CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 50-753

MICROBIOLOGY REVIEW(S)

Division of Anti-Infective Drug Products (HFD-520)
Clinical Microbiology Review Notes #1

NDA # 50-753 **DATE COMPLETED: 1 DEC 97**

APPLICANT(NDA):

Pathogenesis Corp.
201 Elliott Ave.
Suite 150
Seattle, WA 98119

CHEM/THER. TYPE: aminoglycoside

SUBMISSION REVIEWED: Original NDA
PROVIDING FOR: Palliative treatment of cystic fibrosis

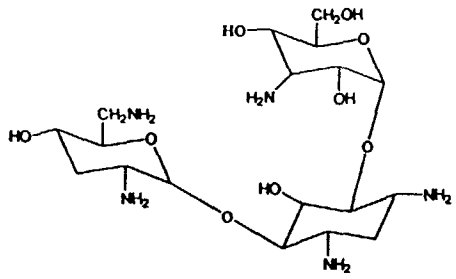
PRODUCT NAMES(S):
Proprietary: TOBI

Non-Proprietary/USAN: tobramycin

CHEMICAL NAME, STRUCTURAL FORMULAS, MOLECULAR FORMULA, MOL. WT.

Tobramycin is an aminoglycoside antibiotic produced by *Streptomyces tenebrarius* as a component of the nebramycin antibiotic complex. Its chemical name is *O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1→4)-*O*-[2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl-(1→6)]-2-deoxy-L-streptamine. The molecular formula is $C_{18}H_{37}N_5O_9$ and the molecular weight is 467.52. The structural formula is as follows:

NDA 50753
TOBI
Pathogenesis



DOSAGE FORMS(S) Inhalation therapy

STRENGTHS: 300 milligrams per single dose ampule

ROUTE(S) OF ADMINISTRATION: by inhalation

PHARMACOLOGICAL CATEGORY: antiinfective

DISPENSED: X Rx ___ OTC

INITIAL SUBMISSION:
Received by CDER: 10 JUL 97
Received by Reviewer: 21 OCT 97 (reassignment)
Review Completed: 1 DEC 97

AMENDMENT(S)
Received by CDER: N/A
Received by Reviewer:
Review Completed:

REMARK(S):

This review was prepared using summary text supplied in electronic format by the applicant and further edited by the reviewer to accommodate the microbiological concerns of the FDA. The text of this REMARKS section includes extensive background information not related to FDA Microbiological concerns but relating to the genetic nature and clinical course of cystic fibrosis. This information was included to provide a basis for understanding the relationship of the cystic

fibrosis patients to their chronic lifetime infections with *P. aeruginosa*. With that understanding, the rationale for microbiological labeling recommendations should be quite clear.

TOBI is intended for maintenance therapy in patients with cystic fibrosis; the patients are chronically infected with *P. aeruginosa*. TOBI is designed to improve pulmonary function, decrease infection, and maintain patient health; it is not intended to cure either the chronic infections or cystic fibrosis. Cystic Fibrosis (CF) is an autosomal recessive disease that affects 30,000 patients in the United States. The CF mutation occurs in the gene encoding a chloride channel protein called the CF transmembrane conductance regulator (CFTR). Patients who are homozygous for defective CFTR genes commonly suffer from chronic recurrent endobronchial infections and sinusitis involving *P. aeruginosa*. Other common manifestations of CF include malabsorption due to pancreatic insufficiency, increased salt loss in sweat, obstructive hepatobiliary disease and reduced fertility. Chronic endobronchial infection with *P. aeruginosa* leads to chronic inflammation of lung tissue with periodic exacerbations. Infection is predominantly limited to the endobronchial space and results in progressive obstructive disease. The inflammatory response does not bring the infection under control, and continued inflammation may cause pulmonary damage, resulting in progressive loss of pulmonary function over time. This pulmonary dysfunction is recognized as responsible for approximately 90% of deaths in CF patients, and the pulmonary dysfunction is largely a result of chronic airway infection.

The microbiological character of *P. aeruginosa* isolates infecting patients with CF is complex. A single sputum sample from a CF patient may contain multiple morphotypes of *P. aeruginosa* and each morphotype may have a different level of *in vitro* susceptibility to a given drug. Isolates recovered from patients following treatment with anti-pseudomonal antibiotics often show increased resistance when compared to the results of susceptibility testing performed prior to treatment. When serial MIC determinations are performed on isolates from a given patient, a decrease in activity may be due to several phenomena, including increased expression of preexisting resistance genes or concurrent infection with another less susceptible isolate of *P. aeruginosa*. By colony counts, the frankly resistant strains (parenteral breakpoint ≥ 16 μg/mL) usually constitute a small proportion of the total *P. aeruginosa* count obtained from a given patient. Following cessation of antibiotic treatment, resistant strains of *P. aeruginosa* from these patients often regain apparent but not true susceptibility to the particular antimicrobial agent used.

Clinical improvement in response to parenteral antibiotic therapy in CF patients has been observed in subjects whose sputum contained strains of *P. aeruginosa* with decreased *in vitro* susceptibility (higher MICs). Subinhibitory concentrations of antimicrobial agents may have beneficial effects. For example, other antimicrobial drugs have been shown to inhibit the expression of various *P. aeruginosa* virulence factors *in vitro*. However, in vivo the nearly intact host defense mechanisms and vigorous immune response of patients with cystic fibrosis force *Pseudomonas aeruginosa* in pulmonary infections into a demonstrably cryptic, microcolony mode of growth that allows its persistence in the face of specific antibodies and antibiotics but limits its toxic activity and its dissemination. This status should probably be considered as a type of colonization in a biofilm which includes mucin.

Mucin production appears to be a virulence factor which influences the colonization of lung tissue with *Pseudomonas aeruginosa* . A pseudomonal mucoid exopolysaccharide appears to alter the surface characteristics of *Pseudomonas aeruginosa* thereby rendering those isolates resistant to nonopsonic phagocytosis. The resistance of mucoid variants to nonopsonic phagocytosis almost certainly provides a survival advantage to these bacteria early in the course of pulmonary infection before opsonic antibody and complement are present in respiratory secretions. Mucin has other effects as well. It has been noted that interaction of bacteria with mucus from patients with cystic fibrosis results in marked induction of expression of several genes, including one that encodes a

lipopolysaccharide biosynthetic enzyme, a gene for a protein responsible for uptake of the ferric pyochelin siderophore, and a gene homologous with a class of iron-responsive repressors. These bacterial genes are not routinely detectable when the bacteria are cultured under normal laboratory conditions. Similarly, expression of resistance genes is frequently delayed; this expression pattern had a profound impact on how to establish susceptibility/resistance breakpoints for TOBI when it is compared with parenteral tobramycin.

NCCLS has established breakpoints to be used for the interpretation of MICs for parenterally administered tobramycin. Values of ≤ 4 μg/mL are considered to be susceptible, MICs of 8 μg/mL are intermediate, whereas those with MICs ≥ 16 μg/mL are resistant. Interpretive categories for the aerosol administration of tobramycin have not yet been established. Tobramycin MIC data collected during the parallel group NDA studies was evaluated in an attempt to establish a susceptibility breakpoint for use with TOBI. The tobramycin MIC of the highest density isolate at baseline isolated from evaluable patients was correlated with the patients' microbiological and clinical responses after 28 days of treatment with TOBI. Although either a microbiological response and/or a clinical response was often observed in patients with highest density *P. aeruginosa* isolates with baseline tobramycin MIC values of up to 128 μg/mL, there was insufficient data to make definitive conclusions.

Another analysis was performed that looked at the clinical response of patients who had any *P. aeruginosa* isolates with tobramycin MICs ≥ 128 μg/mL at Visits 3, 10, or 15. There were four patients that began the trial with *P. aeruginosa* isolates with tobramycin MICs ≥ 128 μg/mL. When these patients were evaluated for FEV_1 response between Visit 3 and Visit 10, none showed improvement. Of the 13 patients who had acquired *P. aeruginosa* isolates with MICs of ≥ 128 μg/mL at Visit 10, seven had a positive clinical response defined as improved FEV_1 between Visit 3 and Visit 10. One additional patient acquired an isolate with a tobramycin MIC > 512 μg/mL at Visit 15. This patient had a positive clinical response between Visit 11 and Visit 16 in spite of having an isolate with an elevated MIC.

Based on the limited results from the parallel group studies, no definitive interpretive criteria for dilution or diffusion susceptibility test methods can be recommended for TOBI therapy of *P. aeruginosa* pulmonary infections in CF patients. However, the data suggest that patients with *P. aeruginosa* isolates with MICs ≥ 128 μg/mL prior to initiation of TOBI therapy may not respond. Although patients may have isolates with MICs of ≥ 128 μg/mL while using TOBI, those patients may still experience a positive clinical response. The few patients with *P. aeruginosa* isolates with MICs ≥ 128 μg/mL evaluated to date, make definite conclusions difficult. Data from ongoing trials may help to delineate the relationship between MIC values and clinical response.

The data from the Phase III studies summarized in this report were analyzed to determine whether treatment with TOBI in patients with CF was associated with changes in tobramycin susceptibility of *P. aeruginosa* and/or changes in the respiratory flora. The population analyses indicated that treatment with TOBI did not affect the susceptibility of the majority of *P. aeruginosa* isolates tested, but that there was a trend toward increasing tobramycin MICs in some isolates. The analysis of all *P. aeruginosa* isolates showed that the reported tobramycin MIC_{50} was unchanged (1 μg/mL) and that the MIC_{90} increased 2-fold (from μg/mL) between baseline and the end of the third on-drug period. The analysis of the highest MIC isolates demonstrated that the MIC_{50} increased 2-fold from μg/mL, and that the MIC_{90} increased 4-fold (from μg/mL) during this same time period. The analysis of the highest density isolates indicated that the tobramycin MIC_{50} was unchanged (1 μg/mL) and that the MIC_{90} increased 4-fold from μg/mL.

In the placebo group, there was essentially no change in the distribution of tobramycin MICs in each of the three *P. aeruginosa* populations evaluated. For all *P. aeruginosa* isolates, the MIC_{50} was 1 μg/mL, and the MIC_{90} was 8 μg/mL. For the highest MIC isolates, the MIC_{50} was 2 μg/mL, and the MIC_{90} was 16 μg/mL. For the highest density isolates, the MIC_{50} was 1 μg/mL, and the MIC_{90} was 4 μg/mL.

In the analyses of changes for individual patients, the majority of patients in both treatment groups had isolates whose MICs remained unchanged or decreased during the study. However, 15.8% of patients treated with TOBI had isolates that exhibited a ≥ 4-fold increase in tobramycin MIC from baseline to the end of the third on-drug period, as compared to 9.1% of the patients who received placebo. The increased MICs of isolates from patients treated with TOBI should be taken in the context of previous findings. Thomassen, *et. al.* found that in 7 (18.9%) of 37 patients, the MICs of *P. aeruginosa* isolates increased over the course of two weeks of parenteral anti-pseudomonal antibiotic therapy in CF patients with pulmonary exacerbations.

During the off-drug period in the third treatment cycle, the tobramycin MIC_{90} value for the highest MIC isolates decreased 2-fold from [redacted] μg/mL (Visit 11). Among 16 patients with *P. aeruginosa* isolates with tobramycin MICs ≥ 128 μg/mL at Visit 10, nine patients treated with TOBI had MICs ≥ 128 μg/mL that decreased ≥ 4-fold at Visit 11. One partial explanation for these findings could include the concept of "adaptive resistance" in which these *P. aeruginosa* isolates with higher MICs return toward lower MICs when antibiotic therapy is discontinued. Clearly, other unidentified phenomena contribute to the observed rise in MICs during therapy and reduction in MICs following active therapy.

Since tobramycin MICs are relatively low among many strains of *Pseudomonas aeruginosa* at the time the strains are isolated and subsequent isolates have relatively high MIC's during active therapy, less susceptible isolates appear to emerge during therapy as was observed during the Phase III trials for TOBI. However, none of these emerging isolates with high MIC's were likely to have recently changed toward resistance during therapy. From the scientific perspective, the mechanisms of expression of high tobramycin MICs was not elucidated for isolates which appeared to develop higher MICs during the clinical trials presented in this application. Thus, quantitation of this phenomenon could not be defined in the clinical trials presently under review. From that perspective, a NOTE in the Microbiology subsection of the package insert will be devoted to appearance of high tobramycin MICs among cystic fibrosis patients in the Phase III studies.

Beyond issues associated with the development of very high MICs, the remainder of the Microbiology subsection of the package insert includes pertinent wording taken from the approved parenteral product labeling.

The signature block for the review follows the appended peripheral information.

CONCLUSIONS and/or RECOMMENDATIONS:

From the microbiological perspective, this application should be approved with the following text in the Microbiology subsection of the package insert.

NDA 50753
TOBI
Pathogenesis
Microbiology

/S/

James R. King
Microbiologist, HFD-520

SMicro/ASheldon RD initialed 12/3/97, Final on 12/4/97 ASS

DepDir/LGavrilovich

12/4/97
12/16/97

cc: Orig. NDA # 50-753
HFD-473
HFD-520/DepDir/LGavrilovich
HFD-635
HFD-520/SMicro/ASheldon
HFD-520
HFD-520/Micro/King
HFD-520/MO/Alexander
HFD-520/Pharm/Ellis
HFD-520/Chem/Pagay
[redacted]

REVIEW FOR HFD-520
OFFICE OF NEW DRUG CHEMISTRY
MICROBIOLOGY STAFF HFD-805

OCT 29 1997

Microbiologist's Review #1 of NDA 50-753
October 29, 1997

A. 1. **APPLICATION NUMBER:** 50-753

APPLICANT: Pathogenesis
201 Elliott Ave. West
Seattle, Washington 98119

2. **PRODUCT NAMES:** Tobramycin solution for inhalation

3. **DOSAGE FORM AND ROUTE OF ADMINISTRATION:** An aerosolized antibiotic product containing 60mg/ml solution of tobramycin. 5 ml of the formulation are packaged in single-use ampules.

4. **METHOD(S) OF STERILIZATION:**

5. **PHARMACOLOGICAL CATEGORY:** 1P; treatment of chronic *P. aeruginosa* infections in patients with Cystic Fibrosis

B. 1. **DATE OF INITIAL SUBMISSION:** April 30, 1997

2. **AMENDMENT:** none

3. **RELATED DOCUMENTS:** IND

4. **ASSIGNED FOR REVIEW:** September 12, 1997

5. **DATE OF CONSULT REQUEST:** August 18, 1997

C. **REMARKS:**

The subject drug product is the first aerosolized antibiotic product for use in cystic fibrosis patients. The formulation is especially designed for cystic fibrosis patient: sterile, non-pyrogenic, and does not contain preservatives or antioxidants.

Tobramycin, USP is an aminoglycoside antibiotic produced by *Streptomyces tenebrarius*. Manufacturing is contracted to
is responsible for drug product solution processing.

D. CONCLUSIONS:

The submission has been reviewed for sterility assurance of drug manufacturing and it is recommended for approval with respect to microbiology.

/S/ 10/29/97

Brenda Uratani, Ph.D.
Review Microbiologist

PK 10/29/97

cc:

NDA 50-753
HFD520/ Div. File
HFD-805/ Uratani
HFD-520/CSO/Duvall-Miller
drafted by: Brenda Uratani, 10/29/97
R/D initialed by P. Cooney, 10/29/97

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 50-753

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Duva
5?

DEC 19 1997

CLINICAL PHARMACOLOGY/BIOPHARMACEUTICS REVIEW

NDA:	50-753
Submission date:	July-10-97
Product:	TOBI™ Tobramycin Inhalation Solution
Sponsor:	PathoGenesis Corporation 201 Elliott Avenue W. Suite 150 Seattle, WA 98119
Type of submission:	Original NDA
Reviewer:	Jenny Zheng, Ph.D.
Date received for review:	7-17-1997

SYNOPSIS:

Sponsor, PathoGenesis, submitted NDA 50-753 to seek approval of TOBI™, a Tobramycin Solution for Inhalation. This dosage form is developed for patients with cystic fibrosis (CF). The strength is 300 mg/5 mL. TOBI™ is intended for repeated, intermittent, (28 days on/28 days off), twice a day therapy in patients with moderate to severe CF disease who are chronically colonized with *P. aeruginosa*. Using this product, a high tobramycin concentration in sputum is intended to be achieved in order to effectively decrease the colonization of common pathogen, *P. aeruginosa*. At the same time, systemic exposure to tobramycin is limited in order to decrease side effects. The Sponsor conducted three studies to measure both sputum and plasma concentrations following the administration of TOBI™. A population pharmacokinetic analysis was also applied to estimate the bioavailability.

RECOMMENDATION:

The human pharmacokinetics section of NDA 50-753 is acceptable. However, the estimate of bioavailability using population pharmacokinetic analysis lacks a solid foundation and the statement in the label regarding bioavailability should be deleted.

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BACKGROUND:

Cystic Fibrosis patients suffer from chronic endobronchial infections, most commonly caused by *Pseudomonas species*. Although the tobramycin MIC_{90} for *P. aeruginosa* is less than 4 μg/mL, it is not uncommon for an isolate from a CF patient to have an MIC well above that value. To be effective, concentrations of tobramycin in the endobronchial space must exceed by several times the MIC of *P. aeruginosa*.

Parenteral tobramycin, an aminoglycoside active against *P. aeruginosa*, is used to treat pulmonary infections in CF patients, but it must penetrate lung mucosa and overcome sputum binding to be effective. As MICs increase, larger parenteral doses are required to achieve effective endobronchial concentrations; these larger doses are associated with significant systemic toxicity.

TOBI™ is designed to achieve concentrations in the endobronchial space far in excess of the MIC for *P. aeruginosa*. At the same time, its low systemic exposure reduces the potential for systemic toxicity.

The local and systemic pharmacokinetics of TOBI™ were calculated from sputum and serum drug concentrations obtained in three clinical trials and the study results are summarized in the following sections.

Study PC-TNDS-001:

Study PC-TNDS-001 is an open labeled, randomized, crossover study design. In this study, the performance of three kinds of nebulizers, UltraNeb 99/100, Sidestream, and PariLC®, were compared. The tobramycin concentrations in sputum at 10, 60 and 120 minutes after the administration using three kinds of nebulizers were measured and compared. The tobramycin concentrations in plasma were also measured. The results are summarized in the following tables:

Table I. Tobramycin concentration in sputum.

Pari LC (n=61)	10 minutes	60 minutes	120 minutes
Mean±SD (μg/g)	665.9±651.8	138.76±250.87	92.67±170.38
Median (μg/g)	433.00	55.80	47.60
Range (μg/g)			
# Pts. (%) w/Sputum Con.>128 μg/g*	53 (87%)		
Probability that each of the nebulizers would deliver a concentration of tobramycin >10 fold MIC to 90% of the patients	92		
Sidestream (n=61)			
Mean±SD (μg/g)	479.2±405.2	115.68±183.12	93.16±124.01
Median (μg/g)	376.4	45.6	37.20
Range (μg/g)			
# Pts. (%) w/Sputum Con.>128 μg/g	55 (90%)		
Probability that each of the nebulizers would deliver a concentration of tobramycin >10 fold MIC to 90% of the patients	91		
UltraNeb (n=61)			
Mean±SD (μg/g)	1447.98±1337.6	387.5±450.91	157.54±184.50
Median (μg/g)	1191.0	233.6	86.60
Range (μg/g)			
# Pts. (%) w/Sputum Con.>128 μg/g	58 (95%)		
Probability that each of the nebulizers would deliver a concentration of tobramycin >10 fold MIC to 90% of the patients	95		

*The resistant P. aeruginosa strains to tobramycin are defined to have MIC>128 mg/mL.

Table II. Tobramycin serum concentrations

	Serum Tobramycin concentrations (μg/mL)	
Pari LC	60 minutes	120 minutes
mean±SD	0.58±0.38	0.44±0.28
range		
number of patients	63	65
Sidestream		
mean±SD	0.74±0.43	0.58±0.29
range		
number of patients	62	62
UltraNeb		
mean±SD	0.78±0.75	0.62±0.68
range		
number of patients	63	62

Table III. The ratio of tobramycin serum to sputum concentrations

	Tobramycin Concentrations		
	60 minutes serum (μg/mL)[1]	60 minutes sputum (μg/g)[2]	Ratio Serum/Sputum (%)[3,4]
Pari LC			
mean±SD	0.58±0.38	138.76±250.87	1.19
median	0.50	55.8	0.97
range			
number of patients	63	63	59
Sidestream			
mean±SD	0.74±0.43	115.68±183.12	2.18±1.42
median	0.60	45.6	1.42
range			
number of patients	62	62	60
UltraNeb			
mean±SD	0.78±0.75	387.5±450.9	0.69±1.24
median	0.60	233.6	0.26
range			
number of patients	63	64	59

1 Sputum LOQ=20 mg/g; Serum LOQ=0.2 mg/mL
2 BQL= Below Quantitative Limits
3 In order to compare serum and sputum concentrations, the comparison was made at the highest concentrations in which both biological matrices were sampled. Similar findings were obtained when the 120 min samples were compared.
4 The ratios were obtained by measuring the individual patient ratios of serum to sputum and calculating the appropriate statistic.

Based on the results from above study, PariLC underwent minor modification and was chosen as the nebulizer for the further studies.

Study PC-TNDS-002 and PC-TNDS-003:

Study PC-TNDS-002 and PC-TNDS-003 are identical study design. They are double-blinded, randomized, placebo-controlled studies. The modified nebulizer, PariLC® Plus was used in the studies. Eligible patients were randomized to treatment either with 300 mg TOBI™ or placebo, then received their first dose at Visit 3. Enrolled patients received study drug twice daily for 28 days, followed by a 28-days off study drug. This 56 day period was considered a treatment cycle, and was repeated twice, for a total of three cycles. A follow-up visit (Visit 11) was conducted for final evaluations. Sputum specimens for tobramycin concentration determination were collected twice during the study, once after the initial dose administration (Visit 3) and again at the end of the third 28 day period (Visit 10). Samples were collected 10 minutes after completion of the aerosol drug administration.

Serum samples were collected pre-dose and one hour post dosing after the first dose at Cycle 1, Visit 3, and the last dose Cycle 3, Visit 10. Additional serum samples for tobramycin concentration determination were collected 1 to 12 hours post dosing on

Visits 5,7,8, and 9. The sputum and plasma concentrations are summarized in the following tables.

Table IV. The summaries of sputum concentration at 10 minutes after the administration

# of observations	Mean µg/gm	SD	Median µg/gm	Geometric mean	Range	# of observed concentrations which are >128 µg/g (%)
240 (visit 3)	1237	1090	959	885		236 (98.3%)
201 (visit 10)	1154	1147	826	783		196 (97.5%)
441 (total)	1199	1116	898	838		432 (98.0)

Table V. The summaries of serum concentration at 1 hour after the administration

# of observations	Mean µg/mL	SD	Median µg/ mL	Range µg/ mL	# of observations which are = 0 or ≥ 1, 2 or 3 µg/mL(%)			
					0	1	2	3
466	1.01	0.58	0.94	0-3.62	9	209	26	4
					1.9%	44.8%	5.6%	0.9%

Table VI. The summaries of ratio of serum to sputum concentration

	Ratio Serum/Sputum*
Mean	0.191
Median	0.010
SD	0.342
Range	
Number of patients	195

*: The mean ratio was calculated as the mean of the individual ratios of serum to sputum. Only those patients with both serum and sputum data on the same visit were included in the analysis of the ratio.

The distribution of sputum and serum concentration are described in the Figure 1 and 2.

Figure 1. The distribution of sputum concentrations at 10 minute following aerosol administration

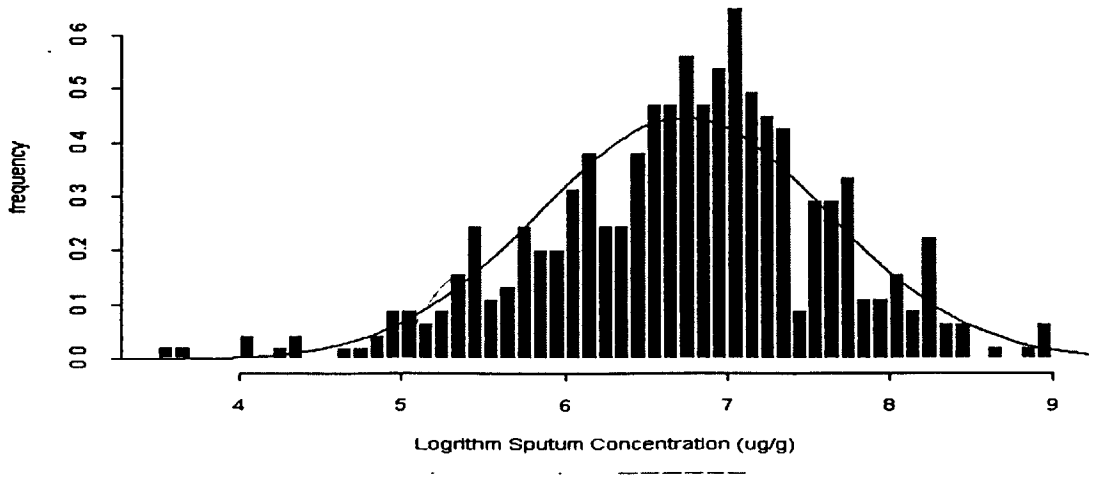
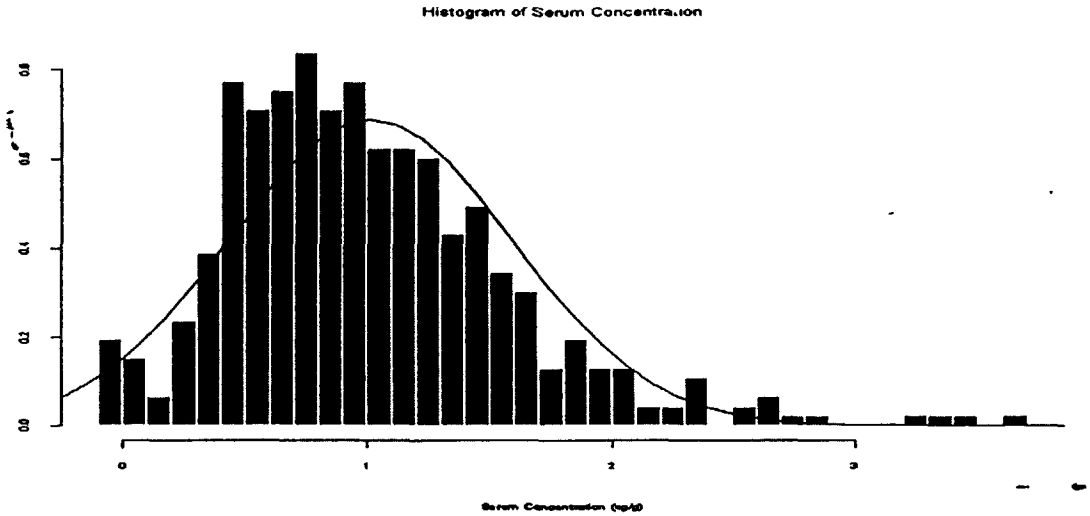


Figure 2. The distribution of serum concentration at 1 hour after aerosol administration



The study results indicated that high tobramycin concentrations can be achieved in the sputum but the variability is very high. However, the systemic exposure to tobramycin is very minimal. The mean serum concentration at 1 hour after the administration is about 1.01 μg/mL.

Pooling the data from studies PC-TNDS-002 and PC-TNDS-003, bioavailability was estimated using population pharmacokinetic analysis. In the analysis, tobramycin was assumed to be absorbed instantaneously (similar to iv when tobramycin reached the lung) The study results indicated that two compartment model was better than one compartment to describe the pharmacokinetics. From the analysis, the apparent clearance, CL/F, was estimated to be 49.6 L/hr. Combined with other pharmacokinetics study of tobramycin in cystic fibrosis patients, in which CL was estimated to be 5.04-7.05 L/h following parenteral administration. Using values of CL/F from these studies and CL from parenteral administration, bioavailability was therefore estimated to be about 11.7%, which is comparable with CL reported in the literature.

Assay:

Formulation:
TOBI™ contains mg/mL tobramycin in % NaCl. Sulfuric acid is added to adjust the pH to 6.0.

Other studies: 17 other pharmacokinetics studies from literature search were included to support the application. The study object for each paper is listed in the following table and the summaries of the results are attached in the Appendix I.

Study #	Route	Assessment
1	IV	PK assessment
2	IM	serum and bronchial secretion
3	IM	serum and bronchial secretion
4	Aerosol	urine sample, bioavailability
5	Aerosol	deposition of tobramycin in pulmonary acini and connecting small airways
6	Aerosol	penetration of tobramycin into respiratory secretions
7	Aerosol/IV	bioavailability and absorption characteristics
8	Aerosol	serum concentration
9	Aerosol	lung distribution; plasma and urine tobramycin
10	IV	PK assessment
11	IV	serum and sputum concentration measurement
12	IV	serum and sputum concentration measurement
13	IV	tobramycin concentration in serum and bronchial secretion
14	Aerosol	lung distribution, sputum concentration
15	Aerosol	sputum and serum concentration measurement
16	Aerosol	PK assessment/bioavailability (urine samples)
17	Aerosol	sputum concentration measurement

COMMENTS:

1. The study design is acceptable to understand the local and systemic exposure to tobramycin, however, the study design is not acceptable to evaluate the pharmacokinetics of tobramycin. Most of samples were collected at one time point which may not be the maximal value.
2. The total but not free tobramycin concentration in sputum were measured. It is known that tobramycin activity is inhibited by the environmental milieu of purulent sputum, i.e., the presence of high concentrations of divalent cations, acidic conditions, increased ionic strength and the presence of macromolecules, which bind the drug.
3. The bioavailability estimate lacks a solid foundation.
    i. The study design is not suitable for the pharmacokinetic analysis. When population pharmacokinetics was analyzed, it was assumed that the tobramycin was absorbed nearly instantaneously. However, the previous study showed that there is a delay in absorption and the peak is at about 1 hour. In this case, the absorption phase can not be well defined because no samples were collected before 1 hour.
    ii. In order to calculate the bioavailability of the tobramycin inhalation, the systemic clearance (CL) was used from the other studies. It is known that CL will be increased in cystic fibrosis patients and the variability is very high. Therefore, it becomes questionable to use the CL from the other studies.

iii. Overall, the fit to the serum concentration data was poor using the proposed model. There is an obvious trend between weight residuals and plasma concentration, indicating the concentrations were overestimated at low concentration and underestimated at high concentrations

LABELING COMMENTS (To be sent to the Sponsor):
The changes are based on the original labeling. The additions to the draft are indicated by underline and the deletions were indicated by strikeout. The
subsection of section should read as follows:

/S/ 12/19/97

Jenny Zheng, Ph.D.
Office Clinical Pharmacology/Biopharmaceutics,
Division of Pharmaceutical Evaluation III

/S/

RD/FT signed by Frank Pelsor, Pharm.D., Team Leader 12/19/97

cc:
Div. File: NDA 50-753
HFD-520 (J. Alexander, MO)
HFD-520 (J. Soreth, MO, TL)
HFD-520 (B. Duvall-Miller, CSO)

HFD-340 (Viswanathan)
✓ HFD-880 (DEPIII File)
✓ HFD-880 (F. Pelsor, TL)
✓ HFD-880 (J. Zheng, Reviewer)
CDR (B. Murphy)

APPENDIX I

1. Assay validation:

2. History of the previous studies:

Safety studies were conducted in the previous study. Generally, no toxicity was reported in the studies.

Subjects	Dose	Toxicity observation
22	600 mg tid for 90 days	No toxicity (Nephro- or oto-)
71	600 mg tid for 56 days	No toxicity
Canada	80 mg tid for 32 months	No
German	80 mg bid for 20 months	No

3. PC-TNDS-001:

Objective:

1. To determine which of the three nebulizer systems could aerosolize sufficient tobramycin sulfate to achieve a sputum tobramycin concentration of 128 μg/g or greater in at least 85% of the patients measured 10 minutes after completion of dose administration;
2. To determine whether a tobramycin delivery system was safe and well-tolerated by the patient.

Study design:

It was a multicenter, open label, randomized, cross over and three-arm study. Three nebulizers, Ultrasonic UltraNeb 100, the PariLC® and Sidestream, will be tested to determine if the tobramycin sputum concentrations of > 128 mg/g can be achieved in at least 85% of patients measured 10 minutes after completion of nebulization. A minimum of 48 hours will elapse between each aerosol administration.

Dose:

Jet nebulizer: 300 mg tobramycin in 5 mL in ¼ normal saline;
Ultrasonic nebulizer: 600 mg tobramycin in 30 mL ½ normal saline. For the ultrasonic nebulizer, the treatment proceeded for 200 inhalations. If the 600 mg tobramycin was delivered in less than 200 inhalations, then another 600 mg tobramycin was added into the reservoir until the 200 inhalation were completed.

Sampling:

Immediately after the administration, the patients rinsed mouths and gargled with normal saline three times. Sputum specimens were collected approximately 10 minutes after rinsing and 1 and 2 hours after completion of the aerosol drug administration. Serum samples were also collected at 1 and 2 hours after treatment.

Assay:

Results: 69 patients at six centers participated in the study and 61 patients, age ranged from years old, completed three aerosolized tobramycin regimens in random order. Four patients were exposed to 600-900 mg tobramycin and nine were exposed to 600-1200 mg following use of the ultrasonic nebulizer. Tobramycin concentration in sputum are summarized in the following table.

Pari LC (n=61)	10 minutes	60 minutes	120 minutes
Mean±SD (μg/g)	665.9±651.8	138.76±250.87	92.67±170.38
Median (μg/g)	433.00	55.80	47.60
Range (μg/g)			
# Pts. (%) w/Sputum Con.>128 μg/g*	53 (87%)		
Probability that each of the nebulizers would deliver a concentration of tobramycin >10 fold MIC to 90% of the patients	92		
Sidestream (n=61)			
Mean±SD (μg/g)	479.2±405.2	115.68±183.12	93.16±124.01
Median (μg/g)	376.4	45.6	37.20
Range (μg/g)			
# Pts. (%) w/Sputum Con.>128 μg/g	55 (90%)		
Probability that each of the nebulizers would deliver a concentration of tobramycin >10 fold MIC to 90% of the patients	91		
UltraNeb (n=61)			
Mean±SD (μg/g)	1447.98±1337.6	387.5±450.91	157.54±184.50
Median (μg/g)	1191.0	233.6	86.60
Range (μg/g)			
# Pts. (%) w/Sputum Con.>128 μg/g	58 (95%)		
Probability that each of the nebulizers would deliver a concentration of tobramycin >10 fold MIC to 90% of the patients	95		

*The resistant P. aeruginosa strains to tobramycin is defined to have MIC>128 mg/mL.

Tobramycin serum concentrations are summarized in the following table:

	Serum Tobramycin concentrations (µg/mL)	
Pari LC	60 minutes	120 minutes
mean±SD	0.58±0.38	0.44±0.28
range		
number of patients	63	65
Sidestream		
mean±SD	0.74±0.43	0.58±0.29
range		
number of patients	62	62
UltraNeb		
mean±SD	0.78±0.75	0.62±0.68
range		
number of patients	63	62

The ratio of Tobramycin serum to sputum concentrations are listed in the following table.

	Tobramycin Concentrations		
	60 minutes serum (µg/mL)[1]	**60 minutes sputum (µg/g)[2]**	**Ratio Serum/Sputum (%)[3,4]**
Pari LC			
mean±SD	0.58±0.38	138.76±250.87	1.19
median	0.50	55.8	0.97
range			
number of patients	63	63	59
Sidestream			
mean±SD	0.74±0.43	115.68±183.12	2.18±1.42
median	0.60	45.6	1.42
range			
number of patients	62	62	60
UltraNeb			
mean±SD	0.78±0.75	387.5±450.9	0.69±1.24
median	0.60	233.6	0.26
range			
number of patients	63	64	59

1 Sputum LOQ=20 mg/g; Serum LOQ=0.2 mg/mL

2 BQL= Below Quantitative Limits

3 In order to compare serum and sputum concentrations, the comparison was made at the highest concentrations in which both biological matrices were sampled. Similar findings were obtained when the 120 min samples were compared.

4 The ratios were obtained by measuring the individual patient ratios of serum to sputum and calculating the appropriate statistic.

Conclusion:

1. Both jet nebulizers can deliver adequate tobramycin concentrations to the lower airways. Recently, the PariLC® has been slightly modified with the addition of one-way flow valves (Pari LC Plus®). In vitro experiments, using a test lung, indicate that this version will deliver up to 20% more drug than the PariLC®. The one-way valves are not in line with the breathing stream, so there is no change in particle size. The

one-way valves also decrease the potential for accidental spillage and allow for the possible addition of an expiratory filter at a later date. For these reasons, the Pari LC Plus® was chosen for use in the Phase III clinical trials.

2. Serum concentrations of tobramycin decreased slightly over the interval 60 to 120 minutes, as demonstrated by the reduction in mean concentrations. However, approximately 10% of the patients had serum concentrations that were higher at the later time point, reflecting a lag in systemic absorption from sputum.

Comments:

1. The sputum to serum samples were collected at specific time points. Therefore, observed concentrations are not necessary the maximum concentration.
2. Using Pari LC, the mean sputum concentration at 10 minutes after administration can be as high as 665.9 μg/mL. But the concentration dropped rapidly to 138.76 μg/mL (21% of the concentration at 10 minutes) at 1 hour after the administration.

4. **PC-TNDS-002 and PC-TNDS-003:**

Title: Phase III Placebo Controlled Clinical Trials to Study the Safety and Efficacy of Tobramycin for Inhalation in Patients with Cystic Fibrosis.

Objective:

To determine the safety and efficacy of TOBI in patients with cystic fibrosis.

Study design:

These two identically designed studies are double-blinded, placebo controlled and randomized. Patients were randomized to receive TOBI or placebo twice daily for 4 weeks, followed by a 4 week period with no treatment. This cycle was repeated twice for a total of three cycles.

Dose:

Pari LC Plus jet nebulizer system was used for dosage administration.

TOBI™ contains 300 mg tobramycin in 5 mL excipient (¼ normal saline, pH 6.0). Placebo was 5 mL of vehicle with 1.25 mg of quinine sulfate added as a flavoring agent to facilitate blinding.

Sampling:

Sputum specimens for tobramycin concentration determination were collected twice during the study, once after the initial dose administration (Visit 3) and again at the end of the third 28 day period (Visit 10). Samples were collected 10 minutes after completion of the aerosol drug administration.

Serum samples were collected pre-dose and one hour post dosing after the first dose at Cycle 1, Visit 3, and the last dose Cycle 3, Visit 10. Additional serum samples for tobramycin concentration determination were collected 1 to 12 hours post dosing on Visits 5,7,8, and 9.

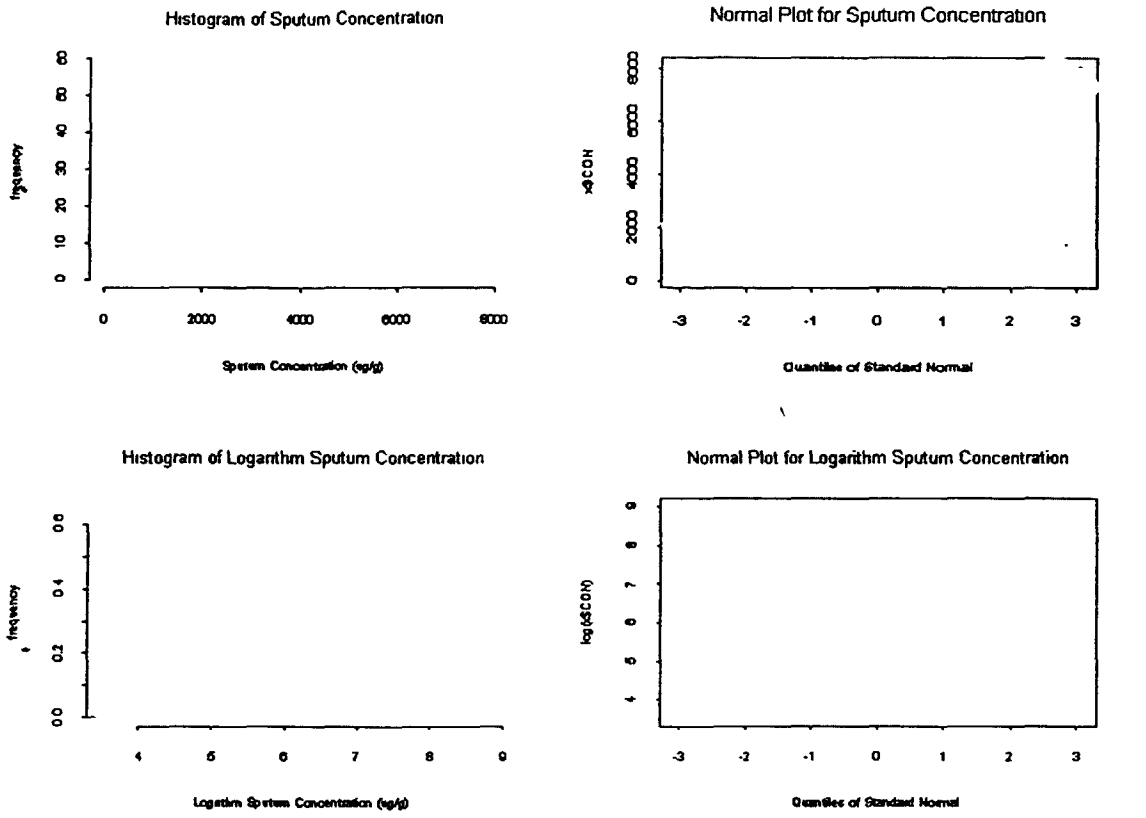
Assay:

Results:

Five hundred twenty (520) patients with moderate to severe cystic fibrosis participated in the study. 258 patients (age: years old; 154 male and 109 female) received TOBI and 257 had measurable sputum and/or serum concentrations collected at specific times. The sputum concentrations after 10 minutes administration are summarized in the following table. The distribution of the sputum concentration was plotted. As shown in the summaries, the variability of sputum concentration is very high. The distribution of sputum concentrations is skewed on the normal scale but normal on the logarithmic scale. The correlation of logarithmic sputum concentration with body weight, age and FEV1 were tested. No correlation was found. Similarly, gender appeared to have no effect on the sputum concentration.

The Summaries of Sputum Concentration at 10 minutes after the administration

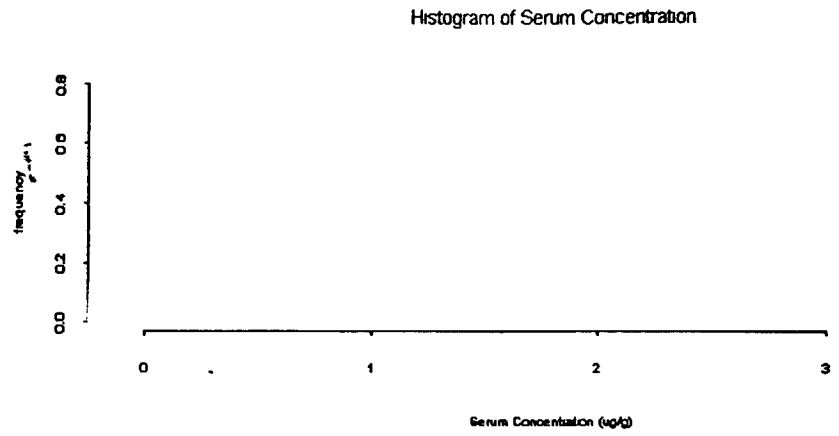
# of observations	Mean µg/gm	SD	Median µg/gm	Geometric mean	Range	# of observed concentrations which are >128 µg/g (%)
240 (visit 3)	1237	1090	959	885		236 (98.3%)
201 (visit 10)	1154	1147	826	783		196 (97.5%)
441 (total)	1199	1116	898	838		432 (98.0)

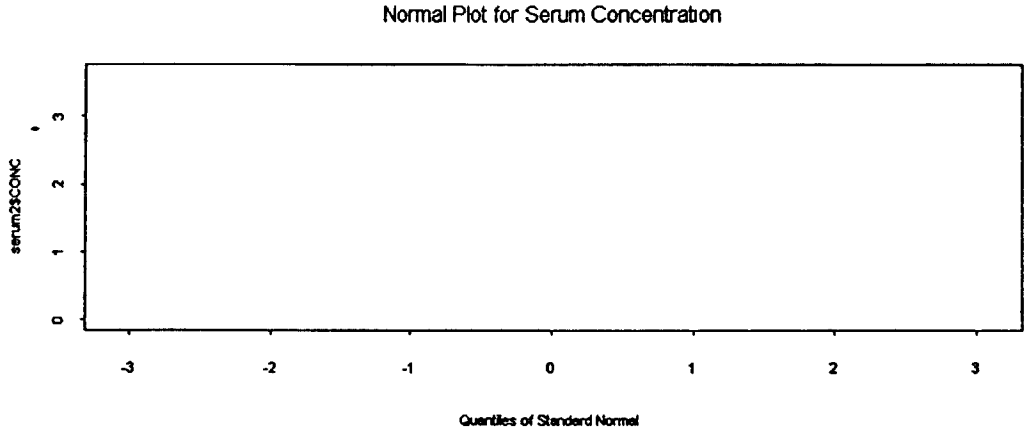


The serum concentrations at 1 hour after the administration were summarized in the following table. In order to have a clear look at the distribution of serum concentration after 1 hour administration the histogram of serum concentrations and their comparison with the normal distribution were plotted by the reviewer and are shown in the following figure. As was seen in the figure, the serum concentration at 1 hour was similar to the normal distribution except at very low or high concentration.

The Summaries of Serum Concentration At 1 hour After the Administration

# of observations	Mean μg/mL	SD	Median μg/mL	Range μg/mL	# of subjects whose concentration = 0 or > 1, 2 or 3 (%)			
					0	1	2	3
466	1.01	0.58	0.94		9 1.9%	209 44.8%	26 5.6%	3 0.6%





The serum and sputum concentrations were compared and shown in the following table.

	Ratio Serum/Sputum*
Mean	0.191
Median	0.010
SD	0.342
Range	
Number of patients	195

*: The mean ratio was calculated as the mean of the individual ratios of serum to sputum. Only those patients with both serum and sputum data on the same visit were included in the analysis of the ratio.

A population pharmacokinetic analysis was conducted using The analysis determined that a two compartment model was better than one compartment model to describe the pharmacokinetics of inhaled tobramycin. The estimated pharmacokinetic parameters were summarized in the table.

Population Pharmacokinetic Parameter Estimates for TOBI Administration
Using the Pari LC Plus Nebulizer PC-TNDS-002/003

Parameter	Population Mean		Inter-individual variability	
	Estimate	Standard error	Estimate	Standard error
CL/F (L/h)	49.6	2.99	0.259	0.0482
V/F (L)	123	5.75	4.82	1.46
Q (L/h)	81.2	10.4	0.579	0.169
Vss/F (L)	490	133	ND[1]	
$\sigma^{2,2}$	0.0782	0.00962		

1 A variance term for Vss/L could not be estimated
2 Residual variance of the model

Conclusion:

1. The mean tobramycin concentration in sputum at 10 minutes after inhalation can be as high as 1199 μg/mL. But the variability is also high. The concentration ranged from μg/mL.
2. The sputum concentrations showed logarithmic distribution.
3. The systemic exposure was low. The mean tobramycin concentration in serum at 1 hour after administration was 1.01 μg/mL, which is far below the toxic concentration level of 12 μg/mL.

Comments:

1. When population pharmacokinetics of tobramycin was analyzed, it was assumed that tobramycin was absorbed nearly instantaneously. However, the previous study showed that there is a delay in absorption and the peak is at about 1 hour. In this case, the absorption phase can not be well defined because no samples were collected before 1 hour.
2. In order to calculate the bioavailability of the tobramycin inhalation, the systemic clearance (CL) was used from the other studies. It is known that CL will be increased in cystic fibrosis patients and the variability is very high. Therefore, it becomes questionable to use the CL from the other studies.
3. Overall, the fit on the serum concentration was poor using the proposed model. There is an obvious trend between weight residuals and plasma concentration, indicating that low concentrations were overestimated and high concentrations were underestimated.
4. Upon the request by reviewer, sponsor provided a plot in which predicted serum concentrations are superimposed on the observed concentrations. Obvious flaw existed in the plot. The tobramycin was given every 12 hours according to the regimen. Therefore, a peak should be expected at about 13 hours. However, in this plot, the serum concentrations continuously dropped until 24 hours.

5. Other Studies:

1. Aarons L., Vozeh S., Wenk M., *et al.*, 1989. Population Pharmacokinetics of Tobramycin. British Journal of Clinical Pharmacology 28: 305-314.

Data from 97 patients (45 female, 52 male) were included in this analysis. Three hundred and twenty-two (322) tobramycin concentrations were available for the data analysis with 1 to 9 samples per individual patient. The patients had an average age of 51 ± 19 years (range) and an average weight of 66 ± 12 kg (range). Creatinine clearance (estimated from serum creatinine) ranged from mL/min (average 67 ± 33) calculated by Cockcroft & Gault equation.

The data analysis was performed utilizing A summary of the results is shown in the following table.

Population Pharmacokinetics Following Intravenous Administration of Tobramycin

Parameter	Population Mean		Inter-Individual Variability	
	Estimate	S.E.	Estimate	S.E.
Cl (mL/min)	0.059[1]	0.002[1]	32%	44%
k_{12} (h^{-1})	0.012	0.003		
k_{21} (h^{-1})	0.027	0.021		
V_1 (L/kg)	0.327[2]	0.014[2]	3%	112%
$\sigma\varepsilon$ [3]	21%	38%		

[1] The estimate (and SE) for clearance was approximated from creatinine clearance. The authors reported a proportionality constant between Cl and creatinine clearance, which when normalized for units, was unity, thus tobramycin is assumed to be completely cleared by renal mechanisms. When calculated, the Cl (L/h) is approximated to be 4.02±1.98 for the average CLcr of 67.9 mL/min.

[2] The estimate (and SE) for V1 was expressed by the author as a proportionality constant for V1 times body weight

[3] Residual intraindividual coefficient of variability of the serum concentration.

2. Alexander MR, Schoell J, Hicklin G, *et al.*, 1982. Bronchial Secretion Concentrations of Tobramycin. American Review of Respiratory Diseases 125: 208-209.

Twenty adult patients, ages 47-73 years, ten (8 males and 2 females) of which had pneumonia and the remaining ten control patients (10 men) were not known to be infected and had normal BUN and creatinine concentrations. At least three consecutive injections of tobramycin were administered intramuscularly or intravenously before sampling at steady state. Patients with pneumonia received varying doses (not identified) by either intravenous (n=9) or intramuscular (n=1) administration at not more than 8 hour intervals, with the exception of one patient who received each dose following hemodialysis. Control patients received 1.7 mg/kg tobramycin by intramuscular administration. Most serum and bronchial secretion specimens were collected at approximately the same time, between 0.5 and 2.5 hours after the last tobramycin dose. Bronchial secretion and serum specimens were assayed using methodology.

Results:

A wide range of serum concentrations were observed in pneumonia patients µg/mL) and in controls µg/mL). In contrast, the bronchial secretion concentrations were generally higher in pneumonia patients (µg/mL) than in controls (µg/mL). The summary of these findings are found in the table:

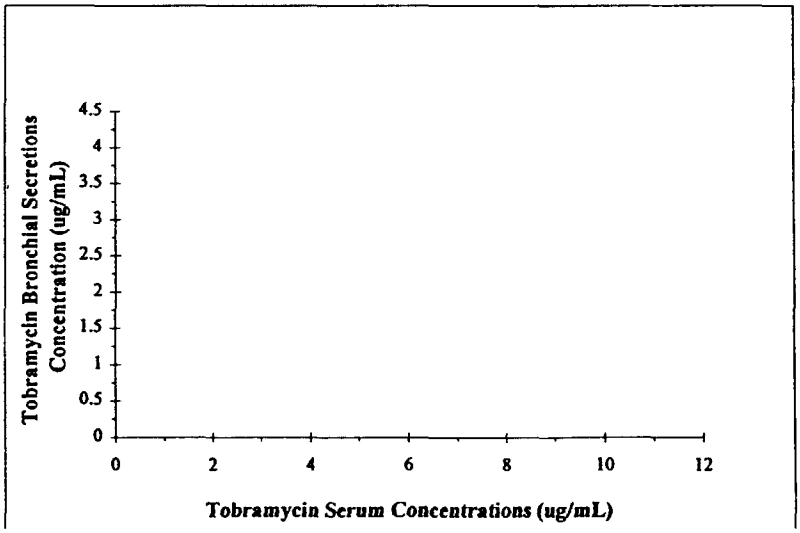
Serum and Bronchial Secretion Concentrations of Tobramycin Following Parenteral Administration

Patient Type		Tobramycin Concentrations (μg/mL)[1]		Ratio
	N	Serum (S)	Bronchial Secretions (BS)	BS/S
Pneumonia Patients	10	4.09 ± 2.02[2]	2.68 ± 1.51	0.66
Controls	10	7.98 ± 2.04	1.37 ± 0.67	0.17

[1] No LOQ reported for either matrix
[2] Mean ± SD

The differences in serum concentrations between groups may be reflective of the lower doses prescribed for the pneumonia patients by their physicians. Even though serum concentrations were considerably lower for the pneumonia patients than concentrations observed in control patients, bronchial secretions were nearly twice as high in pneumonia patients than controls. In order to relate the findings in serum with bronchial secretion results, bronchial secretion concentrations were plotted as a function of serum tobramycin concentration (Figure 0-1).

Figure 0-1
The Relationship Between Serum and Bronchial Secretion Concentrations of Tobramycin In Patients and Controls



These findings demonstrate that pneumonia patients have much higher bronchial secretion concentrations of tobramycin for the same serum concentration and that the relative increase is twice as great in those patients as in the control patients.

3. Alexander MR, Berglund EM, Kasik JE, *et al.*, 1979. The Concentration of Tobramycin in Bronchial Secretions. Chest 75: 675-678.
Fifteen adult male patients were enrolled into the study. None were known to have a pulmonary infection or being treated with antibiotics at the time. Some of the patients had underlying disease, but none were known to have vestibular, auditory, or renal impairment. Three consecutive injections of tobramycin (1.7 mg/kg) were administered intramuscularly at 8-hour intervals. In most patients, serum and bronchial

secretion specimens were collected twice, between 0.8 and 4.0 hours after the last tobramycin dose. Bronchial secretion and serum specimens were assayed using bioassay methodology.

Results:

A nearly three-fold range of tobramycin serum concentrations were observed in these patients (μg/mL); in contrast, the bronchial secretion concentrations were much more variable, with a 12-fold range of values reported. The summary of these findings are found in Table 0-1.

Table 0-1

Relationship of Tobramycin in Serum and Bronchial Secretions Following Intramuscular Administration

Parameters	Tobramycin Concentration (μg/mL)		BS/S Ratio[1]
	Serum (S)	Bronchial Secretions (BS)	(%)
Mean	6.6[2]	3.75[3]	59.6[4]
Standard Deviation	1.3	2.80	38.4
Range			

[1] The ratio was determined as the mean of the individual patient ratios, rather than the ratio of the mean bs value/mean s value.

[2] Mean and SD results not presented in the article, although individual data was included.

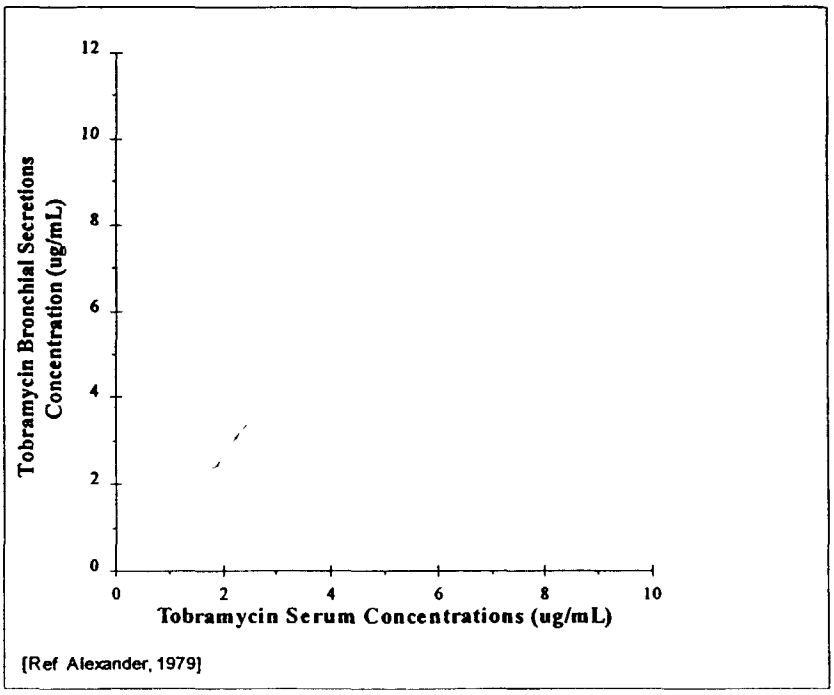
[3] The mean result is reported in the article, however, analysis of the data results in a value of 3.73.

[4] The mean result reported in the literature was "approximately 67%", which may have come from the slope of the serum vs bronchial secretion plot (see Figure 0-2 below).

In order to visualize the relationship of tobramycin serum to bronchial secretion concentrations, bronchial secretion concentrations were plotted as a function of serum tobramycin concentration. The resulting relationship demonstrated that increasing serum concentrations of tobramycin resulted in increasing bronchial secretion concentrations (Figure 0-2).

Figure 0-2

The Relationship between Serum and Bronchial Secretion Concentrations of Tobramycin Following Intramuscular Administration



[Ref Alexander, 1979]

Although the relationship has a low correlation coefficient with large scatter, there is a general trend that with increasing serum concentrations, there is resultant increasing bronchial secretion concentrations. The authors were not able to explain why the results from this study had much higher bronchial secretion to serum ratio than was reported by others. They postulated that possibly the pre-medications to facilitate the collection of the bronchial secretion samples (atropine and lidocaine) may have influenced the bioassay, but did not have any evidence to support the hypothesis.

4. Asmus MJ, Ahrens RC, Stewart BA, *et al.*, 1996. Use of Tobramycin as a Pharmacologic Tracer to Compare Airway Deposition of Drug From a Breath-Actuated and a Standard Jet-Nebulizer. Pediatric Pulmonology Suppl. 13: 303 [abstract].

The standard jet nebulizer was a continuously operating standard Pari LC nebulizer (flow rate 6 L/min) and the breath-actuated nebulizer was a Pari LC nebulizer connected to an electronic pressure sensor that coordinated nebulization with patient inspiration.

Six healthy volunteers inhaled 80, 160 and 320 mg of tobramycin from each device during 6 separate study visits. Inhaled doses > 80 mg were administered by repeat nebulization of an 80 mg/3 mL aliquot. All subjects received a 32 mg oral dose of tobramycin to confirm absence of significant gastrointestinal absorption.

Urine was collected immediately prior to, and for 48 hours after each dose.

Quantification of tobramycin was by EMIT assay (LLQ = 0.5 μg/mL). The relationship between dose and urinary excretion was assessed using ANOVA regression analysis with orthogonal contrasts. Airway deposition of tobramycin from the two nebulizers was calculated from the relative potency.

Results:

Tobramycin measured in the urine following aerosol administration of 80 to 320 mg in two types of nebulizers is summarized in Table 0-2.

Table 0-2
The Relative Bioavailability and Dose Proportionality of Tobramycin Following Aerosol Administration

Nebulizer/Dose		Urinary Excretion (mg)[1]			Mean % Excreted	Y-Intercept
	N[2]	80 mg	160 mg	320 mg	(slope x 10^3)	
Std. Continuous flow	6	3.55	11.0	17.5	6.1	0.943 mg
Breath-Actuated	6	5.17	12.5	19.6	5.5	0.399 mg

[1] No LOQ reported
[2] All patients received all six treatments

Tobramycin excretion into the urine increases proportionally as a function of dose. This is demonstrated for both nebulizers in that urinary excretion plotted as a function of dose did not differ significantly from linearity ($p > 0.05$), with slopes different from zero ($p = 0.001$) and intercepts not different from zero ($p > 0.04$). The relative bioavailability of tobramycin following aerosol administration, as estimated by the slope of the urinary excretion plot, is low (6%). The authors further conclude that the minimal urinary excretion (mean=0.46 mg) following oral administration (32 mg) of tobramycin supports their position that virtually all urinary tobramycin excretion resulted from pulmonary absorption of tobramycin from the aerosol, with unappreciable, if any, contribution from gastrointestinal absorption.

The relative potency of the breath-actuated nebulizer compared to the standard jet nebulizer is 1.26 (95% CI = 0.925-1.78), demonstrating that the breath-activated system delivered an estimated 26% more tobramycin to the lung than the standard jet nebulizer.

5. Baran D, de Vuyst P, and Ooms HA, 1990. Concentration of Tobramycin Given by Aerosol in the Fluid Obtained by Bronchoalveolar Lavage. Respiratory Medicine 84: 203-204.

Twenty adult patients with a variety of lung disorders, including interstitial lung disease, localized lesions, and asbestosis were included in the study. Informed consent was obtained prior to study initiation. Each

patient received a single 80 mg dose of tobramycin administered by jet nebulizer to the respiratory tract (particle size: 74.8% of the total particles between 0.5 and 3.0 microns; flow rate: 6 Liters/min). A bronchoalveolar lavage (BAL) procedure was performed 30 minutes after dose administration. Three 50 mL aliquots of sterile buffered saline were infused into a subsegmental bronchus and withdrawn. The first aspirate specimen was separated, after which all remaining aspirate samples were pooled. A blood sample was drawn 30 min after the BAL and 60 min after tobramycin administration. Tobramycin was assayed in the BAL and blood using a homogenous enzyme immunoassay sensitive to 0.1 ± 0.01 µg/mL.
Results:
The tobramycin concentrations in BAL fluid and blood are summarized in Table 0-3.

Table 0-3

Tobramycin Serum and BAL Concentrations Following Aerosol Administration

	Tobramycin Concentration (µg/mL)[1]		
	Initial BAL sample	Pooled BAL sample	Blood Level
Mean ± SD	2.0 ± 2.26[2]	0.49 ± 0.57	NR[3]
Range			
N	20	20	13

[1] LOQ for BAL and Serum = 0.1 µg/mL
[2] Mean±SD
[3] NR =not reported
[4] BQL = Below Quant. Limit

The mean concentration in the initial BAL sample was 2.0 µg/mL (median 1.1µg/mL) with 18 of the 20 samples equal to or greater than µg/mL. The subsequent pooled samples had a lower mean concentration of 0.49 µg/mL, with 70% at or below µg/mL. These lower concentrations are not unexpected, in that most antibiotic was extracted in the first BAL aspiration. In addition, the pooled samples would have added sample dilution. Corresponding blood concentrations of tobramycin were very low or not detectable.

6. Braude AC, Hornstein A, Klein M, *et al.*, 1983. Pulmonary Disposition of Tobramycin. American Review of Respiratory Diseases 127: 563-565.
Nine patients, 7 male and 2 female, ages 20-77 years (median 59 years) with varying acute or chronic diseases, three of whom had pulmonary fibrosis, were referred for bronchoalveolar lavage (BAL). All patients were receiving tobramycin intravenously in doses ranging from mg/kg body weight as treatment for a variety of suspected or proven gram-negative infections.
Results:
A wide range of serum concentrations µg/mL), tracheal secretion concentrations (µg/mL), and BAL concentrations (µg/mL) were reported as patients received doses ranging from mg every 8 hours and sampling occurred 1 to 8 hours after dosing. In order to relate the findings in serum and BAL, the bronchial lavage tobramycin concentrations corrected for serum creatinine were plotted as a function of serum tobramycin concentration also corrected for serum creatinine. The resulting relationship demonstrated that increasing serum concentrations of tobramycin resulted in increasing BAL concentrations.

7. Cooney GF, Lum BL, Tomaselli M, *et al.*, 1994. Absolute Bioavailability and Absorption Characteristics of Aerosolized Tobramycin in Adults with Cystic Fibrosis. Journal of Clinical Pharmacology 34: 255-259.
Six adult patients with cystic fibrosis, 3 male and 3 female, ages 23-35 years and body weights of 42-61 kg, all had a history of routine hospitalizations for frequent recurrences of acute pneumonitis. Treatment included tobramycin combined with a second antibiotic, ceftazidime, administered intravenously (2 g every 8 hours).
Patients were initiated on an intravenous tobramycin dosing regimen (2.5 mg/kg q 6h for 8 days), and the dose was adjusted to provide tobramycin peak and trough serum concentration ranges of mg/L and 1-

2 mg/L, respectively. Peak and trough values were checked to assure steady state conditions persisted. Once at steady state, serial blood samples were collected at the beginning and end of the 35 minute infusion, at 60-360 minutes after initiation of the infusion, and a baseline blood sample prior to administration of an aerosol dosing regimen of tobramycin. Aerosolized tobramycin was administered as a 7.5 mg/kg/dose regimen using a Microstat Ultra Nebulizer over a period of one hour. Serial blood sampling took place at the beginning and at least two other times during inhalation, and at 90-360 minutes after initiation of the inhalation.

Analysis of the tobramycin solution in the nebulizer determined the actual dose administered. Serum samples were analyzed for tobramycin using an immunofluorescence assay procedure with a sensitivity limit of 0.2 µg/mL and a linear range of µg/mL.

Elimination half-life was estimated using the graphing program By the method of superposition, the inhalation tobramycin serum concentration-time profile was calculated. The actual tobramycin concentration values were the sum of the inhaled drug plus the remaining drug from the final intravenous dose. The superimposed inhalation concentrations over the intravenous concentrations enabled the investigators to measure serum concentrations within the linear range of the assay procedure. Other pharmacokinetic parameters, AUC, Clearance (Cl), steady-state volume of distribution (V_{ss}), and AUMC, were estimated using model independent analysis as described by Gibaldi and Perrier. The absolute bioavailability (F) was calculated from the ratio of the AUC values normalized for dose. The absorption rate constant, K_a, was determined by unweighted least squares log-linear regression of the percent absorbed per unit time values, with the mean absorption time (MAT) calculated from the reciprocal of K_a. Cumulative relative fraction absorbed (CRFA) was also calculated at each time point using the serum concentration, K_e, and $AUC_{(0-\infty)}$ as described by Shargal and Yu.

Results:

All subjects completed the study, with the mean intravenous dose required to maintain the desired tobramycin peak and trough serum concentrations was 2.9 mg/kg every six hours. The mean inhalation tobramycin dose was 5.6 mg/kg, with the mean ratio of the intravenous dose to the inhalation dose being 136 mg:266 mg. Tobramycin serum trough concentrations remained constant in all subjects throughout the 10-14 day course of therapy, and there was no evidence of drug accumulation. Each patient had sputum cultures positive for *P. aeruginosa*, which was sensitive to both antibiotics. All patients responded to treatment without adverse events, with no changes in baseline serum creatinine and blood urea nitrogen laboratory parameters. Therapeutic response was determined by improvement in respiratory function, changes in sputum, and other clinical parameters.

Due to the limited systemic availability of tobramycin following inhalation administration, clearance and volume of distribution estimates for tobramycin were calculated only following intravenous dosing. Absorption estimates from the lung and systemic bioavailability were calculated for tobramycin after inhalation administration. These findings are summarized in Table 0-4 below.

Table 0-4
Summary Tobramycin Pharmacokinetics Following IV Infusion and Inhalation

	Tobramycin Pharmacokinetics by IV Infusion		Tobramycin Pharmacokinetics (5.6 mg/kg) by Inhalation		
	Cl (L/h/kg)	V_{ss} (L/kg)	K_a (hr^{-1})[1]	MAT(min)	F (%)[2]
Mean	0.15	0.26	0.06	60.4	9.1
SEM	0.05	0.10	0.20	104.4	8.3
Range					
N	6	6	6	6	6

[1] Analysis of data suggested that Ka was zero-order.

[2] F calculated by ratio of AUC from oral compared to parenteral

The findings from this study document that tobramycin pharmacokinetics following intravenous administration are in accordance with other published results. In addition, for both Cl and V_{ss}, the results are very consistent between patients. In contrast, the absorption pharmacokinetic parameters for

tobramycin following inhalation administration demonstrate extensive intra-patient variability, with a 300-fold range in K_a and nearly a 10-fold range in MAT. Clearly part of this variability may be due to the limited absorption observed following this route of administration. Evaluation of the amount of bioavailable drug remaining to be absorbed per unit time and the cumulative relative fraction absorbed time plots both suggest that absorption of tobramycin across the alveoli is by a zero-order absorption process.

As mentioned previously, the absolute bioavailability of tobramycin administered by inhalation was very low, and confirms the observation made by Weber *et al* [6]. Weber and colleagues measured serum concentrations after 600 mg doses of tobramycin and found the highest concentrations to be only 1.5 µg/mL. This study clearly documents the poor absolute bioavailability of the drug when administered by aerosolization with only limited uptake of inhaled tobramycin into the systemic circulation.

8. Gappa M, Steinkamp G, Tummler B, *et al.*, 1988. Long-Term Tobramycin Aerosol Therapy of Chronic *Pseudomonas aeruginosa* Infection in Patients with Cystic Fibrosis. Scan J. Gastroent 23 (S143): 74-76.
This clinical trial evaluated the use of aerosolized tobramycin in 14 pediatric and young adult cystic fibrosis patients, ages 9 to 20 years. These patients had a median duration of *Pseudomonas* colonization of 36 months, ranging from months. Tobramycin was administered twice daily as an aerosol preparation (80 mg in a 2 mL volume of intravenous tobramycin solution via a jet nebulizer). Serum samples were collected to assess the systemic absorption of tobramycin.
Results:
Of the 70 measured tobramycin serum concentrations, 50 were below the minimum detectable concentration of 0.1 µg/mL. The highest concentration of 0.4 µg/mL was measured one hour after inhalation. Other markers evaluating nephrotoxicity and ototoxicity documented that there were no abnormalities. The authors concluded that the absence of any toxic side effects during long-term inhalation of tobramycin aerosol was presumably due to the absence of significant systemic absorption.

9. Le Conte P, Potel G, Peltier P, *et al.*, 1993. Lung Distribution and Pharmacokinetics of Aerosolized Tobramycin. American Review of Respiratory Disease 147: 1279-1282.
The study was conducted in three phases: evaluation of the nebulizer efficiency, initial lung distribution and pharmacokinetics in healthy volunteers (HV) and mechanically-ventilated (MV) patients, and the assay of the amount of tobramycin deposited in the lung of lung cancer patients undergoing pneumonectomy (LC).
Tobramycin and technetium labeled- were administered by use of a commercially available pneumatic nebulizer.
Determination of tobramycin deposition in the lung was performed in both HV and MV patients. Tobramycin (300 mg) and technetium-labeled was administered by mouthpiece (HV) and tracheal tube (MV), with ventilation kept constant. The mean duration of aerosolization required to nebulize the total mixture was about 5 minutes. Planar scintigraphy was performed 10 minutes after nebulization. Venous blood samples were then obtained after nebulization at: 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours. Serum was separated and stored at -20°C until analysis. Urine was collected during the following intervals: 0-4, 4-8, 8-12, 12-18, 18-24, 24-48, 48-72 hours post dosing. Bioavailability was defined by the ratio of total urinary excretion to the initial quantity of tobramycin deposit in the nebulizer. In LC patients undergoing pneumonectomy, 300 mg of tobramycin was nebulized 4 to 12 hours before the surgery. A fragment of healthy lung tissue was removed and prepared for measurement of tobramycin by bioassay. Plasma samples were collected 30 minutes after aerosolization and at the time of tissue excising (4 and 12 hours after tobramycin inhalation.
Serum half-life of tobramycin was determined from urinary collection data (amount remaining to be excreted plot) in which the calculated elimination half-life of tobramycin from the urine paralleled the elimination half-life of tobramycin from serum. A monoexponential regression analysis was performed to obtain the slope or elimination rate constant (K), from which the half-life values were calculated ($T_{½}$=0.693/K).
Statistical analysis was performed using Statgraphics® software. Tobramycin concentrations in urine and serum half-lives were compared using Student's t-test. A p value of < 0.05 was considered significant.
Results:

Scintigraphy performed 10 minutes after nebulization of 300 mg tobramycin (and 1 mL of ^{99m}Tc-DTPA) in healthy volunteers demonstrated that there was a homogenous distribution of radioactivity in the whole lungs, with slight deposition on the trachea and bronchi. The mean peak serum concentration of tobramycin was 0.27 ± 0.15 μg/mL with a serum half-life, calculated from urinary excretion results, of 8.96 ± 0.47 hours.

Table 0-5

Summary of the Plasma and Lung Concentrations of Tobramycin Following Aerosolized Tobramycin Administration with Terminal Half-Life and Absolute Bioavailability Estimates

Population	N	Peak Serum Conc (0.5h) (μg/mL)[1]	Lung Conc$_{4h}$ (μg/g)	Lung Conc$_{12h}$ (μg/g)	$T_{1/2}$ (hours)	F (%)
Healthy Volunteers	5	0.27 ± 0.15[2]	—	—	8.96 ± 0.47	4.33 ± 1.99
Mechanically Ventilated Patients[3]	5	BQL[4]	—	—	11.23 ± 2.26	6.62 ± 4.33
Pts with Lung Cancer/Thoracic Surgery	5	0.43[2]	5.57 ± 5.52	—	—	—
Pts with Lung Cancer/Thoracic Surgery	5	0.55[2]	—	3.61 ± 4.34	—	—

[1] LOQ Serum and Urine (F determination) =0.1 μg/mL Lung Tissue Conc = 0.3 μg/mL
[2] Mean ± SD
[3] Patients without respiratory disease
[4] BQL = Below Quant. Limits

Lung uptake of tobramycin in healthy volunteers breathing spontaneously and in mechanically ventilated patients was poor as demonstrated by the low fraction of the dose eliminated in the urine. In part, the low fraction absorbed was due to the amount of the drug solution remaining in the nebulizer and associated tubing (51% without mouthpiece and 83% with a mouthpiece). Some of the tobramycin may have been exhaled without deposition in the lung. In contrast, lung concentrations of tobramycin following this single dose administration reached concentrations of greater than 5 μg/g four hours after administration and remained greater than 3.5 μg/g 12 hours after dosing, suggesting once-daily administration is feasible. This study demonstrated that clinically significant lung concentrations of tobramycin can be reached with systemic plasma concentrations less than 1 μg/mL. Urinary recovery of tobramycin in HV and MV patients was 12.9±5.8 mg and 19.8±12.9 mg, respectively, with absolute bioavailability of 4.33±1.9% and 6.62±4.33%, respectively.

10. Levy J, Smith AL, Koup, JR, *et al.*, 1984. Disposition of Tobramycin in Patients with Cystic Fibrosis: A Prospective Controlled Study. Journal of Pediatrics 105: 117-124.

Twelve adolescent or young adults with cystic fibrosis (CF), between the ages of 8 and 23 years, and six patients with other diseases who were receiving an aminoglycoside for proved or suspected infections caused by *P. aeruginosa* were included in the pharmacokinetic study. All 18 patients had to have a serum creatinine concentration in the normal range. Five of the original study participants were included in the urinary recovery study, with four healthy volunteers (HV) as controls. CF patients, control patients, and healthy volunteers received 75.5±10.2, 55.7±14.7, and 60 mg/m^2 tobramycin, respectively. All patients and controls received at least three doses prior to the pharmacokinetic assessment. All doses of tobramycin were administered intravenously over 5 minutes by constant infusion. Blood samples (n=11) were collected at baseline and 10-360 minutes following the start of the infusion. Urine was collected before the infusion and for the 6 hour duration of the infusion, so as to evaluate the influence of tubular reabsorption on the clearance of tobramycin.

Tobramycin in serum and urine were assayed by a radioenzymatic assay (REA). The serum-concentration-time plots were analyzed using a nonlinear regression analysis program, MULTI. The β and end-of infusion concentration estimates were also used in subsequent model-independent estimates of total body clearance and volume of distribution at steady-state. Half-life of the slowest disposition slope was calculated by $T_{1/2} = 0.693/\beta$. Renal clearance was estimated from the urinary excretion amount over

6 hours/AUC over 6 hours. Statistical analysis between the CF patients and the controls was performed using a two-tailed Student t-test for non-paired observations.
Results:
The pharmacokinetics of tobramycin in patients with cystic fibrosis and in controls are summarized in the following table.

Table 0-6
Comparison of Pharmacokinetics in Cystic Fibrosis Patients and Control Patients

Pharmacokinetic Parameter	Cystic Fibrosis Patients	Control Patients	P-value
Clearance (mL/min/1.73m²)	121.2 ± 14.9[1]	102.2 ± 18.9	< 0.05
$Clearance_{renal}$ (mL/min/1.73m²)	89.5 ± 17.9	81.0 ± 15.8	NS[2]
Vdss (L/1.73m²)	15.5 ± 4.0	14.4 ± 5.2	NS
Vdss (L/kg)	0.31 ± 0.08	0.23 ± 0.07	< 0.05
$t_{½}$ (hours)	1.73 ± 0.42	1.85 ± 0.38	NS

[1] Mean ± SD
[2] Not significant

The urinary collection study attempted to determine the cause of the differences in clearance by exploring both mechanisms associated with GFR and tubular reabsorption processes. There were differences between the groups; no correlation was found between tobramycin clearance and creatinine clearance or iothalamate clearance in patients with CF, however both measures were significant in the control population. In contrast, tobramycin renal clearance in patients with CF was significantly correlated with creatinine clearance but not iothalamate, while in controls the reverse was observed. Volume of distribution of tobramycin was significantly larger in patients with CF than in controls when normalized to body weight, but not body surface area. This difference can be explained as tobramycin appears to be primarily distributed in extracellular fluids, which have a variable relationship with weight according to age and body habitus.

11. McCrae WM, Raeburn JA, and Hanson EJ, 1976. Tobramycin Therapy of Infections Due to *Pseudomonas aeruginosa* in Patients with Cystic Fibrosis: Effect of Dosage and Concentration of Antibiotic in Sputum. Journal of Infectious Diseases 134 Supp: S191-S193.
Seventeen cystic fibrosis patients with respiratory infections, between the ages of 11 months and nearly 17 years of age, were included in the study. All patients were clinically symptomatic with recurrent cough and production of sputum at least during postural drainage in the course of physiotherapy. The sputum in every case gave a profuse growth of mucoid *P. aeruginosa*. Four patients were treated with tobramycin administered intravenously at each of the following dose concentrations: 5, 6, 8, and 10 mg/kg per day. One patient was given 12 mg/kg per day. Tobramycin was administered in three divided doses per day and therapy continued for two weeks. Serum concentrations of tobramycin were measured every other day 1 hour after the injection and just prior to the next injection. Sputum was collected on the same days. Tobramycin was assayed by bioassay methodology.
Results:
The concentration of tobramycin in both serum and sputum samples as a function of dose are summarized in Table 0-7.

Table 0-7
Peak and Trough Serum and Sputum Concentrations of Tobramycin Following Intravenous Doses of 5-12 mg/kg in Cystic Fibrosis Patients

Dose (mg/kg/day)	Number of Patients	Mean Serum Concentrations (μg/mL)[1] Peak	Trough	Sputum Concentration (μg/mL)
5	4	5.7±1.9[2]	0.1±1.8	
6	4	4.5±1.8	0.01±1.8	
8	4	4.9±1.7	0.2±0.2	
10	4	6.8±2.2	0.2±0.2	
12	1	8.7±0.6	0.5±0.2	

[1] LOQ not reported
[2] Mean ± SE

In examining the serum concentration results, there is a trend for increasing concentrations with increasing dose. However, there is considerable variability which in part may be due to the small sample size at each dose level. Similarly, there is little or no relationship between the administered dose and the level of tobramycin in the sputum. The authors postulate that other factors, such as degree of inflammatory reaction to the infection, must influence the level of antibiotic achieved in the sputum.

12. Mendelman PM, Smith AL, Levy J, *et al.*, 1985. Aminoglycoside Penetration, Inactivation, and Efficacy in Cystic Fibrosis Sputum. American Review of Respiratory Disease 132: 761-765.
Ten children with cystic fibrosis (CF), between the ages of 8 and 18 year of age, who weighed between 20.5 and 62 kg with body surface areas ranging from Patients with documented sputum cultures in the prior 2 months yielding predominately *P. aeruginosa* and susceptible to tobramycin were included in the study. All patients were clinically symptomatic with a pulmonary exacerbation of increased cough, fatigue and weight loss. In addition to ticarcillin, all patients received tobramycin 80 mg/m2/dose (approximately 6 to 10.8 mg/kg) administered as a 30 min intravenous infusion at 8 hour intervals. After 4 to 6 doses, a pharmacokinetic assessment was performed to verify therapeutic peak serum concentrations. Serum and simultaneous sputum samples were collected 1.5 to 2.5 hours after initiation of the morning tobramycin infusion. Samples were collected every 5-7 days over a two week course of treatment (all 10 patients) or 3-week course of treatment (4 patients). Tobramycin was assayed by

Results:
The pharmacokinetics of tobramycin in sputum samples from patients with cystic fibrosis are summarized in Table 0-8.

Table 0-8
Comparison of Pharmacokinetics in Cystic Fibrosis Patients and Control Patients

Duration of Treatment (days)	Number of Patients	Mean Sputum Concentrations (μg/mL)[1]		Serum Concentration[1] (μg/mL)
		Bioassay	Radioenzymatic Assay	
5-7	10	1.0[2]		not reported
12-14	10	3.20[2]		not reported
18-21	4	6.0[2]		7.5 [4.5-10.5]

[1] LOQ Sputum (REA) 2 μg/mL Serum not reported
[2] estimate from Figure 1 in article
[3] Mean ± SD
[4] Mean [range]

As can be seen from the results, tobramycin concentrations in sputum increased through 18-21 days of treatment. Tobramycin concentrations in sputum (enzymatic assay) increased from approximately 8 to 68 μg/mL after two weeks to 82 μg/mL after three weeks of treatment. The tobramycin concentrations measured by bioassay were much lower, in part to the inactivation by the sputum itself. The authors emphasized that there was large variability in the sputum concentrations; the range was nearly ten fold μg/mL after 18 days of treatment, while the serum concentration variability was much less μg/mL). This finding suggests that the greater sputum variability was due to factors other than serum pharmacokinetic differences, such as differences in saturable binding to the sputum and changes to the composition and purulence of the sputum with therapy.

13. Mombelli G, Coppens L, Thys JP, *et al.*, 1981. Anti-Pseudomonas Activity in Bronchial Secretions of Patients Receiving Amikacin or Tobramycin as a Continuous Infusion. Antimicrobial Agents and Chemotherapy 19: 72-75.

Seven adults who were obtunded or unconscious neurosurgical patients were entered into the study and received tobramycin. All were hospitalized in the intensive care unit, had a tracheotomy or an endotracheal tube, and manifested acute bronchitis with grossly purulent secretions. All had normal serum creatinine concentrations (<1.2 mg/100 mL) and had not received any antimicrobial drug during the week preceding the study. The microorganisms isolated from the bronchial secretions were *P. aeruginosa*, *Staphylococcus aureus*, or a mixed bacterial flora, mostly enteric bacilli and *S. aureus*. The seven patients received tobramycin 1 mg/kg followed by a continuous intravenous infusion (2-3.5 mg/kg tobramycin) over 8 hours. Blood samples were collected during the infusion at 5 and 7 hours. Bronchial secretions were collected over the last 4 hours of the infusion using hourly catheter aspirations through the tracheo-bronchial tube or tracheotomy. Tobramycin concentrations in serum and the supernatant from the centrifuged bronchial secretions were assayed by a standard bioassay.

Results:

The relationship of tobramycin in serum and bronchial secretions during tobramycin intravenous infusion therapy is summarized in Table 0-9.

Table 0-9
Relationship of Tobramycin in Serum and Bronchial Secretions During Intravenous Infusion Therapy

	Tobramycin Concentration (μg./mL)[1]		
	Serum (S)	Bronchial Secretions (BS)	BS/S Ratio (%)[2]
Mean	3.6	0.71	17.5
Standard Deviation	1.0	0.70	12.3
Range			
N	7	7	

[1] LOQ Not Reported

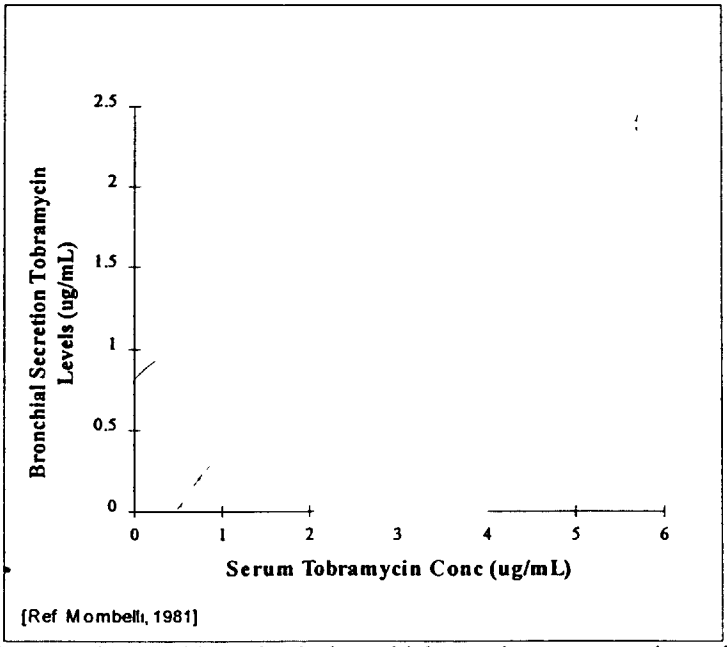
[2] The ratio was determined as the mean of the individual patient ratios, rather than the ratio of the two means (bs /s).

[Ref. Mombelli, 1981]

Due to the design of the study using a continuous infusion of tobramycin, steady state concentrations of the drug provided a reasonable basis to assess the relationship between serum and bronchial secretion tobramycin concentrations. In this case, tobramycin concentrations in the bronchial secretions averaged 17% of that found in the serum at steady state. When the findings are plotted, a linear relationship exists (Figure 0-3).

Figure 0-3
The Relationship between Serum and Bronchial Secretion Concentrations of Tobramycin Following Intravenous Continuous Infusion

The authors point out that the results of studies investigating the penetration of antimicrobial agents into bronchial secretions are often conflicting; in part due to true ability of the antimicrobial agents to penetrate across membranes, but study designs, modes of specimen collections, and assay techniques may also



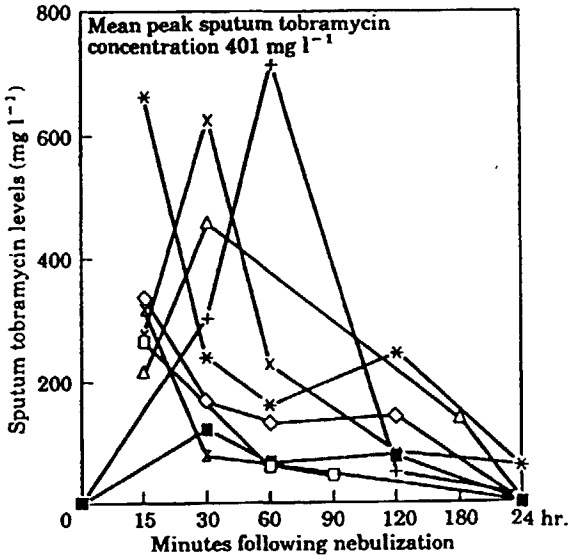
[Ref Mombelli, 1981]

explain some of the discrepancies. In this study, the bronchial secretion concentrations of tobramycin were measured following administration of a continuous intravenous infusion. The constant serum concentrations of tobramycin minimize the variability in the denominator of the bronchial secretion/serum

ratio of tobramycin. As a result, the ratios found in this study were lower than reported elsewhere in the literature.

14. Mukhopadhyay S, Staddon GE, Eastman C, *et al.*, 1994. The Quantitative Distribution of Nebulized Antibiotic in the Lung in Cystic Fibrosis. Respiratory Medicine 88: 203-211.
Twenty-seven cystic fibrosis subjects (15 males, 12 females), 4-23 years of age, were entered into the study. All had ventilation scans with 81m-Krypton and radiolabeled tobramycin, and most had respiratory function tests, lung volume measurements and chest radiographs. Those with respiratory exacerbations and were hospitalized within the three months following the ventilation scans (n=7) were excluded from the study. Sputum samples were produced only by 9 of the patients.
Particle size experiments were conducted on a Malvern MasterSizer using a 3 mL aliquot.
Tobramycin was labeled using 99m-Tc and was administered as a 99m-Tc-(Sn)-tobramycin complex. A 120 mg dose of radio-labeled tobramycin was nebulized to the patients with the Medix World Traveller-Intersurgical nebulizer system, via a mouthpiece. A low-resistance filter was used to remove particles over 0.2 microns, with a >99.9% efficiency. Nebulization was continued until no visible aerosol had been generated for 10 seconds. The mean time of nebulization was 17 minutes (range 15-20 minutes).
Following the aerosol inhalation, the ventilation scan was performed to outline the lung.
Imaging was performed using a gamma camera fitted with a collimator. Inner lung regions were defined with dimensions approximately 1/3 of the total and mid-lung regions. Delivered tobramycin was calculated based upon the total dose minus the residual in the inhalation apparatus. A known amount of radio-labeled tobramycin was used to produce a standard image which was used to calculate the amount of deposition.
Sputum samples were collected at intervals of 15 minutes or longer for the first 120 minutes after nebulization, with a final sample 17-24 hours later. Tobramycin concentrations were measured using a fluoro-immunoassay.
Results:
Particle size distribution analysis for tobramycin resulted in a mass median aerodynamic diameter (MMAD) for tobramycin and radio-labeled tobramycin of 5.42 ± 0.20 μm and 5.28 ± 0.17 μm, respectively.
The quantity of tobramycin reaching the lungs was small, estimated to be about 8.0 mg out of a dose of 120 mg added to the nebulizer, with 60% of the dose still in the nebulizer or on the expiratory filter. Despite the low percent deposition, tobramycin was able to reach all outer and periphery pulmonary areas.
Sputum concentrations were measured 15 minutes through 24 hours after aerosol administration. The results are illustrated in Figure 0-4.

Figure 0-4
Sputum Tobramycin Concentration-Time Course in CF Patients



Peak sputum concentrations were obtained 15-60 minutes following nebulization, with five patients having peaks at 15 minutes, three patients at 30 minutes, and one patient at 60 minutes . The range of peak sputum concentrations were from approximately 200 to 700 µg/mL, with the mean peak level at 401 µg/mL.

15. Smith AL, Ramsey BW, Hedges DL, *et al.*, 1989. Safety of Aerosol Tobramycin Administration for Three Months to Patients with Cystic Fibrosis. Pediatric Pulmonary 7: 265-271; and Weber A, Smith A, Hack B, *et al.*, 1989. Tobramycin Serum Pharmacokinetics after Aerosol Administration. Pediatric Pulmonology Supp 4: 136 [abstract].

Since these two publications describe the same research program, one summary will incorporate both literature citations.

Twenty-two cystic fibrosis (CF) patients (16 males, 6 females), 4-23 years of age, entered and completed the study; two others withdrew. CF was confirmed by a sweat chloride test and sputum cultures yielded *P. aeruginosa* susceptible to tobramycin. All had creatinine clearance > 55 mL/imn/m^2 and normal auditory acuity.

Throughout the 3-month study, patients received reconstituted tobramycin (600 mg in 30 mL saline) via a DeVilbiss 100 Ultrasonic nebulizer three times daily. Patients inhaled the aerosol for 200 inspirations. The volume and concentration of the remaining solution were measured after treatment. General safety, as well as special tests were conducted throughout the study to monitor adverse effects, and laboratory changes, including measurements to assess renal and auditory function.

During each of five visits throughout the 12 weeks, sputum, serum and urine samples were collected. Sputum was collected immediately and again 5 hours after nebulization; urine samples were collected during the six hours following aerosol administration; multiple serum samples were collected but specific times were not reported.

Tobramycin concentrations were measured in urine using an RIA assay, in sputum using an REA assay, and in serum using a modified RIA method.

Results:

Although the study principally examined efficacy and safety parameters of aerosol tobramycin in CF patients, Table 0-10 summarizes the pharmacokinetic results.

Table 0-10
Pharmacokinetics of Tobramycin Following Aerosol Administration

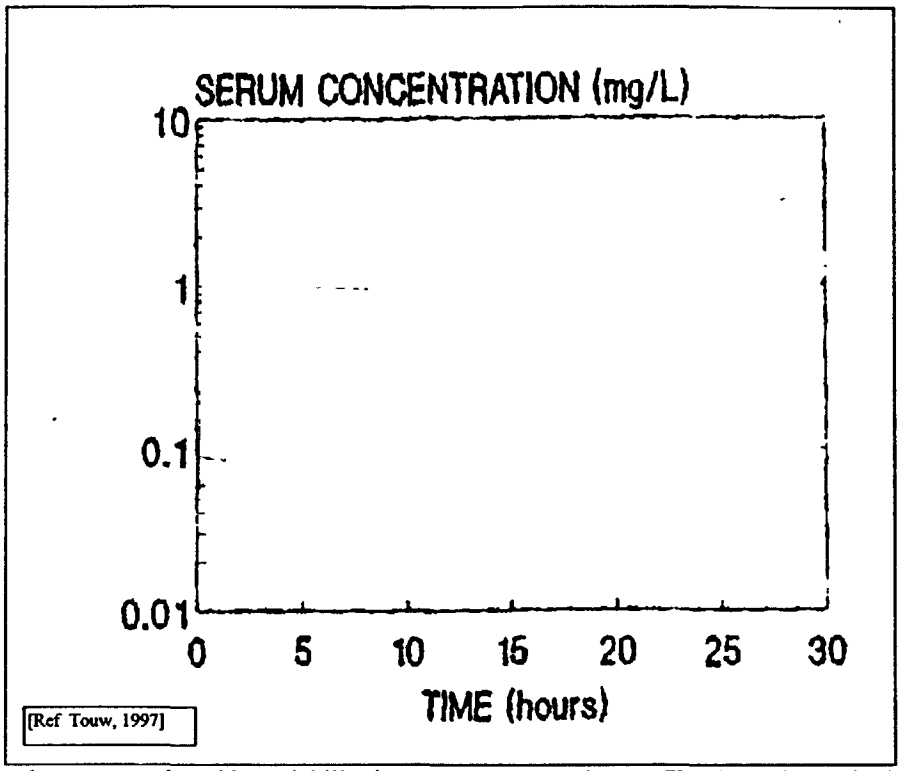
	Dose Delivered		Sputum Tobramycin[1]			Serum Tobramycin[1]		AE[2] (0-6h)		F
	(mg)	(mg/kg)	Peak Conc. (µg/g)	5 Hour Conc. (µg/g)	Total Expectorate d* (mg)	Peak Conc. (µg/mL)	K^3 (h^{-1})	(mg)	(mg/kg)	(% Dose)
Mean	666	17.4	2,300	100	3.47	NR[4]	0.193	4.0	0.283	0.6
SD	195	7.1	1,900	100	4.91	NR	0.074	4.0	0.238	NR
Range										

[1] LOQ Sputum = Not Reported; Serum 0.05 µg/mL; Urine Not Reported
[2] Amt. excreted in urine
[3] Serum elimination rate constant
[4] Not reported by the author
Only limited information about serum concentrations provided, but highest single value was 1.5 µg/mL, with only two other values from a total of 31 were greater than 1.0 µg/mL.

These results demonstrate that for very high doses administered (mean 17.4 mg/kg), peak tobramycin sputum concentrations averaged 2,300 µg/g, while 90% of the peak serum concentrations were below 1.0 µg/mL, and only one reached 1.5 µg/mL. These low serum concentrations resulted in a systemic bioavailability value that was calculated to be 0.6% of the total dose.
Even though the urinary collection time was short and probably incomplete, it is unlikely that the total recovery would be greater than 1-2% of the nebulized dose. Both the peak concentrations and total systemically absorbed tobramycin are indicative of the safety of aerosol tobramycin.

16. Touw DJ, Jacobs F, Brimicombe RW, *et al.*, 1997. Pharmacokinetics of Aerosolized Tobramycin in Adult Patients with Cystic Fibrosis. Antimicrobial Agents and Chemotherapy 41: 184-187; and
Touw DJ, Jacobs F, Brimicombe RW, *et al.*, 1996b. Pharmacokinetics of Aerosolized Tobramycin in Adult Patients with Cystic Fibrosis. Pediatric Pulmonary 22 Supp 13: 294 [abstract].
Since these two publications describe the same research program, one summary will incorporate both literature citations.
Six patients, 4 males and 2 females, ages 17-30 years, with cystic fibrosis and chronic respiratory tract infections due to *P. aeruginosa* were entered into the study. They were not having infectious exacerbations and their pulmonary functions were within 10% of their usual values. All previous treatment with aminoglycosides was stopped at least three days before the study began. The patients received a single 600 mg dose formulated (by the hospital pharmacy) by dissolving 600 mg of tobramycin in 10 mL of water for injection, with the pH adjusted to 6-7 with sulfuric acid and the tonicity adjusted to 270 mosmol/kg with sodium chloride. The solution was aerosolized using an ultrasonic nebulizer (WISTO SENIOR, Wisto, Woerden, The Netherlands). The patients received a single 600 mg dose over at least 15 minutes of inhalation. All retained and undelivered tobramycin in the nebulizer was analyzed for undelivered tobramycin.
Venous blood samples were collected immediately before and after inhalation and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours. Urine was collected for 24 hours. Quantification of tobramycin was by a fluorescence polarization immunoassay (LLQ = 3µg/mL) and a modification of this assay was used to reach much lower limits (LLQ = 0.025µg/mL). Pharmacokinetic calculations, curve-fittings, and simulations were performed using MW/Pharm software (Mediware, Groningen, The Netherlands). Total bioavailability was estimated from the cumulative urinary collection over 24 hours times a 1.176 correction factor to normalize for 100% of the dose (reference of 85% collected in 24 hours (Touw, *et al*, 1996).
Results:
Individual subject serum concentration-time profiles following single 600 mg doses of tobramycin are illustrated in Figure 0-5.

Figure 0-5
Serum Concentration-Time Profile of Tobramycin Administered by Aerosol



These results demonstrate the wide variability in serum concentration profiles following a single dose of tobramycin. Peak serum concentrations generally were observed during the 1-2 hour interval and ranged from μg/mL, a 13-fold difference. The individual and summary pharmacokinetics are listed in Table 0-11.

Table 0-11
The Individual and Summary Pharmacokinetics of Tobramycin in Serum Following Aerosol Administration

	Dose[1] (mg)	AUC (mg.h/L)	CL (L/h)	Vss (L/kg)	Ka (1/h)	Tmax (h)	Cmax (mg/L)	T1/2α (h)	T1/2β (h)	Ae[2] (mg)	F[3] (%)
Mean	374	8.9	6.98	1.74	1.98	1.54	1.27	1.54	13.0	56.4	17.5
SD	48	6.3	2.89	0.96	1.40	0.99	1.07	0.97	5.2	25.5	8.8
CV	12.8	70.8	41.4	55.2	70.7	64.3	84.3	63.0	40.0	45.2	19.9
Range											

[1] The dose represents net delivered dose (600 mg dose in nebulizer - dose recovered in nebulizer after dosing)
[2] The amount excreted in the urine over a 24-hour collection interval
[3] Recomputed value of F when calculated without correction for the efficiency of nebulizer and correction of author computational errors is 11.1 ±5.0%

Tobramycin was widely distributed in the body, was slowly cleared from the body, has a moderate terminal elimination half-life of 13 hours, and was absorbed systemically (absolute bioavailability) to a greater extent in this study than has been reported by others. The authors attribute these differences to a much more sensitive analytical methodology and a nebulizer that was much more efficient in delivering tobramycin than has been reported by others.

In addition to pharmacokinetic analysis. the authors also used computer simulations to estimate serum concentrations in patients with extremely high and low clearance following treatment with 600 mg three

times daily. The simulations resulted in trough serum concentrations ranging from μg/mL. These serum concentrations, both measured and simulated, are a result of significant systemic bioavailability following aerosol administration, and some patients if chronically treated with this regimen, have the potential for toxicity from tobramycin.

17. Weber A, Smith A, Williams-Warren J, *et al.*, 1994. Nebulizer Delivery of Tobramycin to the Lower Respiratory Tract. Pediatric Pulmonary 17: 331-339.

Five nebulizers were chosen based upon their use by cystic fibrosis patients in the author's institution. All were operated in accordance with the manufacturer's instructions. All were run at maximum power. The aerosol characterization was performed by use of a cascade impactor so that aerodynamic median mass diameter (MMAD) of the particles formed from each machine could be determined. The aerosol produced by each nebulizer was characterized twice and the mean value depicted.

The amount of expectorated sputum was determined by weighing, diluting and sonicating the samples. Aliquots were assayed for tobramycin content using a radioenzymatic assay.

Twelve cystic fibrosis patients, 10 males and 2 females, ages 7-28 years, with acceptable renal function (>30 mL/min/1.73m^2), were enrolled in the study. All studies were completed in 8 of the patients. Patients did not receive any aminoglycoside by any route of administration in the two weeks preceding or during the study. A standardized 40 mg dose of tobramycin was attempted to be delivered to the lower respiratory tract by normalizing according to number of respirations and tidal volume of the patient. Sputum was collected in tared cups immediately after aerosol treatment (following gargling and mouth rinse), and 1, 2, 3, and 4 hours after conclusion of aerosol administration. Patients were tested on the Pulmo-Aide and the UltraNeb 100 (with three carrier gases).

All 9 patients had measurable sputum concentrations with all three runs with the UltraNeb 100, while only 7 of 9 patients with CF had measurable tobramycin in sputum following nebulization with the Pulmo-Aide.

Results:

The ranges of sputum tobramycin concentration in the samples obtained immediately after inhalation (approximately 3 minutes) and 4 hours later for each nebulizer and gas is found in Table 0-12.

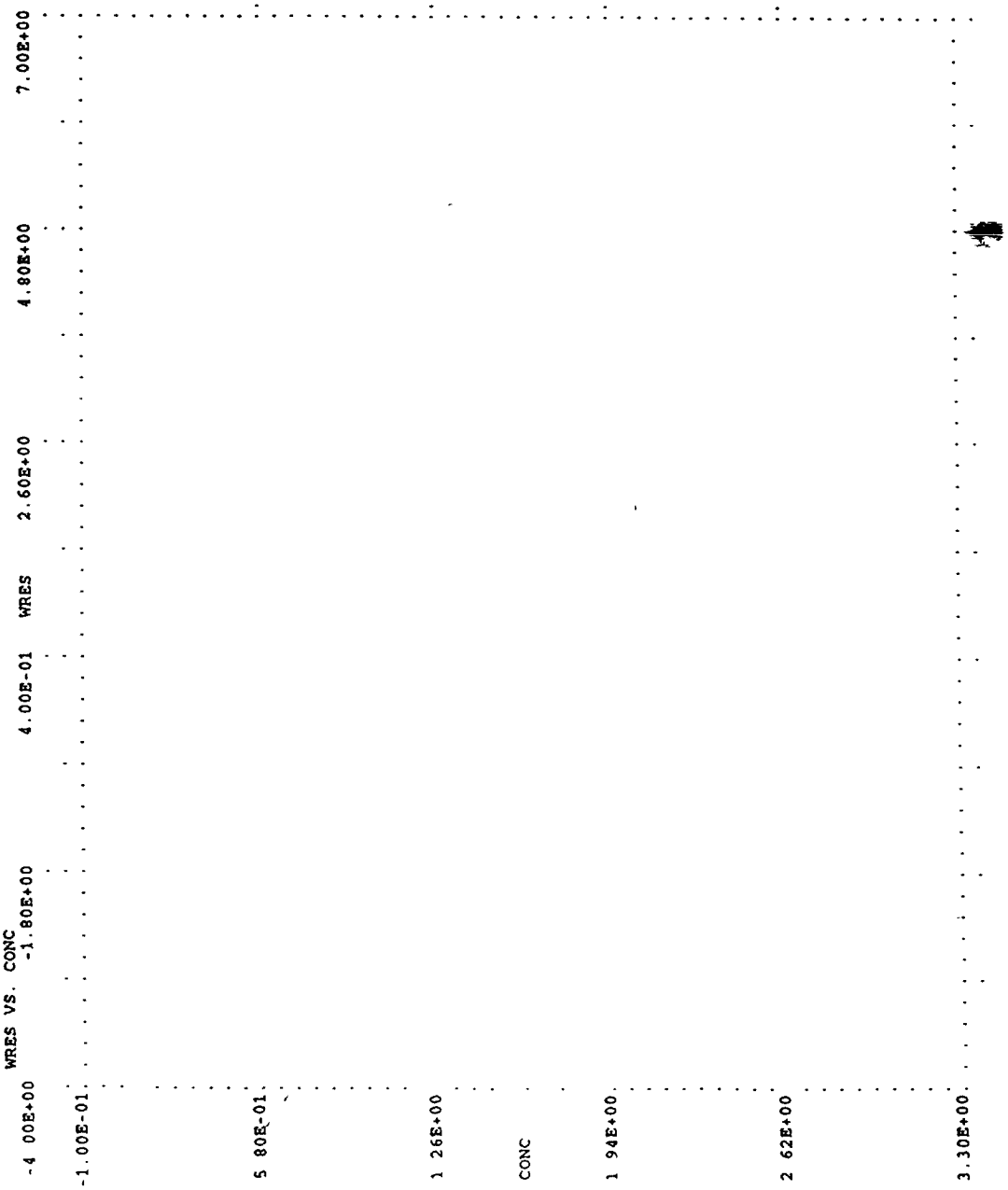
Table 0-12
Effect of Nebulizer and/or Formulation on Tobramycin Delivery

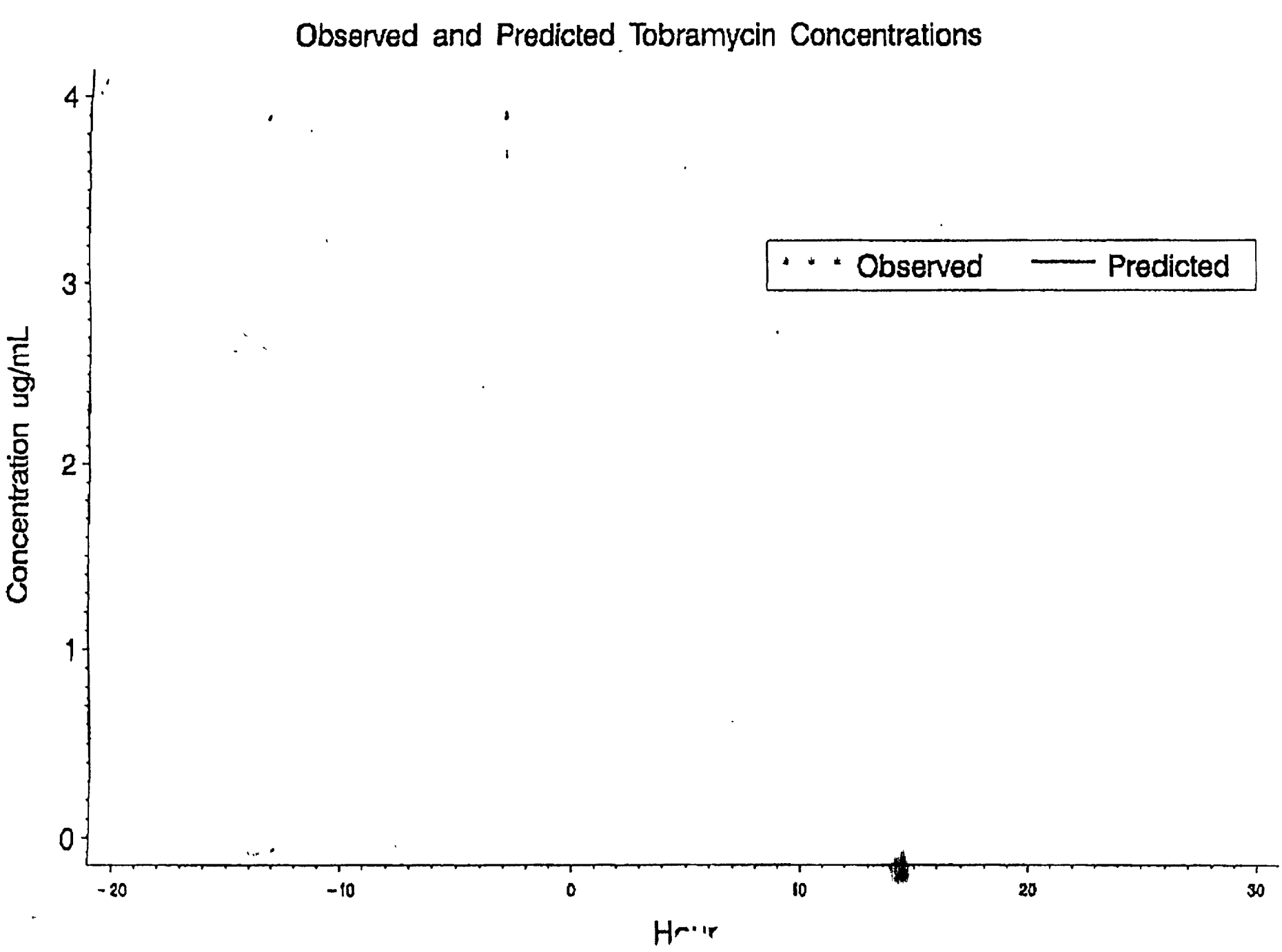
Nebulizer/Carrier Gas	N	Mean Tobramycin Sputum Concentration (μg/g)[1]	
		Time after Aerosolization 0.05 (hours)	Time after Aerosolization 4.0 (hours)
Pulmo-Aide internal fan generated carrier gas	7[2]	0-629	0-57
UltraNeb internal fan generated carrier gas	9	16-1343	12-32
UltraNeb with compressed air	9	34.5-1980	1.2-16
UltraNeb with helox	9	94.2-3385	0-64

[1] LOQ for tobramycin in sputum =1.0 μg/g

[2] All 9 patients received all 4 treatments, however, two pts on the Pulmo-Aide nebulizer did not have measurable concentrations and were not included in the tabulation.

These findings demonstrate that the UltraNeb nebulizer system gave higher and more reproducible results than that observed with the Pulmo-Aide apparatus, and that there were no differences attributed to source or type of carrier gas. The authors concluded the UltraNeb system with an internal fan to supply the carrier gas was the most efficient and convenient nebulizer for consistently delivering tobramycin to the lower respiratory tract.


WRES VS. CONC
WRES
CONC
7.00E+00
4.80E+00
2.60E+00
4.00E-01
-1.80E+00
-4.00E+00
-1.00E-01
5.80E-01
1.26E+00
1.94E+00
2.62E+00
3.30E+00


Observed and Predicted Tobramycin Concentrations
Observed
Predicted
Concentration ug/mL
4
3
2
1
0
-20
-10
0
10
20
30

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 50-753

ADMINISTRATIVE DOCUMENTS

14. PATENT CERTIFICATION

TOBRAMYCIN SOLUTION FOR INHALATION
#50-753

The undersigned certifies that the formulation or composition of Tobramycin Solution for Inhalation is claimed by 5,508,269. This product is the subject of this application, #50-753, for which approval is sought.

Signature Bruce Montgomery May 20 1997
Date

Name Bruce Montgomery, M.D.
Title Sr. Vice President, Research & Development

PATENT INFORMATION

TOBRAMYCIN SOLUTION FOR INHALATION
#50-753

PathoGenesis Corporation submits the following patent information as required by Section 505 (b) of the Food, Drug and Cosmetic Act, as amended, and in compliance with 21 CFR 314.53 (c).

The following patent(s) are relevant to this application:

Patent No:	5,508,269
Date of Patent:	4-16-96
Expiry:	4-15-2013
Claims:	An aminoglycoside formulation for delivery by aerosolization. The concentrated aminoglycoside formulation containing an efficacious amount of aminoglycoside able to inhibit 95-100% of susceptible bacteria. Aminoglycoside formulated in 5 ml. solution of a quarter normal saline having pH between 5.5 and 6.5. The method for treatment of endobronchial infections produced by a formulation delivered as an aerosol having mass medium average diameter predominantly between 1 to 5 μ, produced by a jet or ultrasonic nebulizer.

The owner of the patent is:
PathoGenesis Corporation

1.B. MARKETING EXCLUSIVITY

TOBI™ (Tobramycin Solution for Inhalation) is not eligible for a period of market exclusivity under Title I of the Drug Price Competition and Patent Term Restoration Act of 1984.

However, on October 13, 1994, the FDA granted PathoGenesis' request for Orphan Drug designation for TOBI™ for the treatment of bronchopulmonary infections of *P. aeruginosa* in patients with cystic fibrosis. The exclusivity provisions of the Orphan Drug Act apply to antibiotics. Therefore, if PathoGenesis is the first company to obtain marketing approval for aerosolized tobramycin therapy, it is entitled to seven years of market exclusivity. The exclusivity provided by Orphan Drug Act is automatic without further action by the FDA when the statutory criteria have been met. Nevertheless, PathoGenesis requests written notification recognizing marketing exclusivity when it is earned.

PEDIATRIC PAGE
(Complete for all original applications and all efficacy supplements)

NDA/PLA/PMA # 50-753 Supplement # _____ Circle one: SE1 SE2 SE3 SE4 SE5 SE6

HFD-520 Trade and generic names/dosage form: TOBI (tobramycin solution for inhalation) Action: (AP) AE NA

Applicant PathoGenesis Therapeutic Class 3P

Indication(s) previously approved n/a
Pediatric information in labeling of approved indication(s) is adequate x inadequate ___

Indication in this application Management of cystic fibrosis in patients infected w/ (For supplemen answer the following questions in relation to the proposed indication.) Psuedomonas aeruginosa

___ 1. **PEDIATRIC LABELING IS ADEQUATE FOR ALL PEDIATRIC AGE GROUPS.** Appropriate information has been submitted in this or previous applications and has been adequately summarized in the labeling to permit satisfactory labeling for all pediatric age groups. Further information is not required.

X 2. **PEDIATRIC LABELING IS ADEQUATE FOR CERTAIN AGE GROUPS.** Appropriate information has been submitted in this or previous applications and has been adequately summarized in the labeling to permit satisfactory labeling for certain pediatric age groups (e.g., infants, children, and adolescents but not neonates). Further information is not required.

___ 3. **PEDIATRIC STUDIES ARE NEEDED.** There is potential for use in children, and further information is required to permit adequate labeling for this use.

___ a. A new dosing formulation is needed, and applicant has agreed to provide the appropriate formulation.

___ b. A new dosing formulation is needed, however the sponsor is either not willing to provide it or is in negotiations with FDA.

___ c. The applicant has committed to doing such studies as will be required.
- ___ (1) Studies are ongoing,
- ___ (2) Protocols were submitted and approved.
- ___ (3) Protocols were submitted and are under review.
- ___ (4) If no protocol has been submitted, attach memo describing status of discussions.

___ d. If the sponsor is not willing to do pediatric studies, attach copies of FDA's written request that such studies be done and of the sponsor's written response to that request.

___ 4. **PEDIATRIC STUDIES ARE NOT NEEDED.** The drug/biologic product has little potential for use in pediatric patients. Attach memo explaining why pediatric studies are not needed.

___ 5. **If none of the above apply,** attach an explanation, as necessary.

ATTACH AN EXPLANATION FOR ANY OF THE FOREGOING ITEMS, AS NECESSARY.

/S/ Project Manager 12/8/97
Signature of Preparer and Title Date

cc: Orig NDA/PLA/PMA # 50-753
HFD-520 /Div File
NDA/PLA Action Package
HFD-006/ SOlmstead (plus, for CDER/CBER APs and AEs, copy of action letter and labeling)

NOTE: A new Pediatric Page must be completed at the time of each action even though one was prepared at the time of the last action. (revised)

CERTIFICATION

The undersigned hereby certifies that neither the undersigned nor any person employed by the undersigned, or the entity on whose behalf this Certification is given, to provide services in connection with the New Drug Application ("NDA") to be submitted by PathoGenesis Corporation for its proprietary new drug TOBI™ (Tobramycin Solution for Inhalation) has been, or is proposed to be, debarred under Section 306 (a) or 306 (b) of the Federal Food, Drug and Cosmetic Act. The undersigned further certifies that he, she or it will not employ any person or persons who are, or are proposed to be, debarred under Section 306 (a) or 306 (b) of the Federal Food, Drug and Cosmetic Act to provide services in the future in connection with the above referenced NDA or any other NDA to be submitted by PathoGenesis Corporation.

BY: _____

TITLE: Senior Vice President

DATE: 7 April 1997

Consult #797 (HFD-520)

TOBI tobramycin solution for inhalation

There were no look-alike/sound-alike conflicts or misleading aspects found in the proposed proprietary name.

The Committee has no reason to find the proposed proprietary name unacceptable.

/S/ 6/23/97, Chair
CDER Labeling and Nomenclature Committee

cc. NDA 50-753
HFD-520/Div. file
HFD-520/Chem/S. Pagay
HFD-520/CSO/B. Duvall-Miller

MEMORANDUM OF TELECON

DATE: Friday, December 19, 1997

APPLICATION NUMBER: NDA 50-753; TOBI™ (tobramycin solution for inhalation)

BETWEEN:

Name: Dr. Bill Pitlick, Director, Regulatory Affairs
Dr. Bruce Montgomery, Senior Vice President
Ms. Kelly Otto, Biostatistics
Ms. Jill Van Dalfsen, Microbiology
Ms. Barbara Schaeffler, Project Management
Dr. Lynn Rose, Director, Development
Phone: (201) 467-8100
Representing: PathoGenesis Corporation

AND

Name: Ms. Beth Duvall-Miller, Project Manager
Dr. John Alexander, Medical Officer
Dr. Janice Soreth, Medical Team Leader
Dr. Albert Sheldon, Microbiology Team Leader
Dr. Gary Chikami, Acting Division Director
Division of Anti-Infective Drug Products, HFD-520

SUBJECT: Labeling negotiations

NDA 50-753 was submitted on July 10, 1997. The PDUFA due date for this application is January 9, 1998. Internal labeling meetings began on December 3, 1997. A marked-up revised label was sent to the sponsor on December 11, 1997. PathoGenesis submitted revised marked-up labeling on December 16, 1997 as a response to the Agency's latest version. The TOBI review team met on December 19, 1997 and reviewed this labeling. Following the team's review of the labeling, the sponsor was phoned to discuss the following outstanding issues necessary for agreement of final labeling:

Redacted 1

pages of trade

secret and/or

confidential

commercial

information

/S/

Beth Duvall-Miller
Project Manager

cc:
Original NDA 50-753
HFD-520/Div. File
HFD-520/B. Duvall-Miller

Concurrence only:
HFD-520/SCSO/J. Bona /S/ 1/6/98
HFD-520/MO/J. Alexander /S/ 1/9/98
HFD-520/ActDivDir/G. Chikami 1/9/98

drafted: bdm/December 29, 1997/M:\TELECON\N50753.LBL
Initials r/d:
final: BDM 1/6/98

TELECON

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 50-753

CORRESPONDENCE

PATHOGENESIS

October 20, 1997

Gary Chikami, MD, Acting Director
Division of Anti-Infective Drug Products HFD-520
Center for Drug Evaluation and Research
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20850

RE: NDA 50-753
Drug: Tobramycin Solution for Inhalation

Response to Request for Information

Dear Dr. Chikami,

This information is in response to a facsimile request from Dr. Marianne Mann received at PathoGenesis Corporation on October 7, 1997 regarding information on patients who withdrew from our clinical trials. In addition, we are including an additional analysis promised in response to Dr. Mann's questions of October 10 regarding the microbiological analysis datasets.

First, Dr. Mann requested that we provide a list of all patients who were withdrawn from either the randomized Studies -002 and -003 or the open label study -004 due to the emergence of resistant isolates (either resistant Pseudomonas or any other organism).

NDA 50-753 reported that five patients were withdrawn from the parallel group and open-label studies when tobramycin resistant organisms were identified. However, upon re-examination of the reasons for withdrawal from these studies, it was determined that six patients were withdrawn from study participation by the principal investigators due to the emergence of resistant organisms, including five in the open-label study (004).

Withdrawal from the study was at the discretion of the investigator, based on non-study laboratory results; there was no protocol requirement to withdraw a patient based exclusively on microbiological data. Closer examination of the clinical and microbiology data from each patient suggests it was unlikely that the use of TOBI contributed to the alleged resistance to tobramycin in these patients.

The reasons for withdrawal cited by the investigator, followed by sponsor comments and a table of supporting information as requested by Dr. Mann

PATHOGENESIS CORPORATION
201 ELLIOTT AVENUE WEST SEATTLE, WASHINGTON 98119 TELEPHONE 206 467 8100 FAX 206 282-5065

Second, on October 10, Dr. Mann requested an analysis describing how repeat MIC values differed from (or correlated with) original MIC values at Visits 3, 10, and 11. We responded on the same day by estimating the difference between the original and repeat MIC values at Visits 3, 10, and 11 based on the difference between the MIC_{50} and MIC_{90} for the original versus repeat tests as reported in the submission.

We also calculated the actual difference between the original and repeat MIC values at Visits 3, 10, and 11 per patient per isolate and report the frequency distributions of this difference by visit.

Please call me if there are any further questions at (206) 270-3319.

Sincerely,

William H. Pitlick, Ph.D.
Director, Regulatory Affairs

Enclosures

Attachment 1
Questions from Dr. Mann
NDA 50-753
TOBI™ (tobramycin solution for inhalation)

Text in your application claims:
"One TOBI™ Patient from the parallel group studies and 4 open-label TOBI™ patients were withdrawn from the study when tobramycin resistant organisms were identified by standard Kirby Bauer or MIC Susceptibility criteria and the physician chose alternate antibiotic therapy."

So, at the most, there were 5 TOBI™ patients who were withdrawn for this reason. Dr. Mann is unclear how many placebo patients were withdrawn for this reason and would like some additional information on these subjects.

1. Please provide a list of all patients who were withdrawn from either the randomized Studies -002 and -003 or the open label study -004 due to the emergence of resistant isolates (either resistant Pseudomonas or any other organism).

Please provide the following information on each subject:

A. Patient ID#.
B. Study Drug assignment
C. Was study drug withdrawn during randomized trial or during open-label trial?
D. Total duration on study drug prior to discontinuation. (If discontinuation occurred during the open-label trial, please clarify each patient's duration on placebo followed by days on TOBI™, or total duration of TOBI™ treatment).
E. All MIC data for the relevant resistant pathogen during the study
F. Total days on IV antibiotics for respiratory exacerbations during time on study

For 6 patients, investigators reported resistance as a reason for withdrawal from the study. It should be noted that investigators did not have access to the results of cultures performed at our central laboratory. Thus any microbiology results used in making patient decisions were obtained at local non-study clinical laboratories. (Clinical microbiology laboratories routinely report an MIC of ≥ 8μg/mL associated with *P. aeruginosa* as "resistant.") For a variety of reasons, it is possible that there may be discrepant findings between local laboratories and our central laboratory.

In 5 of the 6 patients withdrawn from study participation, the investigator reported "resistant" *P. aeruginosa* isolates. In 2 of these 5 patients culture at our central laboratory did not confirm these results. In 2 other patients the highest MIC noted by PathoGenesis during the study, 8μg/mL, was noted at a visit before initiation of TOBI (visit 3 for Visit 10 for In 1 of these 5 patients MICs of μg/mL were noted

before initiation of TOBI (Visits 3, 10, 11), as well as an MIC of 64μg/mL after initiation of TOBI (Visit 12). This patient was dropped from the study after a single dose of TOBI.

In 3 of the 6 patients withdrawn from study participation *S. maltophilia*, intrinsically resistant to tobramycin, was isolated. Two of these 3 patients also were reported to have "resistant" *P. aeruginosa*, as described above.

Patient

PI Comments: "Patient became resistant to tobramycin. This patient has developed multiple resistant strains of pseudomonas." Site also provided sponsor with culture and sensitivity results from the , using the Kirby-Bauer method. This report cited "very light growth pseudomonas aeruginosa; very resistant isolate". The same report cited "heavy growth staphylococcus aureus; methicillin resistance".

Sponsor's Comments: Testing performed at reported *P. aeruginosa* isolates with MICs ≤ 1μg/mL during the patient's study participation.

Patient

PI Comments: "Patient needed concurrent aerosolized colistin due to the pseudomonas being resistant to multiple antibiotics."

Sponsor's Comments: Testing performed at reported *P. aeruginosa* isolates with MICs ≤ 2μg/mL during the patient's study participation. However, *S. maltophilia* was present at all visits and was resistant to tobramycin.

Patient

PI Comments: "Following Visit 6, patient was seen by her primary physician due to increase in her pulmonary symptoms. Sputum results from Visit 5 showed a pseudomonas culture that was multiply resistant. Outside of IV antibiotic therapy, the bacteria was only sensitive to colistin."

Sponsor's Comments: Testing performed at reported *P. aeruginosa* at Visit 3 (prior to dosing with TOBI) with an MIC = 8μg/mL. All other MICs used in data analysis were ≤ 4μg/mL during the patient's study participation.

Patient

PI Comments: "Patient experienced pulmonary exacerbation while on cycle 4 of open-label study that required hospitalization. Culture done at our lab grew out MRSA and

Stenotrophomonas maltophilia. Neither organism was susceptible to tobramycin. We felt that using inhaled gentamycin (MRSA is sensitive) was the best choice."

Sponsor's Comments: Testing performed at reported *P. aeruginosa* at Visits 3 and 10 with an MIC = 8μg/mL. *S. maltophilia* was also grown at Visits 11 and 13.

Patient

PI Comments: "Patient is withdrawing from study at time of visit # 13. Patient is beginning colistin therapy in hopes of eradicating his pan resistance which is excluding him from possible lung transplantation."

Sponsor's Comments: Testing performed at reported *P. aeruginosa* at Visit 10 (prior to dosing with TOBI) with an MIC = 8μg/mL. *P. aeruginosa* had MICs ≥ 16μg/mL to all antibiotics except tobramycin and ciprofloxacin. *P. aeruginosa* at Visits 11 and 13 (before and after the first cycle of TOBI) had MICs ≤ 4μg/mL. *S. aureus* was identified at Visits 3 and 10.

Patient

PI Comments: "Patient had resistant organisms in sputum and did not do well in first part of study. She responded well to IV colistin in hospital and wishes to continue with aerosolized colistin".

Sponsor's Comments: Testing performed at reported *P. aeruginosa* at Visits 3, 10 and 11 (all prior to dosing with TOBI) with MICs of 32, 16 and 32 μg/mL respectively. The MIC of *P. aeruginosa* at Visit 12 (after one cycle of TOBI) increased to 64 μg/mL.

ATTACHMENT 1

Pt ID	Study Drug Assigned	Study With-drawn from	# days of study drug (prior to with-drawal)	# days of IV anti-biotics for resp. exac.	Organism*	MIC Data Visit 3	Visit 10	Visit 11	Visit 12	Visit 13	Visit 15
	TOBI/TOBI	004	139	102	PA-A	1	1	0.5		≤ 0.12	
					PA-B	0.5		1			
					PA-C	1		1			
					S Malto		512			64	
	TOBI/TOBI	004	107	16	S Malto	128	>512	2048		2048	2048
					PA-A	1	0.5	1		0.5	0.5
					PA-B	≤ 0.25	≤ 0.25	0.5		0.5	≤ 0.12
					PA-C	1	≤ 0.25	0.25			0.5
					PA-D			0.5			2
					A-Hem Strep	X					
					Coag Neg Staph	X					
					GpD Strep	X					
					S Aureus	X					
					A Fum		X				
	TOBI	002	25	0	PA-A	8	≤ 0.25[1]				
					PA-B	2	4[1]				
					PA-C	2	4[1]				
					Fungus	X					
					S Aureus	X					

[1] Results obtained at Visit 7 (withdrawal visit).

ATTACHMENT 1

	TOBI/TOBI	004	110	103	PA-A	4	8				
					PA-B	4					
					PA-C	8					
					S Aureus	X	X				
					C Albicans		X				
					S Malto-A			2048		1024	
					S Malto-B					2048	
	Placebo / TOBI	004	83/27	36	PA-A	0.5	8	2		2	
					PA-B	4	2	0.25		4	
					PA-C			0.5		2	
					S Aureus	X	X				
	Placebo / TOBI	004	64/1	40	PA-A	32	16	32	1		
					PA-B	1	1	1	64		
					PA-C	1	4		1		
					PA-D		1		0.5		
					Aspergillus	X					
					C Albicans	X	X		X		

* Organisms are abbreviated as follows:
  - PA-A: Pseudomonas aeruginosa, highest concentration
  - PA-B Pseudomonas aeruginosa, second highest concentration
  - PA-C: Pseudomonas aeruginosa, third highest concentration
  - PA-D: Pseudomonas aeruginosa, fourth highest concentration
  - S Malto: Stenotrophomonas maltophilia
  - A-Hem Strep: Alpha-Hemolytic Streptococcus
  - Coag Neg Staph: Coagulase Negative Staphylococcus
  - GpD Strep: Group D Streptococcus
  - S Aureus: Staphylococcus aureus
  - A Fum: Aspergillus fumigatus
  - Fungus: Fungus, not Aspergillus fumigatus
  - C Albicans: Candida albicans
  - S Malto-A: Stenotrophomonas maltophilia, highest concentration
  - S Malto-B: Stenotrophomonas maltophilia, second highest concentration
  - Aspergillus: Aspergillus species

Attachment 2

Follow-up response to Dr. Mann's questions dated 10/10/97.

2. **Please submit an analysis describing how repeat MIC values differed from (or correlated with) original MIC values at Visits 3, 10, and 11. This information is helpful to our understanding of how discrepant the two data sets really are.**

   - The attached SAS output presents the difference between the repeat and original MIC values for each isolate tested in both systems. Each difference is the number of 2-fold dilutions either higher (a positive difference) or lower (a negative difference) the repeat value was from the original. As noted in the previous analysis to answer this question, a bias toward lower MIC's in the original values exists. This bias is most noticeable in the Visit 11 values for the reasons mentioned previously.

Response to FDA Questions from October 10, 1997
Frequency of the Difference Between Repeat and Original MIC Values

Study Visit=03

DIFF	Frequency	Percent	Cumulative Frequency	Cumulative Percent
-8	2	0.2	2	0.2
-7	6	0.5	8	0.6
-6	13	1.1	21	1.7
-5	13	1.1	34	2.7
-4	15	1.2	49	4.0
-3	28	2.3	77	6.2
-2	50	4.0	127	10.3
-1	122	9.9	249	20.1
0	504	40.7	753	60.9
1	361	29.2	1114	90.1
2	98	7.9	1212	98.0
3	18	1.5	1230	99.4
4	3	0.2	1233	99.7
5	2	0.2	1235	99.8
6	1	0.1	1236	99.9
8	1	0.1	1237	100.0

Response to FDA Questions from October 10, 1997
Frequency of the Difference Between Repeat and Original MIC Values

Study Visit=10

DIFF	Frequency	Percent	Cumulative Frequency	Cumulative Percent
-11	1	0.1	1	0.1
-8	2	0.3	3	0.4
-7	5	0.7	8	1.1
-6	7	0.9	15	2.0
-5	9	1.2	24	3.2
-4	9	1.2	33	4.4
-3	4	0.5	37	4.9
-2	12	1.6	49	6.6
-1	70	9.4	119	15.9
0	246	32.9	365	48.8
1	187	25.0	552	73.8
2	134	17.9	686	91.7
3	48	6.4	734	98.1
4	10	1.3	744	99.5
5	1	0.1	745	99.6
8	1	0.1	746	99.7
9	2	0.3	748	100.0

Response to FDA Questions from October 10, 1997
Frequency of the Difference Between Repeat and Original MIC Values

Study Visit=11

DIFF	Frequency	Percent	Cumulative Frequency	Cumulative Percent
-5	1	0.3	1	0.3
-3	2	0.5	3	0.8
-2	8	2.1	11	2.9
-1	19	5.0	30	7.8
0	107	27.9	137	35.8
1	105	27.4	242	63.2
2	107	27.9	349	91.1
3	31	8.1	380	99.2
4	3	0.8	383	100.0

PATHOGENESIS

October 24, 1997

Dr. Gary Chikami
Division of Anti-Infective Drug Products HFD-520
Center for Drug Evaluation and Research
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20850

RE: NDA 50-753
Drug: Tobramycin Solution for Inhalation

Response to Request for Information

Dear Dr. Chikami,

In response to Dr. Pagay's question about particulate matter, please be advised that PathoGenesis has established an additional internal quality control requirement for purchased bulk tobramycin drug substance.

Our procedures now include sampling and visual inspection of all lots for general appearance. This specifically includes a requirement for tobramycin drug substance to be free of foreign matter.

This new requirement relates to a suggestion made by the FDA Investigator during the pre-approval review for NDA #50-753 at

Please call me if there are any further questions at (206) 674-6693.

Sincerely,

Melissa A. Yeager, J.D.
Manager, Regulatory Affairs

cc: Susan P. Bruederle, Investigator, FDA

Susan P. Bruederle, Investigator
US Food & Drug Administration
300 S. Riverside Plaza, Suite 550 South
Chicago, IL 60606

PATHOGENESIS CORPORATION
201 ELLIOTT AVENUE WEST SEATTLE, WASHINGTON 98119 TELEPHONE 206 467 8100 FAX 206 282-5065