

# **SourceCF**

**Serving People with  
Cystic Fibrosis**

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March 11, 2004

Dockets Management Branch  
Food and Drug Administration  
HFA-305, Room 1-23  
12420 Parklawn Drive  
Rockville, MD 20857

Subject: ANDA Suitability Petition for TOFIN<sup>TM</sup> (tobramycin for inhalation)

Dear Sirs,

Please find the attached 2 copies of the subject Suitability Petition.

Contact information is as follows:

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2004-0131

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# SourceCF

Serving People with  
Cystic Fibrosis

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Dockets Management Branch  
Food and Drug Administration  
HFA-305, Room 1-23  
12420 Parklawn Drive  
Rockville, MD 20857

**RE: ANDA Suitability Petition  
TOFIN™ (tobramycin for inhalation)**

Dear Sirs:

This petition is submitted by Michael J. Walters, President and CEO of SourceCF pursuant to 21 C.F.R. §314.93 and §§ 10.20 and 10.30 as provided for in 21 C.F.R. § 314.93 and Section 505(j)(2)(c) of the Federal Food, Drug, and Cosmetics Act (FDC Act) to request the Commissioner of the Food and Drug Administration (FDA) to declare that **TOFIN™ (tobramycin for inhalation)** in suitable for submission as an Abbreviated New Drug Application (ANDA).

## **A. Action Requested**

The petitioner requests that the Commissioner of FDA declare that **TOFIN™ (tobramycin solution for inhalation)** is suitable for submission as an ANDA. The reference listed drug (RLD) product on which the petition is based is TOBI® (tobramycin solution for inhalation). This petition is submitted for a change in concentration of the formulation and a change in the inhaler. This petition submits evidence demonstrating that the changes result in an equivalent respirable dose to that delivered by the RLD. The principle advantage compared to the RLD is that the TOFIN™ formulation, when used as directed, utilizing the eFlow™ Electronic Inhaler by PARI, will yield a **respirable dose equivalent to that of the reference product** in approximately 3 minutes (versus 15 for the referenced product).

## **B. Statement of Grounds**

SourceCF's proposed drug product will provide an equivalent respirable dose of the same active ingredient for the same indications, the management of cystic fibrosis patients with *Pseudomonas aeruginos*, as the approved product, TOBI®, marketed by Chiron Corporation, Emeryville, California. Respirable dose is defined as the fine particle fraction (FPF), with particles ranging from 1um to 5um in diameter, of the delivered dose.

## TOFIN™ Suitability Petition SourceCF

The difference between the proposed and referenced formulations is that the SourceCF product, TOFIN™, is provided in a formulation containing tobramycin at a concentration of 100mg/ml in a 1.9ml volume delivered by the eFlow™ Electronic Inhaler by PARI. By comparison, the reference drug contains tobramycin at a concentration of 60mg/ml in a 5ml volume.

Studies have demonstrated that:

- Increasing the concentration of tobramycin and reducing nebulization time resulted in comparable serum and sputum levels of tobramycin.

In attachment I, a comparison was made of the safety, pharmacokinetics, aerosol delivery characteristics, nebulization time of the reference drug (TOBI® tobramycin solution for inhalation) and a formulation containing 120mg/ml. It was concluded that comparable serum and sputum levels of tobramycin were achieved, and that reduced nebulization time did not change the pharmacokinetics of the drug. The serum and sputum concentrations-time curves for both treatments were virtually superimposable<sup>1</sup>.

- A tobramycin solution containing 120mg/ml is as well tolerated as the reference product:

In attachment I, a comparison was made of the safety, pharmacokinetics, aerosol delivery characteristics, nebulization time of the reference drug, TOBI® (tobramycin solution for inhalation) and a formulation containing 120mg/ml. Safety findings indicate that both formulations were well-tolerated.

- A different inhalation system does not significantly alter pulmonary deposition, systemic absorption and urine recovery of the reference product:

In attachment II, a comparison was made of the safety, pharmacokinetics, aerosol delivery characteristics, nebulization time of the reference drug, TOBI® (tobramycin solution for inhalation) and several dosage strengths utilizing a high efficiency inhaler to demonstrate that an equivalent respirable dose is delivered. It was concluded that one of the studied doses achieved similar actual pulmonary deposition, systemic absorption, and urinary recovery as achieved by the reference product, and that these results in turn were similar to the sputum results obtained in prior pivotal trials supporting the commercial approval of TOBI®.

- When an equivalent respirable dose is delivered, patients are at no increased risk of adverse events due to the change in concentration of product and at no increased risk of adverse events due to a change in the rate of delivery:

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<sup>1</sup> Page 96, lines 8 and 9. In Vitro Study 2, PCT/US02/15999

## **TOFIN™ Suitability Petition SourceCF**

In attachment II, a comparison was made of the safety, pharmacokinetics, aerosol delivery characteristics, nebulization time of the reference drug, TOBI® (tobramycin solution for inhalation) and several dosage strengths utilizing a high efficiency inhaler to demonstrate that an equivalent respirable dose is delivered. It was concluded that there was no statistically significant difference in the incidence of bronchospasm using the dosing parameters described in the study. The study found no evidence that CF patients were at increased risk using the dosing parameters in the study.

### **B. 1. Evidence for Equivalency of Delivered Respirable Dose**

An *in-vitro* comparative study was conducted with TOFIN™ and the eFlow™ Electronic Inhaler by PARI, and the reference product, utilizing the PARI LC PLUS jet nebulizer and a 25psi compressor to demonstrate equivalency in the respirable dose. As seen in Table 1 – APPENDIX 1, an equivalent respirable dose was achieved in the pilot study.

A second *in-vitro* comparative study was conducted using the TOFIN™ formulation and the eFlow™ Electronic Inhaler by PARI, and the reference product utilizing the PARI LC PLUS jet nebulizer and the DeVilbuss Pulmoaide (25psi) compressor to demonstrate equivalency in the respirable dose. As seen in Table 2 – APPENDIX 2, an equivalent respirable dose was achieved in the second study.

A PARI Compass breath simulator<sup>2</sup> was used with tidal volume of 500ml; 15 breaths per minutes; with an inhalation/exhalation ratio of 1:1 to measure the nebulization efficiency of the PARI LC PLUS with TOBI® and TOFIN™ in conjunction with the eFlow™ Electronic Inhaler.

### **B. 2. Equivalence of Physical Performance Parameters**

Antibiotic formulations have been studied to determine the optimum solution characteristics for delivery<sup>3</sup>. Since the physical properties of antibiotic formulations have an effect on nebulization rates, particle size and irritation to the respiratory mucosa, definition was sought on the most important properties: osmolality, viscosity, ionic strength, pH, surface tension. The authors concluded that:

1. From the data in the literature<sup>3,4,5,6</sup> it appears that the ideal antibiotic solution should have an osmolality >150mOsm/kg and < 550mOsm/kg and contain a permeant ion (such as chloride) in a concentration >31mM but <300mM to be readily tolerable to the airway.

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<sup>2</sup> European Nebulizer Standard - <http://www.wmrc.com/businessbriefing/pdf/pharmatech2001/book/dennis.pdf>

<sup>3</sup> Effect of Nebulizer Type and Antibiotic Concentration on Device Performance: Alan Weber, Greg Morlin, Morty Cohen, Judy Williams-Warren, Bonnie Ramsey and Arnold Smith. *Pediatric Pulmonology* 23: 249-260 (1997)

## **TOFIN™ Suitability Petition SourceCF**

2. From the data available it appears that the antibiotic concentration, independent of specific drug, should be 120mg/ml or less in a saline solution between 0.25 and 0.5 normal saline
3. Tobramycin tested (200, 100, 50 and 25) had a pH that was in the range predicted to be tolerable
4. Tobramycin concentrations exceeding 200mg/ml would not be tolerated

In the study summarized in Table 1 - APPENDIX 1, the physical properties of TOFIN™ were equivalent to the reference product and consistent with the findings for optimum solution for nebulization.

In summary, the TOFIN™ formulation possesses equivalent physical performance characteristics and delivers an **equivalent respirable dose of tobramycin** to the of the reference product, TOBI®.

### **B. 3. Evidence for Equivalency of Safety When Using A Different Inhaler To Deliver An Equivalent Respirable Dose of Tobramycin**

A comparison was made of the safety and pharmacokinetics characteristics of the conventional dose and delivery system (5ml ampoule containing 300mg tobramycin and 11.25 sodium chloride in sterile water for injection (TOBI® tobramycin solution for inhalation, Chiron Corporation, Seattle, Washington), pH 6.0 +/- 0.5; administered with the PARI LC PLUS™ jet nebulizer with a DeVilbiss PulmoAide™ compressor (adjusted for a 25 psi output) with three doses of the TOBI® formulation in three experimental doses of TOBI® (30mg, 60mg and 90mg) using the Aerodose™ inhaler device.

The study was designed as an open label, randomized, multicenter, single-dose, unbalanced, four treatment, three period crossover trial. Each patient received three single doses of aerosolized tobramycin; the active drug control during one treatment and two of three experimental doses during the additional two treatments.

Commercial TOBI® 60mg/ml in 5ml solution administered by the PARI LC PLUS™ jet nebulizer and powered by the PulmoAide compressor was the active drug control delivery system. The selection of doses of experimental treatments (TOBI® 30mg in 0.5ml solution, 60mg in 1.0ml solution and 90mg in 1.5ml solution) was based on empirical data on the comparative predicted efficiency of the Aerodose™ inhaler relative to the PARI LC PLUS™.

It was concluded that:

Safety Assessment Results - There was no evidence that CF patients were at increased risk by reason of inhaling single TOBI® doses of 30mg, 60mg or 90mg delivered by the Aerodose™ Inhaler compared to the single TOBI® 300mg dose delivered by the PARI LC PLUS™ jet nebulizer and that:

## TOFIN™ Suitability Petition SourceCF

1. There was no statistically significant differences between the control and any experimental treatment in the incidence bronchospasm and no overall treatment differences in quantitative change in FEV<sub>1</sub> from predose to 30-minute postdose (Table 4 – Appendix 2) despite the differences in mean nebulization times (Table 5 – Appendix 2) between the four regimens.
2. Sputum tobramycin concentrations for TOBI® 90mg delivered by the Aerodose™ inhaler and TOBI® 300mg delivered by the PARI LC PLUS did not differ substantially or consistently
3. Mean C<sub>max</sub> and AUC<sub>0-8</sub> for the TOBI™ 90mg treatment delivered by the Aerodose™ inhaler achieved similar levels as those achieved by the TOBI® 300mg treatment delivered by the PARI LC PLUS™ nebulizer

Systemic Absorption – Serum tobramycin results demonstrated that TOBI® 90mg delivered by the Aerodose™ Inhaler were similar (AUC<sub>0-8</sub>) or nearly similar (C<sub>max</sub>) to those obtained after TOBI® 300mg delivered by the PARI LC PLUS™ jet nebulizer in the present study and in prior studies supporting the commercial product (Table 6 – Appendix 2).

1. The extent of absorption, as measured by serum C<sub>max</sub> results, was significantly greater after the approved TOBI® 300mg regimen than TOBI® 90mg delivered by the Aerodose™ Inhaler in this study and slightly higher than the results reported in the TOBI® NDA. Serum C<sub>max</sub> achieved by the Aerodose™ Inhaler at the TOBI® 90mg dose was virtually identical to the NDA serum concentrations.

This study demonstrated that TOBI® 90mg (but not TOBI® 60mg or TOBI® 30mg) delivered by the Aerodose™ Inhaler achieved similar actual pulmonary deposition, systemic absorption and urinary recovery of tobramycin as achieved by the administration of the TOBI® 300mg dose delivered by the PARI LC PLUS™ nebulizer.

These data indicate that other delivery systems can be customized to provide delivery parameters similar to TOBI® and PARI LC PLUS™. The eFlow™ Electronic Inhaler by PARI currently planned for the delivery of the TOFIN™ product has been customized to produce delivery parameters equivalent to that of TOBI® and the PARI LC PLUS™.

In summary, TOFIN™ delivers an **equivalent respirable dose of tobramycin** to that of the reference product, TOBI®; and that **the data demonstrates that patients are at no increased risk of adverse events due to the change in concentration of product or due to the change in the rate of delivery.**

The equivalency of delivered respirable dose and safety in use will be demonstrated with our new TOFIN™ product delivered in the PARI e-FLOW™ device in an Abbreviated New Drug Application.

## **TOFIN™ Suitability Petition SourceCF**

In summary, the **TOFIN™** product, to be marketed by SourceCF, Huntsville, AL, will be clearly comparable to the **TOBI®** reference product as measured by the three critical parameters: **respirable dose delivered, physical and solution characteristics and nebulization characteristics of the drug solution.**

### **B. 4. Comparison of Labeling of TOFIN™ and TOBI®**

The proposed product labeling for **TOFIN™** is essentially identical to that of the reference drug, but differs only with respect to description of the product, product name, product concentration, product usage, the “how supplied” statement and the specific manufacturer’s information.

**Appendix # 3** contains a side-by-side comparison of the sections differing in labeling content.

**Appendix # 4** contains proposed labeling for **TOFIN™**, Tobramycin Solution for Inhalation, 190 mg and

**Appendix # 5** contains current approved labeling for **TOBI®**, Tobramycin Solution for Inhalation, 300 mg.

### **C. Environmental Impact**

SourceCF requests a categorical exclusion under 21CFR25.31. To the best of the petitioner’s knowledge, no extraordinary circumstances as outlined in 21CFR25.21 exist.

### **D. Economic Impact**

The petitioner does not believe that this is applicable in this case, but SourceCF will supply an economic impact analysis upon request of the Commissioner.

### **E. Certification**

The undersigned certifies that, to the best of his knowledge, this petition includes all information and views on which the petition relies, and that it includes representative data and information known to the petitioner which are unfavorable to the petition.

Very truly yours,



Michael J. Walters  
President/CEO

**TOFIN™ Suitability Petition  
SourceCF**

**APPENDIX # 1**

Table 1 – Physical and Solution Characteristics of TOFIN™ and TOBI® Compared.

<b>Item</b>	<b>SourceCF Product TOFIN™</b>	<b>Reference Drug TOBI®</b>
Dosage Form	Sterile Solution	Sterile Solution
Route of Administration	Inhalation	Inhalation
Respirable Dose	~73 mg <sup>4</sup>	71 mg
Surface Tension	71.27 ± 0.13 mN/m	72.49 ± 0.9 mN/m
Viscosity	1.43 ± 0.17	1.99 ± 0.23
Osmolality	193mOsm/kg	158-183mOsm/kg
Permeant Ion	75mOsm/kg	75mOsm/kg
pH (@ 23°C)	6.05	5.97 <sup>5</sup>
Volume	1.9 mL	5 mL
Total Drug Charge	190 mg	300 mg
How Supplied	To be determined	5 mL LDPE ampoules

Table 2 - Comparison of Delivery Times and Respirable Dose of the TOFIN™ and TOBI® Formulations

<b>Formulation</b>	<b>Device</b>	<b>Respirable Dose</b>	<b>FPF [&lt;5µ]</b>	<b>Delivered Dose</b>	<b>Time</b>	<b>DDR [mg/min]</b>
<b>TOBI®</b> 60mg/mL 5.0 mL	PARI LC PLUS + DeVilbuss PulmoAide	<b>71.2mg</b> 24.0%	62.3%	114.2mg 38.4%	<b>13.3</b>	8.6
<b>TOFIN™</b> 100mg/mL 2.0 mL	eFlow™ Electronic Inhaler	<b>75.7mg<sup>6</sup></b> 38.6%	75.2%	100.7mg 51.4%	<b>3.2</b>	32.1

DDR = Drug Delivery Rate FPF = Fine Particle Fraction

<sup>5</sup> Pilot data only

<sup>5</sup> Lot-No: TOBI® 10K0B

<sup>6</sup> Note: The volume fill was 200mg. TOFIN™ will be 1.9ml versus 2.0ml → ~71.9mg

**TOFIN™ Suitability Petition  
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**APPENDIX # 2**

Table 4 Mean (SD) Relative Change in FEV<sub>1</sub>% Predicted

FEV <sub>1</sub> % Predicted % Parameter	TOBI® 300mg PARI LC PLUS™ (N=51)	TOBI® 30mg Aerodose™ Inhaler (N=34)	TOBI® 60mg Aerodose™ Inhaler (N=32)	TOBI® 90mg Aerodose™ Inhaler (N=33)
Pre-Dose	<b>67.8 (18.4)</b>	65.5 (17.1)	65.4 (16.8)	<b>71.3 (20.0)</b>
30 min Post-Dose	<b>63.7 (17.6)</b>	63.0 (16.7)	62.5 (15.7)	<b>68.7 (19.1)</b>
Relative Change	<b>-6.1 (5.2)</b>	-3.8 (5.4)	-4.2 (6.2)	<b>-3.2 (7.4)</b>

1 Control Treatment = TOBI 300 mg delivered by PARI LC Plus nebulizer  
2 Experimental treatments = TOBI 30, 60, and 90 mg delivered by Aerodose Inhaler  
Relative Change from Predose = 100% X (30 min Post-Dose value less Pre-Dose value)

Table 5 Mean (SD) Nebulization Time

Parameter	TOBI® 300mg PARI LC PLUS™ (N=51)	TOBI® 30mg Aerodose™ Inhaler <sup>1</sup> (N=34)	TOBI® 60mg Aerodose™ Inhaler <sup>1</sup> (N=32)	TOBI® 90mg Aerodose™ Inhaler <sup>1</sup> (N=33)
Nebulization Times (min)	<b>17.7 (4.7)</b>	2.8 (1.0)	5.2 (2.1)	<b>8.0 (2.5)</b>

Control Treatment = TOBI® 300mg delivered by PARI LC PLUS™ nebulizer  
Experimental treatments = TOBI® 30mg, 60mg, and 90mg delivered by Aerodose™ inhaler  
1. Total duration of nebulization excluding fill time.

Table 6. Mean (SD) Serum Tobramycin Concentrations

Serum Pharmacokinetic Parameters	TOBI 300 mg PARI LC Plus (N=49)	TOBI 30 mg Aerodose Inhaler (N=34)	TOBI 60 mg Aerodose Inhaler (N=32)	TOBI 90 mg Aerodose Inhaler (N=33)
C <sub>max</sub> (µg/ml)	<b>1.12 (0.44)</b> (N=49)	0.38 (0.17) (N=30)	0.69 (0.34) (N=32)	<b>0.96 (0.40)</b> (N=32)
T <sub>max</sub> (hr)	<b>1.05 (0.38)</b>	1.14 (0.42)	0.98 (0.28)	<b>1.14 (0.64)</b>
AUC <sub>0-∞</sub>	<b>6.66 (4.32)</b>	6.49 (7.71)	5.11 (4.62)	<b>5.02 (1.63)</b>

1 Control Treatment = TOBI 300 mg delivered by PARI LC Plus nebulizer  
2 Experimental treatments = TOBI 30, 60, and 90 mg delivered by Aerodose Inhaler  
Serum limit of quantification (LOQ): 0.2 µg/ml

**TOFIN™ Suitability Petition  
SourceCF**

**APPENDIX 3**

Side-by-Side Comparison of Differing Labeling Sections for the proposed product, TOFIN™, and the reference drug, TOBI®.

**DESCRIPTION**

**TOFIN™, Tobramycin for Inhalation:**

Tobramycin is a sterile, clear, slightly yellow, non-pyrogenic, aqueous solution with the pH and salinity adjusted specifically for administration by a compressed air driven reusable nebulizer. The chemical formula for tobramycin is C<sub>18</sub>H<sub>37</sub>N<sub>5</sub>O<sub>9</sub> and the molecular weight is 467.52. Tobramycin is O-3-amino-3-deoxy-α-D-glucopyranosyl-(1®4)-O-[2, 6-diamino-2, 3, 6-trideoxy-α-D-ribo-hexopyranosyl-(1®6)]-2-deoxy-L-streptomine.

*Each TOFIN™ (tobramycin for inhalation) single-use 2ml closure system contains 190 mg tobramycin and 4.275 mg sodium chloride in sterile water for injection. Sulfuric acid and sodium hydroxide are added to adjust the pH to 6.0 ± 0.3. All ingredients meet USP requirements. The formulation contains no preservatives.*

**TOBI®, Tobramycin Solution for Inhalation:**

TOBI® is a tobramycin solution for inhalation. It is a sterile, clear, slightly yellow, non-pyrogenic, aqueous solution with the pH and salinity adjusted specifically for administration by a compressed air driven reusable nebulizer. The chemical formula for tobramycin is C<sub>18</sub>H<sub>37</sub>N<sub>5</sub>O<sub>9</sub> and the molecular weight is 467.52. Tobramycin is O-3-amino-3-deoxy-α-D-glucopyranosyl-(1.4)- O-[2,6-diamino-2,3,6-trideoxy-α-D-ribo-hexopyranosyl-(1.6)]-2-deoxy-L-streptomine.

*Each single-use 5 mL ampule contains 300 mg tobramycin and 11.25 mg sodium chloride in sterile water for injection. Sulfuric acid and sodium hydroxide are added to adjust the pH to 6.0. Nitrogen is used for sparging. All ingredients meet USP requirements. The formulation contains no preservatives.*

**DOSAGE AND ADMINISTRATION**

**TOFIN™, Tobramycin for Inhalation:**

The recommended dosage for both adults and pediatric patients 6 years of age and older is 1 single-use ampule (190 mg) administered BID for 28 days. Dosage is not adjusted by weight. All patients should be administered 190 mg BID. The doses should be taken as close to 12 hours apart as possible; they should not be taken less than 6 hours apart.

## **TOFIN™ Suitability Petition SourceCF**

Tobramycin is inhaled while the patient is sitting or standing upright and breathing normally through the mouthpiece of the nebulizer. Nose clips may help the patient breathe through the mouth.

Tobramycin is administered BID in alternating periods of 28 days. After 28 days of therapy, patients should stop tobramycin therapy for the next 28 days, and then resume therapy for the next 28 day on/28 day off cycle.

Tobramycin is supplied as a single-use ampule and is administered by inhalation, using a hand-held eFlow™ Electronic Inhaler. Tobramycin is not for subcutaneous, intravenous or Intrathecal administration.

### Usage

*Tobramycin is administered by inhalation over a 3-4 minute period, using a hand-held eFlow™ Electronic Inhaler. Tobramycin should not be diluted or mixed with dornase alfa in the nebulizer. During clinical studies, patients on multiple therapies were instructed to take them first, followed by tobramycin.*

### **TOBI®, Tobramycin Solution for Inhalation:**

The recommended dosage for both adults and pediatric patients 6 years of age and older is one single-use ampule (300 mg) administered BID for 28 days. Dosage is not adjusted by weight. All patients should be administered 300 mg BID. The doses should be taken as close to 12 hours apart as possible; they should not be taken less than six hours apart. TOBI is inhaled while the patient is sitting or standing upright and breathing normally through the mouthpiece of the nebulizer. Nose clips may help the patient breathe through the mouth. TOBI is administered BID in alternating periods of 28 days. After 28 days of therapy, patients should stop TOBI therapy for the next 28 days, and then resume therapy for the next 28 day on/28 day off cycle.

TOBI is supplied as a single-use ampule and is administered by inhalation, using a hand-held PARI LC PLUS Reusable Nebulizer with a DeVilbiss Pulmo-Aide compressor. TOBI is not for subcutaneous, intravenous or intrathecal administration.

### ***Usage***

*TOBI is administered by inhalation over an approximately 15 minute period, using a hand-held PARI LC PLUS Reusable Nebulizer with a DeVilbiss Pulmo-Aide compressor. TOBI should not be diluted or mixed with dornase alfa (PULMOZYME®, Genentech) in the nebulizer. During clinical studies, patients on multiple therapies were instructed to take them first, followed by TOBI.*

**TOFIN™ Suitability Petition  
SourceCF**

**HOW SUPPLIED**

**TOFIN™, Tobramycin for Inhalation:**

*TOFIN™ is supplied in single-use, 2 ml ampules.*

**TOBI®, Tobramycin Solution for Inhalation:**

*TOBI is supplied in single-use, low-density polyethylene plastic 5 ml ampules.*

**Manufacturing Information**

**TOFIN™, Tobramycin for Inhalation:**

*Manufactured for  
SourceCF  
Huntsville, AL 35805  
By: to be determined*

**TOBI®, Tobramycin Solution for Inhalation:**

*Manufactured for  
CHIRON Corporation  
Emeryville, CA 94608  
by Automatic Liquid Packaging, Inc.,  
Woodstock, IL 60098  
Packaged by Packaging Coordinators Inc.,  
Philadelphia, PA 19114-1123*

**TOFIN™ Suitability Petition  
SourceCF**

**APPENDIX # 4**

**TOFIN™ (Tobramycin for Inhalation)**

**DESCRIPTION**

Tobramycin is a sterile, clear, slightly yellow, non-pyrogenic, aqueous solution with the pH and salinity adjusted specifically for administration by a compressed air driven reusable nebulizer. The chemical formula for tobramycin is C<sub>18</sub>H<sub>37</sub>N<sub>5</sub>O<sub>9</sub> and the molecular weight is 467.52. Tobramycin is O-3-amino-3-deoxy-a-D-glucopyranosyl-(1®4)-O-[2,6-diamino-2,3,6-trideoxy-a-D-ribo-hexopyranosyl-(1®6)]-2-deoxy-L-streptomine.

TOFIN™ (tobramycin for inhalation) single-use 2ml closure system contains 190mg tobramycin and 4.275 mg sodium chloride in sterile water for injection. Sulfuric acid and sodium hydroxide are added to adjust the pH to 6.0 ± 0.5. All ingredients meet USP requirements. The formulation contains no preservatives.

**CLINICAL PHARMACOLOGY**

Tobramycin is specifically formulated for administration by inhalation. When inhaled, tobramycin is concentrated in the airways.

**Pharmacokinetics**

Tobramycin is a cationic polar molecule that does not readily cross epithelial membranes.<sup>1</sup> The bioavailability of tobramycin may vary because of individual differences in nebulizer performance and airway pathology.<sup>2</sup> Following administration, tobramycin remains concentrated primarily in the airways.

**Sputum Concentrations**

Ten (10) minutes after inhalation of the first 300 mg dose of tobramycin, the average concentration of tobramycin was 1237 mg/g (ranging from 35-7414 mg/g) in sputum. Tobramycin does not accumulate in sputum; after 20 weeks of therapy with the tobramycin regimen, the average concentration of tobramycin at 10 minutes after inhalation was 1154 mg/g (ranging from 39-8085 mg/g) in sputum. High variability of tobramycin concentration in sputum was observed. Two (2) hours after inhalation, sputum concentrations declined to approximately 14% of tobramycin levels at 10 minutes after inhalation.

**Serum Concentrations**

The average serum concentration of tobramycin 1 hour after inhalation of a single 300 mg dose of tobramycin by cystic fibrosis patients was 0.95 mg/ml. After 20 weeks of therapy on the tobramycin regimen, the average serum concentration 1 hour after dosing was 1.05 mg/ml.

## TOFIN™ Suitability Petition SourceCF

### Elimination

The elimination half-life of tobramycin from serum is approximately 2 hours after intravenous (IV) administration. Assuming tobramycin absorbed following inhalation behaves similarly to tobramycin following IV administration, systemically absorbed tobramycin is eliminated principally by glomerular filtration. Unabsorbed tobramycin, following tobramycin administration, is probably eliminated primarily in expectorated sputum.

### Microbiology

Tobramycin is an aminoglycoside antibiotic produced by *Streptomyces tenebrarius*.<sup>1</sup> It acts primarily by disrupting protein synthesis, leading to altered cell membrane permeability, progressive disruption of the cell envelope, and eventual cell death.<sup>3</sup> Tobramycin has *in vitro* activity against a wide range of gram-negative organisms including *Pseudomonas aeruginosa*. It is bactericidal at concentrations equal to or slightly greater than inhibitory concentrations.

### Susceptibility Testing

A single sputum sample from a cystic fibrosis patient may contain multiple morphotypes of *Pseudomonas aeruginosa* and each morphotype may have a different level of *in vitro* susceptibility to tobramycin. Treatment for 6 months with tobramycin in two clinical studies did not affect the susceptibility of the majority of *P. aeruginosa* isolates tested; however, increased MICs were noted in some patients. The clinical significance of this information has not been clearly established in the treatment of *P. aeruginosa* in cystic fibrosis patients. For additional information regarding the effects of tobramycin on *P. aeruginosa* MIC values and bacterial sputum density, please refer to CLINICAL STUDIES.

The *in vitro* antimicrobial susceptibility test methods used for parenteral tobramycin therapy can be used to monitor the susceptibility of *P. aeruginosa* isolated from cystic fibrosis patients. If decreased susceptibility is noted, the results should be reported to the clinician.

Susceptibility breakpoints established for parenteral administration of tobramycin do not apply to aerosolized administration of tobramycin. The relationship between *in vitro* susceptibility test results and clinical outcome with tobramycin therapy is not clear.

### CLINICAL STUDIES

Two identically designed, double-blind, randomized, placebo-controlled, parallel group, 24-week clinical studies (Study 1 and Study 2) at a total of 69 cystic fibrosis centers in the United States were conducted in cystic fibrosis patients with *P. aeruginosa*. Subjects who were less than 6 years of age, had a baseline creatinine of > 2 mg/dl, or had *Burkholderia cepacia* isolated from sputum were excluded. All subjects had baseline FEV1 % predicted between 25% and 75%. In these clinical studies, 258 patients received tobramycin therapy on an outpatient basis (see TABLE 1) using a hand-held Pari LC Plus reusable nebulizer with a DeVilbiss Pulmo-Aide compressor.

**TOFIN™ Suitability Petition  
SourceCF**

<b>TABLE 1 Dosing Regimens in Clinical Studies</b>						
	Cycle 1		Cycle 2		Cycle 3	
	28 days	28 days	28 days	28 days	28 days	28 days
Tobramycin regimen n=258	Tobramycin 300 mg BID	No drug	Tobramycin 300 mg BID	No drug	Tobramycin 300 mg BID	No drug
Placebo regimen n=262	Placebo	No drug	Placebo	No drug	Placebo	No drug

All patients received either tobramycin or placebo (saline with 1.25 mg quinine for flavoring) in addition to standard treatment recommended for cystic fibrosis patients, which may include oral and parenteral anti-pseudomonal therapy, b<sub>2</sub>-agonists, cromolyn, inhaled steroids, and airway clearance techniques. In addition, approximately 77% of patients were concurrently treated with domase alfa.

In each study, tobramycin-treated patients experienced significant improvement in pulmonary function. Improvement was demonstrated in the tobramycin group in Study 1 by an average increase in FEV<sub>1</sub>% predicted of about 11% relative to baseline (Week 0) during 24 weeks compared to no average change in placebo patients. In Study 2, tobramycin treated patients had an average increase of about 7% compared to an average decrease of about 1% in placebo patients. The average relative change in FEV<sub>1</sub>% predicted over 24 weeks for both studies.

In each study, tobramycin therapy resulted in a significant reduction in the number of *P. aeruginosa* colony forming units (CFUs) in sputum during the on-drug periods. Sputum bacterial density returned to baseline during the off-drug periods. Reductions in sputum bacterial density were smaller in each successive cycle.

Patients treated with tobramycin were hospitalized for an average 5.1 days compared to 8.1 days for placebo patients. Patients treated with tobramycin required an average of 9.7 days of parenteral anti-pseudomonal antibiotic treatment compared to 14.1 days for placebo patients. During the 6 months of treatment, 40% of tobramycin patients and 53% of placebo patients were treated with parenteral anti-pseudomonal antibiotics.

The relationship between in vitro susceptibility test results and clinical outcome with tobramycin therapy is not clear. However, 4 tobramycin patients who began the clinical trial with *P. aeruginosa* isolates having MIC values <sup>3</sup>128 mg/ml did not experience an improvement in FEV<sub>1</sub> or a decrease in sputum bacterial density.

Treatment with tobramycin did not affect the susceptibility of the majority of *P. aeruginosa* isolates during the 6 month studies. However, some *P. aeruginosa* isolates did exhibit increased tobramycin MICs. The percentage of patients with *P. aeruginosa* isolates with tobramycin MICs <sup>3</sup>16 mg/ml was 13% at the beginning, and 23% at the end of 6 months of the tobramycin regimen.

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### **INDICATIONS AND USAGE**

Tobramycin is indicated for the management of cystic fibrosis patients with *P. aeruginosa*.

Safety and efficacy have not been demonstrated in patients under the age of 6 years, patients with FEV1 <25% or >75% predicted, or patients colonized with *Burkholderia cepacia* (see CLINICAL STUDIES).

### **CONTRAINDICATIONS**

Tobramycin is contraindicated in patients with a known hypersensitivity to any aminoglycoside.

### **WARNINGS**

Caution should be exercised when prescribing tobramycin to patients with known or suspected renal, auditory, vestibular, or neuromuscular dysfunction. Patients receiving concomitant parenteral aminoglycoside therapy should be monitored as clinically appropriate.

Aminoglycosides can cause fetal harm when administered to a pregnant woman. Aminoglycosides cross the placenta, and streptomycin has been associated with several reports of total, irreversible, bilateral congenital deafness in pediatric patients exposed in utero. Patients who use tobramycin during pregnancy, or become pregnant while taking tobramycin should be apprised of the potential hazard to the fetus.

#### **Ototoxicity**

Ototoxicity, as measured by complaints of hearing loss or by audiometric evaluations, did not occur with tobramycin therapy during clinical studies. However, transient tinnitus occurred in 8 tobramycin solution-treated patients versus no placebo patients in the clinical studies. Tinnitus is a sentinel symptom of ototoxicity, and therefore the onset of this symptom warrants caution (see ADVERSE REACTIONS). Ototoxicity, manifested as both auditory and vestibular toxicity, has been reported with parenteral aminoglycosides. Vestibular toxicity may be manifested by vertigo, ataxia or dizziness.

#### **Nephrotoxicity**

Nephrotoxicity was not seen during tobramycin clinical studies but has been associated with aminoglycosides as a class. If nephrotoxicity occurs in a patient receiving tobramycin, tobramycin therapy should be discontinued until serum concentrations fall below 2 mg/ml.

#### **Muscular Disorders**

Tobramycin should be used cautiously in patients with muscular disorders, such as myasthenia gravis or Parkinson's disease, since aminoglycosides may aggravate muscle weakness because of a potential curare-like effect on neuromuscular function.

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### **Bronchospasm**

Bronchospasm can occur with inhalation of tobramycin. In clinical studies of tobramycin, changes in FEV1 measured after the inhaled dose were similar in the tobramycin and placebo groups. Bronchospasm should be treated as medically appropriate.

### **PRECAUTIONS**

#### **Information for Patients**

See the Patient Instructions that are distributed with the prescription.

#### **Laboratory Tests**

##### **Audiograms**

Clinical studies of tobramycin did not identify hearing loss using audiometric tests which evaluated hearing up to 8000 Hz. Tinnitus may be a sentinel symptom of ototoxicity, and therefore the onset of this symptom warrants caution. Physicians should consider an audiogram for patients who show any evidence of auditory dysfunction, or who are at increased risk for auditory dysfunction.

##### **Serum Concentrations**

In patients with normal renal function treated with tobramycin, serum tobramycin concentrations are approximately 1 mg/ml 1 hour after dose administration and do not require routine monitoring. Serum concentrations of tobramycin in patients with renal dysfunction or patients treated with concomitant parenteral tobramycin should be monitored at the discretion of the treating physician.

##### **Renal Function**

The clinical studies of tobramycin did not reveal any imbalance in the percentage of patients in the tobramycin and placebo groups who experienced at least a 50% rise in serum creatinine from baseline (see ADVERSE REACTIONS). Laboratory tests of urine and renal function should be conducted at the discretion of the treating physician.

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### **Carcinogenesis, Mutagenesis, and Impairment of Fertility**

A 2-year rat inhalation toxicology study to assess carcinogenic potential of tobramycin is in progress.

Tobramycin has been evaluated for genotoxicity in a battery of *in vitro* and *in vivo* tests. The Ames bacterial reversion test, conducted with five tester strains, failed to show a significant increase in revertants with or without metabolic activation in all strains. Tobramycin was negative in the mouse lymphoma forward mutation assay, did not induce chromosomal aberrations in Chinese hamster ovary cells, and was negative in the mouse micronucleus test.

Subcutaneous administration of up to 100 mg/kg of tobramycin did not affect mating behavior or cause impairment of fertility in male or female rats.

### **Pregnancy, Teratogenic Effects, Pregnancy Category D**

No reproduction toxicology studies have been conducted with tobramycin. However, subcutaneous administration of tobramycin at doses of 100 or 20 mg/kg/day during organogenesis was not teratogenic in rats or rabbits, respectively. Doses of tobramycin <sup>3</sup>40 mg/kg/day were severely maternally toxic to rabbits and precluded the evaluation of teratogenicity. Aminoglycosides can cause fetal harm (e.g., congenital deafness) when administered to a pregnant woman. Ototoxicity was not evaluated in offspring during nonclinical reproduction toxicity studies with tobramycin. If tobramycin is used during pregnancy, or if the patient becomes pregnant while taking tobramycin, the patient should be apprised of the potential hazard to the fetus. (See WARNINGS).

### **Nursing Mothers**

It is not known if tobramycin will reach sufficient concentrations after administration by inhalation to be excreted in human breast milk. Because of the potential for ototoxicity and nephrotoxicity in infants, a decision should be made whether to terminate nursing or discontinue tobramycin.

### **Pediatric Use**

In pediatric patients under 6 years of age, safety and efficacy of tobramycin have not been studied.

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### **DRUG INTERACTIONS**

In clinical studies of tobramycin, patients taking tobramycin concomitantly with dornase alfa, b-agonists, inhaled corticosteroids, other anti-pseudomonal antibiotics, or parenteral aminoglycosides demonstrated adverse experience profiles similar to the study population as a whole.

Concurrent and/or sequential use of tobramycin with other drugs with neurotoxic or ototoxic potential should be avoided. Some diuretics can enhance aminoglycoside toxicity by altering antibiotic concentrations in serum and tissue. Tobramycin should not be administered concomitantly with ethacrynic acid, furosemide, urea, or mannitol.

### **ADVERSE REACTIONS**

Tobramycin was generally well tolerated during two clinical studies in 258 cystic fibrosis patients ranging in age from 6-63 years. Patients received tobramycin in alternating periods of 28 days on and 28 days off drug in addition to their standard cystic fibrosis therapy for a total of 24 weeks.

Voice alteration and tinnitus were the only adverse experiences reported by significantly more tobramycin-treated patients. Thirty-three patients (13%) treated with tobramycin complained of voice alteration compared to 17 (7%) placebo patients. Voice alteration was more common in the on-drug periods.

Eight (8) patients from the tobramycin group (3%) reported tinnitus compared to no placebo patients. All episodes were transient, resolved without discontinuation of the tobramycin treatment regimen, and were not associated with loss of hearing in audiograms. Tinnitus is one of the sentinel symptoms of cochlear toxicity, and patients with this symptom should be carefully monitored for high frequency hearing loss. The numbers of patients reporting vestibular adverse experiences such as dizziness were similar in the tobramycin and placebo groups.

Nine (3%) patients in the tobramycin group and 9 (3%) patients in the placebo group had increases in serum creatinine of at least 50% over baseline. In all 9 patients in the tobramycin group, creatinine decreased at the next visit.

TABLE 2 lists the percent of patients with treatment-emergent adverse experiences (spontaneously reported and solicited) that occurred in >5% of tobramycin patients during the two Phase III studies.

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<b>TABLE 2 Percent of Patients With Treatment Emergent Adverse Experiences Occurring in &gt;5% of Tobramycin Patients</b>		
<b>Adverse Event</b>	<b>Tobramycin (n=258)</b>	<b>Placebo (n=262)</b>
Cough increased	46.1%	47.3%
Pharyngitis	38.0%	39.3%
Sputum increased	37.6%	39.7%
Asthenia	35.7%	39.3%
Rhinitis	34.5%	33.6%
Dyspnea	33.7%	38.5%
Fever*	32.9%	43.5%
Lung disorder	31.4%	31.3%
Headache	26.7%	32.1%
Chest pain	26.0%	29.8%
Sputum discoloration	21.3%	19.8%
Hemoptysis	19.4%	23.7%
Anorexia	18.6%	27.9%
Lung function decreased†	16.3%	15.3%
Asthma	15.9%	20.2%
Vomiting	14.0%	22.1%
Abdominal pain	12.8%	23.7%
Voice alteration	12.8%	6.5%
Nausea	11.2%	16.0%
Weight loss	10.1%	15.3%
Pain	8.1%	12.6%
Sinusitis	8.1%	9.2%
Ear pain	7.4%	8.8%
Back pain	7.0%	8.0%
Epistaxis	7.0%	6.5%
Taste perversion	6.6%	6.9%
Diarrhea	6.2%	10.3%
Malaise	6.2%	5.3%
Lower resp. tract infection	5.8%	8.0%
Dizziness	5.8%	7.6%
Hyperventilation	5.4%	9.9%
Rash	5.4%	6.1%

\*Includes subjective complaints of fever.

†Includes reported decreases in pulmonary function tests or decreased lung volume on chest radiograph associated with intercurrent illness or study drug administration.

## **OVERDOSAGE**

Signs and symptoms of acute toxicity from overdosage of IV tobramycin might include dizziness, tinnitus, vertigo, loss of high-tone hearing acuity, respiratory failure, and neuromuscular blockade. Administration by inhalation results in low systemic bioavailability of tobramycin. Tobramycin is not significantly absorbed following oral administration. Tobramycin serum concentrations may be helpful in monitoring overdosage.

In all cases of suspected overdosage, physicians should contact the Regional Poison Control Center for information about effective treatment. In the case of any overdosage, the possibility of drug interactions with alterations in drug disposition should be considered.

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### **DOSAGE AND ADMINISTRATION**

The recommended dosage for both adults and pediatric patients 6 years of age and older is 1 single-use closure system (190 mg) administered BID for 28 days. Dosage is not adjusted by weight. All patients should be administered 190 mg BID. The doses should be taken as close to 12 hours apart as possible; they should not be taken less than 6 hours apart.

Tobramycin is inhaled while the patient is sitting or standing upright and breathing normally through the mouthpiece of the nebulizer. Nose clips may help the patient breathe through the mouth.

Tobramycin is administered BID in alternating periods of 28 days. After 28 days of therapy, patients should stop tobramycin therapy for the next 28 days, and then resume therapy for the next 28 day on/28 day off cycle.

Tobramycin is supplied as a single-use closure system and is administered by inhalation, using a hand-held eFlow™ Electronic Inhaler. Tobramycin is not for subcutaneous, intravenous or Intrathecal administration.

### **Usage**

Tobramycin is administered by inhalation over a 3-4 minute period, using a hand-held eFlow™ Electronic Inhaler. Tobramycin should not be diluted or mixed with dornase alfa in the nebulizer.

During clinical studies, patients on multiple therapies were instructed to take them first, followed by tobramycin.

### **REFERENCES**

1. Neu HC. Tobramycin: an overview. [Review]. J Infect Dis 1976; Suppl 134:S3-19.
2. Weber A, Smith A, Williams-Warren J et al. Nebulizer delivery of tobramycin to the lower respiratory tract. Pediatr Pulmonol 1994; 17 (5):331-9.
3. Bryan LE, Aminoglycoside resistance. Bryan LE, Ed. Antimicrobial drug resistance. Orlando, FL: Academic Press, 1984: 241-77.

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**HOW SUPPLIED**

TOFIN™ (Tobramycin for Inhalation) is supplied in a 2ml closure system.

Storage: Tobramycin should be stored under refrigeration at 2-8°C/36-46°F. Upon removal from the refrigerator, or if refrigeration is unavailable, Tobramycin pouches (opened or unopened) may be stored at room temperature (up to 25°C/77°F) for up to 28 days.

Tobramycin should not be used beyond the expiration date stamped on the ampule when stored under refrigeration (2-8°C/36-46°F) or beyond 28 days when stored at room temperature (25°C/77°F).

Tobramycin should not be exposed to intense light. The solution in the ampule is slightly yellow, but may darken with age if not stored in the refrigerator; however, the color change does not indicate any change in the quality of the product as long as it is stored within the recommended storage conditions.

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## APPENDIX # 5

### DESCRIPTION

TOBI® is a tobramycin solution for inhalation. It is a sterile, clear, slightly yellow, non-pyrogenic, aqueous solution with the pH and salinity adjusted specifically for administration by a compressed air driven reusable nebulizer. The chemical formula for tobramycin is  $C_{18}H_{37}N_5O_9$  and the molecular weight is 467.52. Tobramycin is O-3-amino-3-deoxy-β-D-glucopyranosyl-(1.4)-O-[2,6-diamino-2,3,6-trideoxy-β-D-ribo-hexopyranosyl-(1.6)]-2-deoxy-L-streptamine. The structural formula for tobramycin is:  
Each single-use 5 mL ampule contains 300 mg tobramycin and 11.25 mg sodium chloride in sterile water for injection. Sulfuric acid and sodium hydroxide are added to adjust the pH to 6.0. Nitrogen is used for sparging. All ingredients meet USP requirements. The formulation contains no preservatives.

### CLINICAL PHARMACOLOGY

TOBI is specifically formulated for administration by inhalation. When inhaled, tobramycin is concentrated in the airways.

#### Pharmacokinetics

TOBI contains tobramycin, a cationic polar molecule that does not readily cross epithelial membranes.<sup>(1)</sup> The bioavailability of TOBI may vary because of individual differences in nebulizer performance and airway pathology.<sup>(2)</sup> Following administration of TOBI, tobramycin remains concentrated primarily in the airways.

**Sputum Concentrations:** Ten minutes after inhalation of the first 300 mg dose of TOBI, the average concentration of tobramycin was 1237 µg/g (ranging from 35 to 7414 µg/g) in sputum. Tobramycin does not accumulate in sputum; after 20 weeks of therapy with the TOBI regimen, the average concentration of tobramycin at ten minutes after inhalation was 1154 µg/g (ranging from 39 to 8085 µg/g) in sputum. High variability of tobramycin concentration in sputum was observed. Two hours after inhalation, sputum concentrations declined to approximately 14% of tobramycin levels at ten minutes after inhalation.

**Serum Concentrations:** The average serum concentration of tobramycin one hour after inhalation of a single 300 mg dose of TOBI by cystic fibrosis patients was 0.95 µg/mL. After 20 weeks of therapy on the TOBI regimen, the average serum tobramycin concentration one hour after dosing was 1.05 µg/mL.

**Elimination:** The elimination half-life of tobramycin from serum is approximately 2 hours after intravenous (IV) administration. Assuming tobramycin absorbed following inhalation behaves similarly to tobramycin following IV administration, systemically absorbed tobramycin is eliminated principally by glomerular filtration. Unabsorbed tobramycin, following TOBI administration, is probably eliminated primarily in expectorated sputum.

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### Microbiology

Tobramycin is an aminoglycoside antibiotic produced by *Streptomyces tenebrarius*.<sup>(1)</sup> It acts primarily by disrupting protein synthesis, leading to altered cell membrane permeability, progressive disruption of the cell envelope, and eventual cell death.<sup>(3)</sup>

Tobramycin has in vitro activity against a wide range of gram-negative organisms including *Pseudomonas aeruginosa*. It is bactericidal at concentrations equal to or slightly greater than inhibitory concentrations.

### Susceptibility Testing

A single sputum sample from a cystic fibrosis patient may contain multiple morphotypes of *Pseudomonas aeruginosa* and each morphotype may have a different level of in vitro susceptibility to tobramycin. Treatment for 6 months with TOBI in two clinical studies did not affect the susceptibility of the majority of *P. aeruginosa* isolates tested; however, increased minimum inhibitory concentrations (MICs) were noted in some patients. The clinical significance of this information has not been clearly established in the treatment of *P. aeruginosa* in cystic fibrosis patients. For additional information regarding the effects of TOBI on *P. aeruginosa* MIC values and bacterial sputum density, please refer to the **CLINICAL STUDIES** section.

The in vitro antimicrobial susceptibility test methods used for parenteral tobramycin therapy can be used to monitor the susceptibility of *P. aeruginosa* isolated from cystic fibrosis patients. If decreased susceptibility is noted, the results should be reported to the clinician.

Susceptibility breakpoints established for parenteral administration of tobramycin do not apply to aerosolized administration of TOBI. The relationship between in vitro susceptibility test results and clinical outcome with TOBI therapy is not clear.

### INDICATIONS AND USAGE

TOBI is indicated for the management of cystic fibrosis patients with *P. aeruginosa*.

Safety and efficacy have not been demonstrated in patients under the age of 6 years, patients with FEV<sub>1</sub> <25% or >75% predicted, or patients colonized with *Burkholderia cepacia* (see **CLINICAL STUDIES**).

### CONTRAINDICATIONS

TOBI is contraindicated in patients with a known hypersensitivity to any aminoglycoside.

### WARNINGS

Caution should be exercised when prescribing TOBI to patients with known or suspected renal, auditory, vestibular, or neuromuscular dysfunction. Patients receiving concomitant parenteral aminoglycoside therapy should be monitored as clinically appropriate.

Aminoglycosides can cause fetal harm when administered to a pregnant woman. Aminoglycosides cross the placenta, and streptomycin has been associated with several reports of total, irreversible, bilateral congenital deafness in pediatric

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patients exposed in utero. Patients who use TOBI during pregnancy, or become pregnant while taking TOBI should be apprised of the potential hazard to the fetus.

### Ototoxicity

Ototoxicity, as measured by complaints of hearing loss or by audiometric evaluations, did not occur with TOBI therapy during clinical studies. However, transient tinnitus occurred in eight TOBI-treated patients versus no placebo patients in the clinical studies. Tinnitus may be a sentinel symptom of ototoxicity, and therefore the onset of this symptom warrants caution (see **ADVERSE REACTIONS**). Ototoxicity, manifested as both auditory and vestibular toxicity, has been reported with parenteral aminoglycosides. Vestibular toxicity may be manifested by vertigo, ataxia or dizziness.

**In postmarketing experience, patients receiving TOBI have reported hearing loss.** Some of these reports occurred in patients with previous or concomitant treatment with systemic aminoglycosides. Patients with hearing loss frequently reported tinnitus.

### Nephrotoxicity

Nephrotoxicity was not seen during TOBI clinical studies but has been associated with aminoglycosides as a class. If nephrotoxicity occurs in a patient receiving TOBI, tobramycin therapy should be discontinued until serum concentrations fall below 2 µg/mL.

### Muscular Disorders

TOBI should be used cautiously in patients with muscular disorders, such as myasthenia gravis or Parkinson's disease, since aminoglycosides may aggravate muscle weakness because of a potential curare-like effect on neuromuscular function.

### Bronchospasm

Bronchospasm can occur with inhalation of TOBI. In clinical studies of TOBI, changes in FEV<sub>1</sub> measured after the inhaled dose were similar in the TOBI and placebo groups. Bronchospasm should be treated as medically appropriate.

## PRESCRIBING INFORMATION

### Nebulizer Solution — For Inhalation Use Only

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OH  
CH<sub>2</sub>NH<sub>2</sub>  
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®

40-1013-C

### PRECAUTIONS

#### Information for Patients

**NOTE:** In addition to information provided below, a Patient Medication Guide providing instructions

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for proper use of TOBI is contained inside the package.

### Safety Information

TOBI is in a class of antibiotics that have caused hearing loss, dizziness, kidney damage, and harm to a fetus. Ringing in the ears and hoarseness were two symptoms that were seen in more patients taking TOBI than placebo in research studies. Patients with cystic fibrosis can have many symptoms. Some of these symptoms may be related to your medications. If you have new or worsening symptoms, you should tell your doctor.

**Hearing:** You should tell your doctor if you have ringing in the ears, dizziness, or any changes in hearing.

**Kidney Damage:** Inform your doctor if you have any history of kidney problems.

**Pregnancy:** If you want to become pregnant or are pregnant while on TOBI, you should talk with your doctor about the possibility of TOBI causing any harm.

**Nursing Mothers:** If you are nursing a baby, you should talk with your doctor before using TOBI.

### TOBI Packaging

TOBI comes in a single dose, ready-to-use ampule containing 300 mg tobramycin. Each box of TOBI contains a 28-day supply - 56 ampules packaged in 14 foil pouches. Each foil pouch contains four ampules, for two days of TOBI therapy.

### Dosage

The 300 mg dose of TOBI is the same for patients regardless of age or weight. TOBI has not been studied in patients less than six years old. Doses should be inhaled as close to 12 hours apart as possible and not less than six hours apart.

You should not mix TOBI with dornase alfa (PULMOZYME®, Genentech) in the nebulizer.

If you are taking several medications the recommended order is as follows: bronchodilator first, followed by chest physiotherapy, then other inhaled medications and, finally, TOBI.

### Treatment Schedule

You should take TOBI in repeated cycles of 28 days on drug followed by 28 days off drug. You should take TOBI twice a day during the 28 day period on drug.

### How To Administer TOBI

THIS INFORMATION IS NOT INTENDED TO REPLACE CONSULTATION WITH YOUR PHYSICIAN AND CF CARE TEAM ABOUT PROPERLY TAKING MEDICATION OR USING INHALATION EQUIPMENT.

TOBI is specifically formulated for inhalation using a PARI LC PLUS™ Reusable Nebulizer and a DeVilbiss Pulmo-Aide® air compressor. TOBI can be taken at home, school, or at work. The following are instructions on how to use the DeVilbiss Pulmo-Aide air compressor and PARI LC PLUS Reusable Nebulizer to administer TOBI.

You will need the following supplies:

- TOBI plastic ampule (vial)
- DeVilbiss Pulmo-Aide air compressor

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- PARI LC PLUS Reusable Nebulizer
- Tubing to connect the nebulizer and compressor
- Clean paper or cloth towels
- Nose clips (optional)

It is important that your nebulizer and compressor function properly before starting your TOBI therapy.

Note: Please refer to the manufacturers' care and use instructions for important information.

### **Preparing Your TOBI for Inhalation**

1. Wash your hands thoroughly with soap and water.

2a. TOBI is packaged with four ampules per foil pouch.

2b. Separate one ampule by gently pulling apart at the bottom tabs. Store all remaining ampules in the refrigerator as directed.

3. Lay out the contents of a PARI LC PLUS Reusable Nebulizer package on a clean, dry paper or cloth towel. You should have the following parts:

- Nebulizer Top and Bottom (Nebulizer Cup)

Assembly

- Inspiratory Valve Cap
- Mouthpiece with Valve
- Tubing

4. Remove the Nebulizer Top from the Nebulizer Cup by twisting the Nebulizer Top counter-clockwise, and then lifting. Place the Nebulizer Top on the clean paper or cloth towel. Stand the Nebulizer Cup upright on the towel.

5. Connect one end of the tubing to the compressor air outlet. The tubing should fit snugly. Plug in your compressor to an electrical outlet.

6. Open the TOBI ampule by holding the bottom tab with one hand and twisting off the top of the ampule with the other hand. Be careful not to squeeze the ampule until you are ready to empty its contents into the Nebulizer Cup.

7. Squeeze all the contents of the ampule into the Nebulizer Cup.

8. Replace the Nebulizer Top. Note: In order to insert the Nebulizer Top into the Nebulizer Cup, the semi-circle halfway down the stem of the Nebulizer Top should face the Nebulizer Outlet.

9. Attach the Mouthpiece to the Nebulizer Outlet. Then firmly push the Inspiratory Valve Cap in place on the Nebulizer Top. Note: the Inspiratory Valve Cap will fit snugly.

10. Connect the free end of the tubing to the Air Intake on the bottom of the nebulizer, making sure to keep the nebulizer upright. Press the tubing on the Air Intake firmly.

### **TOBI Treatment**

1. Turn on the compressor.
2. Check for a steady mist from the Mouthpiece. If there is no mist, check all tubing connections and confirm that the compressor is working properly.
3. Sit or stand in an upright position that will allow you to breathe normally.
4. Place Mouthpiece between your teeth and on top of your tongue and breathe normally only through your mouth. Nose clips may help you breathe through your mouth and not through your nose. Do not block airflow with your tongue.
5. Continue treatment until all your TOBI is gone.

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and there is no longer any mist being produced. You may hear a sputtering sound when the Nebulizer Cup is empty. The entire TOBI treatment should take approximately 15 minutes to complete. *Note: if you are interrupted, need to cough or rest during your TOBI treatment, turn off the compressor to save your medication. Turn the compressor back on when you are ready to resume your therapy.*

6. Follow the nebulizer cleaning and disinfecting instructions after completing therapy.

### **Cleaning Your Nebulizer**

To reduce the risk of infection, illness or injury from contamination, you must thoroughly clean all parts of the nebulizer as instructed after each treatment. Never use a nebulizer with a clogged nozzle. If the nozzle is clogged, no aerosol mist is produced, which will alter the effectiveness of the treatment. Replace the nebulizer if clogging occurs.

1. Remove tubing from nebulizer and disassemble nebulizer parts.
2. Wash all parts (except tubing) with warm water and liquid dish soap.
3. Rinse thoroughly with warm water and shake out water.
4. Air dry or hand dry nebulizer parts on a clean, lint-free cloth. Reassemble nebulizer when dry, and store.
5. You can also wash all parts of the nebulizer in a dishwasher (except tubing). Place the nebulizer parts in a dishwasher basket, then place on the top rack of the dishwasher. Remove and dry the parts when the cycle is complete.

### **Disinfecting Your Nebulizer**

Your nebulizer is for your use only - Do not share your nebulizer with other people. You must regularly disinfect the nebulizer. Failure to do so could lead to serious or fatal illness.

1. Clean the nebulizer as described above. Every other treatment day, soak all parts of the nebulizer (except tubing) in a solution of 1 part distilled white vinegar and 3 parts hot tap water for 1 hour. You can substitute respiratory equipment disinfectants (such as Control III®) for distilled white vinegar (*follow manufacturer's instructions for mixing*). Rinse all parts of the nebulizer thoroughly with warm tap water and dry with a clean, lint-free cloth. Discard the vinegar solution when disinfection is complete.
2. The nebulizer parts (except tubing) may also be disinfected by boiling them in water for a full 10 minutes. Dry parts on a clean, lint-free cloth.

### **Care and Use of Your Pulmo-Aide Compressor**

Follow the manufacturer's instructions for care and use of your compressor.

**Filter Change:**

1. DeVilbiss Compressor filters should be changed every six months or sooner if filter turns completely gray in color.

**Compressor Cleaning:**

1. With power switch in the "Off" position, unplug power cord from wall outlet.
2. Wipe outside of the compressor cabinet with a

EXAMPLE 3  
IN VIVO STUDY 2

A comparison was made of the safety, pharmacokinetics, aerosol delivery characteristics, and nebulization time of the conventional dose and inhalation delivery system (5 mL ampoule containing 300 mg tobramycin and 11.25 mg sodium chloride in sterile water for injection (TOBI® tobramycin solution for inhalation, Chiron Corporation, Seattle, Washington), pH 6.0; administered with a PARI LC PLUS™ jet nebulizer with a DeVilbiss PulmoAide™ compressor set to deliver an output pressure of 20 psi – the "control delivery system") with a dose of 420 mg Tobramycin Solution for Inhalation at 120 mg/mL (excipient 3.5 mL of 1/4 normal saline adjusted to a pH of 6.0 ± 0.5; 420 mg in 3.5 mL) delivered by the PARI LC PLUS™ jet nebulizer with a Invacare MOBILAIRE™ compressor set to deliver an output pressure of 35 psi (the "experimental delivery system").

The study was designed as an open label, randomized, single-dose, multicenter, two treatment, active-control, and parallel trial. Each patient was administered a single aerosolized dose of study drug with either the control delivery system or the experimental delivery system. In accordance with the study design, a total of 36 eligible male and female patients 12 years of age or older with a confirmed diagnosis of cystic fibrosis were enrolled with a minimum of 4 patients at each site. A 2:1 randomization ratio was employed for assignment of patients to the treatment groups. In the presence of the investigator or study coordinator, each patient was to self-administer either a single dose of 300 mg TOBI® with the control delivery treatment or a single dose of 420 mg Tobramycin Solution for Inhalation with the experimental delivery treatment as listed below.

Control Treatment:

Aerosolized 300 mg TOBI® was delivered by PARI LC PLUS jet nebulizer/DeVilbiss PulmoAide compressor: Preservative free tobramycin for inhalation 60 mg/mL (excipient 5 mL of 1/4 normal saline adjusted to a pH of 6.0 ± 0.5); 300 mg in 5 mL; lot number 03K1C (TOBI® at 60 mg/mL).

Experimental Treatment (420mg Tobramycin Solution for Inhalation or "TSI"):

Aerosolized 420 mg Tobramycin Solution for Inhalation (TSI) was delivered by PARI LC PLUS jet nebulizer/Invacare MOBILAIRE compressor: Preservative  
5 free tobramycin 120 mg/mL (excipient 3.5 mL of 1/4 normal saline adjusted to a pH of  $6.0 \pm 0.5$ ); 420 mg in 3.5 mL.

Both 300 mg TOBI<sup>®</sup> and 420 mg Tobramycin Solution for Inhalation are sterile, non-pyrogenic, preservative-free antibiotics prepared for aerosolization. Each mL of TOBI<sup>®</sup> contains 60 mg tobramycin and 2.25 mg sodium chloride in sterile  
10 water for injection, pH  $6.0 \pm 0.5$  (control treatment). Each mL of TSI contains 120 mg tobramycin and 2.25 mg sodium chloride in sterile water for injection, pH  $6.0 \pm 0.5$  (experimental treatment). ~~Drug supplies for this study were manufactured by Automated Liquid Packaging (ALP), Woodstock, IL. All repackaging, labeling, and distribution for clinical use was provided by Packaging Coordinators, Inc. (PCI),~~  
15 Philadelphia, PA. Study drug and device supplies were shipped from Chiron Corporation, Emeryville, CA for each patient upon enrollment in the study.

The duration of study participation for each patient was approximately two weeks including a brief (one day one week before treatment) screening period, one day treatment period, and a follow-up one-week after treatment. Study treatments  
20 were evaluated for safety and aerosol delivery characteristics up to eight hours post-dose on the day of the single dose treatment administration. The patient was to return to the clinic for a seven day post-treatment follow-up assessment of safety. There were no planned interim safety analyses.

**Criteria for evaluation:**

25 Safety:

- Incidence of bronchospasm defined as FEV<sub>1</sub> decrease of  $\geq 10\%$  and FEV<sub>1</sub> decrease of  $\geq 20\%$  from predose to 30 minutes postdose;
- Relative change and absolute change in airway response (FEV<sub>1</sub>) after single dose of study drug;
- 30 • Laboratory measures of safety (clinical lab tests, spirometry testing);

- Incidence of treatment emergent adverse event:

#### Aerosol Delivery:

- Pharmacokinetic assessment of sputum and serum tobramycin concentrations;
  - Sputum was collected at pre-dose and 15 minutes, 1, 2, 4, and 8 hours after dosing;
  - Serum was collected at pre-dose and 10 minutes, 1, 2, 4, 6, and 8 hours after dosing;
- Nebulization time.

10 **Statistical methods:** All patients who received a dose of study treatment were evaluated for safety and aerosol delivery characteristics.

Rate of bronchospasm measured by the percent of patients with  $\geq 10\%$  and  $\geq 20\%$  relative decrease in  $FEV_1\%$  from pre-dose to 30 minutes post-dose was summarized and compared between treatments using the Fisher's exact test.

15 A two sample t-test was used to compare the relative change in  $FEV_1\%$  from predose to 30 minutes postdose between experimental and control treatments. Summary statistics for relative and absolute change in  $FEV_1$  were tabulated by treatment.

20 Sputum and serum area under curve ( $AUC_{0-8}$ ) and maximum concentrations ( $C_{max}$ ) were summarized and analyzed for treatment differences using a general linear model analysis of variance (ANOVA). Pharmacokinetic parameters were calculated using a non-compartmental model. Sputum and serum concentrations were summarized and graphically illustrated by treatment.

Laboratory measures of safety and incidence of treatment-emergent adverse events were summarized and descriptively compared between treatments.

25 Nebulization time was recorded and summarized for each of the two delivery treatments.

#### Safety Variables

Aerosol delivery variables were tobramycin concentrations in sputum and serum, sputum and serum tobramycin pharmacokinetic parameters, and aerosol

nebulization time. Safety variables were the incidence and severity of bronchospasm, measured as the number of patients experiencing a  $\geq 10\%$  and a  $\geq 20\%$  decrease in forced expiratory volume in one second ( $FEV_1$ ) within 30 minutes after dosing (a  $\geq 20\%$  decrease in  $FEV_1$  was considered clinically significant), the  
5 incidence of treatment emergent adverse events (AEs), clinical laboratory test results, the number of patients with serum tobramycin concentrations  $\geq 4 \mu\text{g/mL}$ , physical examination findings, and vital signs results.

#### Primary Aerosol Delivery Variables

Evaluation of the aerosol delivery characteristics of 420 mg Tobramycin  
10 Solution for Inhalation at 120mg/mL delivered by the PARI LC PLUS<sup>TM</sup>/Invacare MOBILAIRE<sup>TM</sup> delivery system compared to 300 mg TOBI<sup>®</sup> at 60 mg/mL delivered by the FDA-approved PARI LC PLUS<sup>TM</sup>/DeVilbiss PulmoAide<sup>TM</sup> delivery system was based on determination of sputum and serum tobramycin concentrations, calculation of certain sputum and serum pharmacokinetic parameters, and  
15 measurement of nebulization time.

Sputum Tobramycin Concentrations: Sputum samples were expectorated by patients from a deep cough and collected before day 1 dosing (predose) and at 0.25, 1, 2, 4, and 8 h after the end of the nebulization period. Sputum samples were collected as close as possible to specified times and were considered to have been  
20 drawn on time within  $\pm 2$  minutes for the 15-minute posttreatment collection and within  $\pm 10$  minutes for the 1-, 2-, 4-, and 8-hour posttreatment collections. Samples collected outside these intervals were considered protocol deviations. A minimum 100 mg sputum (not saliva) sample was collected before the single dose of study treatment to determine the baseline tobramycin concentration. Immediately after  
25 dosing, patients rinsed their mouths with 30 mL of normal saline, gargled for 5-10 seconds, and expectorated the rinse. This sequence of post-treatment rinsing was repeated for a total of three rinses. Sputum samples were stored at  $-70^\circ\text{C}$  or below until analysis. The concentration of tobramycin was analyzed using reversed-phase high-performance liquid chromatography (HPLC) with ultraviolet detection. Patient  
30 sputum samples were first liquefied with 0.2 N NaOH and diluted with Tris buffer

(20.0 g Trizma base/L). Sputum standard samples were prepared by spiking diluted pooled sputum from CF patients with tobramycin to final concentrations of 0, 20, 40, 100, 200, 400, and 1000  $\mu\text{g/g}$  of sputum. Assay quality control samples were prepared by spiking diluted pooled sputum to contain 40, 300, and 800  $\mu\text{g/g}$ . The

5 internal standard sisomycin (100  $\mu\text{L}$ , 0.15 mg/mL in Tris buffer) was added to 100  $\mu\text{L}$  of each standard, control, and subject sample, followed by 400  $\mu\text{L}$  of acetonitrile and 50  $\mu\text{L}$  of 2,4-dinitrofluorobenzene (0.17 g/mL). The sample reaction mixtures were heated in a dry-block heater for 1 h at 80°C. After addition of 600  $\mu\text{L}$  of 60/40 acetonitrile/water (v/v), 50  $\mu\text{L}$  was analyzed by HPLC. Samples were injected onto a

10 Waters Nova-Pak<sup>®</sup> C-18, 3.9 x 150 mm, 4  $\mu\text{m}$  column connected to a Waters HPLC with 600E pump, 486 or 2487 ultraviolet detector ( $\lambda_{\text{max}} = 360 \text{ nm}$ ) and 717 Plus autosampler. The mobile phase consisted of 0.2% acetic acid in acetonitrile (39/61, v/v), pumped at a rate of 1.5 mL/min for 5 min, 2.0 mL/min for an additional 9 or 10 min, depending on the length of the run. Waters Millennium-32 C/S LC Software

15 (version 3.20) was used to operate the Waters HPLC instruments as well as acquire raw data, process, compute, and report the analytical results. The ratio of the peak height of tobramycin to the internal standard sisomycin (PHR) was calculated. The assay was completed in 8 runs. Retention time ranges of 4.2 to 4.4 min, and 10.8 to 11.8 min were observed for tobramycin and sisomycin, respectively. A linear

20 relationship existed between PHR and concentration from 20 to 1000  $\mu\text{g/g}$  for sputum. The regression model was  $\text{PHR} = Bx + A$  ( $x = \text{tobramycin concentration}$ ), weighted  $1/x$ . The lower limit of quantitation was 20  $\mu\text{g/g}$ . The concentrations of the standard samples were within 97 to 105% of the nominal concentration, with coefficients of variation not higher than 3.4%. The precision of the assay, as

25 reflected by the CV of the quality control samples, was 2.3%, 2.2% and 2.6%, for the 40, 300, and 800  $\mu\text{g/g}$  samples, respectively. The accuracy of the method, reflected by the interassay recoveries of the quality control samples, was 103%, 99%, and 98% for the 40, 300, and 800  $\mu\text{g/g}$  quality control samples, respectively. Overall, this method exhibited suitable accuracy and precision for pharmacokinetic analysis.

Serum Tobramycin Concentrations: Blood samples were collected at predose and at 0.167, 1, 2, 4, 6, and 8 h after the end of the nebulization period. Samples were collected as close as possible to specified times and were considered to have been drawn on time within  $\pm 2$  minutes for the 10-minute posttreatment collection and within  $\pm 10$  minutes for the 1-, 2-, 4-, 6-, and 8-hour posttreatment collections. Samples collected outside these intervals were considered protocol deviations. Serum was harvested and stored at  $-70^{\circ}\text{C}$  or below until analysis. Concentrations of tobramycin in serum were analyzed with a modified fluorescence polarization immunoassay (FPIA) method using the Abbott TDx<sup>®</sup>/TDxFLx<sup>®</sup> System. Samples were added directly to the dilution well of the sample cartridge. The net polarization was acquired by the TDx<sup>®</sup>/TDxFLx<sup>®</sup> apparatus and manually entered into an Oracle database. A weighted four parameter logistic equation was used to calculate the concentrations of tobramycin. The concentrations of tobramycin were reported in terms of free base equivalents. For assaying the subject samples of the study, calibration standards (0.050, 0.100, 0.200, 0.400, 0.600, 0.800, 1.000  $\mu\text{g}/\text{mL}$ ) and quality control samples (0.150, 0.400, and 0.750  $\mu\text{g}/\text{mL}$ ) were prepared in house. The assay was completed in 8 runs. A linear relationship existed between polarization response and concentration from 0.050  $\mu\text{g}/\text{mL}$  to 1.00  $\mu\text{g}/\text{mL}$ . The lower limit of quantitation was 0.050  $\mu\text{g}/\text{mL}$ . The precision of the assay, as reflected by the CV of the quality control samples, was 3.3%, 4.9%, and 4.9% for the 0.150, 0.400, and 0.750  $\mu\text{g}/\text{mL}$  samples, respectively. The accuracy of the method, reflected by the mean interassay recoveries of the quality control samples, was 101%, 103%, and 104% for the 0.150, 0.400, and 0.750  $\mu\text{g}/\text{mL}$  samples, respectively. Overall, this method exhibited suitable accuracy and precision for pharmacokinetic analysis.

Nebulization Time:

Nebulization time was defined as the length of time from the start of the patient's first tidal breath to completion of aerosol administration. Aerosol administration was complete when the nebulizer began to sputter. If aerosol administration was interrupted for any reason, the time of interruption and start and

stop times of continued aerosol administration were recorded. If dosing was interrupted, nebulization time was considered to be not calculable.

Residual Tobramycin in the Nebulizer: The amount of residual tobramycin solution remaining in the nebulizer after completion of aerosol administration was determined by recording pretreatment and posttreatment weight of the nebulizer system including nebulizer, filter valve, and study drug. The research coordinator collected residual study drug remaining in the nebulizer after aerosol administration into a vial labeled with patient information. The vial was returned for measurement of the amount of drug output from the nebulizer and for determination of the extent of the concentration of study drug left in the nebulizer.

#### Safety Variables

Bronchospasm: The study protocol prospectively identified bronchospasm as an adverse airway response to inhalation of aerosolized antibiotic of particular relevance to patients with cystic fibrosis. In order to determine whether current study treatments produced bronchospasm, patients performed spirometry (pulmonary function) tests to measure FEV<sub>1</sub> before and 30 minutes following completion of study treatment administration according to the method described in the protocol. Airway response to the study drug was assessed by evaluating the relative percent change in FEV<sub>1</sub> from predose to 30 minutes after the end of treatment using the following formula.

$$\text{relative FEV}_1 \text{ \% change} = \frac{30 \text{ min postdose FEV}_1 - \text{predose FEV}_1}{\text{predose FEV}_1} \times 100\%$$

Bronchospasm was defined as a decrease in FEV<sub>1</sub> of  $\geq 10\%$  at 30 minutes after dosing, relative to the predose result. A decrease in FEV<sub>1</sub> of  $\geq 20\%$  was considered to represent clinically significant bronchospasm. Moreover, if there was a posttreatment decrease in FEV<sub>1</sub> of  $\geq 30\%$ , spirometry was to be repeated until the FEV<sub>1</sub> decrease was  $< 10\%$  below the predose result. An FEV<sub>1</sub> % decrease  $\geq 30\%$ , and all symptoms associated with the change in pulmonary function, were to be recorded as adverse events. The protocol defined the severity of decrease in FEV<sub>1</sub>

based in part on the National Cancer Institute (NCI) Common Toxicity Criteria Adverse Events Grading Scale. However, slight inconsistencies in the protocol definitions of bronchospasm and of the severity of FEV<sub>1</sub> changes were noted during preparation of the analyses and report. To resolve the differences, the actual system used during the analysis to classify the severity of FEV<sub>1</sub> changes relative to the predose result is listed below.

**TABLE 16. AIRWAY RESPONSE (FEV<sub>1</sub>) (BRONCHOSPASM)**  
**FEV<sub>1</sub> % DECREASE BELOW PREDOSE VALUE**

Severity	Protocol Classification	Analysis Classification
Mild:	≥ 10% - ≤ 20%	≥ 10% - < 20%
Moderate:	> 20% - ≤ 30%	≥ 20% - < 30%
Severe:	> 30%	≥ 30%

#### Clinical Laboratory Tests

At screening, laboratory tests were performed to measure serum creatinine, blood urea nitrogen (BUN), urine protein (proteinuria by dipstick), and to detect pregnancy in females of childbearing potential. If abnormal at screening, serum creatinine, BUN, and urine protein tests were to be repeated before the time of dosing. Final test results were obtained based on specimens drawn at the follow-up visit on day 8 of the study.

After the mean body weight difference between treatment groups became known by Chiron personnel, estimated creatinine clearance was calculated for patients using the Cockcroft-Gault equation below to evaluate renal clearance characteristics of the two groups and to clarify the pharmacokinetic results of the study.

#### Male patients:

estimated creatinine

clearance (mL/min) = (140-age[yr])(body weight[kg])/72\*(serum creatinine

[mg/dL])

Female patients:

estimated creatinine

clearance (mL/min) =  $0.85 * ((140 - \text{age}[\text{yr}])(\text{body weight}[\text{kg}]) / 72 * (\text{serum creatinine} [\text{mg/dL}]))$

5 All abnormal laboratory test results, whether present on entry into the study or arising during the study, were evaluated by the study investigator for clinical significance and relationship to study drug. If the abnormal result was considered unrelated to study drug, the investigator was to identify the probable cause of the result. Laboratory results considered markedly abnormal and clinically significant  
10 were BUN > 16 mmole/l (> 45 mg/dl), serum creatinine > 177  $\mu\text{mole/l}$  (> 2 mg/dl), and proteinuria  $\geq 3+$ .

Other Safety Variables

Serum assay results were screened for tobramycin concentrations  $\geq 4 \mu\text{g/mL}$  from specimens collected from 10 minutes through 8 hours after completion of study  
15 treatments. In parallel, patient records and CRFs were examined for evidence of systemic toxicity potentially related to elevated tobramycin levels. Assay results were not available until after patients' discharge from the study, so screening for unusually high serum tobramycin concentrations and evidence of systemic toxicity was undertaken when all pertinent results were received.

20 Pharmacokinetics

Pharmacokinetic parameters for both sputum and serum tobramycin were derived to characterize aerosol delivery capabilities of control and experimental treatments. The concentration (C) versus time (t) data (Listings 16.2.5.2 and 16.2.5.3) were analyzed by model-independent methods to obtain the pharmacokinetic  
25 parameters. The areas under the plasma concentration-time curve from time zero (predose) to infinity (AUC) and under the first moment of the plasma concentration-time curve (AUMC) were obtained by the trapezoidal rule, extrapolated to infinity. The terminal rate constant ( $\lambda_z$ ) was determined by log-linear regression of the terminal phase. The maximum concentration ( $C_{\text{max}}$ ) and the time to maximum after

the end of the nebulization period ( $t_{\max}$ ) were obtained by inspection. In addition, the following parameters were calculated:

$$\begin{aligned}t_{1/2} &= \ln(2) / \lambda_z \\ CL/F &= D / AUC \\ V_z / F &= CL_{po} / \lambda_z\end{aligned}$$

- 5 where  $t_{1/2}$  is the terminal half-life,  $CL/F$  is the total body clearance, and  $V_z/F$  is the terminal volume of distribution.<sup>1</sup> Since the absolute bioavailability of tobramycin ( $F$ ) in the two formulations used in this study is not known, the calculated clearance and volume of distribution are hybrid parameters that do not account for differences in bioavailability between the two formulations. All parameters were calculated for  
10 serum; only  $AUC$ ,  $C_{\max}$ ,  $t_{\max}$ ,  $\lambda_z$ , and  $t_{1/2}$  were calculated for sputum.

Concentrations below the lower limit of quantitation were treated as zero for all calculations. Since there was an insufficient volume of matrix to assay tobramycin in the following time points, they were excluded from the pharmacokinetic analysis:

TABLE 17. EXCLUSIONS FROM PHARMACOKINETIC ANALYSIS

Matrix	Subject	Time
Serum	01-110	6
	02-116	1
	03-102	0.167, 1, 2
	03-105	0, 0.167, 1, 2, 4, 6, 8
	03-131	0.167
	05-125	4, 8
	06-120	2
Sputum	08-127	2

Data Handling

5 Case report form data were entered in duplicate into a Clintrial™ database by the department of Biostatistics and Clinical Data Management (BCDM) at Chiron Corporation. Data quality assurance was performed using PL/SQL and SAS™ 6.12 or higher software (SAS Institute, Cary, NC). Analysis was performed by Chiron Corporation, using SAS version 6.12 or higher software, based on a predefined  
 10 analysis plan developed by Chiron Corporation. The estimated overall database error rate was 0.xx% with an upper 95% confidence limit of 0.xx%. This upper confidence limit is below the departmental standard of 0.5%.

Statistical Methods and Determination of Sample Size

15 Statistical and Analytical Plans: Serum and sputum pharmacokinetic parameters, the incidence of bronchospasm, and the relative change from predose in

30-minute postdose FEV<sub>1</sub> % predicted were analyzed statistically to assess the significance of any apparent differences between test and reference treatments. All statistical tests described in following sections were two-tailed tests of significance, and the criterion for statistical significance was set at  $\alpha = 0.05$  unless otherwise noted.

#### Primary Aerosol Delivery Analyses:

All patients who received the single dose of test or reference treatment were included in the analysis and evaluation of aerosol delivery characteristics. Aerosol delivery was characterized on the basis of serum and sputum tobramycin concentrations, derived serum and sputum pharmacokinetic parameters, and nebulization time. The effect of treatment (300 mg TOBI vs 420 mg TSI), gender, and age group (less than 18, 18 years or older) on the *AUC*, *C*<sub>max</sub>,  $\lambda_z$ , *CL/F*, and *Vz/F* of tobramycin in serum, and on the *AUC*, *C*<sub>max</sub>, and  $\lambda_z$  of tobramycin in sputum, was analyzed by a three-way analysis of variance. Furthermore, the relationship between body weight and *AUC*, *C*<sub>max</sub>, *CL/F*, and *Vz/F* of tobramycin in serum, and between body weight and *AUC* and *C*<sub>max</sub> of tobramycin in sputum were analyzed by regression analysis. All tests employed a significance level  $\alpha = 0.05$ . All parameters are expressed as the mean  $\pm$  SD. A harmonic half-life was estimated as:

$$\overline{t_{1/2}} = \ln(2) / \overline{\lambda_z}$$

in which  $\overline{\lambda_z}$  is the arithmetic mean of the terminal rate constants at each dose. The standard deviation of the harmonic half-life,  $SD(\overline{t_{1/2}})$ , was obtained as:

$$SD(\overline{t_{1/2}}) = \frac{\ln(2)}{\overline{\lambda_z}} \times \frac{SD(\overline{\lambda_z})}{\overline{\lambda_z}}$$

where  $SD(\overline{\lambda_z})$  is the standard error of the mean terminal rate constant at each dose.

#### Safety Analyses

##### Analysis of Airway Response:

The primary safety variable was the rate of bronchospasm, defined as a  $\geq 10\%$  decrease in FEV<sub>1</sub> from predose to 30 minutes after treatment on day 1 of the study. Secondary safety variables were (a) the rate of clinically significant

bronchospasm, defined as a  $\geq 20\%$  decrease in FEV<sub>1</sub> from predose to 30 minutes after treatment on day 1, and (b) the relative change in FEV<sub>1</sub> from predose to 30 minutes after treatment on day 1. The rates of occurrence of all instances of bronchospasm (FEV<sub>1</sub> % decrease  $\geq 10\%$ ) and of all instances of clinically significant  
5 bronchospasm (FEV<sub>1</sub> % decrease  $\geq 20\%$ ) were analyzed to assess the statistical significance of test vs. reference treatment differences using the Fisher's Exact test. The protocol specified that the treatment difference in the incidence of bronchospasm would be tested for statistical significance using the Cochran-Mantel-Haenszel test. Due to the low incidence of bronchospasm in the enrolled patients, the Fisher's exact  
10 test was used for this analysis since it makes no assumptions regarding the minimum expected cell frequencies. The test vs. reference treatment difference in mean relative change from predose in 30-minute postdose FEV<sub>1</sub> % predicted was tested for statistical significance using the two-sample t-test.

Adverse Events: The total incidence of individual treatment emergent  
15 adverse events (percent of patients who experienced the event at least once during or after study treatment) was evaluated descriptively for any noteworthy differences between test and reference treatments. AEs were also summarized by severity (mild, moderate, severe) and drug relationship (unrelated, possibly related) for test and reference treatments.

#### 20 Disposition of Subjects

A total of 40 patients were screened for the study by the eight investigators. Thirty-eight of the 40 screened patients met entrance criteria, were enrolled in the study (Table 18), and were randomized to one of the two treatments. Enrollment and randomization of the 38 patients at the eight sites was as summarized in Table 18  
25 below:

TABLE 18. ENROLLMENT AND RANDOMIZATION BY SITE AND TREATMENT

Site	300 mg TOBI® PARI LC PLUS™/DeVilbiss PulmoAide™ Delivery System (no. patients enrolled and randomized)	420 mg TSI PARI LC PLUS™/Invacare MOBILAIRE™ Delivery System (no. patients enrolled and randomized)
01	2	2
02	1	3
03	2	6
04	0	2
05	2	4
06	2	3
07	2	2
08	3	2
<b>Total enrolled and randomized</b>	<b>14</b>	<b>24</b>

5 Two of the 40 screened patients failed to meet entrance criteria and were not enrolled in the study: one patient did not meet the protocol inclusion criterion requiring patients to have screening FEV<sub>1</sub> % predicted results that were  $\geq 25$  %; and one patient did not meet the exclusion criterion requiring patients to have not taken inhaled or intravenous aminoglycosides within seven days before study treatment

10 administration. Thirty-eight patients met all study entry criteria and were randomized to treatments. Thirty-seven of the 38 randomized patients received one dose of study treatment (Table 18). One patient was enrolled and randomized but was withdrawn from the study before dosing due to staff inability to establish venous access for predose day 1 (visit 2) blood draws. The 37 randomized and dosed

15 patients constituted the intent to treat (ITT) population. All 37 patients who received study treatments completed the study.

## AEROSOL DELIVERY EVALUATION

### Data Sets Analyzed

All 37 patients in the ITT population (i.e., those who were randomized and received a dose of study treatment) were evaluable for the aerosol delivery objective of the protocol. Twenty four patients received a dose of 420 mg TSI using the PARI LC PLUS™/Invacare MOBILAIRE™ Delivery System, and 13 patients received a dose of 300 mg TOBI® using the PARI LC PLUS™/DeVilbiss PulmoAide™ Delivery System. Patient 08/137 was excluded from all aerosol delivery evaluations due to withdrawal from the study before dosing.

### 10 Demographic and Other Baseline Characteristics

#### Demographic Characteristics:

Nineteen male and 18 female patients, 12 to 44 years of age and diagnosed with cystic fibrosis, constituted the ITT population. Thirty-one patients were Caucasian, four patients were Hispanic, and two patients were black. Gender and race distributions were similar between the 420 mg TSI and 300 mg TOBI® treatment groups. On the average, ITT patients in the 300 mg TOBI® group were approximately 2.7 years older, 4.9 centimeters taller, and 10.7 kilograms heavier at screening (visit 1) than ITT patients in the 420 mg TSI group. A similar treatment difference in mean body weight was apparent before day 1 (visit 2) dosing, and no noteworthy change in mean weight was noted between screening and day 1.

### 20 Analysis of Aerosol Delivery

Primary Aerosol Delivery Analysis: Examination of the mean plasma concentration–time plot for both formulations in serum (Figure 10) indicates that tobramycin is rapidly absorbed: all subjects achieved maximum concentrations in the time span of 10 min to 4 h. An elimination phase was also observed in the concentration-time profiles, with individual estimates of half-life ranging from 1.1 to 6.8 h. In sputum (Figure 11), maximum concentrations were achieved between 15 min and 2 h, and individual estimates of half-life ranged from 0.48 to 9.47 h. These estimates are consistent with previous studies.

Serum and sputum pharmacokinetic parameters are summarized in Tables 19 and 20 as follows.

5  
**TABLE 19 SERUM PHARMACOKINETIC PARAMETERS  
 (MEAN  $\pm$  SD) OF TOBRAMYCIN AFTER ADMINISTRATION  
 OF 300 MG TOBI AND 420 MG TSI**

Parameter	300 mg TOBI	420 mg TSI
$AUC$ ( $\mu\text{g h/mL}$ )	$4.38 \pm 1.97$	$4.41 \pm 1.69$
$C_{\text{max}}$ ( $\mu\text{g/mL}$ )	$0.861 \pm 0.344$	$0.906 \pm 0.542$
Median $t_{\text{max}}$ (h)	1 (1-4)*	1 (0.17-2)
$\lambda_z$ ( $\text{h}^{-1}$ )	$0.250 \pm 0.052$	$0.243 \pm 0.098$
$t_{1/2}$ (h)	$2.78 \pm 0.58$	$2.86 \pm 1.15$
$CL/F$ (L/h)	$88 \pm 62$	$114 \pm 59$
$V_z/F$ (L)	$379 \pm 325$	$511 \pm 278$

10  
**TABLE 20 SPUTUM PHARMACOKINETIC  
 PARAMETERS (MEAN  $\pm$  SD) OF TOBRAMYCIN AFTER  
 ADMINISTRATION OF 300 MG TOBI AND 420 MG TSI**

Parameter	300 mg TOBI	420 mg TSI
$AUC$ ( $\mu\text{g h/g}$ )	$1521 \pm 845$	$1176 \pm 686$
$C_{\text{max}}$ ( $\mu\text{g/g}$ )	$930 \pm 795$	$935 \pm 1040$
Median $t_{\text{max}}$ (h)	0.25 (0.25-2)*	0.25 (0.25-0.25)
$\lambda_z$ ( $\text{h}^{-1}$ )	$0.59 \pm 0.31$	$0.52 \pm 0.37$
$T_{1/2}$ (h)	$1.17 \pm 0.98$	$1.33 \pm 0.95$

15 The serum and sputum concentration-time curves for both treatments were virtually superimposable (Figures 10 and 11; Tables 19 and 20). Serum parameters ( $C_{\text{max}}$ ,  $t_{\text{max}}$ ,  $AUC$ ,  $CL/F$ ,  $V_z/F$ ) showed no statistically significant differences between the treatment groups (Table 19). Sputum parameters ( $AUC$ ,  $C_{\text{max}}$ , and  $\lambda_z$ ) also showed no statistically significant treatment differences (Table 20). Neither age nor

- body weight had a statistically significant effect on these pharmacokinetic parameters. In addition, there were no statistically significant correlations between serum and sputum  $AUC$ , and between serum and sputum  $C_{max}$ . The variability of the pharmacokinetic parameters in serum and in sputum was similar to previous trials.
- 5 In summary, these findings indicate that it is possible to achieve comparable serum and sputum levels of tobramycin to the 300 mg TOBI<sup>®</sup> formulation by using the 420 mg TSI formulation.

#### Secondary Aerosol Delivery Analyses

- Nebulization Time: Nebulization time was substantially reduced during administration of the test 420 mg TSI formulation below that observed during administration of the marketed 300 mg TOBI<sup>®</sup> formulation. Mean  $\pm$  SD total nebulization time was  $9.7 \pm 3.0$  minutes during 420 mg TSI administration compared to  $18.1 \pm 3.6$  minutes during 300 mg TOBI<sup>®</sup> administration (Table 21). These findings indicate that the reduced nebulization times used in the 420 mg TSI treatment did not change the pharmacokinetics of tobramycin relative to the marketed 300 mg TOBI<sup>®</sup> formulation.

**TABLE 21 MEAN (SD) NEBULIZATION TIME**

Parameter [mean (SD)]	300 mg TOBI PARI LC PLUS <sup>a</sup> PulmoAide Compressor (n = 13)	420 mg TSI PARI LC PLUS <sup>b</sup> MOBILAIRE Compressor (n = 24)
Nebulization Time (min)	18.1 (3.6)	9.7 (3.0)
-- No. pts with data	12	23

Source: Table 14.2.2.1.

Notes:

- a Reference treatment = TOBI 300 mg delivered by PARI LC PLUS nebulizer with PulmoAide compressor. Nebulization time for patient 07/132 indeterminate due to interruption in dosing and unrecorded stop/start times.
- b Test treatment = TSI 420 mg delivered by PARI LC PLUS nebulizer with MOBILAIRE compressor. Nebulization time for patient 07/126 indeterminate due to interruption in dosing and unrecorded stop/start times.

Nebulizer Weight: Nebulizer weight changes from before to after dosing indicated that the test 420 mg TSI formulation delivered less product to patients than the marketed 300 mg TOBI® formulation. Mean  $\pm$  SD amounts of product delivered to patients was  $1.86 \pm 0.53$  gm during 420 mg TSI administration and  $2.74 \pm 1.64$  gm during 300 mg TOBI® administration (Table 14.2.2.2), as summarized in Table 11.4-4 below. These findings likely reflect the smaller 3.5 mL volume of TSI formulation in the nebulizer compared to the 5 mL volume of the TOBI® formulation.

**TABLE 22 MEAN (SD) NEBULIZER WEIGHT AND CHANGE IN WEIGHT**

10

Parameter [mean (SD)]	300 mg TOBI <sup>a</sup> PARI LC PLUS PulmoAide Compressor (n = 13)	420 mg TSI <sup>b</sup> PARI LC PLUS MOBILAIRE Compressor (n = 24)
<u>Nebulizer Weight (gm)</u> <u>Predose</u> -- No. patients with data	68.25 (7.30) 13	69.17 (0.61) 24
<u>Postdose</u> -- No. patients with data	65.51 (6.89) 13	67.30 (0.80) 23
<u>Change in weight</u> -- No. patients with data	-2.74 (1.64) <sup>c</sup> 13	-1.86 (0.53) 23

**Notes:**

- a Reference treatment = TOBI 300 mg delivered by PARI LC PLUS nebulizer with PulmoAide compressor.
- b Test treatment = TSI 420 mg delivered by PARI LC PLUS nebulizer with MOBILAIRE compressor. Nebulizer weight for patient 02/116 not recorded after dosing.
- c The posttreatment nebulizer weight for patient 07/132 included the weight of the filter, and the pretreatment to posttreatment change in nebulizer weight was an increase by 2.20 gm. Excluding this erroneous value yields a mean (SD) change of -3.16 ( ) gm.

Discussion

Aerosol delivery findings indicate that it is possible to achieve comparable serum and sputum levels of tobramycin to the 300 mg TOBI® formulation by using the 420 mg TSI formulation. Present findings also indicate that the reduced nebulization times and reduced amount of product delivered to patients during

15

administration of the 420 mg TSI treatment did not change the pharmacokinetics of tobramycin relative to the marketed 300 mg TOBI<sup>®</sup> formulation.

Mean serum tobramycin concentration-time plots for both formulations indicate that tobramycin is rapidly absorbed: all subjects achieved maximum concentrations in the time span of 10 min to 4 h. An elimination phase was also  
5 observed in the concentration-time profiles, with individual estimates of half-life ranging from 1.1 to 6.8 h. In sputum, maximum concentrations were achieved between 15 min and 2 h, and individual estimates of half-life ranged from 0.48 to 9.47 h.

10 The serum and sputum concentration-time curves for both treatments in the present study were virtually superimposable. Serum parameters ( $C_{max}$ ,  $t_{max}$ ,  $AUC$ ,  $CL/F$ ,  $Vz/F$ ) showed no statistically significant differences between the treatment groups. Mean ( $\pm$  SD) serum  $C_{max}$  results for both the 420 mg TSI and the 300 mg TOBI<sup>®</sup> groups ( $0.906 \pm 0.542$   $\mu\text{g/mL}$  vs.  $0.861 \pm 0.344$   $\mu\text{g/mL}$ , respectively) were  
15 consistent with results from previous studies.<sup>5,40,41</sup> The average serum concentration of tobramycin one hour after inhalation of a single 300 mg dose of TOBI<sup>®</sup> by CF patients was 0.95  $\mu\text{g/mL}$ .<sup>5</sup> After 20 weeks of therapy on the TOBI<sup>®</sup> regimen, the average serum tobramycin concentration one hour after dosing was 1.05  $\mu\text{g/mL}$ .

Sputum parameters ( $AUC$ ,  $C_{max}$ , and  $\lambda_z$ ) also showed no statistically  
20 significant treatment differences in the present study. Mean ( $\pm$  SD) sputum  $C_{max}$  results for both the 420 mg TSI and the 300 mg TOBI<sup>®</sup> groups ( $935 \pm 1040$   $\mu\text{g/g}$  vs.  $930 \pm 795$   $\mu\text{g/g}$ , respectively) were consistent with results from previous studies.<sup>5,40,41</sup> Sputum results in the present study were highly variable. By comparison, high  
25 variability of tobramycin concentration in sputum was also observed in both Phase 3 trials.<sup>29,30</sup> Ten minutes after inhalation of the first 300 mg dose of TOBI<sup>®</sup> in the Phase 3 trials, the average concentration of tobramycin in sputum was 1237  $\mu\text{g/g}$  (ranging from 35 to 7414  $\mu\text{g/g}$ ). Tobramycin does not accumulate in sputum; after 20 weeks of therapy with the TOBI<sup>®</sup> regimen, the average concentration of tobramycin at ten minutes after inhalation was 1154  $\mu\text{g/g}$  (ranging from 39 to 8085

µg/g). Two hours after inhalation, sputum concentrations declined to approximately 14% of the tobramycin levels measured at ten minutes after inhalation.

Neither age nor body weight had a statistically significant effect on serum and sputum pharmacokinetic parameters. In addition, there were no statistically significant correlations between serum and sputum *AUC* and between serum and sputum *C*<sub>max</sub>.

Nebulization time for the test 420 mg TSI formulation was substantially reduced below that observed during administration of the marketed 300 mg TOBI<sup>®</sup> formulation (mean ± SD = 9.7 ± 3.0 min vs. 18.1 ± 3.6 min, respectively). Nebulization times for the marketed 300 mg TOBI<sup>®</sup> formulation were consistent with previous studies.<sup>40,41</sup> Therefore, the study achieved a key benchmark with the demonstration that the alternative delivery system, consisting of 3.5 mL of a 120 mg/mL (total 420 mg tobramycin) Tobramycin Solution for Inhalation (TSI) delivered using a PARI LC PLUS<sup>™</sup> jet nebulizer driven by an Invacare MOBILAIRE<sup>™</sup> compressor, reduced nebulization time below 10 minutes on the average.

Finally, present findings indicate that the reduced nebulization times during administration of the 420 mg TSI treatment did not change the pharmacokinetics of tobramycin relative to the marketed 300 mg TOBI<sup>®</sup> formulation.

Safety findings indicate that both a single dose of the 420 mg TSI formulation and a single dose of the marketed 300 mg TOBI<sup>®</sup> formulation were well-tolerated by patients with cystic fibrosis. The incidence of bronchospasm (≥ 10% relative decrease in FEV<sub>1</sub>) was approximately 8% for each treatment (two 420 mg TSI and one 300 mg TOBI<sup>®</sup> patients); a single patient in the 300 mg TOBI<sup>®</sup> group had clinically significant bronchospasm (≥ 10% relative decrease in FEV<sub>1</sub>). The treatment mean relative decrease in FEV<sub>1</sub> was -3.36 ± 5.47% for 420 mg TSI and -2.14 ± 9.62% for 300 mg TOBI<sup>®</sup>.

By comparison, in the Phase III trials of TOBI<sup>®</sup>, the median change in FEV<sub>1</sub> 30 minutes after the first dose of study drug had been administered was -1.8% in the tobramycin group. At Week 20, the median change in FEV<sub>1</sub> was -2.0% in the

tobramycin group. Because up to 95% of CF patients have bronchodilator-responsive airflow obstruction, and the within-subject variability for pulmonary function tests in CF patients has been documented to be greater than in normal patients, a  $\geq 20\%$  decrease in FEV<sub>1</sub> was considered clinically significant.<sup>33</sup> Twelve  
5 of 258 TOBI<sup>®</sup> patients (4.7%) had a  $\geq 20\%$  decrease in FEV<sub>1</sub> with TOBI<sup>®</sup> administration. Only two of these patients documented acute symptoms, and no patients had a  $\geq 20\%$  decrease in FEV<sub>1</sub> more than once with TOBI<sup>®</sup>.

The present study also showed that the incidence of other treatment-related adverse events was very low (2 of 24 TSI patients and 1 of 13 TOBI<sup>®</sup> patients = 8%)  
10 and did not differ between treatments. All three patients reported mild to moderate decreased pulmonary function test results, and one of the three patients also reported severe cough. Among all treatment-emergent AEs, events reported most frequently by 420 mg TSI patients were cough (4 patients = 17%), crepitations and sore throat (13%), and pyrexia, nasal congestion, rhinorrhoea, and sputum increased (8%). AEs  
15 reported most frequently by 300 mg TOBI<sup>®</sup> patients were cough (3 patients = 23%) and sore throat, dyspnoea, and rhinorrhoea (15%). These events were mostly mild to moderate in intensity (two instances of severe cough), were most likely related to patients underlying cystic fibrosis and other medical conditions, and were consistent with previous large Phase 3 study results.<sup>29,30</sup> A single patient experienced serious  
20 non-drug-related symptoms (SAEs) indicative of an exacerbation of CF. None of the patients in the study were withdrawn due to AEs, and no other clinically significant findings were noted in physical examinations, vital signs, or other safety measurements that represented an increase in risk to patients by reason of administration of study treatments.

### 25 Conclusions

The findings of the present study indicate that it is possible to achieve comparable serum and sputum levels of tobramycin to the 300 mg TOBI<sup>®</sup> formulation by using 420 mg TSI formulation. Current findings also indicate that the reduced nebulization times used in the 420 mg TSI treatment did not change the  
30 pharmacokinetics of tobramycin relative to the marketed 300 mg TOBI<sup>®</sup> formulation.

Mean plasma concentration-time plots for both formulations in serum indicate that tobramycin is rapidly absorbed: all subjects achieved maximum concentrations in the time span of 10 min to 4 h. An elimination phase was also observed in the concentration-time profiles, with individual estimates of half-life ranging from 1.1 to 5 6.8 h. In sputum, maximum concentrations were achieved between 15 min and 2 h, and individual estimates of half-life ranged from 0.48 to 9.47 h. These estimates are consistent with previous studies.

The serum and sputum concentration-time curves for both treatments were virtually superimposable. Serum parameters ( $C_{max}$ ,  $t_{max}$ ,  $AUC$ ,  $CL/F$ ,  $V_z/F$ ) showed 10 no statistically significant differences between the treatment groups. Sputum parameters ( $AUC$ ,  $C_{max}$ , and  $\lambda_z$ ) also showed no statistically significant treatment differences. Neither age nor body weight had a statistically significant effect on these pharmacokinetic parameters. In addition, there were no statistically significant correlations between serum and sputum  $AUC$ , and between serum and sputum  $C_{max}$ .

15 During administration of the test 420 mg TSI formulation, nebulization time was substantially reduced below that observed during administration of the marketed 300 mg TOBI<sup>®</sup> formulation (mean  $\pm$  SD = 9.7  $\pm$  3.0 min vs. 18.1  $\pm$  3.6 min, respectively). The apparent treatment difference in change in nebulizer weight likely reflected the different starting volumes of TSI and TOBI<sup>®</sup> formulations in the 20 nebulizer (mean  $\pm$  SD = 1.86  $\pm$  0.53 g vs. 2.74  $\pm$  1.64 g, respectively).

Aerosol delivery findings indicate that it is possible to achieve comparable serum and sputum levels of tobramycin to the 300 mg TOBI<sup>®</sup> formulation by using the 420 mg TSI formulation. Current findings also indicate that the reduced nebulization times during administration of the 420 mg TSI treatment did not change 25 the pharmacokinetics of tobramycin relative to the marketed 300 mg TOBI<sup>®</sup> formulation.

While the preferred embodiments of the invention have been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method of the treatment of a patient in need of such treatment comprising administering to the patient a dose of 4.0 ml or less of a nebulized aerosol formulation comprising from about 60 to about 200 mg/ml of an aminoglycoside antibiotic in less than 10 minutes.
2. A method of Claim 1 wherein the dose comprises less than about 3.75 ml of the nebulized aerosol formulation.
3. A method of Claim 1 wherein the dose comprises 3.5 ml or less of the nebulized aerosol formulation.
4. A method of Claim 1 wherein the aerosol formulation comprises from about 80 to about 180 mg/ml of the aminoglycoside antibiotic.
5. A method of Claim 1 wherein the aerosol formulation comprises from about 90 to about 150 mg/ml of the aminoglycoside antibiotic.
6. The method of Claim 1 wherein the aminoglycoside antibiotic is selected from the group consisting of gentamycin, amikacin, kanamycin, streptomycin, neomycin, netilmicin and tobramycin.
7. A method of Claim 6 wherein the aminoglycoside antibiotic is tobramycin.
8. A method of Claim 7 wherein the dose comprises 3.5 ml or less of a nebulized aerosol formulation comprising from about 80 to about 180 mg/ml of tobramycin.
9. A unit dose device, comprising a container containing less than about 4.0 ml of an aminoglycoside antibiotic formulation comprising from about 60 to

about 200 mg/ml of an aminoglycoside antibiotic in a physiologically acceptable carrier.

10. A unit dose device of Claim 9 which contains less than about 3.75 ml of the aminoglycoside antibiotic formulation.

11. A unit dose device of Claim 9 which contains 3.5 ml or less of the aminoglycoside antibiotic formulation.

12. A unit dose device of Claim 9 wherein the aminoglycoside antibiotic formulation comprises from about 80 to about 180 mg/ml of the aminoglycoside antibiotic.

13. A unit dose formulation of Claim 9 wherein the aminoglycoside antibiotic formulation comprises from about 90 to about 150 mg/ml of the aminoglycoside antibiotic.

14. A unit dose formulation of Claim 9 wherein the aminoglycoside antibiotic is selected from the group consisting of gentamycin, amikacin, kanamycin, streptomycin, neomycin, netilmicin and tobramycin.

15. A unit dose formulation of Claim 14 wherein the aminoglycoside antibiotic is tobramycin.

16. A unit dose device of Claim 9 which contains less than about 4.0 ml of aminoglycoside antibiotic formulation comprising from about 80 to about 180 mg/ml of tobramycin.

17. A system for delivering an aminoglycoside antibiotic formulation to a patient in need of such treatment, comprising a unit dose device comprising a container containing less than about 4.0 ml of an aminoglycoside antibiotic formulation comprising from about 60 to about 200 mg/ml of an aminoglycoside antibiotic in a physiologically acceptable carrier, and means for delivering the

aminoglycoside antibiotic formulation from the unit dose device for inhalation by the patient in aerosolized form in less than 10 about minutes.

These and other aspects of the inventive concepts may be better understood in connection with the following non-limiting examples.

EXAMPLES

EXAMPLE 1

5

IN VIVO STUDY 1

A comparison was made of the safety, pharmacokinetics, aerosol delivery characteristics, and nebulization time of the conventional dose and inhalation delivery system (5 mL ampoule containing 300 mg tobramycin and 11.25 mg sodium chloride in sterile water for injection (TOBI® tobramycin solution for inhalation, Chiron Corporation, Seattle, Washington), pH 6.0; administered with a PARI LC PLUS™ jet nebulizer with a PulmoAide compressor) with 3 doses of TOBI (30 mg tobramycin in 0.5 mL solution, 60 mg in 1.0 mL, and 90 mg in 1.5 mL) using a AeroDose™ inhaler device. 60 mg/mL

15 The study was designed as an open label, randomized, multicenter, single dose, unbalanced, four treatment, three period crossover trial. Each patient was to receive three single doses of aerosolized antibiotic: the active drug control treatment during one treatment period and two of three experimental treatments during two additional treatment periods. Single dose administration during the three treatment periods was to occur at one-week intervals.

20 In accordance with the study design, forty eight eligible male and female patients 12 years of age or older with a confirmed diagnosis of cystic fibrosis were to be enrolled in the study and randomly assigned to one of 12 treatment sequences of three treatments each (one active control and two experimental treatments) with the constraint that the active control treatment was to be administered in either the first or the second of the three treatment periods. Experimental treatments were administered during all three treatment periods. Each patient inhaled a single dose of aerosolized control and two of three experimental treatments in accordance with the present invention as follows.

- control delivery treatment (PARI LC PLUS jet nebulizer + PulmoAide compressor):
  - TOBI 300 mg in 5 mL solution.
- experimental delivery treatments (AeroDose™ inhaler breath actuated nebulizer):
  - 5 • TOBI 30 mg in 0.5 mL solution;
  - TOBI 60 mg in 1.0 mL solution;
  - TOBI 90 mg in 1.5 mL solution.

The duration of study participation for each patient was to be approximately five weeks including a brief (2 days to one week) screening period, three one-week treatment periods, and a one-week telephone follow-up period.

#### Control and Experimental Treatments

Each patient was to self-administer under research staff supervision a total of three single doses of aerosolized tobramycin during the study, one dose per crossover treatment period. Patients were to receive a single dose of the control delivery treatment during period 1 or period 2 of the three treatment periods. In addition, each patient was to receive single doses of two of the three experimental delivery treatments during the remaining two treatment periods. Control and experimental delivery treatments were specified as follows.

#### Control Delivery Treatment:

20 PARI LC PLUS jet nebulizer with PulmoAide compressor: preservative free tobramycin 60 mg/mL (excipient 5 mL of 1/4 normal saline adjusted to a pH of 6.0 ± 0.5); 300 mg in 5 mL.

#### Experimental Delivery Treatments:

- Aerodose with a 3-4 μm mass medium diameter (MMD) aerosol particle size: preservative free tobramycin 60 mg/mL (excipient 0.5 mL of 1/4 normal saline adjusted to a pH of 6.0 ± 0.5); 30 mg in 0.5 mL;

- Aerodose with a 3-4  $\mu\text{m}$  MMD: preservative free tobramycin 60 mg/mL (excipient 1.0 mL of 1/4 normal saline adjusted to a pH of  $6.0 \pm 0.5$ ); 60 mg in 1.0 mL;
- Aerodose with a 3-4  $\mu\text{m}$  MMD: preservative free tobramycin 60 mg/mL  
5 (excipient 1.5 mL of 1/4 normal saline adjusted to a pH of  $6.0 \pm 0.5$ ); 90 mg in 1.5 mL.

Patients were placed upright in a sitting or standing position to promote normal breathing and were instructed to place the nose clips over the nostrils and to breath normally through the mouth until there was no longer any mist produced by the nebulizer. Aerosol delivery was anticipated to take 15 minutes to complete.  
10

A pharmacist or coordinator prepared the 30 mg dose of TOBI by drawing 0.5 mL of the 60 mg/mL TOBI formulation into a one-mL syringe. Each syringe was labeled with the patient identification number. Study drug was dispensed into the medication reservoir as indicated in the Aerodose directions for use. TOBI 60 mg  
15 and 90 mg doses were similarly prepared by drawing two and three 0.5 mL aliquots, respectively, from the TOBI ampoule into two and three one-mL syringes.

#### Aerosol Delivery Systems

The control delivery system (PARI LC PLUS jet nebulizer) was used once per patient during the study for administration of TOBI 300 mg (control treatment).  
20 The experimental delivery system (Aerodose inhaler) was used to deliver only one dose of study treatments.

The control nebulizer, the PARI LC PLUS jet nebulizer with DeVilbiss PulmoAide compressor, generates aerosol by air-jet shear. A detailed comparison of experimental and control devices is provided in Table 1.

DOES NOT  
RESTRICT  
DEVICE

TABLE 1. DEVICE COMPARISON

Device Characteristic	Aerodose Nebulizer	PARI LC PLUS Nebulizer and DeVilbiss PulmoAide Compressor
Aerosol generating principle	Piezoelectric vibration	Air-jet shear
Aerosol characteristics with TOBI - Mass median diameter (MMD) - Output rate - Emitted dose	4.0 $\mu\text{m}$ 8.0 $\mu\text{L}/\text{sec}$ 85%	4.8 $\mu\text{m}$ 3.6 $\mu\text{L}/\text{sec}$ 57%
Dose actuation	Breath-actuated by user inhalation	On/off switch; when on, medication aerosolized continuously
Control of aerosol generation	Breath actuated. An airflow sensor system is used to limit aerosol generation to inhalation	Continuous aerosol output during both inhalation and exhalation
User indicator lights	Green LED flashing for "device ready" and solid for "aerosolization" Red LED for "low battery"	None
Physical characteristics - Size - Weight	3.3" x 2.6" x 1.1"  140 gm	7.5" x 7.5" x 3.0" (nebulizer) 10.1" x 10.5" x 6.5" (compressor) 68 gm (nebulizer) 3,200 gm (compressor)
Power source	Four AAA alkaline batteries	115 VAC, 60 Hz
Power consumption	2.5 watts	90 watts (max.)
Where used	Fully portable	Restricted to power outlets supplying 115 VAC, 60 Hz

Selection of Doses in the Study

Commercial TOBI 60 mg/mL in 5 mL solution administered by PARI LC PLUS jet nebulizer and powered by the PulmoAide compressor was the active drug control delivery system against which potential improvements in aerosol delivery technology by the Aerodose breath actuated nebulizer were compared in this example.

The selection of doses of experimental treatments (TOBI 30 mg in 0.5 mL solution, 60 mg in 1.0 mL, and 90 mg in 1.5 mL) was based on empirical data on the comparative predicted efficiency of the Aerodose inhaler relative to the PARI LC

PLUS nebulizer. The selection of doses was also based on the assumption that TOBI delivered via the PARI LC PLUS jet nebulizer leads to the systemic absorption of approximately 11.7% of the administered dose {Pitlick, Nardella, et al., 1999}. Furthermore, the mean and standard deviation of the serum concentration one hour after inhalation was  $1.0 \mu\text{g/mL} \pm 0.58$ , suggesting a wide range of deposition (Table 5.2 C, Clinical Pharmacology, PathoGenesis NDA, #50,753). Due to design features of the Aerodose inhaler, it was estimated that between 50-70% of the drug would be delivered to the lung. This assumption is based on the predicted efficiency of a nebulized dose.

10 Patients were randomized to treatment sequence groups, and predose procedures were completed including a physical examination (only if abnormal during screening), recheck of inclusion and exclusion criteria, interim history review, spirometry, clinical evaluation, and blood and urine specimens for laboratory tests (only if abnormal during screening). A bronchodilator was to be administered before  
15 dosing if regularly used by the patient. Spirometry was completed 15-60 minutes after the bronchodilator, if applicable.

Patients received a single dose of study treatments during each of three treatment periods separated by an interval of 7 days between treatments. At the time of single dose administration during each period, patients were instructed to sit  
20 upright and use nose clips during aerosol dose administration.

Patients remained at the clinic through completion of 8-hour post treatment procedures (nebulization time, spirometry, and sputum, serum and urine specimens for tobramycin determinations). Patients were then discharged from the clinic and were expected to collect and return their 8-24 hour urine collection at the next visit,  
25 no later than 7 days after their previous visit. Patients were to refrigerate urine collections at all times except during transport.

#### Safety Variables

Safety was assessed by monitoring the incidence of bronchospasm and by the quantitative change in pulmonary function (measured as change in FEV<sub>1</sub> %  
30 predicted), the incidence of treatment emergent adverse events, and the incidence of

unusually high serum tobramycin results ( $\geq 4 \mu\text{g/mL}$ ), the significance of clinical laboratory test results, and the significance of change in clinical evaluation results.

Bronchospasm (Airway Reactivity)

One objective of the study was to compare the rate of occurrence of  
5 bronchospasm (airway reactivity) between control and experimental delivery systems. Bronchospasm was measured by the change in forced expiratory volume in 1 second [FEV<sub>1</sub> (liters)] from before dosing to 30 minutes after dosing during periods 1, 2, and 3. The number and percent of patients who experienced predose to postdose decreases in FEV<sub>1</sub> (liters) that were  $\geq 10\%$  and those that were  $\geq 20\%$  were  
10 determined to assess the comparative incidence of bronchospasm among control and experimental treatments. Decreases in FEV<sub>1</sub> (liters) that were  $\geq 20\%$  were considered clinically significant for the purposes of the study. Additionally, an acute decrease in FEV<sub>1</sub> (liters)  $\geq 30\%$  from before to after treatment was considered a symptom of respiratory distress. In this event, continuation of the patient in the study  
15 was at the discretion of the investigator.

Norms have been developed for FEV<sub>1</sub>. These norms are commonly used in studies of pulmonary patients. This study employed the Knudson equations that use age, gender, and height to predict a patient's FEV<sub>1</sub> values as if the patient was free of pulmonary function disease. The actual FEV<sub>1</sub> value is divided by the normative  
20 value, and the resulting ratio is multiplied by 100 to produce a measure that represents percentage of predicted normal function, commonly called percent predicted. The transformation is:

$$\text{FEV}_1 \% \text{ predicted} = (\text{FEV}_1 \text{ actual value} / \text{FEV}_1 \text{ normative value}) \times 100$$

Relative change in FEV<sub>1</sub> % predicted is defined as the percent change from  
25 predose to 30 minutes postdose in FEV<sub>1</sub> % predicted and is calculated as:

relative change in

$$\text{FEV}_1 \% \text{ predicted} = [(\text{FEV}_1 \% \text{ predicted at 30 minutes postdose}) - \text{FEV}_1 \% \text{ predicted at predose}] / \text{FEV}_1 \% \text{ predicted at predose}] \times 100$$

### Clinical Laboratory Tests

Serum creatinine, blood urea nitrogen (BUN), and dipstick urine protein results were obtained from specimens drawn during screening and before dosing during treatment period 3. Urine dipstick testing was always performed on fresh  
5 specimens. Serum and urine specimens that needed to be retained at the site (e.g., those drawn after shipping pick-up hours or on Friday or Saturday) were frozen until shipment at the next earliest shipping time. Specimens were covered with dry ice for shipping.

All out of range laboratory results were evaluated for clinical significance and  
10 drug relationship by the investigator using the following classification scheme:

- clinically insignificant;
- possible study medication relationship;
- probable study medication relationship;
- unrelated to study medication, related to concurrent illness;
- 15 • unrelated to study medication, related to other concurrent medication;
- other (investigator commentary).

### Aerosol Delivery Variables

Evaluation of the aerosol delivery characteristics of the Aerodose breath actuated nebulizer, compared to characteristics of the FDA-approved PARI LC  
20 PLUS jet nebulizer with PulmoAide compressor, was based on determination of sputum, urine, and serum tobramycin concentrations, calculation of certain sputum and serum pharmacokinetic parameters, and measurement of nebulization time.

### Sputum Tobramycin Concentrations

Before study treatments were administered, patients expectorated sputum  
25 produced from a deep cough into an individual specimen container. Immediately after treatment, patients rinsed their mouths three times with 30 mL of normal saline, gargled for 5-10 seconds, and expectorated the rinse.

Post treatment sputum specimens were collected following the normal saline gargle at 10 minutes and at 1, 2, 4, and 8 hours after completion of the aerosol drug administration for determination of sputum tobramycin concentrations. Sputum specimens were judged to be acceptable if collected within  $\pm 2$  minutes of the scheduled 10-minute posttreatment collection time and within  $\pm 10$  minutes of the scheduled 1-, 2-, 4-, and 8-hour collection times. After collection, specimens were immediately frozen for later determination of tobramycin concentrations in sputum. A minimum of 1 gram of sputum was required for analysis. Tobramycin concentrations in sputum (sputum LOQ = 20.0  $\mu\text{g}/\text{gm}$ ) were measured by using HPLC.

#### Serum Tobramycin Concentrations

Whole blood was drawn by venipuncture, an indwelling heparin/saline lock, or a permanent venous access port at 10 minutes and at 1, 2, 4, and 8 hours after completion of dosing. Blood specimens were judged to be acceptable if collected within  $\pm 2$  minutes of the scheduled 10-minute posttreatment collection time and within  $\pm 10$  minutes of the scheduled 1-, 2-, 4-, and 8-hour collection times. Blood specimens were allowed to clot for 30 minutes and were then centrifuged at 1500 x g for 10 minutes until clot and serum separated. Serum samples (3 mL) were pipetted into plastic vials and frozen immediately for later determination of serum tobramycin concentrations.

Tobramycin concentrations in serum were measured by Abbott TDxFLx<sup>®</sup> assay (Abbott Laboratories, Abbott Park, Illinois) [serum lower limit of quantitation (LOQ) = 0.18  $\mu\text{g}/\text{mL}$ ].

#### Urine Tobramycin Recovery

Urine specimens were collected and combined in a 24-hour collection container during the 12 hours before treatment (-12-0 hour period) and during 0-8 hour and 8-24 hour collection periods after treatment according to instructions provided in the Study Manual. Total urine volume for the collection period was recorded, and a 10 mL aliquot from each urine collection was retained and frozen for later analysis of urine tobramycin concentration.

The recovery of tobramycin in urine (in milligrams) during 0-8 hour and 8-24 hour collection periods was calculated as follows.

$$\begin{aligned} & \text{urine tobramycin recovery } (\mu\text{g}) \\ & = \text{urine volume (mL)} \cdot \text{urine tobramycin concentration } (\mu\text{g/mL}) \end{aligned}$$

5           Urine tobramycin recovery was normalized for each collection period according to TOBI dose as follows.

$$\begin{aligned} & \text{dose-normalized urine tobramycin recovery } (\mu\text{g/mg}) \\ & = [\text{urine tobramycin recovery } (\mu\text{g}) \div \text{TOBI dose (mg)}] \end{aligned}$$

10           The percent of the TOBI dose excreted in urine in the 24-hour period following treatment was calculated as follows.

$$\begin{aligned} & \% \text{ tobramycin excreted in urine} \\ & = [(\text{urinary recovery in } \mu\text{g} \div 1000 \mu\text{g/mg}) \div \text{TOBI dose in mg}] \cdot 100\%. \end{aligned}$$

15           If either the urine volume or the urine tobramycin concentration for a collection interval was missing, then the urine tobramycin recovery was not calculable for that interval. If calculated urine tobramycin recovery was missing for either the 0-8 hour or the 8-24 hour collection interval, then the 0-24 hour urine tobramycin recovery was not calculated. Missing urine tobramycin recovery values were not replaced by estimated values for analysis purposes.

20           Tobramycin concentrations in urine were measured by Abbott TDxFLx<sup>®</sup> assay [urine lower limit of quantitation (LOQ) = 1.0  $\mu\text{g/mL}$ ].

#### Pharmacokinetic Parameters

The maximum tobramycin concentrations ( $C_{\text{max}}$ ) in sputum and serum during the 8-hour posttreatment sampling period were identified for each patient during each treatment period, and the time at which  $C_{\text{max}}$  was observed ( $T_{\text{max}}$ ) was recorded.

25           Area under the concentration-time curve through 8 hours postdose ( $\text{AUC}_{0-8}$ ) was calculated from sputum and serum tobramycin concentrations using the linear

trapezoidal method. Nebulization time (excluding time for refilling) was added to the time between predose and 10 minutes postdose for  $AUC_{0-8}$  calculations.

Area under the concentration-time curve extrapolated to infinity ( $AUC_{0-\infty}$ ) was calculated for sputum and serum as follows.

$$5 \quad AUC_{0-\infty} = AUC_{0-last} + C_{(last)} \div k_{el}$$

where:  $AUC_{0-last}$  is area under the curve from predose through the last non-BQL time

$C_{(last)}$  is the last non-BQL posttreatment concentration result

$k_{el}$  is the elimination rate constant (terminal phase slope)

$$10 \quad \text{and } k_{el} = \log 2 \div T_{1/2}$$

where  $T_{1/2}$  is the elimination half-life for the patient.

Relative systemic bioavailability was calculated based on serum  $AUC_{0-8}$  values for control (TOBI 300 mg delivered by PARI LC PLUS nebulizer) and experimental (TOBI 30 mg, 60 mg, and 90 mg delivered by Aerodose inhaler) groups as follows.

relative bioavailability (%)

$$= \text{experimental group serum } AUC_{0-8} \div \text{control group serum } AUC_{0-8}$$

Missing tobramycin concentrations and those reported as zero or below quantifiable limits (BQL) were not to be replaced with any estimated value.  $C_{max}$  and  $AUC_{0-8}$  were always determinable except in the event that all posttreatment tobramycin concentrations were missing, zero, or BQL. There was no missing sputum  $C_{max}$  and  $AUC_{0-8}$  values among the 49 patients who completed the study (refer to report section 9.3.1 for details). Four completing patients had indeterminate serum  $C_{max}$  and  $AUC_{0-8}$  values due to BQL serum results for each posttreatment sampling time (refer to report section 9.4.1 for details).

#### Nebulization Time

The timing (duration) of nebulization began with the patient's first tidal breath after the device was in place and continued until the device aerosolized no more

TOBI solution. Nebulization time did not include any interruptions or time needed for instillation of drug into the nebulizer between the repeat filling of the AeroDose™ inhaler. The length of any interruption in nebulization and the reason for interruption were recorded.

## 5 Safety Analyses

Reductions in FEV<sub>1</sub> % predicted  $\geq 10\%$  and  $\geq 20\%$  were used as indicators of the occurrence of bronchospasm (airway reactivity). McNemar's test for paired comparisons (replacing the Cochran-Mantel-Haenszel (CMH) test) was used for control vs. experimental treatment comparisons of the incidence of patients with  
10 predose to 30-minute postdose decreases in FEV<sub>1</sub> % predicted that were  $\geq 10\%$  and  $\geq 20\%$ . In addition, pairwise t-tests were used to compare mean relative change in spirometry FEV<sub>1</sub> % predicted from predose to postdose between each experimental treatment and the control treatment. All statistical analyses were performed using two-sided tests conducted at a 0.05 significance level (i.e.,  $\alpha = 0.05$ ). Since all  
15 statistical tests were exploratory in nature, no adjustment of p-values was made for multiple testing. Changes from predose to postdose in vital signs, body weight, and the incidence of abnormal and/or clinically significant laboratory and physical examination results were summarized and evaluated descriptively.

Individual patient serum tobramycin results were monitored for unusually  
20 high values ( $\geq 4 \mu\text{g/mL}$ ) that might potentially indicate the occurrence of systemic toxicity.

## Aerosol Delivery Analyses

The natural logarithms of AUC<sub>0-8</sub>, AUC<sub>0-∞</sub>, and C<sub>max</sub> based on sputum and serum tobramycin concentrations were to be statistically analyzed using a mixed-  
25 effect repeated-measure analysis of variance model containing treatment, sequence, period, and carryover as fixed effects and patient as a random effect. In the planned analysis of variance model, sequence and carryover (treatment by period interaction) effects were confounded. The actual model used for the analysis was therefore modified by dropping the sequence term so that the assessment of carryover (i.e.,  
30 treatment by period interaction) could proceed. When AUC<sub>0-∞</sub> values were

calculated, large outlier values were noted, and the analysis for this parameter was dropped.

Three hypotheses regarding whether the experimental delivery treatment of 30 mg, 60 mg, or 90 mg TOBI was equivalent to the control delivery treatment of 300 mg TOBI were to be tested in the model. The experimental treatment to control ratio for each of the log AUC and  $C_{max}$  parameters was estimated with 90 percent confidence intervals (CIs). Upper and lower limits for the CIs were then obtained by back transformation (i.e., by exponentiating the log values of the upper and lower limits) to the original scale of the parameter. If the CIs for the ratio of experimental and control treatments contained the value of 1.0, it was concluded that the treatments were not significantly different at the  $\alpha = 0.1$  for the 90% CIs.

If demographic or baseline characteristics showed important apparent differences between the three experimental AeroDose™ groups compared to all patients, then the discrepant factor and its interaction with the delivery treatment factor were to be added to the mixed-effect model. Exploratory evaluations of age, gender, body weight, and baseline pulmonary function (FEV<sub>1</sub> percent predicted) demonstrated no important effects on pharmacokinetic results.

#### Disposition of Patients

A total of 56 patients were screened for the study by the nine investigators. Fifty-three patients met entrance criteria, were enrolled in the study, and were randomized to one of the 12 sequences of treatment administration identified in the randomization code. A total of 3 patients failed to meet entrance criteria and were not enrolled in the study: 2 patients had screening FEV<sub>1</sub> % predicted results that were below the 40 % criterion required for entry, and one patient exhibited disqualifying serum creatinine, BUN, and/or proteinuria.

Accrual of the 53 randomized patients at 9 sites was as follows: 3 sites randomized 8 patients each, 2 sites randomized 7 patients, 3 sites randomized 4 patients, and one site randomized 3 patients. Fifty two patients received at least one dose of study treatments, and one patient was enrolled and randomized but withdrew from the study before the first study treatment due to increased productive cough

with a significant decline in forced expiratory volume (FEV) since screening (both events and associated hyperventilation were considered SAEs due to hospitalization of the patient: included in study database).

5 Of the 52 patients who received study treatments, 49 patients completed the study, and 3 patients withdrew after having received one dose of study treatment. Two of the withdrawn patients discontinued the study during the control treatment period (TOBI 300 mg administered by PARI LC PLUS nebulizer), and one patient withdrew during the TOBI 90 mg by AeroDose™ inhaler treatment period.

#### Baseline Characteristics

10 Enrolled patients had documented laboratory (sweat chloride  $\geq 60$  mEq/L by quantitative pilocarpine iontophoresis test (QPIT) and/or genotype with 2 identifiable mutations) and clinical evidence consistent with a diagnosis of cystic fibrosis. Patients met all inclusion and exclusion criteria except for one patient whose pulmonary function entrance requirement (FEV<sub>1</sub>  $\geq 40\%$  of predicted based on  
15 gender, age, and height) was waived (the patient's screening FEV<sub>1</sub> % predicted was 39.87%). The average FEV<sub>1</sub> % predicted of all randomized patients was 66.4% at screening with a range from approximately 40% to 116%.

Patients reported no known local or systemic hypersensitivity to aminoglycosides. Patients had taken no loop diuretics, no form of aminoglycoside  
20 within 7 days before study treatments, and no investigational medications within 2 weeks before study treatments.

Female patients had a negative pregnancy test before study treatments, and all patients had serum creatinine  $\leq 2.0$  mg/dL, BUN  $< 40$  mg/dL, and  $< 2+$  proteinuria at visit 1 screening, as required by the protocol. Screening or repeat serum creatinine  
25 and BUN results were within the normal ranges for these tests before study treatments. Screening or repeat urine protein results were positive 1+ in 3 patients, but this result did not preclude participation of these patients in the study.

No disqualifying medical history or physical examination findings were noted at visit 1 screening. Screening and visit 1 predose vital signs were unremarkable for  
30 nearly all patients. One patient exhibited low systolic and diastolic blood pressures

at (72/49 mmHg), but these results did not preclude participation of the patient in the study.

### Safety Evaluation

#### Extent of Exposure

5           Forty-nine patients received all 3 single doses of study treatments according to the randomization code, and 3 patients who withdrew from the study received one dose of study treatment. These 52 patients were included in the safety evaluation. Fifty-one of the 52 patients received a single dose of TOBI 300 mg, and 34, 32, and 10 33 of the 52 patients received a single dose of TOBI 30 mg, 60 mg, and 90 mg, respectively. Three of the 49 completing patients had to stop treatment due to inhaler malfunction and subsequently repeated the treatment period at a later date. As a result, these 3 patients received a partial dose of TOBI during the period in which the malfunction occurred (the amount of the partial dose was not recorded) and a full dose of TOBI during the repeated period.

#### 15 Pulmonary Function Results

##### Bronchospasm

          In one aspect, the study compared the rate of occurrence of bronchospasm (airway reactivity) between control and experimental delivery systems. The occurrence of bronchospasm was determined quantitatively based on the percent 20 change in FEV<sub>1</sub> (liters) from before dosing to 30 minutes after dosing in each of the 3 treatment periods. For the purposes of the study, predose to postdose reductions in FEV<sub>1</sub> (liters)  $\geq 10\%$  and  $\geq 20\%$  were defined as bronchospasm; reductions in FEV<sub>1</sub> (liters)  $\geq 20\%$  were considered clinically significant.

          Fifteen patients (9 male and 6 female) experienced 24 instances of 25 bronchospasm during the study. Two instances of clinically significant bronchospasm were observed (decline in FEV<sub>1</sub> (liters)  $\geq 20\%$ : patient 105-1034 after TOBI 300 mg and patient 102-1040 after TOBI 60 mg). No statistically significant pairwise differences in the overall incidence of bronchospasm were noted between control and experimental treatments. No clear relationship appeared to exist between

the incidence of bronchospasm and TOBI dose or delivery system (see Table 2 below).

Table 2. Incidence of Acute Bronchospasm by Treatment

Bronchospasm Parameter	TOBI 300 mg PARI LC PLUS <sup>1</sup>	TOBI 30 mg Aerodose inhaler <sup>2</sup>	TOBI 60 mg Aerodose inhaler <sup>2</sup>	TOBI 90 mg Aerodose inhaler <sup>2</sup>
	(N = 51)	(N = 34)	(N = 32)	(N = 33)
FEV <sub>1</sub> Decrease ≥ 10%	9 (17.6%)	5 (14.7%)	6 (18.8%)	4 (12.1%)
FEV <sub>1</sub> Decrease ≥ 20%	1 (2.0%)	0 (0.0%)	1 (3.1%)	0 (0.0%)
Bronchospasm was defined by protocol as a decrease in FEV <sub>1</sub> (liters) ≥ 10% and ≥ 20% from predose to 30 minutes postdose. Declines ≥ 20% were considered clinically significant.				
1 Control (C) treatment = TOBI 300 mg delivered by PARI LC PLUS nebulizer.				
2 Experimental (E) treatments = TOBI 30, 60, or 90 mg delivered by Aerodose inhaler.				

5 One patient 34 experienced clinically significant bronchospasm at 30 minutes after completing the TOBI 300 mg dose during treatment period 1 (visit 2). This 32-year old male patient's FEV<sub>1</sub> was 2.55 L before dosing and 1.98 L (decline in FEV<sub>1</sub> (liters) ≥ 20%) at 30 minutes after dosing. He experienced moderate chest tightness that resolved spontaneously. This patient also experienced a second episode of  
10 bronchospasm 30 minutes after TOBI 60 mg during period 2. The FEV<sub>1</sub> was 2.47 L before dosing and 2.14 L (decline in FEV<sub>1</sub> (liters) ≥ 10% but < 20%) at 30 minutes after dosing. No symptomatology was reported at the time of this event. No prestudy aminoglycoside use was noted for this patient.

15 One patient experienced one instance of clinically significant bronchospasm 30 minutes after TOBI 60 mg during period 3 (visit 4) of the crossover. This 36-year old male patient's FEV<sub>1</sub> was 2.26 L before dosing and 1.75 L (decline in FEV<sub>1</sub> (liters) ≥ 20%) at 30 minutes after dosing (Archival Listing 3), but he reported no other symptomatology at this time. No prestudy aminoglycoside use was noted for this patient. This episode of bronchospasm appeared due in part to an

uncharacteristically high predose FEV<sub>1</sub> value. The 30-minute posttreatment value was similar to that obtained during period 2 when the change in FEV<sub>1</sub> did not meet the definition of bronchospasm.

Among the 13 patients who experienced clinically non-significant  
5 bronchospasm, one patient experienced a decline in FEV<sub>1</sub> (liters)  $\geq 10\%$  but  $< 20\%$  after all three study doses were administered, 6 patients experienced a decline in FEV<sub>1</sub> (liters)  $\geq 10\%$  after two doses of study medication, and 6 patients experienced a single instance of bronchospasm. Table 3 below lists instances of bronchospasm by patient, treatment period, and TOBI dose.

Table 3. Patient Dosing Regimen and Acute Bronchospasm

Site- Patient ID/Gender	Period 1 (Visit 2) TOBI Dose Received	Period 2 (Visit 3) TOBI Dose Received	Period 3 (Visit 4) TOBI Dose Received
108-1048 <sup>b</sup> / Female	300 <sup>c</sup>	30 <sup>c</sup>	60
109-1015 <sup>b</sup> / Male	300	30 <sup>c</sup>	60
107-1027 / Male	300	30 <sup>c</sup>	90 <sup>c</sup>
103-1038 <sup>b</sup> / Female	300 <sup>c</sup>	60	30
105-1034 / Male	300 <sup>de</sup>	60 <sup>c</sup>	30
107-1026 / Female	300	60 <sup>c</sup>	90
102-1009 <sup>b</sup> / Female	300 <sup>c</sup>	90 <sup>c</sup>	30
102-1040 <sup>b</sup> / Male	300	90	60 <sup>d</sup>
106-1050 <sup>b</sup> / Female	30 <sup>ce</sup>	300	90
102-1007 <sup>b</sup> / Male	60 <sup>c</sup>	300 <sup>c</sup>	30
104-1021 / Male	60 <sup>c</sup>	300 <sup>c</sup>	30
108-1044 / Male	60	300 <sup>c</sup>	30 <sup>c</sup>
105-1047 / Female	60	300 <sup>c</sup>	90
106-1022 <sup>b</sup> / Male	90 <sup>ce</sup>	300	30
106-1041 <sup>b</sup> / Male	90 <sup>ce</sup>	300 <sup>c</sup>	60 <sup>c</sup>

Bronchospasm is defined as a decrease in FEV<sub>1</sub> (liters)  $\geq 10\%$  and  $\geq 20\%$  from predose to 30 minutes postdose. Declines  $\geq 20\%$  were considered clinically significant.

<sup>b</sup> The patient used a bronchodilator before dosing with study medication.

<sup>c</sup> Bronchospasm (not clinically significant): decrease in FEV<sub>1</sub> (liters)  $\geq 10\%$  but  $< 20\%$ .

<sup>d</sup> Bronchospasm (clinically significant): decrease in FEV<sub>1</sub> (liters)  $\geq 20\%$ .

<sup>e</sup> The patient also reported "lung function decrease" (COSTART term) as an AE during the designated treatment period.

Three of the 15 patients with bronchospasm reported treatment-related symptoms at the same time. One patient 15 experienced moderate wheezing (coded as asthma) after TOBI 30 mg during period 2, one patient 4 experienced moderate chest tightness (coded as chest pain as reported previously) after TOBI 300 mg during period 1, and one patient 41 experienced increased cough after TOBI 60 mg during period 3. All events resolved either spontaneously (chest tightness), with treatment (wheezing), or by holding and restarting therapy (increased cough). None of the adverse events led to a serious outcome.

Four of the 15 patients with bronchospasm (and one patient without bronchospasm) reported "lung function decreased" (COSTART term) as an adverse event. In addition to the 4 patients with bronchospasm identified in Table 3 above, one patient who experienced no bronchospasm, reported lung function decreased  
5 once after TOBI 60 mg and once after TOBI 90 mg delivered by the AeroDose™ inhaler.

Initial instances of bronchospasm occurred more frequently during period 1 than during periods 2 or 3 of the crossover. Nine of the 15 patients first experienced bronchospasm during the first treatment period (visit 2), five patients during the  
10 second treatment period, and one patient during the third treatment period.

Patients who routinely used a bronchodilator were permitted to continue to do so during the study. Bronchodilator doses were to be administered 15 to 60 minutes prior to study treatments. Nine of the 15 patients who experienced bronchospasm during the study used a bronchodilator prior to administration of study treatment.

15 Relative Change in FEV<sub>1</sub> % Predicted

The magnitude of the relative change in FEV<sub>1</sub> % predicted was calculated as a quantitative measure of the effect of TOBI treatments on pulmonary function during the study. There were no statistically significant differences among the 4 treatments and no evidence of the presence of period or carryover (treatment by  
20 period interaction) effects. Results of pairwise comparisons between control and experimental treatments are summarized in Table 4. Since the overall treatment difference was not statistically significant, the significant p-value for the TOBI 300 mg vs. TOBI 30 mg comparison in Table 4 below ( $p = 0.019$ ) should not be interpreted as conclusive evidence of a difference. Figure 1 graphically illustrates the  
25 mean relative changes in FEV<sub>1</sub> % predicted from before to 30 minutes after dosing for each of the treatments.

TABLE 4. MEAN (SD) RELATIVE CHANGE IN FEV<sub>1</sub> % PREDICTED

FEV <sub>1</sub> % Predicted (%) Parameter	TOBI 300 mg PARI LC PLUS <sup>1</sup> (n = 51)	TOBI 30 mg Aerodose inhaler <sup>2</sup> (n = 34)	TOBI 60 mg Aerodose inhaler <sup>2</sup> (n = 32)	TOBI 90 mg Aerodose inhaler <sup>2</sup> (n = 33)
Predose	67.8 (18.4) n = 51	65.5 (17.1) n = 34	65.4 (16.8) n = 32	71.3 (20.0) n = 33
30 minutes postdose	63.7 (17.6) n = 51	63.0 (16.7) n = 34	62.5 (15.7) n = 32	68.7 (19.1) n = 32
Relative change from predose <sup>3</sup>	-6.1 (5.2) n = 51	-3.8 (5.4) n = 34	-4.2 (6.2) n = 32	-3.2 (7.4) n = 32
P-value for crossover:		Treatment: 0.141	Period: 0.199	Carryover: NC
Pairwise contrasts: C vs. E p-value (paired t-test):		0.019	0.058	0.083
<sup>1</sup> Control ( C ) treatment = TOBI 300 mg delivered by PARI LC PLUS nebulizer. <sup>2</sup> Experimental ( E ) treatments = TOBI 30, 60, or 90 mg delivered by Aerodose inhaler. <sup>3</sup> Relative change from predose = 100 % • ((30 minute postdose value – predose value)/predose value). NC = carryover (treatment by period interaction) effect not statistically significant and was dropped from final model.				

### Safety Conclusions

Nine males and six females experienced treatment-induced bronchospasm during the study. There was no difference in the rate of occurrence of TOBI induced bronchospasm between control and experimental delivery systems regardless of dose. The occurrence of bronchospasm was rarely associated with patient symptoms. All but four of the patients experiencing drug-induced bronchospasm had been prescribed bronchodilators prior to the study suggesting that they had a history of airway reactivity. The disproportionate number of males versus females experiencing airway reactivity is unusual in light of the fact that enrollment was approximately 60% female and 40% male. The pivotal trials showed that gender had

no influence on drug induced airway reactivity. However, it would be difficult to base any conclusions on this finding due to the small patient numbers in this study.

Treatment-emergent adverse events occurred in all treatment groups regardless of causality. The most common treatment-emergent experiences were associated with Respiratory and Body as a Whole systems. The most common individual events were cough increased, rhinitis, sputum increased, asthma, chest pain, and headache. These events were also common to the patient's pretreatment symptoms reflecting the patients underlying disease. For the majority of treatment-emergent adverse events, there were no meaningful differences between TOBI doses or between the PARI LC PLUS nebulizer and the AeroDose™ inhaler.

The serious adverse events (SAEs) reported were primarily associated with an exacerbation of the patients underlying disease states. The one treatment-related SAE involved a possible sensitivity reaction that, if documented, would have occurred regardless of device or dose.

Review of the clinical chemistry, vital signs, and physical findings did not reveal any clinically significant safety issues associated with the dose or delivery system used to administer TOBI.

All the patients were on multiple concurrent medications appropriate to their disease state (cystic fibrosis), other underlying illnesses, and age throughout the study. The concurrent medications did not appear to have any influence on the safety profile of the study drug or either device during the study. Overall, no clinically significant or unexpected safety issues for TOBI were identified in the study. The study showed that there were no meaningful differences in the safety profiles of administering TOBI via the PARI LC PLUS delivery system in comparison with the Aerodose delivery system regardless of dose.

#### Aerosol Delivery Results

#### Data Analysis

Forty-nine of the 52 dosed patients completed the study and were evaluable for pharmacokinetics by reason of having completed at least 2 doses of study treatments. These 49 patients also constituted the "completers" subset of patients

For the AeroDose™ inhaler, mean sputum tobramycin concentrations increased with increasing TOBI dose at each measurement time during the 8-hour postdose period. Mean sputum concentrations for the TOBI 90 mg treatment with the AeroDose™ inhaler were similar throughout the 8-hour period to those obtained  
5 for the TOBI 300 mg treatment with the PARI LC PLUS nebulizer.

By 2 hours after the end of TOBI 30 mg and by 8 hours after TOBI 60 mg, 90 mg, and 300 mg treatments, sputum concentrations were below LOQ in at least half of the patients. Period effects on sputum tobramycin concentrations were not observed.

10 After TOBI administration using the AeroDose™ inhaler, maximum plasma concentrations ( $C_{max}$ ) and area under the plasma concentration time profile ( $AUC_{0-8}$ ) increased linearly with dose (Table 5 below and Figures 3 and 4), suggesting linear pharmacokinetics. Dose normalized  $C_{max}$  and AUC values were comparable among AeroDose™ dose levels, indicating dose proportionality (based on AUC values).

15 Comparing devices, mean  $C_{max}$  and  $AUC_{0-8}$  for the TOBI 90 mg treatment delivered by the AeroDose™ inhaler achieved similar levels as those obtained by the TOBI 300 mg treatment delivered by the PARI LC PLUS nebulizer. The dose normalized  $C_{max}$  and  $AUC_{0-8}$  results were higher during AeroDose™ treatments than during the PARI LC PLUS treatment, indicating that the AeroDose™ inhaler  
20 exhibited higher efficiency. The bioavailability of the AeroDose™ device was about 3-fold higher than that of the PARI LC PLUS nebulizer.

Exploratory analyses suggested that sputum pharmacokinetic results were unaffected by characteristics present before treatments began (age, gender, body weight, FEV<sub>1</sub> % predicted at screening) and were unaffected by events noted after the  
25 start of treatments (device failure, occurrence of bronchospasm defined as a decrease  $\geq 10\%$  in FEV<sub>1</sub>, and relative change in FEV<sub>1</sub> % predicted).

TABLE 5. MEAN (SD) SPUTUM TOBRAMYCIN PHARMACOKINETIC PARAMETERS

Sputum Pharmacokinetic Parameters	TOBI 300 mg PARI LC PLUS <sup>a</sup> (n = 49)	TOBI 30 mg Aerodose inhaler <sup>b</sup> (n = 34)	TOBI 60 mg Aerodose inhaler <sup>b</sup> (n = 32)	TOBI 90 mg Aerodose inhaler <sup>b</sup> (n = 32)
<b>C<sub>max</sub> (µg/gm)</b>	985.65 (839.34)	329.05 (311.30)	577.83 (538.42)	958.00 (952.30)
-- No. pts with data:	49	34	31	32
-- E vs C p-value <sup>c</sup> :		< 0.001	0.002	0.856
-- E/C (90% CIs) <sup>d</sup> :		(0.23, 0.41)	(0.43, 0.75)	(0.72, 1.30)
<b>Dose-normalized C<sub>max</sub> (µg/gm)/mg</b>	3.29 (2.80)	10.97 (10.38)	9.63 (8.97)	10.64 (10.58)
-- No. pts with data:	49	34	31	32
-- E/C (90% CIs) <sup>d</sup> :				(2.82, 5.13)
<b>T<sub>max</sub> (hr)</b>	0.26 (0.38)	0.24 (0.24)	0.38 (0.76)	0.33 (0.41)
-- No. pts with data:	49	34	31	32
<b>T<sub>1/2</sub> (hr)</b>	6.41 (24.09)	2.04 (1.31)	12.89 (42.61)	13.02 (36.91)
-- Median T <sub>1/2</sub> (hr)	1.71	1.78	2.06	1.60
-- No. pts with data:	41	15	21	24
<b>AUC<sub>0-8</sub> (hr•µg/gm)</b>	1471.16 (1278.22)	360.79 (422.23)	804.78 (722.83)	1275.23 (1358.52)
-- No. pts with data:	49	34	31	32
-- E vs C p-value <sup>c</sup> :		< 0.001	< 0.001	0.465
-- E/C (90% CIs) <sup>d</sup> :		(0.19, 0.28)	(0.45, 0.69)	(0.72, 1.14)
<b>Dose-normalized AUC<sub>0-8</sub> (hr•µg/gm)/mg</b>	1.90 (4.26)	12.03 (14.07)	13.41 (12.05)	14.17 (15.10)
-- No. pts with data:	49	34	31	32
-- E/C (90% CIs) <sup>d</sup> :				(2.78, 4.12)
<b>AUC<sub>0-∞</sub> (hr•µg/gm)</b>	1996.36 (2013.70)	638.68 (586.85)	1661.66 (2334.89)	5544.88 (14831.0)
-- No. pts with data:	41	15	21	24
<p>a Control ( C ) treatment = TOBI 300 mg delivered by PARI LC PLUS nebulizer.  b Experimental ( E ) treatments = TOBI 30, 60, or 90 mg delivered by Aerodose inhaler.  c Pairwise contrast: TOBI 300 mg PARI LC PLUS group vs TOBI (30,60,90 mg) Aerodose groups.  d Back-transformed 90% confidence intervals around the mean of the log ratio of E and C treatments.  Sputum limit of quantifiability (LOQ): 20 µg/gm.</p>				

Differences among the treatment groups in C<sub>max</sub> and AUC<sub>0-8</sub> (Table 5 above; Figures 3 and 4) were statistically significant (p < 0.001) with no evidence of period

or carryover (treatment by period interaction) effects. In pairwise comparisons,  $C_{\max}$  and  $AUC_{0-8}$  were significantly greater for TOBI 300 mg than for TOBI 30 mg and for TOBI 60 mg but not for TOBI 90 mg (90% CIs for  $C_{\max} = (0.72, 1.30)$ ; for  $AUC_{0-8} = (0.72, 1.14)$ ).

5           The AeroDose™ inhaler was more efficient, regardless of TOBI dose, than the PARI LC PLUS nebulizer based on dose normalized sputum  $C_{\max}$  and  $AUC_{0-8}$  results. Dose normalized means for these pharmacokinetic parameters were similar among AeroDose™ treatments but approximately 3-fold higher than the dose normalized results after TOBI 300 mg delivered by the PARI LC PLUS nebulizer (see Table 5).

10           The time to maximum sputum tobramycin concentrations ( $T_{\max}$  in Table 5 above) was similar for all treatment groups and averaged between 0.24 and 0.38 hours for AeroDose™ doses compared to 0.26 hours for the TOBI 300 mg treatment using the PARI LC PLUS. Elimination half-life (median  $T_{1/2}$  in Table 5) was also similar among AeroDose™ treatments, averaging 1.60 to 2.06 hours, compared to  
15 1.71 hours for TOBI 300 mg.

Exploratory analyses revealed no substantial association between sputum pharmacokinetic results and patient characteristics present before treatments (age, gender, body weight, pulmonary function [ $FEV_1$  % predicted] at screening) or emergent events after the start of treatments (device failure, occurrence of  
20 bronchospasm [decrease  $\geq 10\%$  in  $FEV_1$  from predose to 30 minutes postdose], relative change in  $FEV_1$ ).

#### Serum Tobramycin Concentrations and Pharmacokinetic Parameters

Forty-four (44) of 49 completing patients had no measurable serum tobramycin concentrations before dosing in any of the 3 treatment periods, and five  
25 patients exhibited measurable predose serum tobramycin above the lower LOQ in the periods indicated in Table 6 below.

**TABLE 6. MEASURABLE TOBRAMYCIN IN PREDOSE SERUM  
SPECIMENS**

Patient	Treatment* Sequence	Previous Treatment Period			Measurable <sup>b</sup> Predose Tobramycin during Period Listed -- Tobramycin Concentration (µg/mL)
		TOBI Dose (mg)	8-hour Serum Tobramycin Concentration (µg/mL)	Serum T <sub>1/2</sub> (hr)	
107-1030	C-1-2	prestudy	na <sup>c</sup>	na <sup>c</sup>	Per 1 -- 0.70
107-1027	C-1-3	300	< 0.20	1.68	Per 2 -- 0.29
105-1034	C-2-1	prestudy	na <sup>c</sup>	na <sup>c</sup>	Per 1 -- 0.28
		300	1.00	7.75	Per 2 -- 0.23
103-1019	1-C-2	30	0.35	10.85	Per 2 -- 0.20
102-1007	2-C-1	prestudy	na <sup>c</sup>	na <sup>c</sup>	Per 1 -- 0.77
		60	0.75	7.71	Per 2 -- 1.38
		300	0.96	10.62	Per 3 -- 0.60

a Treatments: C=Control TOBI 300 mg using PARI LC PLUS; 1=TOBI 30 mg using Aerodose inhaler; 2=TOBI 60 mg using Aerodose inhaler; 3=TOBI 90 mg using Aerodose inhaler.  
b Measurable tobramycin in serum: tobramycin concentration > LOQ (0.2 µg/mL).  
c na = not available before the start of Period 1.

Table 6 also identifies predose serum specimens for periods 2, 3, or both that had measurable tobramycin in 4 of the 5 patients. These findings are also reflected in non-zero mean amounts of predose tobramycin concentrations in periods 2 and 3. Three of the 5 patients exhibited measurable serum tobramycin after having received TOBI 300 mg during the immediately preceding study period.

These measurable predose results may represent carryover from previous treatment or non-specific assay interference, but the low frequency and magnitude of the results suggests that a substantial effect on posttreatment analyses was unlikely.

After each of the four TOBI treatments, serum tobramycin concentrations gradually increased, reaching a maximum at one hour after dosing (Figure 5), and declined thereafter with median half-lives ranging from 2.73 to 4.27 hours (Table 7 below).

For the Aerodose inhaler, mean serum tobramycin concentrations increased with increasing TOBI dose at each time during the 8-hour posttreatment period, but mean values for TOBI 90 mg were less at each posttreatment time than those seen for TOBI 300 mg using the PARI LC PLUS nebulizer.

5 By 4 hours after the end of TOBI 30 mg and by 8 hours after TOBI 60 mg and 90 mg treatments, serum concentrations were below LOQ in at least half of the patients [median (50<sup>th</sup> percentile) serum concentrations = 0.0 µg/mL]. More than half of the TOBI 300 mg patients remained above the serum LOQ at 8 hours posttreatment. There was no apparent pattern of change in posttreatment serum  
10 tobramycin concentrations from period to period for any of the 4 treatments, and there was no clear indication of the presence of a carryover (treatment by period interaction) effect in posttreatment results.

#### Serum Pharmacokinetic Parameters

After TOBI administration using the Aerodose inhaler, mean  $C_{max}$  and AUC  
15 results increased linearly with dose after the administration of the 30, 60, and 90 mg doses (Table 7), suggesting linear pharmacokinetics. Dose normalized AUC results were similar among the Aerodose dose levels, suggesting dose proportionality.

Comparing devices,  $C_{max}$  and  $AUC_{0-8}$  for the TOBI 90 mg dose using the Aerodose inhaler were not as high as results achieved by the TOBI 300 mg dose  
20 using the PARI LC PLUS nebulizer. However, the dose-normalized parameters were higher for the Aerodose inhaler at all three TOBI dose levels, indicating better efficiency of the new device. Similar to the sputum data, the relative bioavailability was approximately 3-fold higher for the Aerodose inhaler as compared to the PARI nebulizer. The variability based on AUCs was similar for both devices.

25 Exploratory analyses suggested that serum pharmacokinetic results were unaffected by characteristics present before treatments began (age, gender, body weight, FEV<sub>1</sub> % predicted at screening) and were unaffected by events noted after the start of treatments (device failure, occurrence of bronchospasm defined as a decrease  $\geq 10\%$  in FEV<sub>1</sub>, and relative change in FEV<sub>1</sub> % predicted).

TABLE 7. MEAN (SD) SERUM TOBRAMYCIN CONCENTRATIONS  
BY TIME AND PHARMACOKINETIC PARAMETERS

Serum Pharmacokinetic Parameters	TOBI 300 mg PARI LC PLUS <sup>a</sup> (n = 49)	TOBI 30 mg Aerodose inhaler <sup>b</sup> (n = 34)	TOBI 60 mg Aerodose inhaler <sup>b</sup> (n = 32)	TOBI 90 mg Aerodose inhaler <sup>b</sup> (n = 32)
<b>C<sub>max</sub> (µg/mL)</b>	1.12 (0.44)	0.38 (0.17)	0.69 (0.34)	0.96 (0.40)
-- No. pts with data:	49	30	32	32
-- E vs C p-value <sup>c</sup> :		< 0.001	< 0.001	0.027
-- E/C (90% CIs) <sup>d</sup> :		(0.29, 0.36)	(0.53, 0.66)	(0.75, 0.96)
<b>Dose-normalized C<sub>max</sub> (µg/mL)/mg</b>	0.0037 (0.0015)	0.0127 (0.0058)	0.0116 (0.0056)	0.0106 (0.0045)
-- No. pts with data:	49	30	32	32
-- E/C (90% CIs) <sup>d</sup> :				(2.52, 3.25)
<b>T<sub>max</sub> (hr)</b>	1.05 (0.38)	1.14 (0.42)	0.98 (0.28)	1.14 (0.64)
-- No. pts with data:	49	30	32	32
<b>T<sub>1/2</sub> (hr)</b>	3.42 (1.63)	6.75 (5.31)	4.16 (2.34)	3.10 (1.10)
-- Median T <sub>1/2</sub> (hr)	3.14	4.27	3.42	2.73
-- No. pts with data:	49	11	28	31
<b>AUC<sub>0-8</sub> (hr•µg/mL)</b>	4.96 (2.24)	1.43 (1.43)	2.98 (1.92)	3.94 (1.52)
-- No. pts with data:	49	30	32	32
-- E vs C p-value <sup>c</sup> :		< 0.001	< 0.001	0.165
-- E/C (90% CIs) <sup>d</sup> :		(0.18, 0.25)	(0.46, 0.62)	(0.75, 1.03)
<b>Dose-normalized AUC<sub>0-8</sub> (hr•µg/mL)/mg</b>	0.0166 (0.0075)	0.0478 (0.0477)	0.0496 (0.0319)	0.0438 (0.0169)
-- No. pts with data:	49	30	32	32
-- E/C (90% CIs) <sup>d</sup> :				(2.51, 3.21)
<b>AUC<sub>0-∞</sub> (hr•µg/mL)</b>	6.66 (4.32)	6.49 (7.71)	5.11 (4.62)	5.02 (1.63)
-- No. pts with data:	49	11	28	31
<p>a Control ( C ) treatment = TOBI 300 mg delivered by PARI LC PLUS nebulizer.  b Experimental ( E ) treatments = TOBI 30, 60, or 90 mg delivered by Aerodose inhaler.  c Pairwise contrast: TOBI 300 mg PARI LC PLUS group vs TOBI (30,60,90 mg) Aerodose groups.  d Back-transformed 90% confidence intervals around the mean of the log ratio of E and C treatments.  Serum limit of quantifiability (LOQ): 0.2 µg/mL.</p>				

Differences among treatment groups in serum C<sub>max</sub> and AUC<sub>0-8</sub> (Table 7 above; Figures 6 and 7) were statistically significant (p < 0.001) with no period or

carryover effects in the overall analyses. In pairwise comparisons,  $C_{\max}$  and  $AUC_{0-8}$  were significantly greater for TOBI 300 mg using the PARI LC PLUS than for TOBI 30 mg and TOBI 60 mg using the Aerodose inhaler ( $p < 0.001$  in each comparison).  $C_{\max}$  was statistically significantly higher ( $p = 0.027$ ) for TOBI 300 mg compared to  
5 the TOBI 90 mg dose, and  $AUC_{0-8}$  was slightly but not significantly ( $p = 0.165$ ) greater for TOBI 300 mg than for TOBI 90 mg.

The Aerodose inhaler was more efficient, regardless of TOBI dose, than the PARI LC PLUS nebulizer based on dose normalized sputum  $C_{\max}$  and  $AUC_{0-8}$  results. Dose normalized means for these pharmacokinetic parameters were similar among  
10 Aerodose treatments but approximately 3-fold higher than the dose normalized results after TOBI 300 mg delivered by the PARI LC PLUS nebulizer (Table 7).

$T_{\max}$  (Table 7) was similar for the four treatments, averaging between 0.98 and 1.14 hours for Aerodose treatments and 1.05 hours for the TOBI 300 mg treatment using the PARI LC PLUS. Median  $T_{1/2}$  ranged from 2.73 to 4.27 hours among the  
15 Aerodose dose levels, compared to 3.14 hours for TOBI 300 mg using the PARI LC PLUS nebulizer. Median  $T_{1/2}$  results using the Aerodose inhaler appeared to decrease with increasing TOBI dose, but this was considered an artifact related to greater frequency of missing  $T_{1/2}$  values (due to more BQL results) at lower TOBI dose levels.

20 Exploratory analyses revealed no substantial association between serum pharmacokinetic results and patient characteristics present before treatments (age, gender, body weight, pulmonary function [ $FEV_1$  % predicted] at screening) or emergent events after the start of treatments (device failure, occurrence of bronchospasm [decrease  $\geq 10\%$  in  $FEV_1$  from predose to 30 minutes postdose],  
25 relative change in  $FEV_1$ .

#### Urinary Recovery of Tobramycin

Thirty-nine (39) of 49 completing patients had no measurable urine tobramycin concentrations before dosing in any of the 3 treatment periods, and 10  
30 patients exhibited measurable predose urine tobramycin above the lower LOQ in the periods indicated in Table 8 below.

**TABLE 8. MEASURABLE TOBRAMYCIN IN PREDOSE URINE  
SPECIMENS**

Patient	Treatment* Sequence	Previous Period			Measurable <sup>b</sup> Predose Tobramycin during Period Listed – Urine Tobramycin Concentration (µg/mL)
		TOBI dose (mg)	8-24 hour Urine Tobramycin Concentration (µg/mL)	Serum T <sub>1/2</sub> (hr)	
103-1005	C-1-2	prestudy	na <sup>d</sup>	na <sup>d</sup>	Per 1 -- 3.80
		300	3.92	4.80	Per 2 -- 2.06
		30	2.48	not estimable	Per 3 -- 1.20
103-1039	C-1-3	prestudy	na <sup>d</sup>	na <sup>d</sup>	Per 1 -- 1.82
		300	6.76	1.87	Per 2 -- 2.58
104-1024	C-1-3	300	5.14	3.16	Per 2 -- 1.48
107-1027	C-1-3	prestudy	na <sup>d</sup>	na <sup>d</sup>	Per 1 -- 3.14
		300	6.04	1.68	Per 2 -- 1.58
104-1020	C-2-1	prestudy	na <sup>d</sup>	na <sup>d</sup>	Per 1 -- 1.74
		300	13.40	2.93	Per 2 -- 2.28
		60	5.80	12.96	Per 3 -- 1.30
109-1014	C-2-3	60	<1.0	4.06	Per 3 -- 13.22
106-1025	1-C-2	300	5.14	3.80	Per 3 -- 2.70
103-1012	2-C-3	300	2.26	3.63	Per 3 -- 1.16
101-1002	3-C-1	300	7.82	3.37	Per 3 <sup>c</sup> -- 1.12
103-1006	3-C-2	prestudy	na <sup>d</sup>	na <sup>d</sup>	Per 1 -- 2.72
		90	10.10	3.14	Per 2 -- 3.10
		300	8.06	4.48	Per 3 -- 2.08

a Treatments: C=Control TOBI 300 mg using PARI LC-PLUS; 1=TOBI 30 mg using Aerodose inhaler; 2=TOBI 60 mg using Aerodose inhaler; 3=TOBI 90 mg using Aerodose inhaler.  
b Measurable tobramycin in urine: tobramycin concentration > LOQ (1.0 µg/mL).  
c Dosing interrupted by inhaler malfunction.  
d na = not applicable; previous urine specimens were not collected.

Table 8 shows that measurable urine tobramycin was recovered before dosing in periods 2, 3, or both for all 10 patients. Nine of the 10 patients had measurable predose urine tobramycin after TOBI 300 mg treatment during the preceding study period. One patient exhibited measurable tobramycin in both predose serum and

predose urine, and these events both followed TOBI 300 mg administration during the previous period.

Although carryover effect cannot be ruled out, the overall results suggest that such an effect is unlikely. The elimination half-life in sputum ranged from 1.60 to 2.06 hours, and in serum ranged from 2.73 to 4.27 hours, with no substantial differences between the four treatments. Additionally, the amount of tobramycin excreted in urine was larger during the 0-8 hour period compared to the 8-24 hour period, consistent with the short  $T_{1/2}$  of tobramycin. More importantly, in clinical Phase III studies in patients, multiple daily administrations did not result in any accumulation. Therefore it can be concluded that such carryover effect is most likely due to nonspecificity of the assay.

Consistent with the serum data, the amount of tobramycin excreted in urine was higher for TOBI 300 mg compared to TOBI 90 mg (Table 9 below). However, the percent of dose excreted in urine was 3-fold higher for the Aerodose inhaler at all dose levels (16 to 18%) as compared to the PARI LC PLUS nebulizer.

TABLE 9. MEAN (SD) URINARY RECOVERY OF TOBRAMYCIN BY  
TIME

Urine Tobramycin Recovery	TOBI 300 mg PARI LC PLUS <sup>a</sup> (n = 49)	TOBI 30 mg Aerodose inhaler <sup>b</sup> (n = 34)	TOBI 60 mg Aerodose inhaler <sup>b</sup> (n = 32)	TOBI 90 mg Aerodose inhaler <sup>b</sup> (n = 32)
<b>Collection Interval Before and After Dosing:</b>				
-12-0 hr predose (µg) -- No. pts with data	305.1 (1412.0) 48	122.8 (340.7) 33	67.9 (192.8) 32	615.5 (3202.5) 31
0 – 8 hr postdose (µg) -- No. pts with data	15003.0 (7116.2) 48	4835.6 (2649.6) 34	8490.3 (3159.6) 32	12304.8 (5352.7) 32
Dose-normalized (µg)/mg -- No. pts with data -- E/C (90% CIs) <sup>d</sup> :	50.0 (23.7) 48	161.2 (88.3) 34	141.5 (52.7) 32	136.7 (59.5) 32 (2.50, 3.62)
8 – 24 hr postdose (µg) -- No. pts with data	3072.1 (2271.2) 47	794.1 (853.1) 34	1367.4 (1118.8) 31	2095.2 (1818.7) 31
Dose-normalized (µg)/mg -- No. pts with data -- E/C (90% CIs) <sup>d</sup> :	10.2 (7.6) 47	26.5 (28.4) 34	22.8 (18.6) 31	23.3 (20.2) 31 (2.44, 3.48)
Total 0 – 24 hour (µg) -- No. pts with data	18113.2 (8303.4) 46	5629.7 (2993.6) 34	9802.7 (3771.0) 31	14588.1 (6044.9) 31
Dose-normalized (µg)/mg -- No. pts with data -- E/C (90% CIs) <sup>d</sup> :	60.4 (27.7) 46	187.7 (99.8) 34	163.4 (62.8) 31	162.1 (67.2) 31 (2.23, 3.27)
Percent of Dose Excreted (%) <sup>c</sup>	6.0	18.8	16.3	16.2

- a Control ( C ) treatment = TOBI 300 mg delivered by PARI LC PLUS nebulizer.  
b Experimental ( E ) treatments = TOBI 30, 60, or 90 mg delivered by Aerodose inhaler.  
c % excreted = [(urinary recovery in  $\mu\text{g} \div 1000 \mu\text{g}/\text{mg}) \div \text{Dose in mg}] \cdot 100\%$ .  
Urine limit of quantifiability (LOQ): 1.0  $\mu\text{g}/\text{mL}$  urine.

For the Aerodose inhaler, mean 24-hour recovery of tobramycin from the urine increased with increasing TOBI dose during the study (Table 9 above; Figure 8). Tobramycin recovery appeared to be dose proportional for the Aerodose inhaler, as mean 24-hour recovery normalized for dose was similar among Aerodose treatments.

Comparing devices, mean recovery for the TOBI 90 mg treatment was less than that seen for TOBI 300 mg using the PARI LC PLUS nebulizer. However, a greater percentage of the administered TOBI dose was recovered in the urine of patients who were dosed with the Aerodose inhaler (18.8%, 16.3%, and 16.2%, respectively), irrespective of TOBI dose, than was recovered from patients who were dosed with the PARI LC PLUS nebulizer (6.0% of the administered TOBI 300 mg dose).

The largest amount of tobramycin was recovered during the first 8 hours after dosing. There was no apparent pattern of period-to-period change in posttreatment urine tobramycin recovery for any of the 4 treatments. Although a potential carryover could not be ruled out in approximately 20% of the patients due to recovery of measurable tobramycin in predose urine, there was no clear indication of the presence of a carryover (treatment by period interaction) effect in posttreatment results.

The percent of administered dose recovered in urine over 24 hours postdose does not represent the delivered dose in the lung or absolute bioavailability. It is understood that a substantial amount of lung deposited dose still remains in the body at 24 hours postdose.

#### Nebulization Time

Mean total nebulization time increased with increasing TOBI dose (Table 10 below; Figure 9) and was substantially less when the Aerodose inhaler was used at each TOBI dose level (mean  $\pm$  SD for TOBI 30 mg =  $2.8 \pm 1.0$  min; TOBI 60 mg =

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5.4 ± 2.1 min; TOBI 90 mg = 8.0 ± 2.5 min) than when the PARI LC PLUS nebulizer was used (TOBI 300 mg = 17.7 ± 4.7 min).

TABLE 10. MEAN (SD) NEBULIZATION TIME

Parameter	TOBI 300 mg PARI LC PLUS <sup>1</sup> (n = 51)	TOBI 30 mg Aerodose inhaler <sup>2</sup> (n = 34)	TOBI 60 mg Aerodose inhaler <sup>2</sup> (n = 32)	TOBI 90 mg Aerodose inhaler <sup>2</sup> (n = 33)
Nebulization Time <sup>3</sup> (min)	17.7 (4.7)	2.8 (1.0)	5.2 (2.1)	8.0 (2.5)
— No. pts with data	51	34	32	32
1 Control ( C ) treatment = TOBI 300 mg delivered by PARI LC PLUS nebulizer. 1 Experimental ( E ) treatments = TOBI 30, 60, or 90 mg delivered by Aerodose inhaler. 2 Total duration of nebulization excluding fill time.				

### Conclusions

5 The Aerodose inhaler substantially reduced the amount of time required to nebulize the administered TOBI dose, compared to the approved PARI LC PLUS nebulizer, and nebulization time increased with increasing TOBI dose (TOBI 300 mg delivered by PARI LC PLUS mean = 17.7 minutes vs. 2.8 minutes, 5.4 minutes, and 8.0 minutes for TOBI 30 mg, 60 mg, and 90 mg, respectively).

10 Sputum tobramycin concentrations throughout the 8-hour sampling period after dosing increased with increasing TOBI dose through 90 mg delivered by the Aerodose inhaler, but results for TOBI 90 mg and TOBI 300 mg delivered by the PARI LC PLUS nebulizer did not differ substantially or consistently. Sputum tobramycin results were highly variable, with coefficients of variation approaching or exceeding 100% for each treatment at all time points. On average, sputum  
15 concentrations reached their maximum at 10 minutes after each of the 4 treatments. By 2 hours after TOBI 30 mg and by 8 hours after TOBI 60 mg, 90 mg, and 300 mg, sputum concentrations were below the lower limit of quantifiability (LOQ) in at least half of the patients.

20 The mean of the maximum sputum concentration was significantly greater after TOBI 300 mg (mean = 985.65 µg/gm) than after TOBI 30 mg (329.05 µg/gm:

p < 0.001) and TOBI 60 mg (577.83  $\mu\text{g}/\text{gm}$ : p = 0.002) but not TOBI 90 mg (958.00  $\mu\text{g}/\text{gm}$ : p = 0.856; 90% CIs for the ratio of TOBI 90 mg / TOBI 300 mg  $C_{\text{max}}$  = 0.72, 1.30). The Aerodose inhaler was more efficient than the PARI LC PLUS nebulizer based on sputum  $C_{\text{max}}$  results adjusted for TOBI dose administered (TOBI 300 mg  
5 with PARI LC PLUS: dose-normalized mean  $C_{\text{max}}$  = 3.29 ( $\mu\text{g}/\text{gm}$ )/mg; TOBI 30, 60, and 90 mg with Aerodose = 10.97, 9.63, and 10.64 ( $\mu\text{g}/\text{gm}$ )/mg, respectively).

Mean sputum  $T_{\text{max}}$  was virtually identical for TOBI 300 mg (mean = 0.26 hr) and TOBI 30 mg (0.24 hr) but was slightly less than  $T_{\text{max}}$  for TOBI 60 mg (0.38 hr) and TOBI 90 mg (0.33 hr).

10 Mean sputum  $\text{AUC}_{0-8}$  was significantly greater after TOBI 300 mg (mean = 1471.16  $\text{hr}\cdot\mu\text{g}/\text{gm}$ ) than after TOBI 30 mg (360.79  $\text{hr}\cdot\mu\text{g}/\text{gm}$ : p < 0.001) and TOBI 60 mg (804.78  $\text{hr}\cdot\mu\text{g}/\text{gm}$ : p < 0.001) but not TOBI 90 mg (1275.23  $\text{hr}\cdot\mu\text{g}/\text{gm}$ : p = 0.465; 90% CIs for the ratio of TOBI 90 mg / TOBI 300 mg  $\text{AUC}_{0-8}$  = 0.72, 1.14). The greater efficiency of the Aerodose inhaler was also seen in dose-normalized  
15  $\text{AUC}_{0-8}$  results (TOBI 300 mg with PARI LC PLUS = 4.90 [ $\text{hr}\cdot\mu\text{g}/\text{gm}$ ]/mg; TOBI 30, 60, and 90 mg with Aerodose = 12.03, 13.41, and 14.17 [ $\text{hr}\cdot\mu\text{g}/\text{gm}$ ]/mg, respectively).

No inferential analyses of sputum  $\text{AUC}_{0-\infty}$  were performed due to high variability that increased with increasing TOBI dose.

20 Serum tobramycin concentrations also increased with increasing TOBI dose at each time during the 8-hour posttreatment observation period. Mean serum tobramycin concentrations reached their maximum at one hour after each treatment. By 4 hours after TOBI 30 mg and by 8 hours after TOBI 60 mg and TOBI 90 mg, serum concentrations were below LOQ in at least half of the patients. More than half  
25 of the TOBI 300 mg patients remained above the serum LOQ at 8 hours posttreatment.

Mean serum  $C_{\text{max}}$  was significantly greater after TOBI 300 mg (mean = 1.12  $\mu\text{g}/\text{mL}$ ) than after the other 3 treatments (TOBI 30 mg = 0.38  $\mu\text{g}/\text{mL}$ , p < 0.001; TOBI 60 mg = 0.69  $\mu\text{g}/\text{mL}$ , p < 0.001; TOBI 90 mg = 0.96  $\mu\text{g}/\text{mL}$ , p = 0.027). The  
30 Aerodose inhaler was also more efficient than the PARI LC PLUS nebulizer based

on serum  $C_{max}$  results adjusted for TOBI dose administered (TOBI 300 mg with PARI LC PLUS: dose-normalized mean  $C_{max} = 0.0037$  ( $\mu\text{g/mL}$ )/mg; TOBI 30, 60, and 90 mg with Aerodose = 0.0127, 0.0116, and 0.0106 ( $\mu\text{g/mL}$ )/mg, respectively).

Mean serum  $T_{max}$  was similar for the 4 treatments (mean = 1.05 hr, 1.02 hr, 5 0.98 hr, and 1.14 hr for TOBI 300 mg, 30 mg, 60 mg, and 90 mg, respectively).

Mean serum  $AUC_{0-8}$  was significantly greater after TOBI 300 mg (mean = 4.96  $\text{hr}\cdot\mu\text{g/mL}$ ) than after TOBI 30 mg (1.43  $\text{hr}\cdot\mu\text{g/mL}$ ,  $p < 0.001$ ) and TOBI 60 mg (2.98  $\text{hr}\cdot\mu\text{g/mL}$ ,  $p < 0.001$ ) but not TOBI 90 mg (3.94  $\text{hr}\cdot\mu\text{g/mL}$ ,  $p = 0.165$ ; 90% CIs for the ratio of TOBI 90 mg / TOBI 300 mg  $AUC_{0-8} = 0.75, 1.03$ ). The greater 10 efficiency of the Aerodose inhaler was also seen in dose-normalized  $AUC_{0-8}$  results (TOBI 300 mg with PARI LC PLUS = 0.0166 [ $\text{hr}\cdot\mu\text{g/mL}$ ]/mg; TOBI 30, 60, and 90 mg with Aerodose = 0.0478, 0.0496, and 0.0438 [ $\text{hr}\cdot\mu\text{g/mL}$ ]/mg, respectively).

Serum  $AUC_{(0-\infty)}$  was not analyzed statistically due to high variability but generally appeared to increase as the TOBI dose increased.

15 Recovery of tobramycin from the urine within 24 hours after dosing increased with increasing TOBI dose during the study (expressed in mg [ $\text{mg} = \mu\text{g}/1000$ ], mean urine tobramycin recovery = 18.1 mg, 5.6 mg, 9.8 mg, and 14.6 mg after TOBI 300 mg, TOBI 30 mg, TOBI 60 mg, and TOBI 90 mg doses, respectively). Most of the tobramycin was recovered within the first 8 hours after dosing. Normalized for dose, 20 urine tobramycin recovery within 24 hours was 6.0%, 18.8%, 16.3%, and 16.2% of the administered TOBI 300 mg, TOBI 30 mg, TOBI 60 mg, and TOBI 90 mg doses, respectively.

Results of the present study showed that TOBI 300 mg delivered by the PARI LC PLUS nebulizer (the control delivery system) and TOBI 30 mg, 60 mg, and 90 25 mg delivered by the Aerodose inhaler (the experimental delivery system) were safe and well-tolerated by male and female cystic fibrosis patients. Fifteen patients (9 male and 6 female) experienced 24 instances of bronchospasm (decline in  $FEV_1$  (liters)  $\geq 10\%$ ). There were no statistically significant differences between control and any experimental treatment in the incidence of bronchospasm. There were no

overall treatment differences in quantitative change in FEV<sub>1</sub> from predose to 30-minute postdose measurement times.

The study found no evidence that CF patients were at increased risk by reason of inhaling single TOBI doses of 30 mg, 60 mg or 90 mg compared to the single  
5 TOBI 300 mg dose delivered by the PARI LC PLUS jet nebulizer. The most frequently reported treatment emergent adverse events (cough increased, rhinitis, sputum increased, chest pain, asthma, and headache) and the SAEs reported by 4 of the patients were primarily associated with patients' underlying CF disease and related medical conditions. The incidence of these events before and after study  
10 treatments was substantially similar, suggesting that neither TOBI dose levels nor control and experimental inhalers altered ongoing symptomatology associated with patients' underlying medical conditions. There were also no clinically significant safety issues reflected in clinical laboratory test results, vital signs, or physical findings.

15 In this example, the Aerodose inhaler substantially reduced the time required for nebulization of all three dose levels (30 mg, 60 mg, and 90 mg) of TOBI compared to the nebulization time for the approved TOBI 300 mg delivery system using the PARI LC PLUS jet nebulizer. Average nebulization times were 2.8, 5.4, and 8.0 minutes using the Aerodose inhaler to deliver TOBI 30 mg, 60 mg, and 90  
20 mg, respectively vs. 17.7 minutes using the PARI LC PLUS nebulizer to deliver TOBI 300 mg. The Aerodose inhaler therefore cut nebulization time of the TOBI 90 mg dose by more than 50% compared to the PARI LC PLUS nebulizer in the present study, and nebulization times for lower TOBI doses were reduced by even greater amounts. Present nebulization time results in CF patients  $\geq 12$  years of age with  
25 baseline FEV<sub>1</sub> % predicted  $\geq 40\%$  were consistent with those obtained after single doses of TOBI 60 mg using the Aerodose inhaler (mean = 5.7 minutes) but slightly less than TOBI 300 mg results using the PARI LC PLUS nebulizer (mean = 20.4 minutes) in the TOBI gamma scintigraphy study of tobramycin deposition in the lungs of healthy adult male and female volunteers of Example 2, *infra*.

This example demonstrates that TOBI 90 mg (but not TOBI 60 mg or TOBI 30 mg) delivered by the Aerodose inhaler achieved similar actual pulmonary deposition, systemic absorption, and urinary recovery of tobramycin as that achieved by administration of the TOBI 300 mg dose delivered by the PARI LC PLUS nebulizer. Normalized for TOBI dose, the Aerodose inhaler was substantially more efficient than the PARI LC PLUS nebulizer in the delivery of aerosolized tobramycin to the lungs and to the systemic circulation.

Pulmonary deposition of tobramycin was measured by determination of sputum tobramycin concentrations and by calculation of sputum pharmacokinetic parameters. Maximum sputum tobramycin concentrations were reached by 10 minutes after administration of each treatment, and concentrations were below the LOQ in half or more of the patients at 2 hours after TOBI 30 mg and at 8 hours after TOBI 60 mg, 90 mg, and 300 mg. The extent of pulmonary deposition of tobramycin, as measured by maximum sputum concentrations and sputum  $AUC_{0-8}$  results, increased with increasing TOBI dose through 90 mg, but TOBI 90 mg and TOBI 300 mg did not differ statistically (mean sputum  $C_{max}$  = 958.00 and 985.65  $\mu\text{g}/\text{gm}$ ; mean sputum  $AUC_{0-8}$  = 1275.23 and 1471.16  $\text{hr}\cdot\mu\text{g}/\text{gm}$ , respectively). Mean sputum  $C_{max}$  results after TOBI 30 mg and 60 mg doses were significantly less than that of the TOBI 300 mg dose. Present sputum  $C_{max}$  results achieved after the single TOBI 300 mg dose were slightly less than sputum tobramycin concentrations achieved 10 minutes after a single TOBI 300 mg dose (mean sputum tobramycin concentration = 1237  $\mu\text{g}/\text{gm}$ , median = 1090  $\mu\text{g}/\text{gm}$ ) in two large previously conducted Phase III pivotal trials.

The results of this example demonstrate that at least one of the three TOBI doses (TOBI 90 mg) delivered by the experimental Aerodose inhaler achieved similar actual sputum tobramycin concentrations and that these results in turn were similar to sputum results obtained in the prior pivotal studies supporting the commercial TOBI product. It is also important that present sputum results demonstrated that the experimental Aerodose inhaler was substantially more efficient, regardless of TOBI dose, in delivery of aerosolized tobramycin to the lung

20 (19)  
5.97

than the PARI LC PLUS jet nebulizer. Dose-normalized sputum  $C_{max}$  was 10.97, 9.63, and 10.64 ( $\mu\text{g}/\text{gm}$ )/mg for TOBI 30 mg, 60 mg, and 90 mg delivered by Aerodose inhaler, respectively, compared to 3.29 ( $\mu\text{g}/\text{gm}$ )/mg for TOBI 300 mg delivered by PARI LC PLUS. Similarly, dose-normalized sputum  $AUC_{0-8}$  was  
5 12.03, 13.41, and 14.17 [ $\text{hr}\cdot\mu\text{g}/\text{gm}$ ]/mg for TOBI 30 mg, 60 mg, and 90 mg delivered by Aerodose inhaler, respectively, compared to 4.90 [ $\text{hr}\cdot\mu\text{g}/\text{gm}$ ]/mg for TOBI 300 mg delivered by PARI LC PLUS.

Systemic absorption of tobramycin was measured by determination of serum tobramycin concentrations and by calculation of serum pharmacokinetic parameters.  
10 Maximum serum tobramycin concentrations were reached at one hour after each of the four TOBI treatments, and concentrations were below LOQ in half or more of the patients by 4 hours after TOBI 30 mg and by 8 hours after TOBI 60 mg and 90 mg. More than half of the patients at TOBI 300 mg had measurable serum tobramycin at 8 hours postdose. The extent of absorption of tobramycin, as measured by serum  
15  $C_{max}$  results, increased with increasing TOBI dose, as  $C_{max}$  was significantly greater after TOBI 300 mg (mean = 1.12  $\mu\text{g}/\text{mL}$ ) than after each of the lower TOBI doses (mean = 0.38, 0.69, and 0.96  $\mu\text{g}/\text{mL}$  for TOBI 30 mg, 60 mg, and 90 mg doses, respectively). Serum  $C_{max}$  for TOBI 300 mg in the present study was slightly higher (mean  $\pm$  SD = 1.10  $\pm$  0.44  $\mu\text{g}/\text{mL}$  with a mean  $T_{max}$  of 1.05 hr) than the mean serum  
20 tobramycin concentration reported at one hour after TOBI 300 mg in the TOBI NDA (0.95  $\pm$  0.50  $\mu\text{g}/\text{mL}$ ). Serum  $C_{max}$  achieved by the Aerodose inhaler at the TOBI 90 mg dose level in the current study was virtually identical to the NDA serum concentrations one hour after TOBI 300 mg (mean = 0.96  $\pm$  0.37  $\mu\text{g}/\text{mL}$ ), although it was significantly ( $p = 0.027$ ) less than the current TOBI 300 mg.

25 Thus, present serum tobramycin results demonstrated that TOBI 90 mg delivered by the Aerodose inhaler were similar ( $AUC_{0-8}$ ) or nearly similar ( $C_{max}$ ) to those obtained after TOBI 300 mg delivered by the PARI LC PLUS nebulizer in the present study and in the prior pivotal studies supporting the TOBI commercial product. Present serum results also demonstrated that the experimental Aerodose  
30 inhaler was substantially more efficient, regardless of TOBI dose, in delivery of

aerosolized tobramycin to the systemic circulation than the PARI LC PLUS jet nebulizer. Dose-normalized serum  $C_{max}$  was 0.0127, 0.0116, and 0.0106 ( $\mu\text{g/mL}$ )/mg for TOBI 30 mg, 60 mg, and 90 mg delivered by Aerodose inhaler, respectively, compared to 0.0037 ( $\mu\text{g/mL}$ )/mg for TOBI 300 mg delivered by PARI LC PLUS. Similarly, dose-normalized serum  $AUC_{0-8}$  was 0.0478, 0.0496, and 0.0438 [ $\text{hr}\cdot\mu\text{g/mL}$ ]/mg for TOBI 30 mg, 60 mg, and 90 mg delivered by Aerodose inhaler, respectively, compared to 0.0166 [ $\text{hr}\cdot\mu\text{g/mL}$ ]/mg for TOBI 300 mg delivered by PARI LC PLUS. The greater efficiency of the Aerodose inhaler observed in present serum tobramycin results is consistent with greater efficiency and less wastage of the tobramycin dose observed in earlier studies.

Urinary recovery of tobramycin was measured by determining the cumulative amount of tobramycin recovered in urine collected for 24 hours after dosing. The amount of urinary tobramycin recovered within 24 hours postdose increased with increasing TOBI dose (expressed in mg [ $\text{mg} = \mu\text{g}/1000$ ], mean urine tobramycin recovery = 5.6 mg, 9.8 mg, 14.6 mg, and 18.1 mg tobramycin after TOBI 30 mg, 60 mg, 90 mg, and 300 mg). The results were not tested statistically, and it was not possible to determine whether TOBI 90 mg and TOBI 300 mg results for 24-hour recovery of urine tobramycin were similar or different.

Normalized for dose by dividing the mean amount of tobramycin recovered by the nominal amount of TOBI administered, urinary recovery of tobramycin was approximately 18.8 %, 16.3 %, 16.2 %, and 6.0 % of the administered TOBI 30 mg, 60 mg, 90 mg, and 300 mg doses, respectively.

During the study, measurable tobramycin (i.e., above the lower limit of quantifiability [LOQ] of the assay) was detected in 12-hour predose urine collections in a total of 10 patients, including 5 patients before the first dose of study treatments in period one and all 10 patients before the second or third doses in periods 2 and 3 or both. Similarly, measurable tobramycin was detected in predose serum specimens in a total of 5 patients, including 3 patients before the first dose of study treatments in period one and 4 patients before dosing in periods 2, or 3, or both. A single patient had measurable tobramycin in both urine and serum.

Substantial variability is known to occur among patients in the rate and extent of uptake, renal accumulation, and elimination of aminoglycoside antibiotics, even in patients with normal renal function. Each of these factors may lengthen the amount of time that measurable concentrations of aminoglycoside antibiotics may be detected in serum and urine. The present study employed a prestudy washout interval of 7 days from previous prescription aminoglycoside antibiotic use and a 7-day interval between the 3 single doses of TOBI, an aminoglycoside antibiotic, during the crossover treatment periods. It is plausible that prestudy and on-study washout intervals in the study may have been too short for complete elimination of residual tobramycin previously administered, if any. Measurable amounts of tobramycin for these patients would have had little effect on study results, since the amounts and concentrations detected were very small in nearly all cases, and no unusually high serum or urine tobramycin results were noted during the study.

The Aerodose inhaler was a safe and efficient aerosolization and delivery device for TOBI during the study.

#### EXAMPLE 2

#### SCINTIGRAPHY STUDY

In order to assess the *in vivo* lung deposition of 300 mg tobramycin (TOBI<sup>®</sup>) inhaled using the PARI LC PLUS<sup>™</sup> jet nebulizer / DeVilbiss PulmoAide<sup>®</sup> compressor delivery system (current commercial delivery system) compared with the deposition of 60 mg tobramycin (TOBI<sup>®</sup>) using the AeroDose<sup>™</sup> inhaler in accordance with the present invention, a gamma scintigraphy study was performed. The imaging technique of gamma scintigraphy is a well-established method<sup>10-12</sup> that provides precise quantification of drug delivered to the lungs<sup>13</sup>. It also provides an assessment of the distribution of deposited drug in different lung regions (peripheral, intermediate and central lung regions corresponding to small airways, medium sized airways and large airways, respectively<sup>14</sup>). Gamma scintigraphy is the only non-invasive method currently available for obtaining this type of information.

The study of this example was designed as an open label, randomized, single center, single dose, two period crossover Phase I study of aerosol delivery

characteristics and safety of two inhalation devices in healthy adult volunteers. A maximum of 14 healthy male or non-pregnant, non breast-feeding female volunteers aged 18 to 65 years of age were to receive in random order two single doses of aerosolized antibiotic mixed with a sterile radiotracer (technetium bound to diethylenetriaminepentaacetic acid:  $^{99m}\text{Tc}$  DTPA) separated by a washout interval of a minimum of 44 hours between doses. Radiolabeled aerosols consisted of a single 300 mg dose in a 5 mL solution of TOBI delivered by the control delivery system (PARI LC PLUS jet nebulizer with a PulmoAide compressor) and a single 60 mg dose in a 1 mL solution of TOBI delivered by the experimental delivery system (Aerodose inhaler).

Aerosol delivery characteristics of control and experimental delivery systems were compared on the basis of lung deposition of radiolabeled tobramycin determined by gamma scintigraphy, time to complete nebulization of aerosolized doses, serum concentrations of tobramycin determined by Abbott TDxFLx assays, and serum tobramycin pharmacokinetic parameters.

The safety of control and experimental TOBI delivery systems was compared on the basis of changes in pulmonary function, the incidence of treatment emergent adverse events, and the occurrence of clinically significant laboratory and clinical evaluations and of unusually high serum tobramycin concentrations.

The duration of study participation for each subject was to be approximately five weeks including a screening period of up to 3 weeks in duration, two treatment periods of approximately 9 hours each separated by a minimum 44-hour washout interval, and a follow-up period through 2 weeks after the end of dosing.

#### Treatments

TOBI<sup>®</sup> was administered by inhalation as a single 300 mg dose and as a single 60 mg dose to each subject during the study. The 300 mg dose was supplied as a commercial ampoule of TOBI. The 60 mg dose of tobramycin solution was prepared by study site personnel by withdrawing 1.0 mL of solution from the 300 mg / 5 mL commercial ampoule of TOBI into two unit dose syringes containing 0.5 mL each.

Sterile  $^{99m}\text{Tc}$  DTPA was added as a radiotracer to both 300 mg and 60 mg solutions at the study site prior to instillation into the nebulizer. Sufficient  $^{99m}\text{Tc}$  DTPA was added to both the 300 mg and the 60 mg dose so that no more than 10 MBq  $^{99m}\text{Tc}$  DTPA was delivered to the subject with each single dose administered.

5 Using control and experimental aerosol delivery systems, each subject was to self-administer two single aerosolized doses of radiolabeled ( $^{99m}\text{Tc}$  DTPA) TOBI, one dose in each of two crossover treatment periods, according to the randomization scheme for the study. Subjects were instructed to use nose clips and breathe in a normal breathing pattern while inhaling the medication according to the instructions  
10 for use for each inhaler.

Control and experimental treatment delivery systems were specified as follows.

Control Treatment Delivery System: PARI LC PLUS jet nebulizer with DeVilbiss PulmoAide compressor delivering 300 mg (5 mL) of TOBI.

15 Experimental Treatment Delivery System: Aerodose inhaler delivering 60 mg (1 mL) of TOBI.

When the PARI LC PLUS nebulizer was used, 5 mL radiolabeled TOBI was added to the drug reservoir and nebulized without interruption until the nebulizer reservoir was dry. The PARI system was configured such that exhalation by the  
20 subject did not result in escape of radioactive aerosol into the surrounding atmosphere. Exhaled droplets were collected using a filter attached to the side of the inhaler by a T-piece. In addition, a scavenger filter was placed above the inhaler, which was in turn connected to a vacuum pump. The scavenger system was used to collect any radiolabeled droplets escaping from the inhaler.

25 When the Aerodose inhaler was used, one 0.5 mL aliquot of radiolabeled TOBI was added to the drug reservoir and nebulized to dryness. A second 0.5 mL dose was then added to the reservoir and nebulized to dryness. The inhaler was surrounded with an exhaled air collection box. Air was drawn through a filter at the back of the box using a vacuum pump.

Start and stop times of nebulization for both the Aerodose and PARI LC PLUS nebulizers were to be recorded in CRFs. Nebulization time for the Aerodose inhaler was not to include the time needed to refill the drug reservoir according to the protocol.

- 5 Enrolled volunteers were randomly assigned to two treatment sequence groups as illustrated below according to a randomization scheme.

PARI 300 mg / Aerodose 60 mg:

- period 1: PARI LC PLUS with TOBI 300 mg
- period 2: Aerodose with TOBI 60 mg

10 Aerodose 60 mg / PARI 300 mg:

- period 1: Aerodose with TOBI 60 mg
- period 2: PARI LC PLUS with TOBI 300 mg

All subjects randomly assigned to a single treatment sequence group received control and experimental treatments in the same order during the study, while  
15 subjects assigned to the other treatment sequence group received treatments in the reverse order. Table 11, below shows the two sequences of treatment administration employed during the study via the randomization process.

**TABLE 11. TREATMENT SEQUENCE GROUPS AND SEQUENCE OF TREATMENTS IN THE STUDY**

Treatment Sequence Group <sup>1</sup>	Treatment Period 1	Treatment Period 2
C-E <sup>2</sup>	C	E
E-C	E	C

<sup>1</sup>Subjects were randomly assigned to the two treatment sequence groups.  
<sup>2</sup>C and E refer to control and experimental treatments administered during the study as follows:  
C = PARI LC PLUS jet nebulizer (60 mg/mL; 300 mg in 5 mL)  
E = Aerodose inhaler (60 mg/mL; 60 mg in 1.0 mL)

- 20 Before dosing, TOBI formulations were radiolabeled with <sup>99m</sup>Tc-DTPA in preparation for gamma scintigraphy to determine posttreatment tobramycin deposition in the lungs. Subjects practiced the inhalation procedure with both control

and experimental devices filled with normal saline. When the investigator was satisfied that the subject could reproducibly perform the correct inhalation technique, the inhaler was filled with the radiolabeled formulation, and the subject inhaled the radiolabeled dose until the nebulizer was dry and nebulization was stopped.

5 Immediately following inhalation of radiolabeled, aerosolized tobramycin, scintigraphic images were recorded to determine radioactivity associated with lung and oropharyngeal tobramycin deposition and with external items such as nebulizer parts, mouthpieces, filters, and tissues used by subjects. If not previously done within the last 5 years, a posterior lung ventilation scan was also performed during  
10 the study after subjects inhaled the radioactive inert gas  $^{81m}\text{Kr}$  to determine the lung outlines and facilitate the determination of regional deposition of radiolabeled tobramycin.

#### Deposition of Tobramycin

Assessment and comparison of tobramycin deposition patterns between PARI  
15 LC PLUS and Aerodose delivery systems was a primary objective of the study. Deposition patterns of inhaled, radiolabeled tobramycin were determined using scintigraphic imaging methodology. Lung, oropharyngeal, and (if necessary) abdominal radioactivity was measured from images obtained immediately after inhalation of each single dose of radiolabeled tobramycin using a gamma camera  
20 (General Electric Maxicamera) with a 40 cm field of view and fitted with a low energy parallel hole collimator. Images were obtained as described below:

- posterior view of the chest;
- anterior view of the chest;
- right lateral view of the oropharynx;
- 25 • anterior and posterior abdominal views if necessary, i.e., if activity had spread through the intestine, beyond the field of view in either of the chest images;
- items external to the body of the subject as follows:
  - for the PARI LC PLUS system:
    - nebulizer cup

- mouthpiece
  - exhalation filter and T-piece
  - scavenger filter
  - any tissues used by the subject
- 5
- for the Aerodose system:
    - Aerodose inhaler
    - exhaled air containment box and filter
    - any tissues used by the volunteer

10 Additionally, a posterior lung ventilation scan was performed using the radioactive inert gas, krypton ( $^{81m}\text{Kr}$ ), to determine the lung outlines. The lung outlines were used to divide lung images of each subject into central, intermediate, and peripheral lung zones in order to determine the amount of aerosolized tobramycin deposited in each of these zones<sup>17</sup>. Lung ventilation scans taken for subjects who participated in earlier studies were acceptable for use for this study  
15 provided the scan was obtained within the last five years and the subject had no record of serious lung disease in the intervening period.

Deposition zones of interest on scintigraphic images were additionally drawn around the oropharynx, esophagus, and stomach (including any activity in the small intestine). The counts obtained within all regions of interest were corrected for  
20 background radioactivity, radioactive decay, and for tissue attenuation<sup>18</sup>. In regions where both anterior and posterior images were recorded, the geometric mean of counts in both images was calculated prior to correction for tissue attenuation. Determination of the percentage of the dose deposited in the oropharynx included activity adhering to the mouth and oropharynx together with any swallowed activity  
25 detected in the esophagus, stomach, and intestine.

All images were recorded using Micas X *plus* software installed on a UNIX based computer system. Images were stored on digital audio tape (DAT) for subsequent analysis and archiving. Scintigraphic data were analyzed by Pharmaceutical Profiles Ltd. (PPL) in accordance with the PPL Standard Operating

Procedure N 1013 "Lung Quantitative Data Analysis". The data were summarized to obtain the following parameters:

- whole lung deposition (% of metered dose);
- central lung zone deposition (% of metered dose);
- 5 • intermediate lung zone deposition (% of metered dose);
- peripheral lung zone deposition (% of metered dose);
- ratio of peripheral to central zone deposition (lung penetration index);
- oropharyngeal deposition (including esophagus and stomach) (% of metered dose);
- 10 • inhaler deposition (PARI LC PLUS or AeroDose) (% of metered dose);
- radioaerosol in exhaled air (filters) (% of metered dose);
- radioaerosol on PARI LC PLUS mouthpiece, T-piece, scavenger filter and subject tissues (% of metered dose);
- radioaerosol on Aerodose exhaled air collection box and subject tissues (% of
- 15 metered dose).

The counts in each area were expressed as a percentage of the metered dose that was determined from the sum of the total body counts in addition to those deposited on the inhaler and the exhalation filter. Since the volume of TOBI placed into each of the two inhalers was different, direct comparisons of the percentage

20 deposition values was problematic. To aid interpretation of the data, the percentage deposition values were multiplied by the nominal metered dose (300 mg for the PARI LC PLUS and 60 mg for the Aerodose inhaler) to obtain amounts of drug deposited in milligrams for each of the deposition parameters listed above.

#### Nebulization Time

25 Assessment and comparison of nebulization time between PARI LC PLUS and Aerodose delivery systems was another objective of the study. Elapsed time from the start of nebulization (defined as the subject's first tidal breath after the inhaler was in place) until no more TOBI solution was aerosolized by the inhaler was

measured by staff at the site using a stopwatch. Nebulization time was not to include time needed for instillation of drug into the nebulizer between the repeat filling of the Aerodose inhaler. The length of any interruption in nebulization and the reason for interruption were recorded.

5           Serum tobramycin concentrations were determined for the present study, and pharmacokinetic parameters were calculated, to provide preliminary estimates of the bioavailability of 60 mg TOBI delivered by the Aerodose system in comparison with that of the marketed 300 mg TOBI formulation. Additionally, unusually high serum tobramycin results ( $\geq 4 \mu\text{g/mL}$ ) were considered an important measure of safety  
10 during the study.

Venous blood samples (8 mL) for the determination of serum tobramycin concentrations were collected by intravenous cannula or by venipuncture before each single dose of TOBI and at 30 minutes and 1, 2, 4, and 8 hours after completion of dosing. The first one mL of blood withdrawn from the cannula was discarded, and  
15 the subsequent 7 mL was withdrawn into serum sampling tubes. Cannulae were frequently flushed with saline during the course of the treatment day.

Blood samples were centrifuged at approximately 1600 g for 10 minutes at 4°C. The resulting serum fraction was split into two aliquots by pipetting into two pre-labeled polypropylene screw cap tubes. Tubes were stored at -20°C for each  
20 study period and were then transferred to a -70°C freezer.

The maximum tobramycin concentration ( $C_{\text{max}}$ ) and the time to reach  $C_{\text{max}}$  ( $T_{\text{max}}$ ) were the observed values. The elimination rate constant ( $k_{\text{el}}$ ; used to calculate  $\text{AUC}_{0-\infty}$ ; see next paragraph) was calculated as the negative slope of the log plasma concentration vs. time plot using the last two measurable concentrations. Use of  
25 more than two concentrations at or after  $T_{\text{max}}$  is preferred for calculation of the elimination rate constant; however, several subjects had only two measurable tobramycin concentrations at the terminal phase after TOBI 60 mg using the Aerodose inhaler. The alternate method of calculating  $k_{\text{el}}$  using the last two measurable concentrations was employed for all subjects for both period 1 and  
30 period 2.

Area under the curve through 8 hours postdose ( $AUC_{0-8}$ ) and extrapolated to infinity ( $AUC_{0-\infty}$ ) were calculated for serum tobramycin concentrations using the linear trapezoid rule. Actual nebulization time was added to the time between predose and 30 minutes after the end of inhalation when calculating  $AUC_{0-8}$ .  $AUC_{0-\infty}$  was extrapolated from the last measurable concentration to infinite time by adding the quantity equal to the last measurable concentration divided by the elimination rate constant ( $k_e$ ).

#### Statistical Methods Planned in the Protocol

Scintigraphic data were analyzed in accordance with the current version of the PPL Standard Operating Procedure N 1013 "Lung Quantitative Data Analysis". Manipulation and calculation of radioactivity counts were accomplished using a custom written region of interest program, where regions of interest were central, intermediate, peripheral, and stomach/intestines if necessary. Numerical data were downloaded automatically from the Park Medical computer into a customized spreadsheet.

Due to the small number of subjects in the study, statistical analysis was performed only on whole lung deposition data and on selected pharmacokinetic parameters. All other study data were summarized descriptively. Descriptive summaries for quantitative data included sample size, mean, standard deviation, median, minimum, maximum, and/or range values as appropriate. Descriptive summaries for qualitative or categorical data included number and percent of subjects with the characteristic. All clinical data manipulations, analyses, summaries, and transformations employed SAS version 6.12<sup>20-22</sup>.

#### Aerosol Delivery Analyses

Whole lung deposition was the primary endpoint for the analysis. The Wilcoxon one-sample, matched-pairs, signed ranks test was used to determine whether differences between the whole lung deposition patterns (percent and amount of metered dose deposited) for the two inhalers were significant. The significance level was set at  $\alpha = 0.05$ .