CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 50-753

ENVIRONMENTAL ASSESSMENT AND/OR FONSI

PATHOGENESIS

October 9, 1997

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

RE: NDA 50-753, **SECTION 3.4: ENVIRONMENTAL ASSESSMENT**

Dear Dr. Chikami,

Pursuant to 21 CFR 25.31, PathoGenesis Corporation is requesting categorical exclusion from the requirement for preparation of an Environmental Assessment for Tobramycin Solution for Inhalation (TOBI™), NDA 50-753.

PathoGenesis certifies that Tobramycin Solution for Inhalation, subject of application 50-753, meets the criteria for the categorical exclusion as defined in 21 CFR 25.31 based on the fact that approval will not increase the use of the active moiety.

I certify that the information presented is true, accurate, and complete to the best of the knowledge of PathoGenesis Corporation. PathoGenesis Corporation recognizes its obligations and responsibilities, and intends to comply with all applicable Federal, State, and Local regulations and ordinances to provide a safe and healthy workplace for its employees and to protect the environment. Environmental, Health and Safety Policies have been developed by PathoGenesis to comply with these requirements.

Sincerely,

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

201 Elliott Avenue West Seattle, Washington 98119 Telephone 206 467 8100 Fax 206 270-3324

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:NDA 50-753

PHARMACOLOGY REVIEW(S)

Review and Evaluation of Pharmacology and Toxicology Data
Division of Anti-Infective Drug Products, HFD-520

DEC 19 1997

NDA #: 50,753-000 and M-002

SPONSOR: PathoGenesis Corporation
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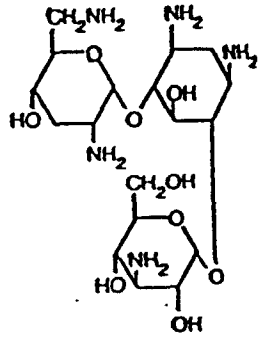
Seattle, WA 98119
(206) 467-8100; FAX (206) 282-5065

AUTHORIZED REPRESENTATIVE: William Pitlick
Director, Regulatory Affairs
(206) 270-3319

DRUG NAMES: Tobramycin for Inhalation (TOBI)

CATEGORY: Aminoglycoside antibiotic

STRUCTURAL FORMULA:



RELATED SUBMISSIONS: IND IND IND
While PathoGenesis does not have permission to cross reference this IND or the NDA for Nebcin (50,477), Lilly has provided them with a number of nonclinical toxicity study reports for tobramycin that were submitted under IND

NUMBER OF VOLUMES: 13

CONTAINS INTEGRATED TOX SUMMARY IN LIEU OF FINAL REPORT: NO

DATE CDER RECEIVED: M-002 was received on 4/10/97 and -000 was received on 7/11/97.

DATE ASSIGNED: M-002 was assigned approximately 4/11/97 and -000 was assigned on 7/14/97.

DATE REVIEW STARTED: 7/10/97

DATE 1ST DRAFT COMPLETED: 12/18/97

DATE REVIEW ACCEPTED BY TEAM LEADER: December 19, 1997

REVIEW OBJECTIVES: To ascertain whether the nonclinical studies submitted by the sponsor adequately demonstrate the potential toxicity of tobramycin (with special focus on the lung) when administered via inhalation and to determine if this drug product meets safety standards allowing it to be approved for marketing.

PROPOSED DOSAGE FORM AND ROUTE OF ADMINISTRATION:

INDEX OF NONCLINICAL STUDIES SUBMITTED TO THIS NDA (and location of review):

14-Day Inhalation Toxicity Study of Tobramycin in the Rat and Guinea Pig (SC950011)
IND

14-Day Inhalation Toxicity Study of Tobramycin in the Rat (N001328A)
IND

6-Month Inhalation Toxicity Study of Tobramycin in the Rat (N001328B)
reviewed below

Mutagenicity Test with Tobramycin in the *Salmonella typhimurium-Escherichia coli* Mammalian Microsome Reverse Mutation Assay with Confirmatory Assay (17452-0-409R)
reviewed below

Mutagenicity Test on Tobramycin in the L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay with Confirmatory Assay (17452-0-431R)
reviewed below

Mutagenicity Test on Tobramycin: Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation with a Confirmatory Assay with Multiple Harvests (17452-0-437Z)
reviewed below

Mutagenicity Test on Tobramycin: Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation with a Confirmatory Assay with Multiple Harvests (18080-0-437Z reviewed below

Mutagenicity Test on Tobramycin in an *In Vivo* Mouse Micronucleus Assay (17452-0-455CO)
reviewed below

REVIEWS OF NONCLINICAL STUDIES:

6-Month Inhalation Toxicity Study of Tobramycin in the Rat (N001328B)

Report dated 3/18/97, U.S. GLP

Vol. 2.6 p. 1395- Vol. 2.7 p. 2042

Animals: Male and female Sprague-Dawley rats, approximately 7 weeks old and 114-225 g at the initiation of dosing, 15/sex/group were to be sacrificed at the end of the dosing period, 5/sex/group were kept for an additional 4 week recovery period prior to sacrifice.

Diet: Certified Purina Rodent Chow and tap water were available *ad libitum* except during aerosol exposure and for 12 hours prior to obtaining blood from each rat for clinical chemistry and hematologic analysis during weeks 4, 8, and 12 and prior to sacrifice and necropsy.

Drug Dose and Route of Administration: Tobramycin (60 mg/ml) was dissolved in 1/4 normal saline (0.225% NaCl) for administration via nose-only inhalation of the aerosolized solution (nominal tobramycin concentration in aerosol mg/L). The vehicle control group was exposed to aerosolized 1/4 normal saline. Groups of animals were exposed to aerosolized drug or vehicle daily for 6 months as follows:

1. Vehicle, 180 min/day
2. 60 mg/ml tobramycin, 20 min/day
3. 60 mg/ml tobramycin, 60 min/day
4. 60 mg/ml tobramycin, 180 min/day

The aerosolized solutions were generated using a Pari LC Plus jet nebulizer. Air flow rate for the exposure system was set at approximately 500 ml per minute per exposure port. Validation experiments demonstrated that a consistent concentration of tobramycin was delivered within a single port during a period of about 160 minutes (relative standard deviation of approximately 12-17%) and that concentrations between exposure ports were similar (relative standard deviation approximately 9.5%). Particle size was 3.0 - 3.3 μm (measured either gravimetrically or analytically) with a standard deviation of about 0.5 - 0.7 μm over the course of the 6-month exposure period. The mean aerosol concentration of tobramycin in the test atmosphere was 0.59-0.67 mg/l with relative standard deviations of 16-19%.

Length of Study: Rats were exposed to aerosolized tobramycin daily for 6 months. Some animals from each dose group were kept for an additional 4 weeks without treatment to assess the potential reversibility of any lesions. The rats in the recovery group were also used for toxicokinetics. On day 1, and during weeks 4, 8, 12, and 26, 1 ml blood samples were drawn from the retro-orbital plexus of these animals within 5 minutes after the end of their daily exposures.

Results: No drug-related mortality occurred in this study. One female in the low dose group died by accident (positional suffocation during exposure) and one male in the low dose group was found dead (pericardial hemorrhage that did not appear related to tobramycin) on day 159. No drug-related clinical signs were observed in the rats. Mean body weights were significantly reduced (approximately 7%) in the high-dose male and female rats at the end of the 6-month exposure. Mean body weight was also lower in the mid dose males, but it was not statistically significant. There appeared to be no tobramycin-related toxicologically significant effects on hematologic parameters or serum chemistry, with the exception of decreased total serum protein and globulin observed in all drug-treated male rats that may be related to chronic nephropathy.

Lung weights significantly increased in a dose-responsive fashion compared to control in all tobramycin groups. Absolute lung weight was increased by about 18%, 28%, and 45% in the low, mid, and high dose groups. The increase was associated with hyperplasia of bronchiolar epithelium and infiltration of macrophages. Mean absolute kidney weight was significantly higher than control in the high dose male rats. High dose female rats also had higher mean absolute kidney weights although the increase was not statistically significant compared to control. Chronic nephropathy (including renal tubular degeneration, mineralization, protein casts in tubular lumina, and compensatory tubular regeneration) was observed at a greater frequency in rats from the high dose group compared to control. Although chronic nephropathy is frequently observed in Sprague-Dawley rats (particularly males) as they age, tobramycin treatment appeared to shorten the time to appearance of this condition, suggesting some systemic toxicity. The following table depicts the incidence of treatment-related microscopic lesions following 6 months of exposure to aerosolized tobramycin or vehicle:

Treatment-Related Microscopic Lesions in Rats After 6 Months of Exposure to Aerosolized Tobramycin

Lesion	Control		20 min		60 min		180 min	
	M	F	M	F	M	F	M	F
Degeneration of Olfactory Epithelium (Nose)	0	0	0	1	0	7	15	15
Hyperplasia of Squamous Epithelium (Larynx)	0	0	3	6	9	15	15	15
Hyperplasia of Bronchioli and Chronic Interstitial Inflammation	2	0	0	0	3	5	15	15
Infiltration of Alveolar Macrophages	1	0	14	14	15	15	15	15
Chronic Nephropathy	6	1	8	2	6	6	15	9

n = 15 for each group except for low exposure males and females where n = 14

The microscopic lesions listed in the table above were still observed in the majority of the rats in the "reversal group" 4 weeks after their exposure to aerosolized tobramycin ceased. However, hyperplasia of laryngeal squamous epithelium and infiltration of alveolar macrophages were observed at lower incidences in the low and mid dose rats in the "reversal group" than they had been in the rats sacrificed immediately after the treatment period.

The investigator regarded the 20 min exposure to be the NOAEL in this 6-month study because squamous cell hyperplasia of laryngeal epithelium and infiltration of alveolar macrophages were considered to be nonspecific effects that can be elicited by inhalation of any particulate aerosol.

Toxicokinetics:

Serum Tobramycin Levels (μg/ml) in Rats Immediately After Exposure (mean $\pm$ SE)

Week of Study	Exposure Time (minutes)		
	20	60	180
Males			
1	5.9 $\pm$ 0.9	16.8 $\pm$ 1.9	44.6 $\pm$ 12.7
4	2.4 $\pm$ 0.6	5.1 $\pm$ 1.3	17.7 $\pm$ 4.0
8	5.0 $\pm$ 1.7	8.7 $\pm$ 1.5	16.0 $\pm$ 4.4
12	1.6 $\pm$ 0.2	4.4 $\pm$ 0.9	18.21 $\pm$ 5.6
26	4.0 $\pm$ 1.1	8.2 $\pm$ 2.1	13.2 $\pm$ 1.3
Females			
1	5.3 $\pm$ 0.8	18.5 $\pm$ 2.7	20.5 $\pm$ 7.0
4	3.3 $\pm$ 1.5	12.8 $\pm$ 4.2	17.2 $\pm$ 4.1
8	2.0 $\pm$ 0.9	8.0 $\pm$ 5.2	18.8 $\pm$ 6.2
12	3.2 $\pm$ 0.9	5.3 $\pm$ 0.4	23.1 $\pm$ 7.8
26	4.1 $\pm$ 1.2	7.8 $\pm$ 3.1	14.5 $\pm$ 2.5

The mean deposited doses for this study calculated by the investigators were 4.9, 14.3, and 57.5 mg/kg, combining male and female rats for the 20, 60 and 180 minute exposure groups, respectively. The actual deposited doses changed over the course of the study as the body weights of the rats increased, but the exposure times remained constant. The equation below was used by the investigators to estimate deposited doses for each group of animals.

$$\frac{\text{Test Article Aerosol Concentration (mg/l)} \times \text{Minute Volume (l/min)} \times \text{Exposure Duration (min)} \times \text{Deposition Fraction}}{\text{Body Weight (kg)}}$$

Assumptions:

Test Article Aerosol Concentration- mg/l

Minute Volume for Rats- ml/min

Deposition Fraction- 50% (it should be noted that the Pulmonary Division of the CDER assumes a deposition fraction of 10% for the rat)

Using this equation (assuming test aerosol concentration=0.62 mg/l; rat body weight of 300 g and deposition factor of 10%), the reviewer calculates the approximate deposited doses in rats to be 1.24, 3.72, and 11.16 mg/kg for the 20, 60, and 180 minute exposure groups, respectively. This is less than calculated by the investigators primarily because of the difference in the deposition factor that was used (50% vs. 10%). The Pulmonary Division of the CDER usually assumes a deposition fraction of 100% in the human, thus the assumption would be that the entire 300 mg/kg dose of tobramycin would be deposited. With twice daily doses of 300 mg each, this would be 12 mg/kg/day deposited in the lungs of a 50 kg adult and 30 mg/kg/day deposited in the lungs of a 20 kg child. However, blood samples from clinical studies indicate that tobramycin is not readily absorbed from the lungs of most patients (much of it may be bound to sputum). The serum levels of the drug are relatively low in the patients, averaging about 1 μg/ml one hour after inhalation of a 300 mg dose of tobramycin (serum half life of this drug is about 2 hours in humans when it is given IV). The serum concentrations of tobramycin in the rats were much higher than what has been measured in humans after dosing via inhalation. Consequently, the nominal doses of tobramycin estimated for rats and humans using the deposition factors above do not appear to be useful for comparing systemic exposure to this drug when it is aerosolized and given via inhalation. The human subjects receiving recommended doses of TOBI appear to have been exposed to much lower systemic tobramycin levels compared to the rats in this study.

Mutagenicity Test with Tobramycin in the *Salmonella typhimurium-Escherichia coli* Mammalian Microsome Reverse Mutation Assay with Confirmatory Assay (17452-0-409R)

Report dated 10/16/96, U.S. GLP

Vol. 2.9, pp. 2590-2626

Strains Used: *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvr*A

Method: The plate incorporation method was used to investigate tobramycin's mutagenic potential in bacteria. Bacteria were combined with test substances ± S-9 and molten overlay agar (0.7% agar with 0.5% NaCl supplemented with either 0.05 mM histidine or 0.05 mM tryptophan depending on bacteria used) then plated on minimal agar (Vogel-Bonner minimal medium E with 1.5% agar and 0.2% glucose). Triplicate plates were incubated for approximately 48 hours at 37°C before revertants were counted. S-9 mix derived from Aroclor 1254-induced Sprague-Dawley rats was added to the overlay agar as applicable. Positive controls in the absence of S-9 were 2-nitrofluorene (1 μg/plate for TA98), sodium azide (2 μg/plate for TA100 and TA1535), ICR-191 (2 μg/plate for TA1537) and 4-nitroquinoline-N-oxide (1 μg/plate for WP2*uvr*A). The positive control in the presence of S-9 was 2-aminoanthracene (2.5 μg/plate for TA98, TA100, TA1535 and TA1537 and 25 μg/plate for WP2*uvr*A). TA100 and WP2*uvr*A were used in a dose range finding study to establish the

cytotoxic limits for tobramycin in *Salmonella* and *E. coli* in the presence and absence of S-9. The amounts of drug per plate used for the *Salmonella* strains in the first mutagenicity study were 5, 10, 25, 50, 100, and 333 μg with S-9 and 10, 25, 50, 100, 333, and 667 μg in the absence of metabolic activation. The latter set of doses were used ± S-9 in the confirmatory *Salmonella* mutagenicity study. The amounts of drug per plate used for *E. coli* WP2*uvr*A were 0.05. 0.1, 0.5, 1, 5, and 25 μg ± S-9 for the initial mutagenicity study. For the confirmatory E. coli study, the doses were the same as the first study in the presence of S-9, but the high dose of 25 μg was eliminated in the absence of metabolic activation and a dose of 0.01 μg was added. The highest concentrations used for testing caused reductions in the number of revertants on selective agar and a diminution of the bacterial lawn in non-selective plates. The solvent for the tobramycin was deionized water with sufficient sulfuric acid to adjust the pH of the tobramycin stock solution to 6.5-7.0.

Results: Acceptable numbers of bacterial revertants were observed on control plates in all strains for both mutagenicity studies and positive controls performed adequately. In the first mutagenicity assay, no increases in the number of bacterial revertants ≥2-fold greater than control was observed in any strain of bacteria at any dose level of tobramycin regardless of metabolic activation. In the absence of S-9, TA98 had an increase in the average number of revertants 1.9 times greater than control at 100 μg (25 vs. 13), but not 333 μg. At the latter dose level, the number of revertants was about half of control, indicating cytotoxicity despite the lack of a noticeable effect on the bacterial lawn at 333 μg. In the second mutagenicity assay, none of the bacterial strains exhibited an increase in revertants ≥2-fold greater than control with the exception of TA98 in the absence of S-9. At 50 μg, there was a 2-fold increase in revertants compared to control (24 vs. 12) and at 100 μg, there was 2.5-fold increase (30 vs. 21). At 333 ug, no increase in revertants compared to control was observed, but this concentration of tobramycin slightly reduced the bacterial lawn on non-selective plates, indicating cytotoxicity. In the presence of S-9, a 1.9-fold increase in revertants over control was observed at 100 μg (40 vs. 21), but no such increase was seen at 333 μg, despite the lack of cytotoxicity at either tobramycin concentration.

The reviewer does not believe that the small increase in revertants observed in the single *Salmonella* strain, TA98, is biologically significant. The actual numbers of revertants on the plates was still within the historical control range for this bacterial strain despite the small increase compared to the concurrent control. Additionally, there was no strong dose-response relationship for the increase in revertants. Bacterial mutagenicity assays are not ideal for studying anti-infective compounds due to the cytotoxicity of these agents in the tester strains- only relatively low doses of such compounds can be tested. Within the limitations of this assay, the reviewer considers the results negative for the induction of bacterial revertants.

Mutagenicity Test on Tobramycin in the L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay with Confirmatory Assay (17452-0-431R)

Report dated 5/30/96, U.S. GLP

Vol. 2.9, pp. 2627-2659

Method: Mouse lymphoma L5178Y cells (clone 3.7.2C) were cultured in growth medium consisting of RPMI 1640 supplemented with Pluronic® F68, L-glutamine, sodium pyruvate, antibiotics (not specified), and 10% heat-inactivated horse serum. The cells were treated with test agents in Fisher's medium with the same supplements, but the concentration of serum was only 5%. Treated cells were cloned in growth medium without Pluronic® F68 and with 20% horse serum and 0.24% agar. Cloning medium for selection of mutants contained 3 μg/ml of 5-trifluorothymidine.

Tobramycin was not particularly cytotoxic to the mouse lymphoma cells, so concentrations up to 5 mg/ml were tested (0.5, 1, 2, 3, 4, and 5 mg/ml) in the presence and absence of S-9 from Aroclor 1254-treated male Sprague-Dawley rats. The vehicle for tobramycin was tissue culture grade water with sufficient sulfuric acid to adjust the pH of the tobramycin stock solution to 6.5-7.0. The positive controls were methyl methanesulfonate (0.06, 0.12, and 0.18 mM) for studies without S-9 and methylcholanthrene (2 and 4 μg/ml) in the presence of metabolic activation. Cells were treated for 4 hours with test compounds, then washed, resuspended in growth medium and incubated at 37°C for about 2 days to allow for expression of the TK-/- phenotype if mutation has occurred. The cells were then suspended in cloning medium and colonies were counted 10-14 days later using an Artek Model 880 colony counter.

Results: In the first assay without S-9, the 4 and 5 mg/ml samples had to be discarded due to contamination and an excessive number of viable colonies compared to reasonable expectations. Tobramycin did not induce mutations at the TK locus of L5178Y mouse lymphoma cells at concentrations up to 3 mg/ml without metabolic activation in the first assay or 5 mg/ml in the first assay with activation and the second assay ±S-9. This is the highest concentration of test substance generally tested in this assay. Positive controls performed as expected and relative suspension growth (about 50-100% of control at concentrations of tobramycin tested ±S-9) and cloning efficiency were adequate.

Tobramycin was not mutagenic or clastogenic in L5178Y mouse lymphoma cells at concentrations up to 5 mg/ml with or without metabolic activation.

Mutagenicity Test on Tobramycin: Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation with a Confirmatory Assay with Multiple Harvests (17452-0-437Z)

Report dated 6/5/96, U.S. GLP

Vol. 2.9, pp. 2660-2695

Method: CHO cells were cultured at 37°C in McCoy's 5a medium supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin and streptomycin. Cytotoxicity testing of CHO cells was performed in the presence of 10 μM bromo deoxyuridine (BrdU) to stain the DNA. In the absence of metabolic activation, cells were treated with tobramycin and BrdU for 24 hours, with Colcemid (0.1 μg/ml) present for the last 2 hours of treatment before cytotoxicity (cell cycle delay and reduction mitotic index) were determined. In the presence of metabolic activation (S-9 from Aroclor 1254-treated male Sprague-Dawley rats), CHO cells were treated with tobramycin for 6 hours with BrdU, then washed, resuspended in medium with BrdU and incubated for another 17.8 hours, with Colcemid present for the last 2 hours.

Tobramycin was not particularly cytotoxic to the cells, so concentrations up to 5 mg/ml were tested (0.5, 1.25, 3.75 and 5 mg/ml) in the presence and absence of S-9. The vehicle for tobramycin was sterile deionized water with sufficient sulfuric acid to adjust the pH of the tobramycin stock solution to 6.5-7.0. The positive controls were mitomycin C (0.5 and 1 μg/ml for 12 hour harvest or 0.05 and 0.1 μg/ml for 24 hour harvest) for studies without S-9 and cyclophosphamide (5 and 12.5 μg/ml for 12 hour harvest or 5 and 10 μg/ml for 24 hour harvest) in the presence of metabolic activation. In the absence of S-9, CHO cells were treated with tobramycin for 12 or 24 hours in the first assay and 24 or 48 hours in the confirmatory assay. Colcemid was present for the last 2 hours of incubation. Cells were harvested, swelled with 0.075M KCl, dropped on slides, fixed with methanol:glacial acetic acid, and stained with Hoechst 33258 fluorescent stain and Giemsa Azure B so that chromosomes could be evaluated. In the presence of S-9, cells were treated for 6 hours with test compounds, then washed, resuspended in growth medium and incubated at 37°C for 12, 24 or 48 hours prior to harvest with Colcemid present for the last 2 hours. For control purposes, another set of cultures was incubated with tobramycin for 6 hours without S-9, and treated as above with 12 or 24 hour harvests in the first assay and 24 or 48 hour harvests in the second. One hundred cells from each replicate culture per dose level were analyzed when possible. For positive controls, at least 25 cells were analyzed. The percentage of cells with chromosomal aberrations, polyploidy, and endoreduplication were determined.

Results: In both trials, signs of toxicity were not observed in the cultures. Tobramycin did not induce an increase in chromosomal aberrations, polyploidy, or endoreduplication at either the 12 or 24 hour harvest in the absence of metabolic activation in the first trial. In the confirmatory assay, CHO cells were harvested 24 and 48 hours after the initiation of treatment with tobramycin. Again, no increase in chromosomal aberrations was observed. Mitomycin C induced an increase in chromosomal aberrations in the absence of metabolic activation.

In the presence of S-9, significant increases in chromosomal aberrations, polyploidy or endoreduplication were not observed in either the first (12 and 24 hour harvests) or confirmatory (24 and 48 hour harvests) assays. Cyclophosphamide induced an increase in chromosomal aberrations in the presence of metabolic activation.

Tobramycin was not clastogenic to CHO cells in the presence or absence of metabolic activation at concentrations up to 5 mg/ml.

Mutagenicity Test on Tobramycin: Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation with a Confirmatory Assay with Multiple Harvests (18080-0-437Z)

Report dated 1/8/97, U.S. GLP

Vol. 2.10, pp. 2696-2729

Method: This was a repeat of the study above using a heat-stressed solution of tobramycin for inhalation (TOBI). The concentration of the solution to be tested was 62 mg/ml and it was added to culture medium to obtain dose levels approximately 0.5, 1.25, 2.5, 3.75 and 5 mg/ml.

Results: The heat-stressed tobramycin solution did not induce chromosome aberrations in the absence or presence of metabolic activation at harvest times of 12 and 24 hours in the first assay or 24 and 48 hours in the confirmatory assay. Positive controls performed adequately.

Mutagenicity Test on Tobramycin in an *In Vivo* Mouse Micronucleus Assay (17452-0-455CO)

Report dated 6/5/96, U.S. GLP

Vol. 2.10, pp. 2730-2759

Method: Male (29.0-35.3 g) and female (21.8-27.7 g) mice were dosed intraperitoneally with tobramycin at doses of 90, 180, and 360 mg/kg. These doses were selected based upon the results of a dose-range finding study that demonstrated significant mortality at intraperitoneal doses of tobramycin $\geq$400 mg/kg. The vehicle was deionized water with sufficient sulfuric acid to adjust the pH of the dosing solution to 6.97. The dose volume was 15 mg/kg. The positive control, cyclophosphamide, was administered via oral gavage at 80 mg/kg (dose volume of 15 mg/kg). Each dose group had 5 animals per sex per time point. Tobramycin-treated mice were sacrificed 24, 48, and 72 hours after dosing. Positive control animals were sacrificed 24 hours after dosing. Bone marrow was harvested from both femurs of each mouse and pooled in bovine serum. Several slides were made for

each animal. Cells on the slides were fixed in methanol and stained with May-Grunwald solution followed by Giemsa. The slides were coded for analysis. One thousand polychromatic erythrocytes (PCEs) per animal were scored. The ratio of PCEs to normochromatic erythrocytes (NCEs) was also determined.

Results: Tobramycin was not associated with a significant increase in micronucleated PCEs harvested from mouse bone marrow compared to control at intraperitoneal doses up to 360 mg/kg at any harvest time point. The positive control, cyclophosphamide, significantly increased the percentage of mouse bone marrow PCEs with micronuclei compared to negative control (0.17 ± 0.04 vs. 3.10 ± 0.38 for the 24 hour time point).

LABELING:

The ***Carcinogenesis, Mutagenesis, Impairment of Fertility*** and ***Pregnancy*** sections should read as follows:

SUMMARY AND EVALUATION:

Rats exposed to an aerosolized 60 mg/ml solution of tobramycin for 14 days for 30, 60, or 120 minutes demonstrated no drug-related effects other than a non-dose-dependant increase in lung weights. Immediately after exposure on day 1, the mean serum levels of tobramycin for each group were approximately 7.2, 11.4, and 15.8 μg/ml with no sign of accumulation in serum on day 14. No microscopic lesions were observed in the lungs or other organs of the rats. Guinea pigs with the same dose groups and length of treatment demonstrated a non-dose-dependant increase in kidney weights, but microscopic changes were not observed in this organ. Serum tobramycin levels immediately after exposure on day 1 were approximately 4.4, 9.2, and 9.1 μg/ml for the 30, 60 and 120 minute groups respectively. Again, there was no evidence of drug accumulation in the serum on day 14. Microscopic lesions were not observed in the lungs or cochleae of the guinea pigs. Ulcers of the laryngeal or tracheal mucosa (graded minimal to slight) were observed in guinea pigs at a greater incidence in the 120 minute group than the other dose groups, but these appeared to be nonspecific lesions associated with aerosol administration as opposed to tobramycin-related damage.

Rats exposed to aerosolized 60 or 100 mg/ml solutions of tobramycin 6 hours per day for 14 days demonstrated no clinical signs of tobramycin toxicity. Serum levels immediately after exposure were approximately 14.6 and 22.5 μg/ml, respectively, and there was no evidence of accumulation in serum on day 14. Microscopic examination of the respiratory system revealed tobramycin-related lesions including necrosis of the olfactory epithelium in the nasal turbinates (particularly the posterior); mixed inflammatory cell infiltration in the nasal turbinates, larynx, and trachea; hyperplasia of laryngeal and bronchial/bronchiolar epithelium and tracheal mucosa; and accumulation of alveolar macrophages in the lung. Differences in the incidence and severity of these lesions were minimal between the 60 and 100 mg/ml tobramycin treatment groups.

Aerosolized tobramycin (60 mg/ml solution) inhaled by rats daily for 6 months for 20, 60, or 180 minutes per day did not cause drug-related mortality in these animals. Drug-related effects observed in this study included an increase in lung weights associated with hyperplasia of bronchiolar epithelium and infiltration of macrophages. Dose related increases in microscopic lesions of the respiratory system such as degeneration of olfactory epithelium (nose), hyperplasia of bronchioli, and chronic interstitial inflammation were observed, with few or no observations in the low dose group and all high dose rats affected. Chronic nephropathy (including renal tubular degeneration, mineralization, protein casts in tubular lumina, and compensatory tubular regeneration) was observed at a greater frequency in rats from the high dose group compared to control. Although chronic nephropathy is frequently observed in Sprague-Dawley rats (particularly males) as they age, tobramycin treatment appeared to shorten the time to appearance of this condition, suggesting some systemic toxicity. The systemic exposure to tobramycin received by these animals appeared to be greater than what humans receive when using TOBI as recommended. Serum levels in the high dose group (180 minutes of exposure per day) immediately after dosing averaged approximately 20 μg/ml over the course of the study. Average serum levels for the 20 and 60 minute exposure groups were 3.7 and 9.6 μg/ml, respectively. The average serum level of tobramycin in humans one hour after inhalation therapy was 1 μg/ml, so the animals in all of

the TOBI studies submitted in this NDA appear to be getting much greater systemic exposure to tobramycin than human when the drug is administered via inhalation.

Tobramycin is not well absorbed from the GI tract. Tobramycin does not undergo biotransformation, is freely filtered by the kidney and is excreted in the urine. Acute toxicity studies conducted by Lilly in support of the Nebcin IND and NDA and obtained by the current sponsor used other routes of administration such as intravenous and subcutaneous. In mice and rats, the LD50s of tobramycin using these dosing routes were:

Route	Mice	Rats
IV	53-107 mg/kg	131-134 mg/kg
SC	416-484 mg/kg	928-1020 mg/kg

Clinical signs and symptoms of tobramycin toxicity included clonic convulsions, decreased and labored respiration, hypoactivity, ataxia and prostration. Tobramycin can interfere with nerve conduction in a variety of species, including humans. Like other aminoglycosides, exposure to sufficient doses tobramycin can cause renal impairment and hearing loss (via damage to the nerve or to the cochlear hair cells). These effects are well known and the label for this product contains appropriate warnings regarding neuro-, oto- and renal toxicities. These toxicities should be limited in the clinic due to the relatively low blood levels observed in cystic fibrosis patients after TOBI administration, however, one should consider that the drug is to be used chronically.

Reproduction toxicology studies were not conducted with TOBI (i.e., via inhalation), but the sponsor acquired reports of studies that were conducted with tobramycin by Lilly using the rat and rabbit. These were submitted to the Division in the mid 1970s to support IND The studies were conducted according to the guidelines in place at that time. Additionally, the methods used are generally comparable to current ICH recommendations (the sponsor submitted an "expert report" evaluating the acceptability of these studies by current standards). However, the pharm/tox reviewer notes some deviations from current recommendations including: (1) testicular weights and histopathology were not obtained in drug-treated males, (2) serum levels of tobramycin were not measured during the reproduction studies, and (3) the acquisition of developmental landmarks and fertility of the F1 offspring were not evaluated. In the rat, tobramycin did not impair fertility in males or females at subcutaneous doses up to 100 mg/kg/day, nor were these doses associated with pre- or post-implantation loss. In rats and rabbits, subcutaneous doses of tobramycin up to 100 mg/kg (rats) or 20 mg/kg (rabbits) given during organogenesis were not associated with visceral or skeletal malformations in offspring. Doses $\geq$40 mg/kg were toxic to the rabbit dams (as is frequently observed with anti-infective compounds), leading to abortion or death in many animals and precluding the evaluation of teratogenicity. In rats, the 100 mg/kg dose was not associated with clinical signs of toxicity, but microscopic changes ("reparative nephrosis") were observed in the kidneys of the dams. Tobramycin did not appear to affect late gestation or parturition in a peri/postnatal study in rats (up to 100 mg/kg administered from gestation day 14 to postpartum day 21). There were no indications of fetotoxicity (similar fetal body weights and survival across treatment groups) and no external malformations were observed in the offspring. It should be noted that ototoxicity, which has been observed in the offspring of pregnant women taking streptomycin, was not evaluated in these reproduction toxicity studies

Appendices:
IND

with tobramycin. Although some of the methodology could have been better, the reviewer believes that the data from these studies are sufficient to write an adequate label for tobramycin. Despite the lack of toxicity observed in the offspring of rats and rabbits dosed subcutaneously with tobramycin, TOBI will be conservatively labeled Pregnancy Category D (a class label for the aminoglycosides) due to the human data demonstrating streptomycin-induced ototoxicity in pediatric patients exposed to this drug *in utero*.

Tobramycin was not mutagenic in the presence or absence of metabolic activation in the Ames bacterial reversion test conducted with *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 (concentrations up to μg/plate) and *E. coli* WP2*uvr*A (concentrations up to μg/plate). At concentrations up to 5 mg/ml, tobramycin did not induce mutations at the TK locus of L5178Y mouse lymphoma cells or induce chromosome breaks in these cells or CHO cells. Additionally, a heat-stressed solution of tobramycin was not clastogenic in CHO cells. *In vivo*, tobramycin was not associated with an increase in micronucleated polychromatic erythrocytes in bone marrow from mice dosed with up to 360 mg/kg intraperitoneally. Thus, tobramycin does not appear to be a genotoxic compound.

RECOMMENDATION: The pharmacologist has no objection to the approval of this NDA for TOBI (tobramycin for inhalation) for the management of patients with cystic fibrosis who are infected with *P. aeruginosa*. As part of a Phase IV commitment, the sponsor will be submitting data

The protocol for this study has been reviewed by the CDER Executive Carcinogenicity Assessment Committee was found acceptable. The study is in progress.

/S/

Amy L. Ellis, Ph.D.
Pharmacologist, HFD-520

Concurrence Only:
HFD-520/REOsterberg 12/19/97
HFD-520/LGavrilovich 12/22/97

Orig. NDA
cc:
HFD-520
HFD-520/Pharm Team Ldr/Osterberg
HFD-520/Pharm/Ellis
HFD-590/Pharm/McMaster
HFD-520/MO/Alexander
HFD-590/MO/M. Mann
HFD-520/Chem/Pagay
[redacted]
HFD-520/Micro/King

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 50-753

STATISTICAL REVIEW(S)

STATISTICAL REVIEW AND EVALUATION

DEC 5 1997

NDA#:	50-753
APPLICANT:	Pathogenesis Corp.
NAME OF DRUG:	Tobramycin® Solution for Inhalation (TOBI®)
INDICATION:	Improvement of Pulmonary Function in Cystic Fibrosis Patients
TYPE OF REVIEW:	Clinical
DOCUMENTS REVIEWED:	Volume 5.1, 9.11, 9.13, 9.22
MEDICAL INPUT:	John Alexander, M.D. (HFD-520), Marianne Mann, M.D. (HFD-590)

STATISTICAL REVIEW AND EVALUATION

NDA#: 50-753

1. Background

1.1 Overall Objectives

The applicant submitted two randomized, double blind, placebo controlled clinical trials with Tobramycin (TOBI).

The primary objective of these trials was to determine the effects of repeated, intermittent, short-term therapy with Tobi on pulmonary function tests (PFT's) and colony forming units (CFU's) of Pseudomonas aeruginosa. The study population was patients with cystic fibrosis (CF) who were chronically colonized with P. aeruginosa.

1.2 Summary of Study Design

The two trials were of identical design and used the same inclusion/exclusion criteria. They differed only in the sites at which patients were studied. Both studies were double-blind, randomized, two-arm, parallel, placebo-controlled multi-center trials. Both studies were designed to have subjects treated for three cycles, each cycle consisting of four weeks on therapy followed by four weeks off therapy. 334 subjects were enrolled in trial 003 and 244 in trial 002. Patients were to be $\geq$ 6 years old, have clinical confirmed cystic fibrosis, have FEV_1 levels between 25% and 75% of those predicted on the basis of age and sex, and to have P. aeruginosa in sputum within 6 months of screening.

Patients were randomly assigned to one of the two treatment arms in 1:1 ratio. Randomization was implemented by a minimization algorithm whose purpose was to balance on six factors: 1) age group (6-12 years, 13-18 years, >18 years), 2) concomitant recombinant human dornase alpha (rhDNase) therapy (yes or no), 3) sputum production (yes or no), 4) P. aeruginosa minimum inhibitory concentration (MIC) to tobramycin ($<$ or $\geq$ 8 μg/mL), 5) FEV_1 % predicted (25-50% or 50-75%), and 6) site. The important details of the minimization algorithm are discussed in technical appendix A.1. 29 of the 69 sites were specified to constitute the sites for protocol 002 and the other 40 sites were specified to constitute the sites for protocol 003.

Patients who failed the screening test were withdrawn but were eligible for re-screening as new entrants to the study. Subjects withdrawn before drug administration were not counted in the scoring algorithms used to achieve balance.

Patients were withdrawn from the study for patient choice, adverse event, or initiation of unapproved therapy (rhDNase or Thairapy® Vest). Withdrawn patients were supposed to return for a follow-up visit.

1.3 Patient Accounting and Baseline Characteristics

In trial 002, 223 patients began treatment from 29 centers. Of these, 109 were randomized to Tobi, and 114 to placebo. In trial 003, 297 patients began treatment from 40 centers. Of these, 149 were randomized to Tobi, and 148 to placebo. The study populations in the two arms across both trials were 49-58% male, had a mean age of 20-21 years with a range of . 65-74% used rhDNase therapy, 45-49% had FEV_1 below 50% of the Knudsen predicted value, 83-88% had tobramycin MIC for *P.aeruginosa* < 8 μg/mL. Table 1.3 A gives the number of patients still in the trial at each cycle.

TABLE 1.3 A
SUBJECTS COMPLETING EACH CYCLE

	Tobi	Placebo
TRIAL 002		
Enrolled	122	122
Baseline Data	119	117
Began Treatment	109	114
Completed Cycle 1	105	106
Completed Cycle 2	99	101
Completed Cycle 3	96	100
TRIAL 003		
Enrolled	164	170
Baseline Data	156	157
Began Treatment	149	148
Completed Cycle 1	144	141
Completed Cycle 2	138	136
Completed Cycle 3	136	132

Table 1.3 B summarizes the primary reasons for discontinuation from treatment.

TABLE 1.3 B
REASONS FOR TREATMENT DISCONTINUATION

	TRIAL 002		TRIAL 003	
	TOBI	Placebo	TOBI	Placebo
Randomized	109	114	149	148
Completed 3 cycles	96 (88%)	100 (88%)	136 (91%)	132 (89%)
Discontinued Reason	13	14	13	16
Adverse event	0	1	3	1
Medical complaint	4	6	4	9
Died	0	1	0	1
Lost to follow-up	2	2	1	0
Other	7	4	5	5
Discontinued Time				
Cycle 1	4	8	5	7
Cycle 3	6	5	6	5
Cycle 3	3	1	2	4

1.4 Summary of Methods of Assessment

Patients were seen every 2 weeks from 4 weeks before the start of cycle 1 through week 8 (=beginning of cycle 2) and every 4 weeks thereafter until week 24 (=end of cycle 3 + 4 weeks). At each visit, pulmonary function tests (PFT's) were conducted and sputum samples were collected from those patients who produced sputum. Bacterial densities in log CFU's (colony forming units) were measured from these samples. Audiology data for ototoxicity determinations were obtained every four weeks.

The applicant converted two PFT's (FEV_1 and FVC) to percent of predicted by using the Knudsen equations to determine the predicted PFT from age, height, and sex and dividing the observed PFT by this predicted value.

1.5 Summary of Statistical Analysis

1.5.1 Primary Efficacy Analyses

An analysis of variance (ANOVA) was used to assess the treatment effect on each of the PFT's expressed as percent of predicted and on log CFU. The applicant computed the relative change from baseline to end of treatment in PFT's and absolute change from baseline to end of treatment in log(CFU). The ANOVA did not adjust for covariates (i.e. it was a Student t-test).

In addition, a multivariate (or repeated measures) analysis of variance (MANOVA) was used to assess the treatment effect on each of the PFT's and on log CFU. The applicant computed the relative change from beginning to end of each 28 day cycle, thereby summarizing each subject's time course of responses as three numbers. The MANOVA was performed on these three numbers as dependent variables with tests for treatment effect, cycle effect, and treatment-cycle interaction. Treatment effects on the changes within each of the three cycles were also compared by the Student t-test.

1.5.2 Secondary Efficacy Analyses

Use of non-aerosol anti-Pseudomonal antibiotics was analyzed by calculating the number of days a patient was on such antibiotics over each cycle and over the entire 168 day study period. The Wilcoxon rank sum test was used to test for differences in these numbers of days between the two treatment arms. Cox proportional hazards regression was used to compare time until first use of antibiotics.

Number of days hospitalized for any reason and for lower respiratory tract disease and number of days missed from work or school were also compared by the Wilcoxon test. (In what follows, all references to hospitalization will mean for lower respiratory illness, unless stated otherwise; all references to IV antibiotic use will mean IV or oral anti-Pseudomonal antibiotics even if the words oral and anti-Pseudomonal are omitted.)

Investigator's subjective assessment of patients as "unchanged", "better", or "worse" were compared by the Cochran-Mantel-Haenszel test using sites as strata.

Subgroup analyses were performed by age group, gender, level of baseline FEV_1 as % of predicted, concomitant use of rhDNase, and concomitant use of anti-Pseudomonal antibiotics.

Analyses of safety data paid particular attention to several events. Arms were compared for drug-induced bronchospasm, defined as a 15% decrease in FEV_1 at 30 minutes after administration of drug, by signed rank tests on the changes in FEV_1 at 30 minutes after drug at visits 3 and 10. Arms were compared for ototoxicity by a Fisher exact test on the proportion of subjects with a change in audiology $\geq$ 20 dB at any time in the study. Repeated measures ANOVA on serum creatinine values was used to compare arms for nephrotoxicity.

The emergence of resistance was studied by measuring minimum inhibitory concentrations (MIC), MIC_{50}, and MIC_{90} for tobramycin at each visit. The same three quantities were also computed for eight other antibiotics (gentamicin, ticarcillin, ceftazidime, trimethoprim, chloramphenicol, amikacin, aztreonam, and ciprofloxacin). Only descriptive statistics were computed for these quantities. In addition, the percentages of patients with tobramycin MIC $\geq$ 128μg/mL and $\geq$ 8 μg/mL were compared between arms at baseline and at the end of each study cycle.

2. Summary of Applicant's Results

2.1 Primary Efficacy Variables:

Both trials showed statistically significant improvement in change of PFT's and of log(CFU) from baseline (visit 3) to end of treatment (visit 10). These results are summarized in table 2.1 A. In this table, change in PFT's is percent change from baseline; change in log(CFU) is absolute change from baseline. P-values are computed by Student t-test.

TABLE 2.1 A
RELATIVE CHANGES IN PRIMARY ENDPOINTS

	Protocol 002			Protocol 003		
	Mean Change		P-value	Mean Change		P-value
Endpoint	TOBI	Placebo	for Diff.	TOBI	Placebo	for Diff.
FEV_1%Pred	12.02	-.52	<.001	8.70	-2.72	<.001
FVC%Pred	8.72	-.89	.001	7.07	-1.55	<.001
log(CFU)	-.87	.30	<.001	-.62	.37	<.001

As can be seen from figures 6.4 and 6.5 in the applicant's Integrated Summary of Efficacy (vol. 9.11), both PFT's in both protocols rose between visit 3 and visit 4 in the TOBI arm and remained elevated in these arms until the end of the study, without returning to baseline during the two-week off-drug phases of each cycle.

In contrast, as can be seen from figure 6.6 in vol. 9.11, in both protocols the log(CFU) decreased in the TOBI arm during the on-drug phase and returned to baseline during the off-drug phase of each cycle. Moreover, the decrease during the on-drug phase was less in each successive cycle.

In both trials, the repeated measures analysis also showed a statistically significant increase in FEV_1 and FVC percent predicted for TOBI compared to placebo, with p-value < .001. There were significant treatment-cycle effect as well: cycle 1 showed a significant increase in FEV_1 from value at the beginning of the cycle for TOBI compared to placebo but the other cycles did not show an increase from the value at the beginning of the cycle for either treatment. The FDA reviewer notes that the applicant failed to observe that these data together with figures 6.4 and 6.5 actually support the inference of a sustained TOBI effect. Because the FEV_1 and FVC did not decrease back to baseline from its level at the end of cycle 1 but rather remained constantly elevated relative to placebo levels between cycles, there was little change between beginning and end of later cycles.

The repeated measures analysis also showed a statistically significant decrease in colony forming units (CFU's) for TOBI relative to placebo (p < .001 in each protocol). Because at the end of each cycle, log CFU returned nearly to baseline for TOBI, the tests for a treatment effect in each cycle were also

significant. These significant effects showed be interpreted as the converse to the non-significant effects found for PFT's in the second and third cycles. Significant treatment effects in change from beginning to end of later cycles are evidence that the TOBI is not sustained during the off-drug phase of the previous cycle.

The applicant also performed subgroup analyses stratifying by age, gender, disease severity, concurrent rhDNase use, or concurrent anti-Pseudomonal antibiotic use. Details are not reproduced here because no suggestions of treatment-covariate interactions were found.

2.2 Secondary Efficacy Variables:

The two protocols differed noticeably on the endpoint of time to hospitalization for lower respiratory illness. The results of Cox proportional hazards regression for risk of hospitalization are given in table 2.2 A. This table gives the percent of patients hospitalized by the end of the study and the point estimates and 95% confidence intervals for the TOBI/placebo relative risk of hospitalization for lower respiratory illness. Here relative risks < 1 are favorable to TOBI. It also gives the mean number of days spent in the hospital and the p-value for treatment effect on the number of days spent in the hospital.

TABLE 2.2 A
RELATIVE RISKS & DURATIONS OF HOSPITALIZATION
FOR LOWER RESP. ILLNESS, TOBI/PLACEBO

	Protocol 002		Protocol 003		Pooled	
	TOBI	Placebo	TOBI	Placebo	TOBI	Placebo
% Hospitalized	28%	45%	43%	45%	37%	45%
Relative Risk	.557		.891		.744	
95% Con. Limits	.356-.871		.631-1.258		.567-.975	
Days Hospitalized	46	98	38	63	46	98
P-value for Days	.008		.57		.03	

For percent of patients hospitalized by the end of the study, there was a statistically significant reduction in risk with TOBI in protocol 002 alone and in both protocols together, but for protocol 003, the relative risk might have been anywhere from .631 to 1.258. Likewise, the cumulative number of days spent in the hospital was statistically significantly lower for

TOBI than for placebo in protocol 002 and in both protocols pooled together but was not significantly different in protocol 003.

The two protocols also differed, although to a lesser extent, on the endpoint of time to IV anti-Pseudomonal antibiotic use. The results of Cox proportional hazards regression for risk of IV antibiotic use are given in table 2.2 B along with the mean number of days on such IV or oral antibiotics and the p-value for treatment effect on the number of days.

TABLE 2.2 B
RELATIVE RISKS & DURATIONS OF ANTI-PSEUDOMONAL ANTIBIOTIC USE, TOBI/PLACEBO

	Protocol 002		Protocol 003		Pooled	
	TOBI	Placebo	TOBI	Placebo	TOBI	Placebo
% Antibiotic Use	33%	54%	43%	50%	39%	52%
Relative Risk	.512		.746		.640	
95% Con. Limits	.339-.773		.533-1.045		.494-.830	
Days Antibiotics	20	30	28	34	25	32
P-value for Days	.001		.08		<.001	

For percent of patients with antibiotic use, there was a statistically significant reduction in risk with TOBI in protocol 002 alone and in both protocols together. For protocol 003, the relative risk might have been anywhere from .533 to 1.045, a confidence which is more supportive of a true relative risk less than 1 than was the interval for relative risk of hospitalization. Likewise, the cumulative number of days with antibiotic use was statistically significantly lower for TOBI than for placebo in protocol 002 and in both protocols pooled together. In protocol 003, the mean number of days on IV-PO antibiotics was less, with a p-value of .08. Again, this was a larger TOBI effect than was seen in this protocol for days of hospitalization.

Investigator assessment of improvement, scaled as worse, unchanged, or better, did show statistically significantly better responses for TOBI than placebo at the end of study assessment, with 33% vs 18% better and 14% vs 23% worse in protocol 002 (p-value = .005), and with 34% vs 17% better and 13% vs 24% worse in protocol 003 (p-value <.001).

2.3 Emergence of Resistance

Both protocols were pooled together for the purpose of analyzing increases in minimum inhibitory concentration (MIC) to tobramycin and other aminoglycosides of Pseudomonas isolates from patients. Increased resistance to patients on the TOBI arm was demonstrated by several criteria. 16% of TOBI patients experienced at least a four-fold increase in the MIC of their most resistant isolate between visits 3 and 10, compared to only 9% of placebo patients. MIC_{50}, based on the highest MIC isolate in each individual, started at the same value for TOBI as for placebo, but doubled by visit 10 for TOBI while remaining unchanged for placebo. MIC_{90} increased four-fold for TOBI and remained unchanged for placebo. No tests of statistical significance were reported with respect to the above findings. The percent of patients with MIC > 8 μg/mL increased from 14% to 26% with TOBI compared to an increase from 11% to 18% with placebo. This latter difference in treatment arms was statistically significant with a p-value of .03 by Fisher's exact test. Similarly, the percent of patients with MIC $\geq$ 128 μg/mL increased from 2% to 7% with TOBI compared to an increase from 2% to 4% with placebo. This latter difference in treatment arms had a p-value of .14 by Fisher's exact test.

3. Summary of Applicant's Conclusions

The applicant has concluded that TOBI, administered at 300 mg bid in intermittent 28-day treatment periods, is a safe and effective agent for the improvement of pulmonary function in patients with cystic fibrosis. TOBI treatment was associated with an improvement in FEV_1 % predicted of 11% relative to placebo. Improvements in PFT's were demonstrated regardless of age, gender, disease severity, concurrent rhDNase use, or concurrent anti-Pseudomonal antibiotic use.

Treatment with TOBI was also associated with a decrease in the incidence of several adverse experiences. No safety problems with the exceptions of tinnitus and voice alteration were observed.

4. Statistical Reviewer's Comments and Analyses

There are several issues in the applicant's report that need to be addressed. These include 1) the choice of clinically meaningful primary endpoints, 2) the method of assigning subjects to arms of the study, 3) the emergence of resistance, 4) the use of an appropriate summary test for primary endpoints measured over time, and 5) the determination of an appropriate level of baseline resistance above which usage should not be recommended.

With respect to the first point, the applicant used laboratory parameters, FEV_1, FVC, and log(CFU), as the primary efficacy measures. The FDA clinical reviewer has recommended that more clinically meaningful measures would be the times to first hospitalization for lower respiratory illness and to first use of anti-Pseudomonas IV or oral antibiotics. These alternative endpoints are discussed in detail in section 4.1 below.

With respect to the second point, the applicant used a minimization algorithm to assign patients to treatment. The use of minimization makes the calculation of p-values using standard methods difficult to justify. Technical comments about this issue are discussed in section 4.5 below.

With respect to the third point, the FDA clinical reviewers have expressed concern about the emergence of resistance to TOBI and other aminoglycosides with continued use of TOBI. This issue is discussed in detail in section 4.2 below.

With respect to the fourth point, the applicant has conducted an analysis that can either overstate or understate the magnitude of the treatment effect on the improvement in PFT's and CFU's over the duration of the study. An FDA re-analysis that does not suffer from these potential misstatements is given in section 4.3 below.

With respect to the fifth point, the applicant has observed that no improvement was found in the four subjects with baseline MIC of 128 and have taken that fact as supporting a cutpoint of 128. The FDA re-analysis has looked in more detail at several response variables as functions of treatment and baseline MIC. This is given in section 4.4 below.

4.1 Analyses of Times to Hospitalization and Antibiotic Use

The FDA medical reviewer considers times to hospitalization for lower respiratory illness and for IV or oral anti-Pseudomonal antibiotic use to be more clinically relevant measures than the applicant's choice of primary endpoints. (In what follows, all references to hospitalization will mean for lower respiratory illness, unless stated otherwise; all references to IV antibiotic use will mean IV or oral anti-Pseudomonal antibiotics even if the words oral and anti-Pseudomonal are omitted.) The FDA statistical reviewer has computed Kaplan-Meier curves for both of these endpoints for all subjects and for subjects subdivided by 1) protocol, 2) age category, 3) sex, 4) baseline FEV_1 category, 5) prior rhDNase use, 6) baseline MIC category, 7) hospitalization within 6 months prior to study, 8) anti-Pseudomonal antibiotic, and 9) number of patients per site. The last criterion is not truly a covariate of the patient but may be a surrogate for patient health if sicker patients tend to go to more active centers. On the other hand, it may be a surrogate for the experience level of center. Approximately half of the patients were recruited at centers with at least 9 patients so subgroups were defined as those from centers with ≥ 9 patients and those from centers with ≤ 8 patients.

The curves for all subjects and subdivided by protocol are given in figures 4.1 i-iv below. These curves show an apparent superiority of TOBI relative to placebo in delaying times to both hospitalization and IV antibiotic use when all subjects are pooled and in protocol 002 alone. In protocol 003, there is no difference in the times to hospitalization and a smaller difference than in protocol 002 for the times to IV antibiotic use.

In order to determine whether these observed differences were statistically significant, the FDA reviewer computed both

Wilcoxon-Gehan and log-rank tests, as described in technical appendix A.2. The p-values for these tests are given in table 4.1 A. Regardless of the test used, TOBI showed statistically significant decreases in time to hospitalization and in time to IV antibiotic use in protocol 002 alone and in both protocols pooled together. In protocol 003 alone, there was no difference in times to hospitalization (as can be seen in figure 4.1 ii below) and the observed difference (seen in figure 4.1 iv below) in times to IV antibiotic use had p-values of .11 to .22.

TABLE 4.1 A
P-VALUES FOR TESTS OF TREATMENT EFFECT ON TIMES TO HOSPITALIZATION, ANTIBIOTIC USE

	WILCOXON TEST PROTOCOL			LOG-RANK TEST PROTOCOL		
ENDPOINT	002	003	BOTH	002	003	BOTH
DROP-OUTS CENSORED						
HOSPITALIZATION	.029*	.60	.11	.13	.44	.16
IV ANTIBIOTIC USE	.0015*	.18	.004*	.016*	.22	.019*
DROP-OUTS AS FAILURES						
HOSPITALIZATION	.02*	.49	.06	.11	.38	.12
IV ANTIBIOTIC USE	.001*	.11	.003*	.016*	.17	.016*

There is one additional issue to be addressed with respect to these time to event analyses. A small number of subjects are lost to follow-up before the end of the trial. Table 4.1 B shows the number of subjects who were censored with respect to either time to hospitalization or time to IV/PO antibiotic use at times ranging from day 1 to day 138. (All of the patients censored for IV antibiotic use were also censored for hospitalization. The other three patients censored on hospitalization were observed to need IV antibiotics before censoring.)

TABLE 4.1 B
EARLY CENSORING ON HOSPITALIZATION/IV ANTIBIOTIC USE

	# Censored before Day 140			
	Hospitalization		IV Antibiotic Use	
Protocol	TOBI	Placebo	TOBI	Placebo
002	9	10	9	8
003	7	9	6	9

For these 37 patients, there is a question as to whether they should be considered as censored or as failures at the time

of loss to follow-up. The Kaplan-Meier curves, the Wilcoxon and log-rank tests, and proportional hazards regressions all assume that when a subject is censored, the time until his failure has the same distribution as the time until failure for those subjects still being observed. If subjects are lost to follow-up because they moved to another city or because the trial came to an end, then this censoring assumption is appropriate. If subjects are lost to follow-up during the trial because they are sicker than those subjects remaining in the trial, then the censoring assumption is invalid. Since such subjects would be expected to fail sooner than the subjects still being observed, one may do a conservative correction for such drop-outs by treating them all as having failed on the last day they were observed. The results of this more conservative analysis are given in table 4.1 A above, under the heading 'Drop-outs as Failures'. There is no consequential changes in the conclusions with this conservative analysis.

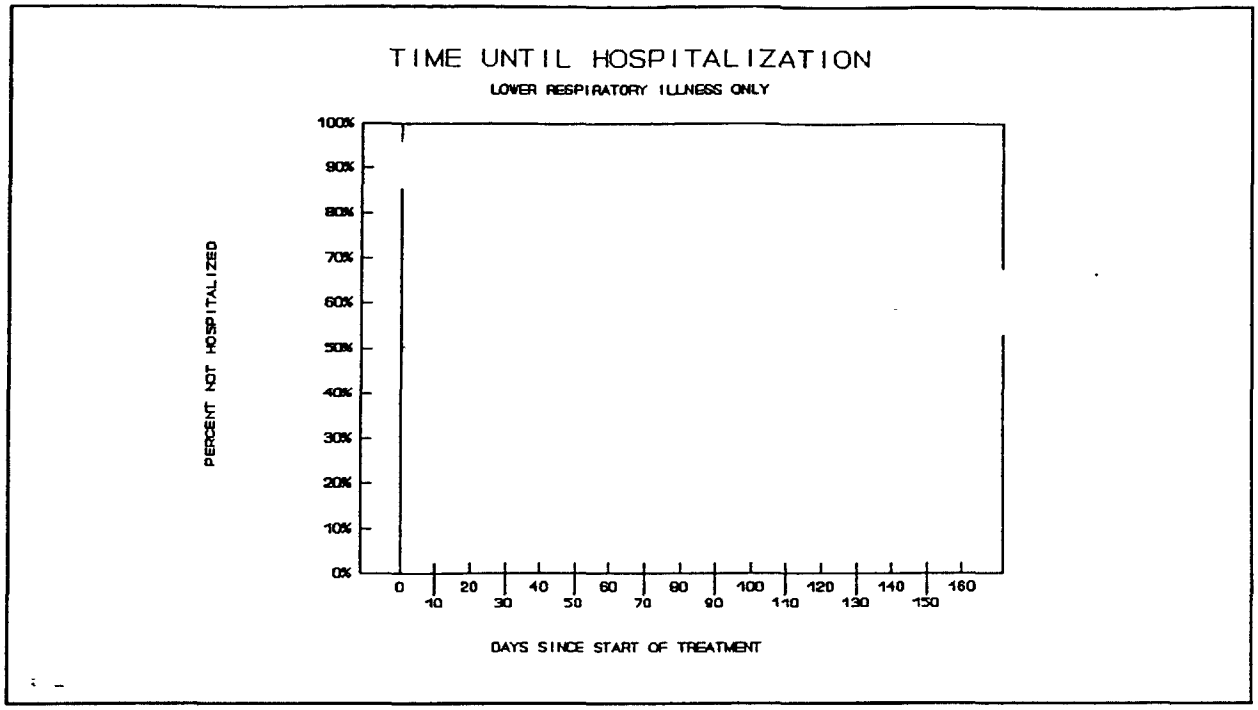


Figure 4.1 i

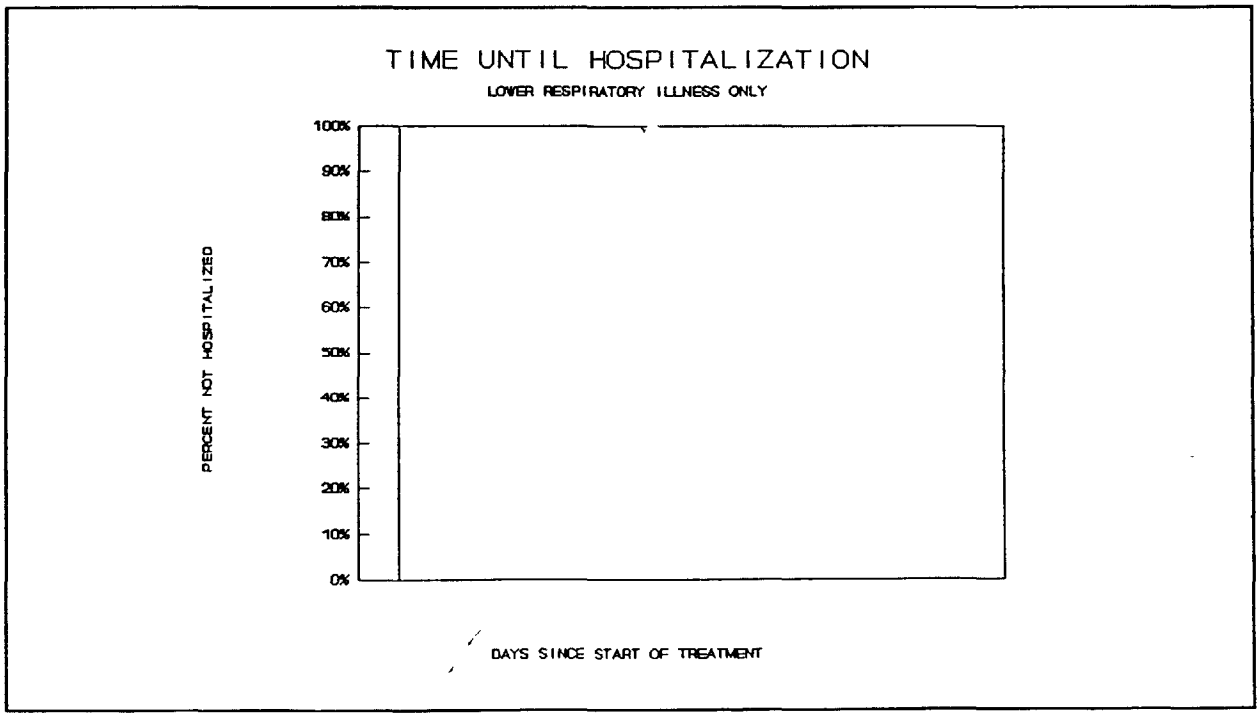


Figure 4.1 ii

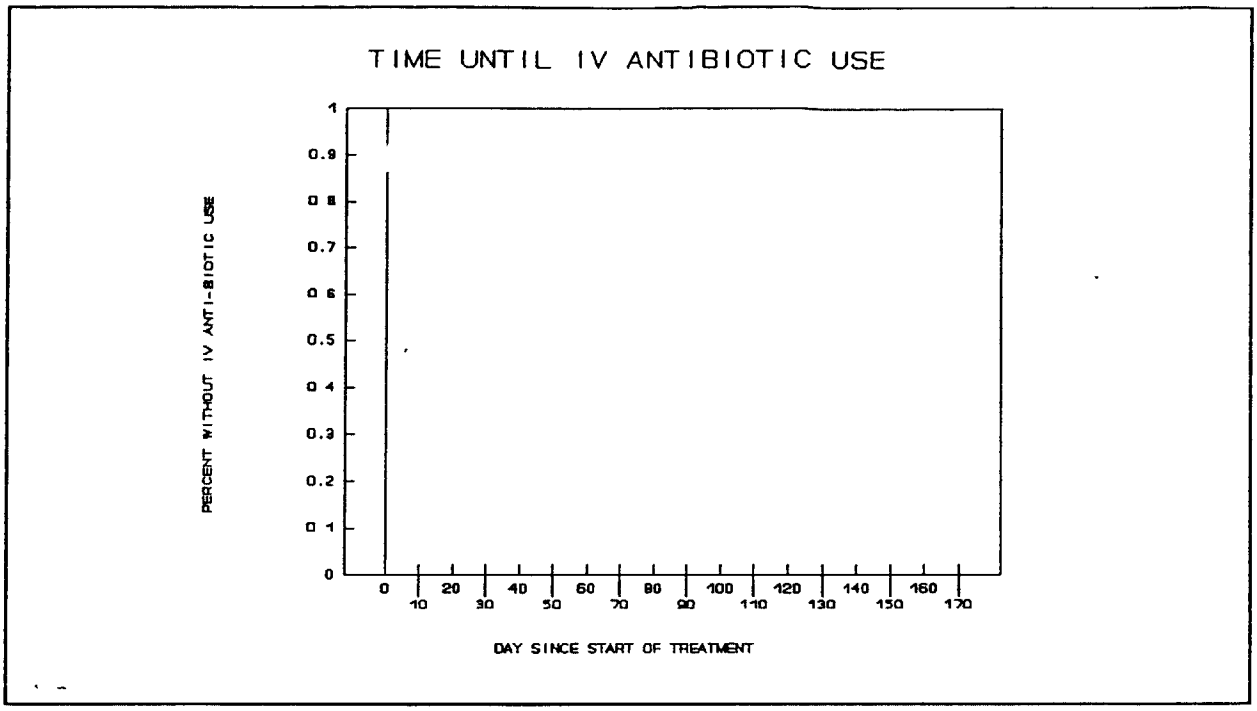


Figure 4.1 iii

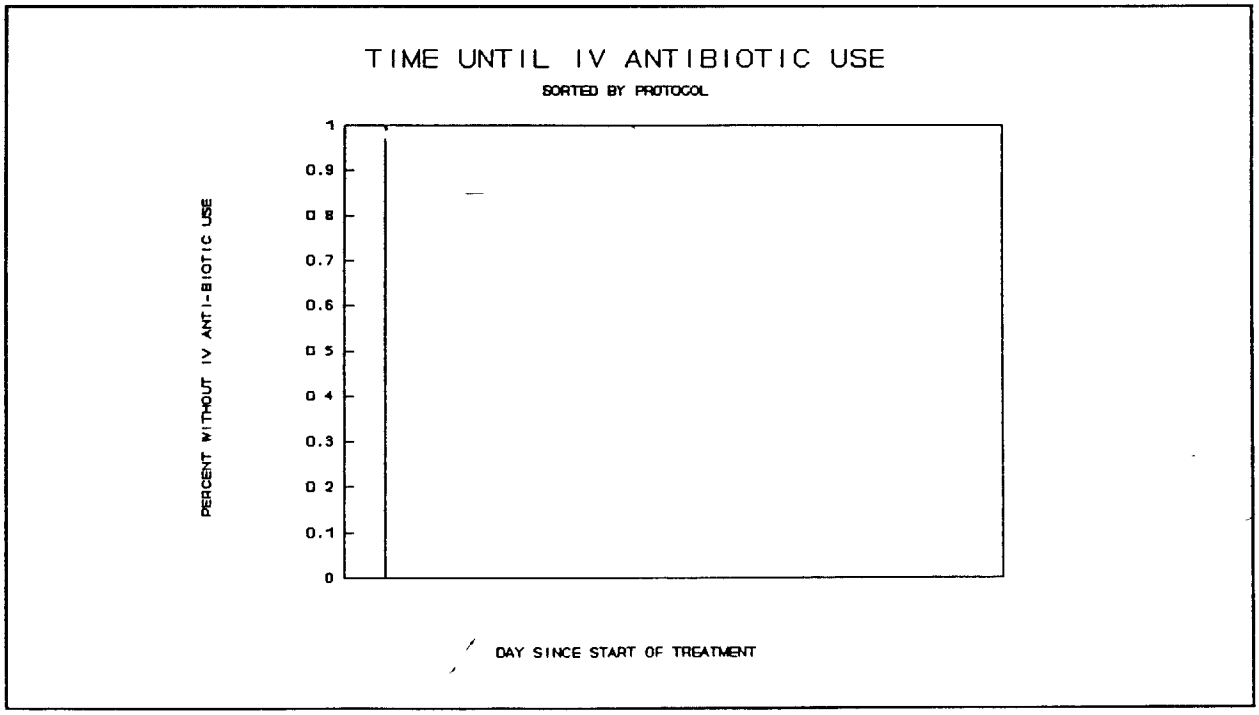


Figure 4.1 iv

Because there was no difference in the inclusion-exclusion criteria in the two protocols, both protocols were pooled together prior to be subdivided on the basis of baseline covariates. In the interest of brevity, the curves for times to hospitalization and antibiotic use stratified by 1) age category, 2) sex, 3) baseline FEV_1 category, 4) prior rhDNase use, 5) baseline MIC category, 6) hospitalization within 6 months prior to study, 7) anti-Pseudomonal antibiotic use within 6 months prior to study, and 8) number of patients per site are not reproduced here. They may be briefly summarized as follows.

For time to hospitalization for lower respiratory illness, the largest treatment effect was seen in the 6-12 years old age group with little treatment effect in the other two age groups. There was a treatment difference observed in females but not in males; with baseline FEV_1 > 50% predicted but not with lower baseline FEV_1; with baseline MIC < 8 but not greater; with prior rhDNase use but not without. There was a slight treatment difference in subjects without prior hospitalization and in those without prior antibiotic use but a larger treatment effect in those with prior hospitalization or with prior antibiotic use. There was an observed TOBI superiority among subjects with prior rhDNase use but a slight TOBI inferiority among subjects without prior rhDNase use. There was a treatment effect among subjects recruited from centers with 9 or more subjects but no treatment effect among subjects recruited from centers with 8 or fewer subjects. These subgroup analyses are exploratory and should not be taken as confirming covariate-treatment interactions.

For time to IV antibiotic use, the longer times for the TOBI arm were more obvious at all levels of each of the covariates examined with two exceptions. As with time to hospitalization, there was a treatment effect among subjects recruited from centers with 9 or more subjects but no treatment effect among subjects recruited from centers with 8 or fewer subjects. Also, there was a treatment effect among subjects with prior rhDNase use but none among subjects without prior rhDNase use. Unlike the case for time to hospitalization, there was overall less suggestion of interactions between treatment and baseline covariate.

In order to pursue the issue of potential treatment-covariate interactions, exploratory proportional hazards

regressions were run, using these covariates and their interactions with treatment as predictor variables. The results are given in tables 4.1 C and D below. In these tables, hazard ratios less than one correspond to longer times until hospitalization or IV antibiotic use with TOBI than with placebo. Upper confidence bounds below 1 correspond to treatment differences which were statistically significant even within subgroups. The reviewer tested for differences in treatment effect between the strata by forming t-statistics for the differences in the hazard ratios between strata, using the standard errors for the log hazard ratios estimated by the proportional hazards regression.

The important point from these tables is that there is only one statistically significant differences in the hazard ratios for time to hospitalization or time to IV antibiotic use between any of the levels of baseline covariates tested. The estimated hazard ratios were consistently less than one (longer times for TOBI). Within subgroups, statistical significance was not usually achieved for times to hospitalization (upper limits were usually > 1) but frequently was achieved for times to IV antibiotic use (upper limits < 1). The one baseline covariate which did have a statistically significant interaction with treatment was size of the site. Patients recruited from sites with at least 9 patients had statistically significantly greater benefit from TOBI.

TABLE 4.1 C

PROPORTIONAL HAZARDS REGRESSION FOR TIME TO HOSPITALIZATION

INTERACTIONS WITH BASELINE COVARIATES

	Hazard Ratio	95% Con Limits Lower	Upper	P-value for Difference in Treatment Effect between Strata
SEX=M	0.885	0.595	1.314	.32
SEX=F	0.662	0.439	0.999	
FEV_1<.5	0.886	0.610	1.289	.24
FEV_1>.5	0.628	0.406	0.971	
MIC<8	0.741	0.539	1.020	.76
MIC>8	0.823	0.442	1.532	
6-12	0.614	0.340	1.110	.36[1]
13-18	0.897	0.514	1.564	.67[2]
>18	0.771	0.518	1.146	
No rhDNase Use	1.22	0.61	2.42	.13
rhDNase Use	0.68	0.50	0.94	
No Prior Hosp	0.595	0.344	1.028	.28
Prior Hosp	0.845	0.607	1.176	
No Prior AB	0.682	0.334	1.392	.74
Prior AB	0.777	0.571	1.057	
<9 Pat/Site	1.045	0.672	1.625	.057
≥9 Pat/Site	0.596	0.411	0.865	

[1] Comparison of 6-12 with 13-18

[2] Comparison of 13-18 with >18

TABLE 4.1 D
PROPORTIONAL HAZARDS REGRESSION FOR TIME
TO IV ANTIBIOTIC USE
INTERACTIONS WITH BASELINE COVARIATES

	Hazard Ratio	95% Con Limits Lower	Upper	P-value for Difference in Treatment Effect between Strata
SEX=M	0.668	0.465	0.960	.71
SEX=F	0.608	0.417	0.886	
FEV_1<.5	0.613	0.434	0.866	.87
FEV_1>.5	0.640	0.431	0.951	
MIC<8	0.603	0.452	0.806	.60
MIC>8	0.720	0.393	1.321	
6-12	0.549	0.311	0.969	.51[1]
13-18	0.711	0.426	1.188	.69[2]
>18	0.626	0.438	0.895	
No rhDNase Use	0.86	0.47	1.57	.27
rhDNase Use	0.59	0.44	0.79	
No Prior Hosp	0.493	0.307	0.789	.22
Prior Hosp	0.700	0.512	0.957	
No Prior AB	0.454	0.238	0.866	.27
Prior AB	0.676	0.509	0.898	
<9 Pat/Site	0.803	0.547	1.180	<.001
≥9 Pat/Site	0.526	0.369	0.750	

[1] Comparison of 6-12 with 13-18
[2] Comparison of 13-18 with >18

The proportional hazards regression assume that the hazard ratio is a constant over time. The FDA reviewer checked this assumption for the above regressions by plotting the non-simultaneous 95% confidence limits for the log hazard ratio at time t, estimated by the difference between TOBI and placebo values of log(-log(Kaplan-Meier survival curve for given arm and covariate level)). If the log hazard ratio is in fact constant over time, then it should be possible to draw a horizontal line between these upper and lower limits. In most cases, it did

appear possible to draw such a horizontal line. In the interest of brevity, these plots are not reproduced here.

4.2 Development of Resistance During Treatment

The FDA clinical reviewers have expressed concern that resistance to Tobramycin and related antibiotics will develop over time with the use of TOBI. In particular, increases in MIC (minimum inhibitory concentration) to absolute values over 8 μg/mL and to values 8 times larger than baseline have been identified as having clinical importance. Furthermore, if such increases occur more frequently in the TOBI arm, it would be clinically useful to identify any baseline covariates which may be predictive of such large increases. The FDA statistical reviewer has analyzed the development of increased resistance by comparing the absolute MIC's at the end of the study and the changes in MIC from baseline to end of study.

The applicant reported that there were quality control problems with some of the initial MIC measurements. Therefore, the data used in the FDA analyses were all repeat measurements of MIC plus all original measurements made after July 13, 1996 (the date at which the quality control problem was corrected). For subjects who had multiple MIC measurements at a given visit, the maximum value at that visit was used in the analyses described below. Visit 3 was used as the baseline. The maximum of visit 10 and visit 11 was used as the end of study measurement. Some analyses were also done using only visit 10 data or only visit 11 data to determine whether conclusions were sensitive to which visit was used as end of study.

Table 4.2 A gives the percent of subjects who experienced 2 fold, 4 fold and 8 fold increases from baseline in MIC by arm and the percent who had final MIC >= 2, 4 or 8 μg/mL. Results of chi-square tests for differences between the arms are also given. The table shows that the TOBI arm had statistically significantly larger percent of subjects with 2, 4, and 8 fold increases in MIC, relative to baseline, with 11-14% more TOBI subjects than placebo subjects experiencing the given increase.

TABLE 4.2 A

PERCENTS WITH 2, 4, 8-FOLD INCREASES BY ARM
WITH P-VALUES FOR DIFFERENCE BETWEEN ARMS

	TOBI	PLACEBO	P-VALUE
8 FOLD INCREASE	17%	5.7%	<.0001
4 FOLD INCREASE	32%	18%	.0003
2 FOLD INCREASE	53%	42%	.02
FINAL MIC $\geq$ 8 μg/mL	31%	16%	<.0001
FINAL MIC $\geq$ 4 μg/mL	54%	36%	<.0001
FINAL MIC $\geq$ 2 μg/mL	68%	56%	.007

It would be clinically useful to be able to identify subjects more likely to experience such increases beforehand. Table 4.2 B shows the frequency of 8-fold increases from baseline by arm and the difference between placebo and TOBI percents, stratified by several baseline covariates: sex, age group, hospitalization within 6 months of study entry, antibiotic use within 6 months of study entry, baseline FEV_1 as a percent of predicted, and protocol. For each covariate, the FDA reviewer performed Mantel-Haenszel tests for a treatment effect, adjusted for the baseline covariate and for differences in the treatment effect at different levels of the covariate (i.e. chi-square test for homogeneity of treatment effects). The TOBI and placebo arms were always statistically significantly different. However, no statistically significant difference in treatment effects at different levels of the covariate was found for any of the covariates tested. In other words, none of the covariates was a statistically significant predictor of the size of the difference between TOBI and placebo.

TABLE 4.2 B

PERCENT WITH 8-FOLD INCREASE IN MIC FROM BASELINE
BY ARM AND BASELINE COVARIATE

	TOBI	PLAC	DIFFERENCE
AGE			
6-12	16.1%	3.3%	12.8%
13-18	20.6%	9.0%	11.7%
>18	16.4%	5.2%	11.2%
PRIOR HOSPITALIZATION			
NO	16.5%	7.9%	8.6%
YES	18.4%	3.7%	14.7%
PRIOR ANTIBIOTIC USE			
NO	16.7%	8.7%	8.0%
YES	17.6%	4.7%	13.0%
BASELINE MIC			
<=1	23.9%	7.7%	16.2%
2-4	19.6%	6.9%	12.7%
>=8	9.4%	4.2%	5.2%
BASELINE FEV_1			
<50%	17.1%	4.7%	12.4%
>50%	17.6%	6.7%	10.9%
SEX			
M	14.7%	6.8%	7.8%
F	21.1%	4.6%	16.5%
PROTOCOL			
002	14.7%	6.1%	8.5%
003	19.3%	5.4%	13.9%

The general pattern was the same with visit 10 data only, with visit 11, and with the maximum of visit 10 and visit 11 serving as the end of study data. There was always a statistically significantly larger increase in MIC with TOBI than with placebo. The general pattern of difference between the choices of visit for study endpoint can be seen in figure 4.2 i.

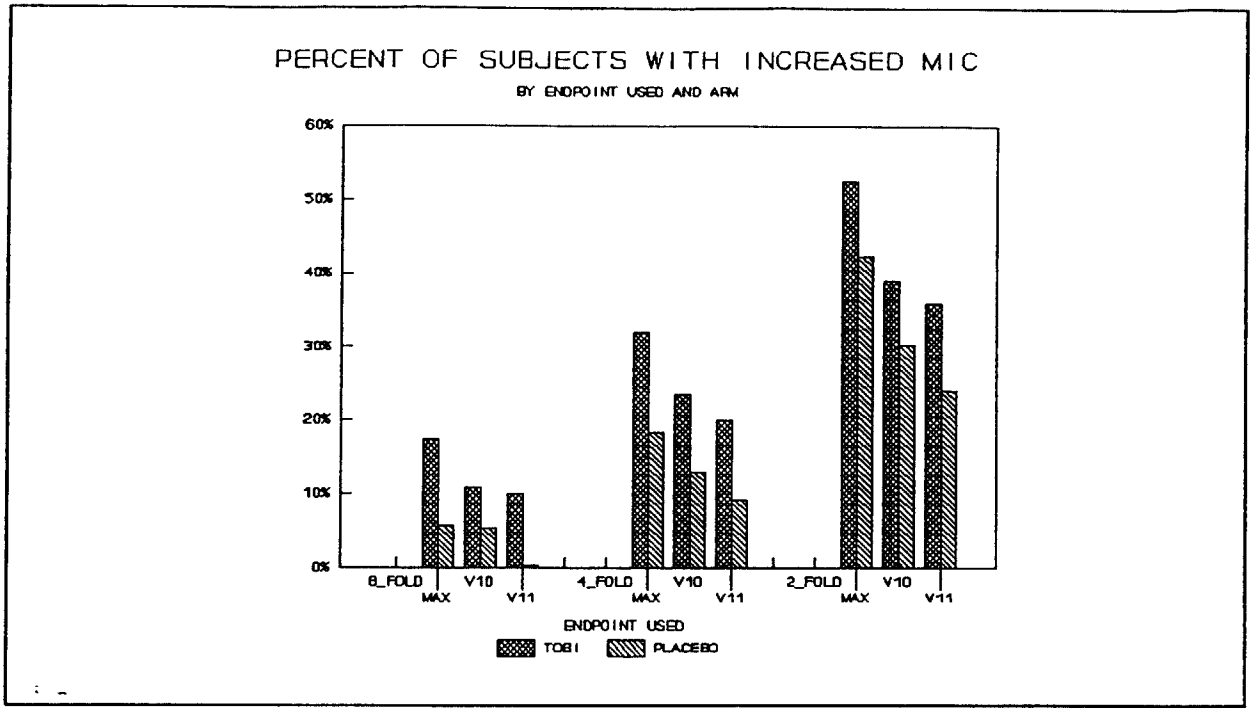


Figure 4.2 i

One additional concern with the increase in resistance has to do with concomitant use of IV antibiotics. As shown in section 4.1 above, placebo subjects use more IV antibiotics than do TOBI subjects. Since use of IV antibiotics is also expected to increase MIC, one might expect the greater use by placebo subjects to partially mask the effect of TOBI use on increasing MIC. The FDA statistical reviewer doubts that this is a legitimate concern. If TOBI is more effective in reducing the effects of Pseudomonas infection, then lower concomitant use of IV antibiotics is an inherent part of TOBI treatment. The only comparison of legitimate clinical interest is the ITT comparison of increase in MIC among all subjects assigned to TOBI to increase in MIC among all subjects assigned to placebo, regardless of concomitant medications. For microbiological summary, it is of interest to observe the differences between patients with and without concomitant IV antibiotic use. Table 4.2 C gives the incidence rates of 8-fold increases in baseline MIC, stratified by this variable. In this analysis, the FDA reviewer also performed Mantel-Haenszel tests for differences in the treatment effect between the two strata of concomitant antibiotic use (chi-square test for homogeneity). For neither 8-

fold, nor 4-fold, nor 2-fold increase was there any statistically significant change in the TOBI-placebo difference between the stratum with concomitant use and the stratum without concomitant use. (The p-values for these tests of interaction between treatment and antibiotic use stratum are not included in the table. Only the p-values for treatment effect within each stratum are given.) Except for 2-fold increase in the stratum with no concomitant use, there were statistically significantly higher rates in the TOBI arm in both strata.

TABLE 4.2 C
INCIDENCE OF 8-FOLD, 4-FOLD, 2-FOLD INCREASE IN MIC
STRATIFIED BY CONCOMITANT IV ANTIBIOTIC USE

Concomitant Antib Use	TOBI Rate	Plac Rate	Diff	P_value for TOBI - Plac
8-Fold Increase				
No	15.1%	5.5%	9.6%	0.01
Yes	21.0%	5.9%	15.1%	0.001
4-Fold Increase				
No	34.0%	18.1%	15.9%	0.003
Yes	29.0%	18.5%	10.5%	0.06
2-Fold Increase				
No	53.5%	45.7%	7.8%	0.19
Yes	51.0%	39.3%	11.7%	0.07

One additional issue should be mentioned in this section. The above analyses all focus on the percent of subjects who experienced increases of a given, clinically relevant, magnitude. To give a more balanced view of changes in MIC in the two arms, the FDA reviewer also compared the mean change in MIC for all subjects. Subjects on TOBI increased their MIC by an amount estimated to be 1.46 times larger than the mean increase in MIC for placebo subjects. With 95% confidence, the increase in MIC for TOBI subjects was 1.11 to 1.92 times as large as for placebo subjects, a statistically significant difference. Comparisons of mean increase in MIC for TOBI and placebo, stratified by sex, age group, baseline FEV_1, baseline MIC, hospitalization or IV antibiotic use in the 6 months prior to study were also examined. The pattern of approximately 1.5 times larger increases with TOBI than with placebo was generally constant across all covariate strata. These analyses were all done by computing Student t confidence intervals for the differences between arms in the log of change in MIC.

Finally, there is the issue of whether use of TOBI increases resistance to other anti-Pseudomonal antibiotics, especially to other aminoglycosides. To address this issue, the FDA reviewer computed the box plots for change in MIC between visits 3 and 10 for amikacin, aztreonam, ceftazidime, chloro, ciprofloxacin, gentamicin, ticarcillin, and trimethoprim. These boxplots are given as figure 4.2 ii. In these figures, the midline of the boxes is the median of change in MIC, the top and bottom of the boxes are the first and third quartiles (for several drugs, the third quartile coincides with the median so the top and middle of the box are the same), the upper and lower spikes extend to what would be the 5th and 95th percentiles if the log of change in MIC had a gaussian distribution. The plus signs are individual subjects with exceptionally large or small changes in MIC. (For ceftazidime, the placebo has median = lower quartile; for trimethoprim, both arms have median = upper quartile.) It appears from this plot that only amikacin, gentamicin and possibly ceftazidime show a shift upward for TOBI relative to placebo.

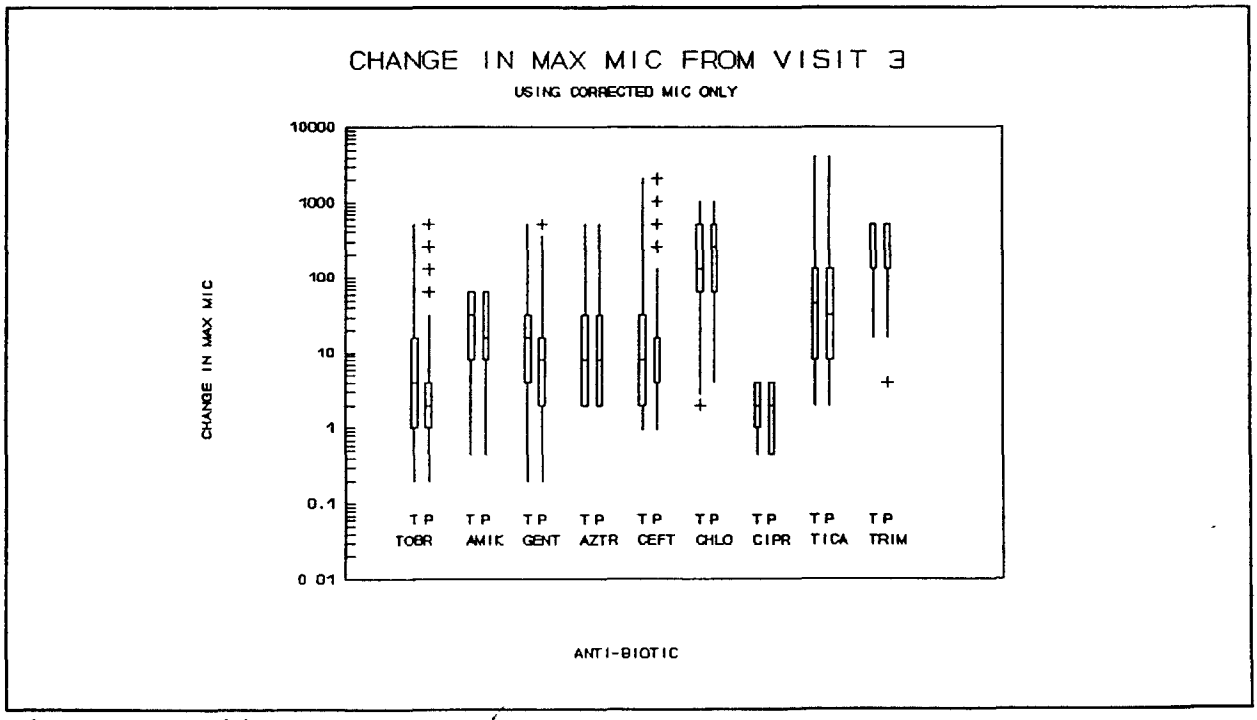


Figure 4.2 ii

4.3 FDA Re-Analysis of the PFT and CFU measures:

The repeated measures analysis used by the applicant is not the most informative method of analyzing the PFT and log(CFU) data over time. This analysis produces an estimate of treatment effect which can get larger when the treatment actually gets smaller. Specifically, if the primary measure were to show an improvement for TOBI patients in the first half of each cycle and then return to baseline during the off-treatment half of each cycle, the estimated treatment effect would be larger than if the improvement at the beginning of cycle 1 persisted throughout the off-treatment halves of each cycle. However, an improvement that persists through both halves of all cycles is better for the patient than repeated occurrences of improvement in the first half of each cycle and return to baseline in the second half of each cycle. The FDA statistical reviewer has re-analyzed these data by using the average change from baseline over the entire course of the study as the summary statistic for the PFT's and log(CFU's), rather than performing a repeated measures ANOVA. The average change is computed using linear interpolation of the PFT or the log(CFU) between successive observations. This equivalent to computing the area under the curve of change from baseline by the trapezoidal rule, obtaining a quantity measured in liter-weeks or log count-weeks, and then dividing by the number of weeks to obtain a quantity measured in liters or log counts. The percent average change for PFT's is this last quantity divided by the baseline to obtain a quantity measured in percent of baseline. Since there is substantial variation in PFT's due to age, both average change and percent average change were analyzed.

A small number of subjects had missing data from visits 3 or 10. For the former, the average of visit 1 and 2 measurements were used as baseline. For the latter, a linear interpolation of visit 11 and the next earlier visit was used for visit 10. If visit 11 data was also missing, the area under the curve out to the last recorded visit was used. To get average change over the whole study this area was divided by the 20 weeks intended for the study, regardless of whether the area was computed out to week 20 or not.

The primary test for treatment effect on PFT's and on log(CFU) used the difference between the TOBI arm and the placebo arm in mean average change of FEV_1, mean average change of FVC, mean average change of log(CFU), mean percent average change of FEV_1, and mean percent average change of FVC. Because the applicant used a dynamic minimization algorithm to assign subjects, the statistical significance of the observed differences was determined by using a randomization based test, as described in technical appendix A1. The results of these comparisons are given in table 4.3 A.

TABLE 4.3 A
MEAN CHANGES OVER 24 WEEKS IN PFT'S, LOG(CFU'S)
AND RANDOMIZATION-BASED P-VALUES FOR TREATMENT EFFECT

	Mean Change TOBI	Placebo	P-value for Difference
Prot 002			
FEV_1	.126 liters	-.016	<.001
FVC	.114 liters	-.010	.0011
FEV_1%	11.18 %	.18 %	.002
FVC%	7.42 %	.71 %	.006
LOGCFU	-2.22 log count	.08	<.001
Prot 003			
FEV_1	.097 liters	-.028	<.001
FVC	.098 liters	-.008	.0013
FEV_1%	7.15 %	-.90 %	<.001
FVC%	5.38 %	.29 %	.0011
LOGCFU	-2.09 log count	.28	<.001

The FDA reviewer also compared these three efficacy measures within strata of age group, sex, prior rhDNase use, prior hospitalization, prior IV antibiotic use, baseline FEV_1, and baseline MIC. None of these analyses showed any conspicuous treatment- covariate interactions. For illustrative purposes, the box plots of mean change in FEV_1 and mean change in log(CFU) for TOBI and placebo, stratified by age group are given below as figures 4.3 i and ii. In these figures, the midline of the boxes is the median of FEV_1 or log(CFU), the top and bottom of the boxes are the first and third quartiles, the upper and lower spikes extend to what would be the 5th and 95th percentiles if the response variable had a gaussian distribution. The plus signs are individual subjects with exceptionally large or small

responses. As one can see, there is overlap between the distributions of FEV_1 and log(CFU) for TOBI and placebo but also a clear shift between the two arms. The direction and size of the shift is similar across all age groups. The plots for FEV_1, FVC, and log CFU stratified by other baseline covariates all showed the same consistency of shift from TOBI to placebo. These other plots are not reproduced here.

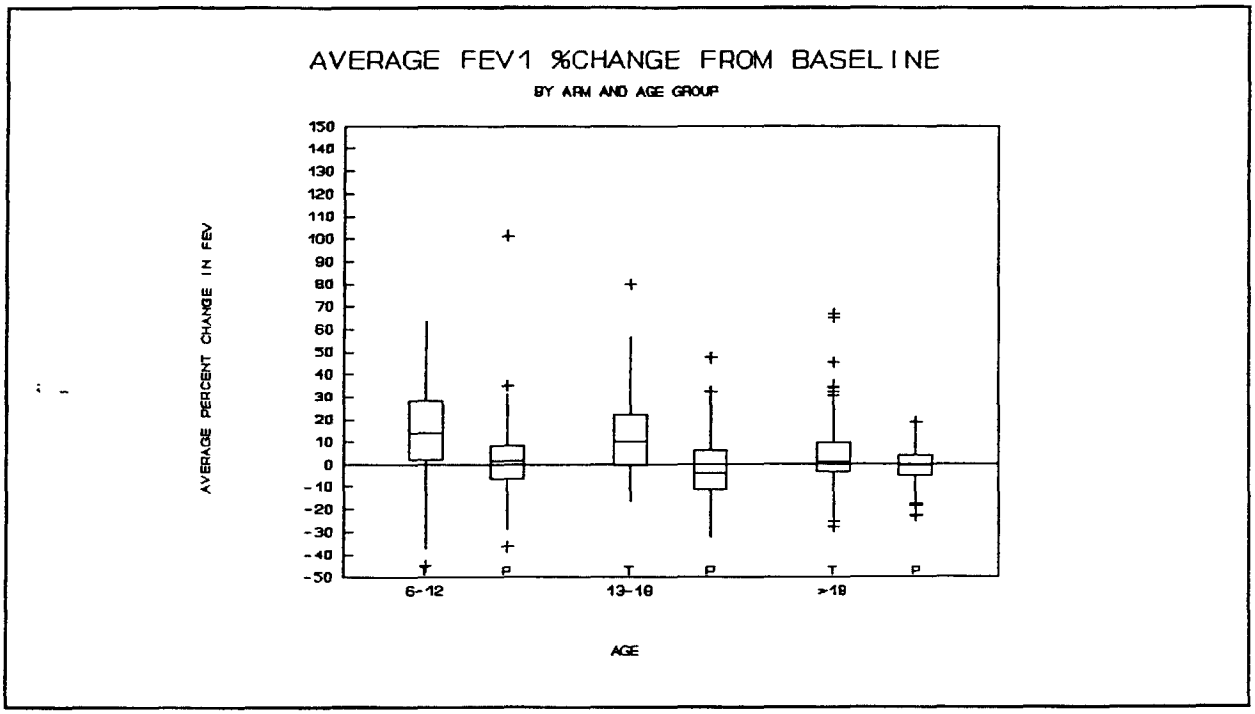


Figure 4.3 i

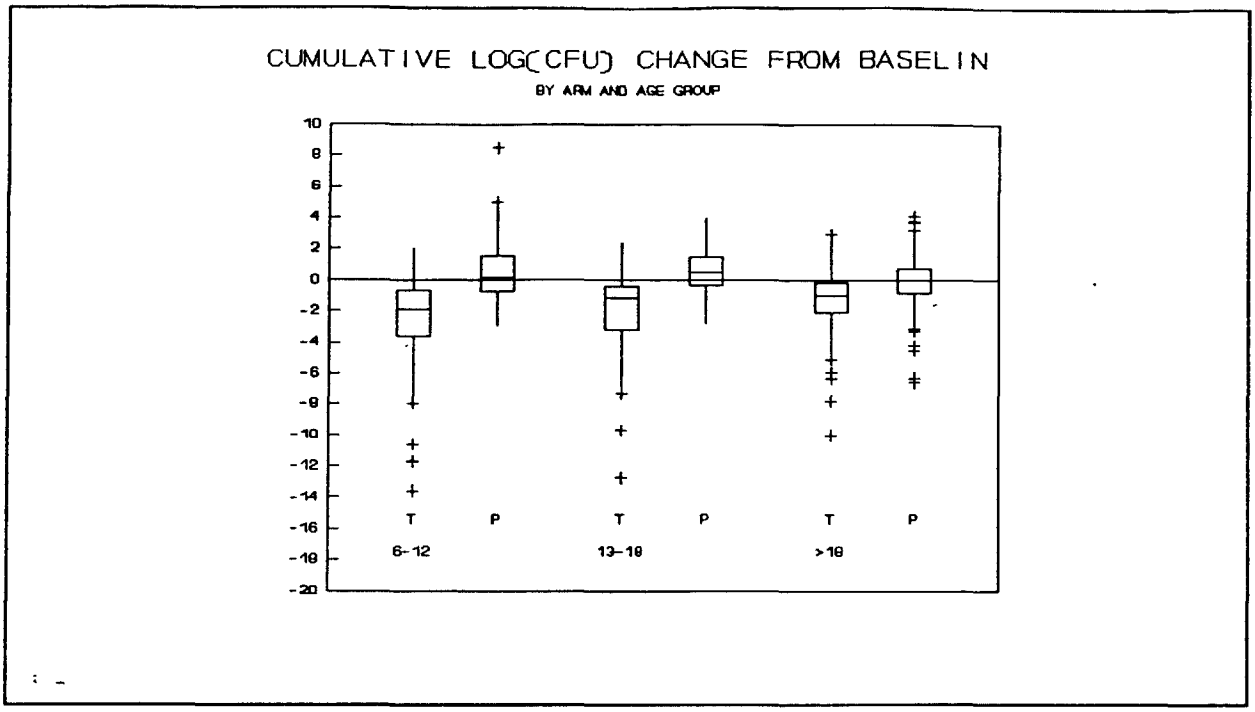


Figure 4.3 ii

4.4 Influence of Baseline MIC on Responses

The FDA medical reviewers consider that the indication for this drug will include recommendations for which individuals will benefit from the drug determined by baseline resistance. The study is not designed to address this issue in any detail, since only baseline MIC > 8 or <8 was used as a design variable. Any more detailed modelling of the treatment effect as a function of baseline resistance is exploratory. Various estimates of treatment effect at different levels of baseline resistance and confidence intervals for these effects given in the remainder of this section are dependent on unverified model assumptions. All inferences drawn from these results should be regarded as noticeably less credible than the conclusions of an overall treatment effect based on the randomization test. (See sections 4.1 and 4.3 above.)

To explore the question of where an appropriate cutpoint for response occurs as a function of baseline resistance, the FDA reviewer has performed some analyses beyond those described in

previous sections to explore possible treatment interactions with this covariate. First, the reviewer ran polynomial regressions of average change in log(CFU), average change in FEV_1, and cumulative days on IV antibiotic use, on log(baseline MIC), separately for each arm. (The mathematical details of this are included in technical appendix A.3.) The differences in the predicted responses for the two arms and the 95% confidence intervals for these differences were plotted in figures 4.4 i-iii. Theses plots show the estimated TOBI response minus the estimated placebo response plotted against baseline MIC. Similar plots were also computed by grouping baseline MIC into the following five categories: <=1, >1 and <=2, >2 and <8, >=8 and <16, and >=16. The mean and standard deviation for the same four response variables were computed for each arm in each of the five categories and the 95% confidence interval for the difference between mean TOBI and mean placebo response was computed. This analysis does not depend on the modelling of the data used in the polynomial regression but is much less informative about the predicted responses above baseline MIC of 32.

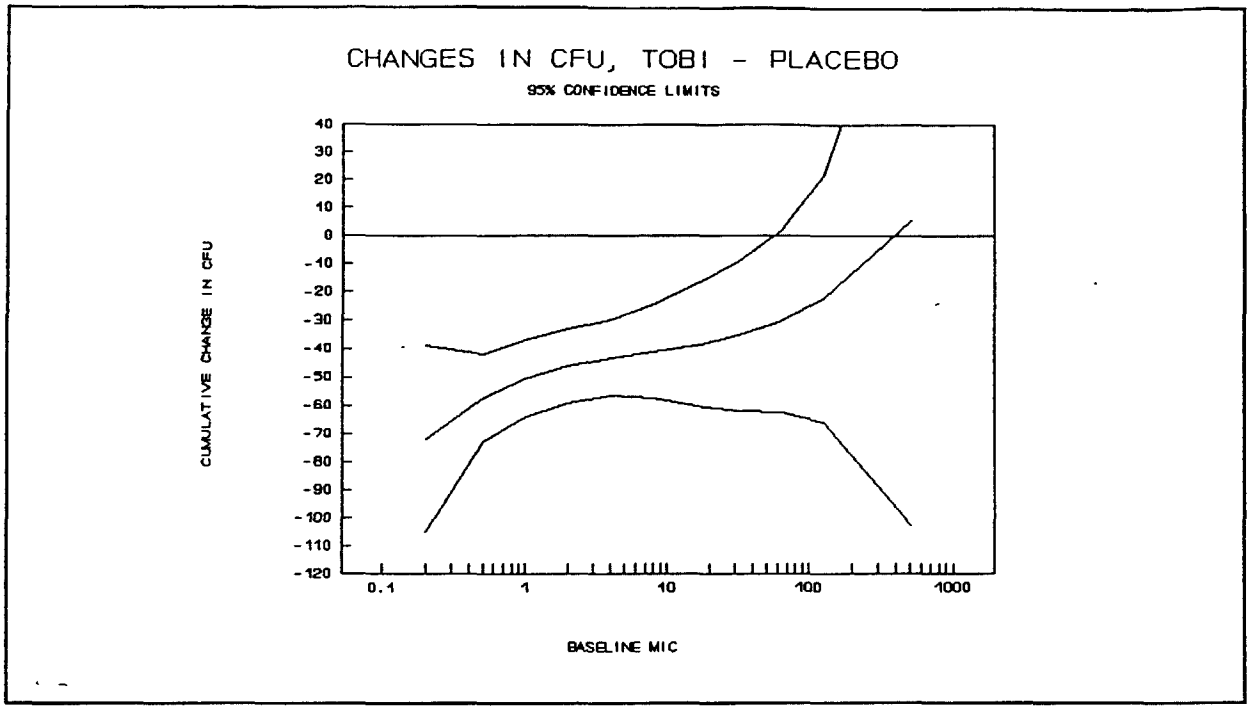


Figure 4.4 i

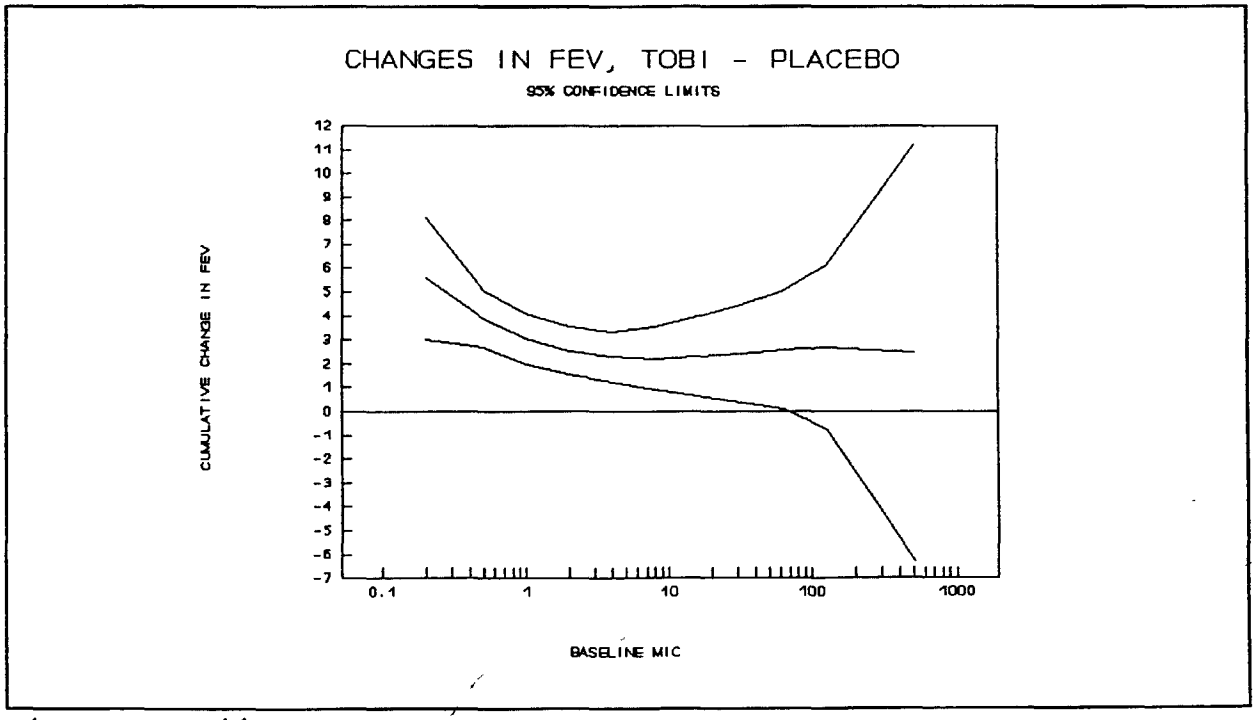


Figure 4.4 ii

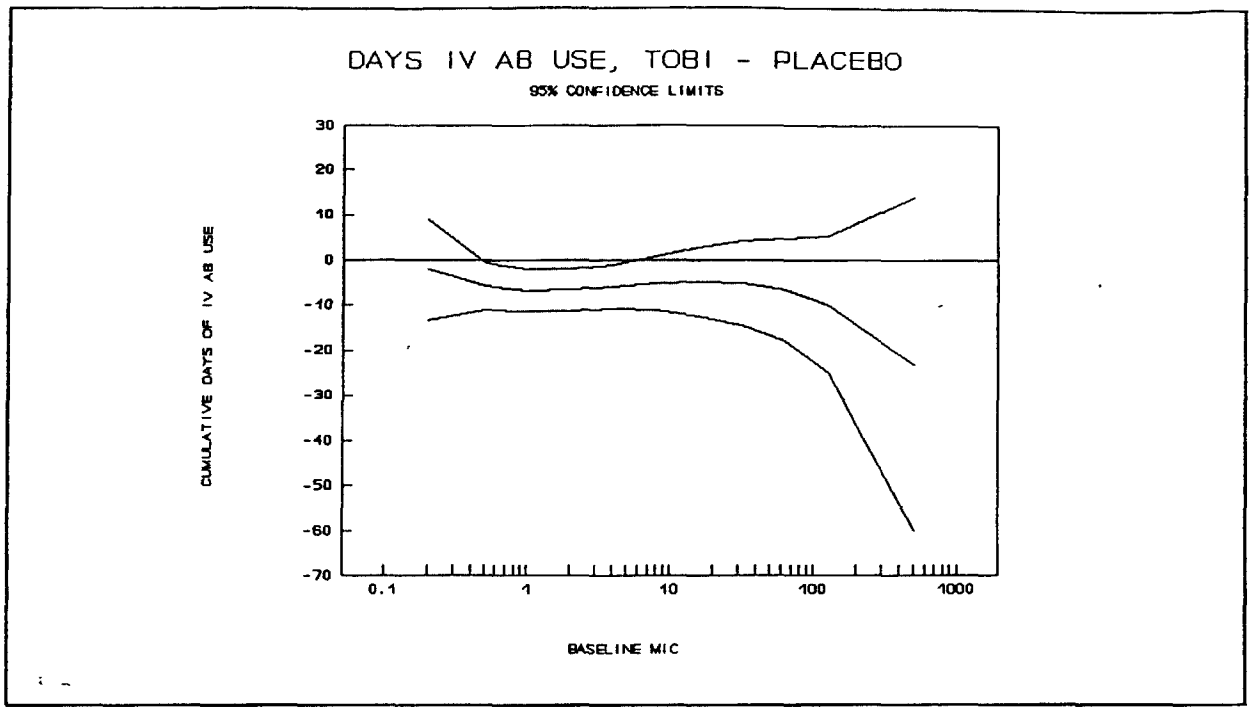


Figure 4.4 iii

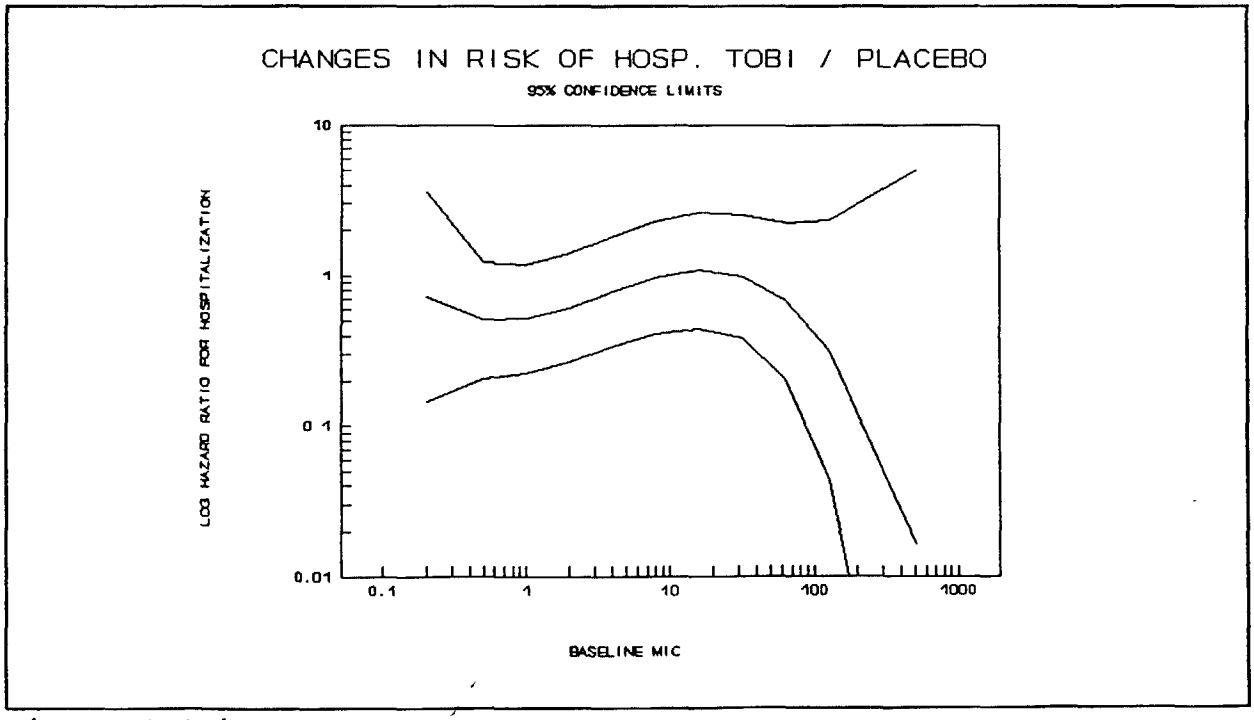


Figure 4.4 iv

The reviewer also ran proportional hazards regressions depending on group and baseline MIC for the time to hospitalization for lower respiratory illness and for the time to IV antibiotic use. The plot of the estimated hazard ratio, TOBI to placebo, and its 95% confidence limits, for time until hospitalization plotted against baseline MIC is given in figure 4.4 iv. Here, values of the hazard ratio less than 1 correspond to longer waiting times for TOBI. The plot for time until IV antibiotic use looks similar and is not given here.

It is difficult to draw very firm conclusions from these plots. There is a suggestion in the plots for log(CFU), days of IV antibiotic use, and time to hospitalization, that the difference between TOBI and placebo begins to diminish around baseline MIC of 16 μg/mL. One can, on the other hand, argue almost as convincingly that a baseline MIC of 128 μg/mL is required before a clear loss of treatment effect is noticed. The less parametric plots mentioned in the paragraph just preceding the figures are not reproduced here but they don't look dramatically different from these plots. One's conclusions don't change from looking at these plots.

4.5 Minimization Method of Assignment

The applicant's assertion that this is a randomized trial needs to be qualified. The applicant actually assigned subjects to treatment arms by a minimization algorithm, which forced the assignment of subjects to a given arm in order to achieve near balance among treatments at all three levels of age category, for both sexes, for both levels of prior rhDNASE treatment, for both categories of baseline MIC (above and below 8), for both categories of baseline FEV_1 predicted, for both categories of sputum production, and for site. This procedure entails many more deterministic (i.e., based on previous assignments) assignments than does blocked randomization. There is a small literature about this method. This literature does not provide adequate description of the operating characteristics of the minimization method.

The only known virtue of the minimization method is to provide a cosmetic balance on treatments with respect to certain pre-specified baseline covariates, something standard

randomization would also do for 95% of baseline covariates. Minimization does not guarantee balance with respect to baseline covariates not in the pre-specified group. The major drawback to minimization is that it achieves balance on some covariates at the cost of making it impossible to know if standard methods of analysis (e.g. t-tests, ANCOVA, or log-rank tests) yield correct p-values.

The applicant's original analysis attempts to evade the difficulties with respect to minimization by postulating that the PFT's and log CFU's are independent random variables with the same distribution for all subjects on the same treatment arm, and same baseline covariates. This is not an unreasonable assumption to make if one has no other basis for inference, e.g. if this were an observational study. The applicant has not attempted to check the validity of the assumption that the distributions of PFT's and log(CFU's) depend only on treatment and the six covariates mentioned. It rests entirely on biological plausibility. Similar caveats apply to any confidence intervals based on data obtained from minimization designs.

At the request of the FDA, the applicant has provided an alternative method of testing the null hypothesis of no treatment effect that attempts to take into account the restrictions on randomization imposed by minimization. The results of this analysis are presented in section above.

The FDA statistical reviewer has performed an independent computation of the re-randomization distributions of both the applicant's designated primary endpoints and for the FDA clinical reviewer's preferred primary endpoints. Specifically, the FDA statistical reviewer has computed the p-values for all of the following test statistics: 1) the difference in treatment means for the PFT's and log(CFU's) and 2) the log-rank statistics for time to first hospitalization for lower respiratory illness and for time to first IV use of an anti-Pseudomonas antibiotic. For the two times to first event, two test statistics were computed: the Wilcoxon statistic and the Savage or log-rank statistic. (See the technical appendix for details.)

5. Statistical Reviewer's Summary

The applicant's demonstration of a statistically significant improvement in PFT's and in log(CFU's) in both protocols was robust to different methods of examining the data. A statistically significant difference was found whether looking at the average change over the entire duration of the study or at repeated measures analysis of the sequence of measurements.

The demonstration of a treatment benefit on the two directly measured clinical endpoints, time until hospitalization for lower respiratory illness and time until IV or PO anti-Pseudomonal antibiotic use, was more problematic. With respect to both of these endpoints, protocol 002 showed a statistically significant improvement for the TOBI patients. Both protocols pooled together showed a statistically significant improvement for the TOBI patients for time until IV antibiotic use. However. there was almost no difference even in the estimated survival curves for time to hospitalization between the arms in protocol 003, much less a statistically significant one. There was a difference in favor of TOBI for time to IV antibiotic use in protocol 003 but there was no convincing demonstration that this was more than chance variation, with a p-value for treatment effect of .18. None of these conclusions changed if one considered early dropouts as failures rather than as censored.

There were a number of apparent treatment interactions with baseline covariates. None of these interactions was statistically significant when tested in a proportional hazards model. There did appear to be a statistically significantly larger treatment effect among subjects recruited from larger centers. It is impossible to say whether this finding is an artifact resulting from multiple tests or is the result of a real difference in patients on some unknown variable for which size of center is a surrogate.

There is a statistically significantly higher incidence of increased tobramycin resistance with TOBI. Approximately 10% more TOBI patients experienced four fold or eight fold increases in MIC over the course of the study than did placebo patients.

None of the four TOBI patients with baseline MIC > 128 experienced improvement in their PFT's. Highly model dependent

analyses did not suggest that a possible decrease in efficacy at lower levels of baseline MIC. However, the confidence intervals for the estimates of treatment effect as a function of baseline MIC were quite wide, reflecting the lower amount of data at high values of baseline MIC.

TECHNICAL APPENDICES

A.1 Re-randomization Tests

The applicant assigned subjects according to a minimization algorithm using five baseline covariates (listed in section 1.2 above) and site. Minimization scores were computed to track how the number assigned to each arm deviated from the target 1:1 ratio. These scores were computed for each of the 80 (=3+2+2+2+2+69) marginal levels of the six covariates, including site. They were also computed for each of the 48 (=2*2*2*2*3) cells formed by cross-tabulation of the first five covariates, excluding site. Subjects after the first one were randomized only if the minimization scores did not compel their assignment to a particular arm.

All subjects in both protocols were assigned as one group. This would appear to make the assignment of subjects to protocol 002 was dependent on the pattern of assignment of subjects to protocol 003.

Re-randomizations were based on computing the difference in the treatment means of the response variables for 10,000 replications for all subjects randomized, regardless of whether they were subsequently treated or measured. Subjects who were randomized but not treated or measured contribute missing values to the test statistics.

The FDA reviewer also tested for dependence between the two protocols. As mentioned above this was a concern because the minimization algorithm was not explicitly stratified by protocol. However, imbalance in sites was more heavily weighted in the minimization scores. Scatter plots of 10,000 re-randomized pairs of test statistics in protocols 002 and 003 for various responses showed no association between the protocol 002 test statistics and the protocol 003 test statistics. It is thus reasonable to treat the protocols as truly independent.

A.2 Tests for Time to Hospitalization and IV Antibiotic Use

Two test statistics were used for each endpoint tested: the Wilcoxon statistic and the Savage or log-rank statistic. The Wilcoxon statistic is computed as follows. Each subject observed

to fail is assigned the rank of his time to failure among all times to failure or censoring. Each subject censored before failure is assigned the average rank of all times to failure or censoring longer than his among all times. These scores are averaged over each treatment arm. The final Wilcoxon statistic is the average for the TOBI arm minus the same average for the placebo arm.

The Savage statistic is computed the same way, except that one starts with Savage scores instead of ranks. The Savage statistic is considered to be more powerful at detecting treatment effects with highly skewed distributions of times; the Wilcoxon statistic is considered to be more powerful at detecting treatment effects with more nearly symmetric distributions of times.)

A.3 Polynomial Regressions of Responses on Baseline MIC

The polynomial regressions mentioned in section 4.4 above were computed using the first three Chebyscheff polynomials of log baseline MIC, rescaled to range from -1 to +1. That is, the three arguments of the regressions were t, $2t^2 - 1$, and $4t^3 - 3t$, where $t = 2 * (x - \min x)/(\max x - \min x) - 1$. Here x = log of baseline MIC. The Chebyscheff polynomials applied to predictors rescaled to lie in the interval -1 to +1 tend to give more reasonable looking results than other polynomial fits.

The proportional hazards regressions were run using a treatment arm indicator and six polynomial predictors. The first three predictors were equal to the three Chebyscheff polynomials if the treatment was TOBI and were equal to zero if the treatment was placebo; the second three predictors were equal to the three Chebyscheff polynomials if the treatment was placebo and were equal to zero if the treatment was TOBI.

/S/

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