilead Reference No. PC-205-2001)
Conducting laboratory and location: (b) (4)
Date of study initiation: 8/25/08
GLP compliance: OECD, UK
QA report: yes (X) no ( )
Drug, lot #, % purity: Aztreonam, 07/01400, 99.9%; L-lysine monohydrate 0200 4922, 100%

Methods

Doses: 0 (vehicle, 30 mM NaCl), 35, 70, and 140 mg/kg. These targeted inhaled doses were the same as those used for the 90 day repeat dose toxicity study. Estimated achieved inhaled doses were determined by washing the apparatus after dosing and collecting expired air from the dogs using an impinger and collection filter. The estimated achieved inhaled dose was the total amount of drug nebulized, minus the amount found in the apparatus washout solution and expired air. The mean estimated achieved inhaled doses for the low, mid, and high dose groups were 40.0, 101.5, and 162.7 mg/kg, respectively.
Species/strain: Beagle dogs
Number: 4 males
Route, formulation: Inhalation of nebulized solution. The concentration of aztreonam in 30 mM NaCl (vehicle) for the low, medium, and high dose groups was 19, 38, and 75 mg/ml, respectively. Each ml of the high dose solution contained 52.5 mg of lysine monohydrate with 75 mg of aztreonam and the solutions prepared for the lower dose groups contained lysine monohydrate in the same proportion.
Age: approximately 11 months
Weight: 6.8-7.8 kg
Study Design/Methodology: Dogs were implanted with peritoneal radio transmitters that allowed monitoring of blood pressure, heart rate, lead II ECG, and core body temperature. QT intervals were corrected for heart rate using 2 different formulas, Van de Water's and Fridericia's. Telemetry readings were analyzed every 15 minutes for one hour prior to dosing, then 1.5, 2, 3, 5, 7, 12, 16, and 23 hours after the start of dosing. The dogs were acclimated to the dosing apparatus and to wearing the Vivometric Lifeshirt System®, also used for monitoring cardiac and respiratory parameters which are stored in a memory card located in a transmitter pack inside the jacket. Heart rate and ECG data were monitored during dosing using the Lifeshirt. The data collected by this method were analyzed every 15 minutes for one hour prior to dosing, then during dosing (10, 30, 45, and 60 minutes after the start of dosing). Respiratory parameters (rate, tidal volume, minute volume) collected using the Lifeshirt were analyzed for the same time points as the cardiac parameters collected by either the Lifeshirt or implanted radiotransmitter. Animals were

restrained in slings during dosing, but they were not restrained at any other time. Arterial blood samples for blood gas determinations were collected prior to dosing, during dosing (about 40 minutes after initiation), and 1 hour after the end of dosing. A closed face mask with a mouth tube connected to the De Vilbiss ultrasonic nebulizer was used to administer test articles. The dogs received each dose one time with at least a 3 day washout period in between, with dosing order based on a Latin Square design. Dogs received aerosolized test solution for one hour. In between test article treatment days, dogs were exposed to aerosolized 0.9% saline for one hour each day so that they remained acclimated to the dosing apparatus.

Results: There were no treatment-related clinical signs observed in any of the dogs. No treatment-related changes in cardiovascular parameters were observed following any dose of aztreonam when measured either by the peritoneal radio transmitter or the Lifeshirt. No treatment-related changes in body temperature were measured by the radio transmitter and no treatment-related changes in respiratory parameters were measured by the Lifeshirt. There were no changes in blood gas values that appeared toxicologically relevant.

Genotoxicity:

Aztreonam: Testing for Mutagenic Activity with *Salmonella typhimurium* TA 1535, TA 100, TA 1537 and TA 98 and *Escherichia coli* WP2*uvr*A (pKM101)

Study no.: 785401 (Gilead Reference No. TX-205-2008)

AND

Degraded and Non-Degraded AZLI: Testing for Mutagenic Activity with *Salmonella typhimurium* TA 1535, TA 100, TA 1537 and TA 98 and *Escherichia coli* WP2*uvr*A (pKM101)

Study no.: 785857 (Gilead Reference No. TX-205-2011)

Conducting laboratory and location (for both bacterial mutagenicity studies): (b) (4)

Dates of study initiation: 7/8/08 and 10/31/08
GLP compliance: OECD and UK GLPs
QA report: yes (X) no ( )

Key findings: Aztreonam was lethal to the bacteria used for these assays at concentrations $\geq$ 1.7 µg/plate. Due to excessive cytotoxicity, the Ames assay was not considered suitable for testing the genotoxic potential of aztreonam or AZLI. The assay was canceled after the results of the initial cytotoxicity tests with *S. typhimurium* TA 1535 and TA 100 were available.

Aztreonam: Chromosomal Aberrations Assay with Chinese Hamster Ovary Cell Cultures *In Vitro*

Key findings: Under the conditions of this assay, aztreonam did not induce structural chromosome aberrations in CHO cells regardless of metabolic activation. Polyploidy and endoreduplication were observed after 22 hours of treatment with 3483.4 µg/ml of aztreonam in the absence of S-9. However, this concentration was significantly cytotoxic.

Study no.: 785417 (Gilead Reference No. TX-205-2009)
Conducting laboratory and location: (b) (4)
Date of study initiation: 7/8/08
GLP compliance: OECD and UK GLPs
QA report: yes (X) no ( )
Drug, lot #, and % purity: Aztreonam, Batch No. 02005007, 85.0% pure

Methods

Cells: Chinese hamster ovary (CHO 10 B_4) cells were cultured in Ham's F-10 medium.

Doses used in definitive study: The initial assay conducted with a 6 hour incubation ± S-9 used concentrations up to 5000 µg/ml. The second assay with 6 and 22 hour incubations in the absence of metabolic activation and 6 hour incubations with S-9 used concentrations of 435.4, 870.9, 1741.7, 2612.6, 3483.4 and 4354.3 µg/ml.

Basis of dose selection: The 5000 µg/ml concentration used in the initial assay precipitated during incubation and was excessively cytotoxic. Thus, the high concentration was lowered for the second assay. Cytotoxicity was determined by counting the number of cells in the treated cultures compared to negative controls and observing the cells in culture and on the slides for evidence of metaphase and signs of necrosis.

Negative controls: Ham's F-10 medium

Positive controls: Methyl methanesulfonate (MMS) was the positive control in the absence of S-9 (20 and 30 µg/ml for the 6 hour treatment and the 22 hour treatment with the 24 hour harvest; 10 and 30 µg/ml for the 22 hour treatment with the 48 hour harvest) and cyclophosphamide was the positive control in the presence of S-9 (20 and 40 µg/ml for the first assay and 30 and 40 µg/ml for the second). The S-9 mix was prepared from the livers of adult male Fischer rats that had been induced with Aroclor 1254.

Incubation and sampling times: CHO cells were treated with test article, vehicle, or MMS in the absence of metabolic activation for 6 hours or 22 hours. Treatments in the presence of S-9 lasted for 6 hours. The first assay only used 6 hour incubations and 24 hour harvests. The second assay used both 24 and 48 hour harvests following the 22 hour incubations. Colcemid was added for the final 2 hours prior to harvest. Harvested cells were swollen, fixed, and dropped on slides (3 per culture) and stained with Giemsa for evaluation of chromosome aberrations and determination of mitotic index. Slides were coded so that evaluation was conducted in a blinded

manner. One hundred cells per culture (50 per slide when possible) were analyzed for the presence of chromosome aberrations. Based on observations when scoring the slides for chromosome aberrations, an extra evaluation of the numbers of cells that were diploid, polyploid, or endoreduplicated was conducted in approximately 300 metaphase cells per concentration level from the 22 hour aztreonam treatment in the absence of S-9 harvested at 48 hours.

Results

Study validity: All aztreonam and negative control cultures were treated in duplicate. The study was valid. No cultures were contaminated. The vehicle control cultures grew as expected and the background numbers of aberrations were within the 95% confidence limits of the historical negative control data. Positive controls induced significant ($p \leq 0.01$) increases in the frequency of cells with chromosome aberrations in both the presence and absence of S-9 in at least the higher of the 2 concentrations tested.

Study outcome: Both clastogenicity assays showed that aztreonam did not induce structural chromosome aberrations in CHO cells under the conditions of testing regardless of metabolic activation.

Concentrations selected for analysis in the first assay were 625, 1250 and 2500 µg/ml. No significant increase in the frequency of cells with chromosome aberration was observed at any of these concentrations regardless of metabolic activation. The 5000 µg/ml dose level was not appropriate for analysis due to excessive cytotoxicity- no metaphases were available for assessment.

In the confirmatory assay, the concentrations selected for analysis were 1741.7, 2612.6 and 3483.4 µg/ml. Again, no significant increase in the frequency of cells with chromosome aberrations was observed at any of these concentrations (± S-9). The 4354.3 µg/ml concentration level contained no metaphases for evaluation at 24 or 48 hour harvests. Reduced cell counts were observed with 22 hours of treatment with aztreonam at 2612.6 and 3483.4 µg/ml (reduction > 50% of vehicle control at the latter concentration) at the 24 hour harvest and at 3483.4 µg/ml at the 48 hour harvest. The 6 hour incubation with 2612.6 µg/ml was not cytotoxic and 3483.4 µg/ml was less cytotoxic than seen following the 22 hour incubation.

The extra polyploidy assessment of the aztreonam cultures from the second assay at the 48 hour harvest demonstrated both polyploidy and endoreduplication at the highest concentration, 3483.4 µg/ml (no S-9), consistent with observations made by the individuals who scored the cultures for chromosome aberrations. Numerical aberrations were not observed in the chromosomes from the 2 lower aztreonam concentrations that were assessed.

Degraded and Non-Degraded AZLI: Chromosomal Aberrations Assay with Chinese Hamster Ovary Cell Cultures *In Vitro*

Key findings: Under the conditions of this assay, AZLI (degraded or not) did not induce structural chromosome aberrations in CHO cells regardless of metabolic activation. Polyploidy and endoreduplication were observed after 22 hours of treatment with 5000µg/ml of non-degraded AZLI (no S-9). However, this concentration was significantly cytotoxic. Numerical aberrations were not observed when cultures were treated with degraded AZLI for the same length of time under the same conditions.

Study no.: 785862 (Gilead Reference No. TX-205-2012)
Conducting laboratory and location: (b) (4) (CHO assay) and (b) (4) (analysis of solutions)
Date of study initiation: 10/28/08
GLP compliance: OECD and UK GLPs in UK; US GLP in US
QA report: yes (X) no ( )
Drug, lot #, and % purity: Aztreonam lysine for inhalation (AZLI), Batch No. 04008 was used after appropriate storage or after being heated to 74ºC for 5 days. The concentrations of aztreonam and impurities were analyzed by (b) (4) and provided in the report.

This is the certificate of analysis for the product at the time of manufacturing (not used in this assay):

Name:	Aztreonam Lysinate for Inhalation Drug Product
Batch (Lot) Number:	Lot # 04008
Formulation Description:	Lyophilized Material in 2 mL Amber Vial
Formulation Strength:	75 mg Aztreonam, 52.5 mg Lysine monohydrate
Packaging configuration:	Single Use Vial
Analytical Reference Number:	030511
Manufacture Date:	November 05, 2004

Storage/Special Handling: Store between 2-8 °C
Special Handling: Protect from light

(b) (4)

NOTE: This document is a certificate of analysis of the GMP testing performed on the lot specified above and does not constitute approval and release of the manufacturing batch.

This is the certificate of analysis for expired, but appropriately stored product (used in this assay as nondegraded AZLI):

REPORT OF ANALYSIS

Forced Degradation Study

Product:	Aztreonam Lysine For Inhalation	Gilead Sciences, Inc. Lot #	04008 (Control)
Contact:	(b) (4)	(b) (4) Study #	8018-001
Client:	Gilead Sciences, Inc.	(b) (4) Protocol #	P7987.01
Address:	2025 First Ave.	Storage Condition	5 °C
	Suite PH	(b) (4) Sample Number	8018-001-00002
	Seattle, WA 98121	Study Start Date	09-Oct-2008
		Dates of Testing	15-Oct-2008 through 20-Oct-2008
Phone:	206-832-2073	Control Sample Batch Size	(b) (4)
Fax:	206-728-5095	Study Report Date	28-Oct-2008
		Amendment Date	NA

Control Sample

(b) (4)

[redacted]

This is the certificate of analysis for product degraded by heat (used in this assay as degraded AZLI):

REPORT OF ANALYSIS

Forced Degradation Study

Product: Aztreonam Lysine For Inhalation	
Contact: (b) (4)	
Client: Gilead Sciences, Inc.	
Address: 2025 First Ave. Suite PH Seattle, WA 98121	
Phone: 206-832-2073	
Fax: 206-728-5095	
Gilead Sciences, Inc. Lot #	04008 (Degraded)
(b) (4) Study #	8018-001
(b) (4) Protocol #	P7987.01
Degradation Conditions	74 °C for 5 Days
(b) (4) Sample Number	8018-001-00001
Degradation Start Date	09-Oct-2008
Degradation End Date	14-Oct-2008
Dates of Testing	15-Oct-2008 through 20-Oct-2008
Storage Condition after degradation	5 °C
Force Degraded Batch Size	(b) (4)
Study Report Date	28-Oct-2008
Amendment Date	NA

Degraded Sample

(b) (4)

The degraded AZLI was spiked with additional aztreonam (b) (4) from Lot # F1D056. Vials containing approximately 127.5 mg of degraded AZLI were reconstituted with 0.255 ml of a water for injection containing 3 mg/ml of the (b) (4) Vials containing approximately 127.5 of nondegraded AZLI were reconstituted with 0.255 mg of water for injection. This was to obtain an AZLI concentration of approximately 500 mg/ml. AZLI contains about a (b) (4) ratio of aztreonam free base:lysine. The reconstituted solutions were used “as is” for the highest dose

levels in the assay and diluted as necessary for the lower dose levels. Fifty µl of these solutions were added to culture medium to obtain a final volume of 5 ml for incubation. The Appendix to the study report contains information from (b) (4) regarding the exact amounts of aztreonam and degradation products contained in 5 vials each of nondegraded and degraded AZLI.

Methods

Cells: Chinese hamster ovary (CHO 10 B_4) cells were cultured in Ham's F-10 medium.

Doses used in definitive study: The initial assay conducted with a 6 hour incubation ± S-9 used AZLI concentrations up to 5000 µg/ml. The second assay with 22 hour incubations in the absence of metabolic activation and 6 hour incubations with S-9 used concentrations of 313, 625, 1250, 2500 and 5000 µg/ml.

Basis of dose selection: The limit dose for the CHO assay is 5000 µg/ml. Cytotoxicity was determined by counting the number of cells in the treated cultures compared to negative controls and observing the cells in culture and on the slides for evidence of metaphase and signs of necrosis.

Negative controls: Water for injection

Positive controls: Methyl methanesulfonate (MMS) was the positive control in the absence of S-9 (20 and 30 µg/ml for the 6 hour treatment and the 22 hour treatment with the 24 hour harvest; 10 and 30 µg/ml for the 22 hour treatment with the 48 hour harvest) and cyclophosphamide was the positive control in the presence of S-9 (20 and 30 µg/ml for the first assay and 20 and 40 µg/ml for the second). The S-9 mix was prepared from the livers of adult male Fischer rats that had been induced with Aroclor 1254.

Incubation and sampling times: CHO cells were treated with test article, vehicle, or MMS in the absence of metabolic activation for 6 hours or 22 hours. Treatments in the presence of S-9 lasted for 6 hours. The first assay only used 6 hour incubations and 24 hour harvests. The second assay used both 24 and 48 hour harvests following the 22 hour incubations. Colcemid was added for the final 2 hours prior to harvest. Harvested cells were swollen, fixed, and dropped on slides (3 per culture) and stained with Giemsa for evaluation of chromosome aberrations and determination of mitotic index. Slides were coded so that evaluation was conducted in a blinded manner. One hundred cells per culture (50 per slide when possible) were analyzed for the presence of chromosome aberrations. Based on observations when scoring the slides for chromosome aberrations, an extra evaluation of the numbers of cells that were diploid, polyploid, or endoreduplicated was conducted in approximately 300 metaphase cells per concentration level from the 22 hour aztreonam treatment in the absence of S-9 harvested at 48 hours.

Results

Study validity: All aztreonam and negative control cultures were treated in duplicate. The study was valid. No cultures were contaminated. The vehicle control cultures grew as expected and

the background numbers of aberrations were within the 95% confidence limits of the historical negative control data. Positive controls induced significant ($p \leq 0.01$) increases in the frequency of cells with chromosome aberrations in both the presence and absence of S-9 in at least the higher of the 2 concentrations tested.

Study outcome: Both clastogenicity assays showed that AZLI (degraded or not) did not induce structural chromosome aberrations in CHO cells under the conditions of testing regardless of metabolic activation.

Concentrations selected for analysis in the first assay were 1250, 2500, and 5000 µg/ml. No significant increase in the frequency of cells with chromosome aberration was observed at any of these concentrations, regardless of metabolic activation or whether the AZLI was degraded. Cytotoxicity was observed in one 5000 µg/ml culture with non-degraded AZLI with S-9, but there were sufficient metaphase cells for analysis.

In the second assay, the concentrations selected for analysis were 1250, 2500, and 5000 µg/ml except for the 22 hour treatment/24 hour harvest with non-degraded AZLI (no S-9) which used concentrations of 625, 1250, and 2500 µg/ml for analysis. The 5000 µg/ml culture treated for 22 hours and harvested after 24 hours did not contain enough metaphase cells for assessment. Signs of cytotoxicity were observed at 2500 µg/ml. At the 48 hour harvest following 22 hours of incubation with 5000 µg/ml non-degraded AZLI, there were also indications of cytotoxicity, but sufficient metaphase cells for analysis. There was no cytotoxicity when cultures were treated with degraded AZLI regardless of S-9 and treatment length. As in the initial assay, no significant increase in the frequency of cells with chromosome aberrations was observed at any of these concentrations.

The extra polyploidy assessment of the aztreonam cultures from the second assay at the 48 hour harvest demonstrated both polyploidy and endoreduplication at the highest concentration of non-degraded AZLI, 5000 µg/ml, consistent with observations made by the individuals who scored the cultures for chromosome aberrations. Numerical aberrations were not observed in the chromosomes from the 2 lower non-degraded AZLI concentrations that were assessed or at any concentration of degraded AZLI.

Aztreonam Mouse Lymphoma Mutation Assay

Key findings: Under the conditions of this assay, aztreonam did not induce mutations at the TK locus in mouse lymphoma L5178Y $TK^{+/-}$ cells regardless of metabolic activation.

Study no.: 785511 (Gilead Reference No. TX-205-2010)
Conducting laboratory and location: (b) (4)
Date of study initiation: 7/24/08
GLP compliance: OECD and UK GLPs
QA report: yes (X) no ( )
Drug, lot #, and % purity: aztreonam, Batch No. 02005007, 100.9 % pure

Methods

Cells: Mouse lymphoma L5178Y $TK^{+/-}$ cells cultured in RPMI 1640 medium.

Doses used in definitive study: For the 4 hour treatment times (+S-9) in the first assay: 2, 4, 6, 8, and 10 mM
For the 4 hour confirmatory assay with S-9: 2, 4, 6, 8, and 10 mM
For the 24 hour treatment time without S-9: 3, 4, 5, 6, 7, 8, 9, and 10 mM

10 mM is equivalent to 4354.3 µg/ml

Basis of dose selection: A dose-range finding study was performed using amounts of aztreonam ranging from 0.5-5000 µg/ml with 4 hour (+ S-9) and 24 hour (no S-9) incubations. A significant decreases in relative suspension growth (RSG) were observed at 5000 µg/ml with the 24 hour treatment; RSG was 5.6%. Additionally, the pH of the medium was reduced by approximately 1 unit (or slightly greater) at 5000 µg/ml. Thus, the high dose for mutagenicity testing was set slightly below this level.

Negative controls: RPMI 1640 medium was the vehicle control.

Positive controls: Methyl methanesulfonate (MMS) and ethyl methanesulfonate (EMS) were the positive controls in the absence of S-9 (10 µg/ml MMS and 250 µg/ml EMS for the 4 hour incubation; 5 µg/ml MMS and 150 µg/ml EMS for the 24 hour incubation) and 3-methylcholanthrene was the positive control in the presence of S-9 (2.5 µg/ml).

The S-9 mix was prepared from the livers of male Fischer 344 rats that had been induced with Aroclor 1254.

Incubation and sampling times: Duplicate cultures were treated with test articles and cultures were treated in quadruplicate with vehicle. The initial assay (+ S-9) used a 4 hour exposure period, followed by a 2 day expression period. The second assay repeated the 4 hour exposure period to test aztreonam in the presence of S-9, but used a 24 hour exposure period in the absence of metabolic activation. Cultures were adjusted to approximately 3×10^5 cells/ml on Day 1 or 2, depending on the length of the exposure period. Cultures with the 5 highest concentrations of test article (+S-9) that had adequate RSG were chosen for mutant selection. To determine cloning efficiency, each culture was diluted to approximately 8 cells/ml and plated into two 96-well dishes with 200 µl of suspension per well (to give about 1.6 cells/well). To determine mutation frequency, cells were suspended in medium containing TFT at a concentration of 1×10^4 cells/ml and plated as above to provide about 2000 cells/well. Cloning efficiency was determined after at least 9 days of incubation and mutation frequency was determined after at least 12 days. The numbers of large (covers more than ¼ of the well floor) and small (covers less than ¼ of the well floor) colonies on each plate were counted. Each well was scored only once, so if a well contained more than one type of colony, it was scored as containing a large type. The method used to calculate mutation fraction requires enumeration of the empty wells and those that contain a cell colony.

Results

Study validity: The study was valid. Suspension growth following test article treatment and cloning efficiencies following the expression period were acceptable. Mutation frequencies for

the negative and positive controls were within acceptable ranges based on historical data. Additionally, the mutant colonies in the positive control cultures had the expected distribution between large and small.

Study outcome: Aztreonam did not induce mutations in cultured L5178Y $TK^{+/-}$ mouse lymphoma cells in the presence or absence of metabolic activation under the conditions of this study. Mutation frequencies in the cells treated with aztreonam were comparable to concurrent and historical controls and did not meet the criteria for a positive response.

Degraded and Non-Degraded AZLI: Mouse Lymphoma Mutation Study

Key findings: Under the conditions of this assay, AZLI did not induce mutations at the TK locus in mouse lymphoma L5178Y $TK^{+/-}$ cells regardless of metabolic activation.

Study no.: 786143 (Gilead Reference No. TX-205-2013)
Conducting laboratory and location: (b) (4) (mouse lymphoma cell assay) and (b) (4) (analysis of solutions)
Date of study initiation: 11/12/08
GLP compliance: OECD and UK GLPs
QA report: yes (X) no ( )
Drug, lot #, and % purity: Aztreonam lysine for inhalation (AZLI), Batch No. 04008 was used after appropriate storage or after being heated to 74ºC for 5 days. The concentrations of aztreonam and impurities were analyzed by (b) (4) and provided in the report. The certificates of analysis for the AZLI can be found above, in the review of the CHO study conducted with non-degraded and degraded AZLI. This review also discusses the method used to reconstitute AZLI which was also used for the current study. The Appendix to this mouse lymphoma mutation study report contains information from (b) (4) regarding the exact amounts of aztreonam and degradation products contained in 5 vials each of nondegraded and degraded AZLI. This is the same (b) (4) report that was appended to the AZLI CHO study.

Methods

Cells: Mouse lymphoma L5178Y $TK^{+/-}$ cells cultured in RPMI 1640 medium.

Doses used in definitive study: For the 4 hour treatment times (±S-9) in the first assay: 500, 1000, 2000, 3000, 4000, and 5000 µg/ml
For the 4 hour confirmatory assay with S-9: 2000, 3000, 4000, and 5000 µg/ml
For the 24 hour treatment time without S-9: 250, 500, 1000, 2000, 3000, 4000, and 5000 µg/ml

Basis of dose selection: Doses were set based on the results of the mouse lymphoma study conducted with aztreonam. Additional testing with AZLI showed that pH would not be shifted unacceptably by the highest concentration.

Negative controls: Water for injection was the vehicle control.

Positive controls: Methyl methanesulfonate (MMS) and ethyl methanesulfonate (EMS) were the positive controls in the absence of S-9 (10 µg/ml MMS and 250 µg/ml EMS for the 4 hour incubation; 5 µg/ml MMS and 150 µg/ml EMS for the 24 hour incubation) and 3-methylcholanthrene was the positive control in the presence of S-9 (2.5 µg/ml).

The S-9 mix was prepared from the livers of male Fischer 344 rats that had been induced with Aroclor 1254.

Incubation and sampling times: Duplicate cultures were treated with test articles and cultures were treated in quadruplicate with vehicle. The initial assay (± S-9) used a 4 hour exposure period, followed by a 2 day expression period. The second assay repeated the 4 hour exposure period to test AZLI in the presence of S-9, but used a 24 hour exposure period in the absence of metabolic activation. Cultures were adjusted to approximately 3 X 10^5 cells/ml on Day 1 or 2, depending on the length of the exposure period. Cultures with the 4 or 5 highest concentrations of test article (±S-9) that had adequate RSG were chosen for mutant selection. To determine cloning efficiency, each culture was diluted to approximately 8 cells/ml and plated into two 96-well dishes with 200 µl of suspension per well (to give about 1.6 cells/well). To determine mutation frequency, cells were suspended in medium containing TFT at a concentration of 1 X 10^4 cells/ml and plated as above to provide about 2000 cells/well. Cloning efficiency was determined after at least 9 days of incubation and mutation frequency was determined after at least 12 days. The numbers of large (covers more than ¼ of the well floor) and small (covers less than ¼ of the well floor) colonies on each plate were counted. Each well was scored only once, so if a well contained more than one type of colony, it was scored as containing a large type. The method used to calculate mutation fraction requires enumeration of the empty wells and those that contain a cell colony.

Results

Study validity: The study was valid. Suspension growth following test article treatment and cloning efficiencies following the expression period were acceptable. The high dose, 5000 µg/ml (equal to the limit dose for this assay), had 72-78% relative total growth compared to the vehicle control when 24 hour incubations were conducted and AZLI (degraded or not) was not cytotoxic when 4 hour incubations were used. Mutation frequencies for the negative and positive controls were within acceptable ranges based on historical data. Additionally, the mutant colonies in the positive control cultures had the expected distribution between large and small.

Study outcome: AZLI did not induce mutations in cultured L5178Y $TK^{+/-}$ mouse lymphoma cells in the presence or absence of metabolic activation under the conditions of this study. Mutation frequencies in the cells treated with AZLI were comparable to concurrent and historical controls and did not meet the criteria for a positive response.

FINAL CONCLUSIONS AND RECOMMENDATIONS FOR NDA 80-814:

As stated in her review of the original submission, the pharmacologist does not object to the approval of NDA 50-814 for Cayston™ (aztreonam for inhalation). Labeling recommendations can be found in this review and they have not changed with the exception that

the mutagenicity data newly submitted by the sponsor should be added. The paragraph in Section 13.1 that currently reads:

(b) (4)

should be changed to:

"Genetic toxicology studies performed *in vitro* demonstrated that aztreonam did not induce structural chromosome aberrations in CHO cells and did not induce mutations at the TK locus in mouse lymphoma L5178Y $TK^{+/-}$ cells. Likewise, genetic toxicology studies performed *in vivo* did not reveal evidence of mutagenic potential."

cc: K Hyon, Project Manager DAIOP
J Alexander, Medical Officer Team Leader, DAIOP

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/

AMY L ELLIS
12/07/2009

WENDELYN J SCHMIDT
12/09/2009