

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

201688s000

MICROBIOLOGY REVIEW(S)

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
 Novartis Pharmaceuticals Corporation
 NDA 201—688 SN000

Clinical Microbiology Review #9
 Peter Coderre, PhD
 17 October 2012

Applicant:

Novartis Pharmaceuticals Corporation
 Drug Regulatory Affairs
 One Health Plaza
 East Hanover, NJ 07936-1080

Contact:

John Noh, PhD
 Tel: 862-778-1689
 Fax: 973-781-2565
 Email address: john.noh@novartis.com

Submission Reviewed: NDA 201—688 SN000

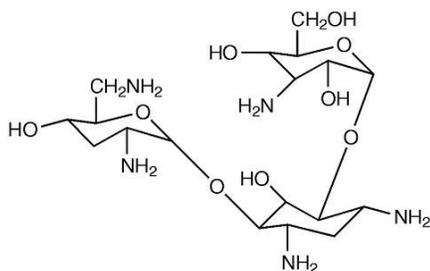
Indication: Management of Cystic Fibrosis (CF) patients infected with *Pseudomonas aeruginosa*

Product Names:

Proprietary: TOBI Podhaler™
 Non-proprietary/USAN: Tobramycin Powder for Inhalation (TPI)

Chemical Name: *O*-3-amino-3-deoxy-(α)-D- glucopyranosyl-(1->4)- *O*-[2,6-diamino-2,3,6-trideoxy-(α)-D- *ribo*-hexopyranosyl-(1->6)]-2-deoxy-L-streptamine

Molecular Formula: C₁₈ H₃₇ N₅ O₉; MW: 467.52

Structural Formula:

Dosage Form and Duration of Treatment: Dry powder for inhalation repeated cycles of 28 days on drug dose of 300 mg twice daily followed by 28 days off drug

Route of Administration: Inhalation by Nektar T-326 Dry Powder Inhaler Device

Dispensed: Rx

Initial Submission Dates:

Received by CDER:	20 December 2011
Received by Reviewer:	21 December 2011
Initial Review Completed:	07 August 2012
Amended Review Completed:	17 October 2012

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
17 October 2012

Related Documents: IND 64,409

Remarks:

On 05 September 2012, the Anti-infective Drugs Advisory Committee (AIDAC) voted to approve NDA 201—688.

The recommendations of the AIDAC were considered by the Division and subsequently, the Division decided to approve TOBI Podhaler for the treatment of *Pseudomonas aeruginosa*.

As a result, this Reviewer submits modifications to the Microbiology subsection of the package insert and proposes two Post Marketing Requirements (PMRs).

Conclusions and Recommendations:

From the Microbiology perspective, the primary concern regarding the data in this submission is the occurrence of the reduced susceptibility of *Pseudomonas aeruginosa* to tobramycin while on tobramycin inhalation powder therapy and the consequences this may have on successfully reducing manifestations of the disease such as decreased FEV₁. A second safety concern is the potential that these less susceptible *P. aeruginosa* may be transmitted to others in the immediate environment of the cystic fibrosis patient. I believe these to be **safety concerns** based on the following data:

- Low log₁₀ reduction rates of *P. aeruginosa* CFUs in sputum during therapy;
- Large increases in tobramycin MICs for *P. aeruginosa* clinical isolates during therapy;
- Increased resistance of bacteria to tobramycin and other antibiotics during therapy; and
- Increased emergence of other pathogens during therapy.

Low log₁₀ reduction rates of *P. aeruginosa* CFUs in sputum during therapy.

The log₁₀ reduction rates of *P. aeruginosa* CFUs in sputum during therapy in [Studies 2301](#) and [2302](#) are inconsistent. In [Study 2301](#), contrary to expected results, there are *greater* reductions in CFUs in the placebo/TIP/TIP treatment arm than the TIP/TIP/TIP treatment arm. However, in [Study 2302](#), the log₁₀ reduction rate among patients in the TIP treatment arm are greater than the log₁₀ reduction rate among patients in the TOBI treatment arm. Also, log₁₀ reduction rates among patients in both the TIP and TOBI treatment arms of [Study 2302](#) are *lower* than the log₁₀ reduction rates seen in historical data from the TOBI patients from the original clinical trials performed for the approval of TOBI.

Large increases in tobramycin MICs for *P. aeruginosa* clinical isolates during therapy.

Large increases in tobramycin MICs for *P. aeruginosa* clinical isolates during therapy occurred. In some instances, the MICs increased three or more dilution steps and as high as > 512 mg/ml in the TIP treatment arm. These large increases were not present in either the placebo/TIP/TIP or the TOBI comparator arms. Poor compliance among TIP patients may offer one explanation for the observed data.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
17 October 2012

Increased resistance to tobramycin and other antibiotics develop during therapy. Increased resistance to tobramycin and other antibiotics was observed during therapy. In [Study 2301](#), between 5.4% (muroid colony types) and 18.6% (small colony types) of isolates were tobramycin resistant; in [Study 2302](#), between 6.7% (muroid colony types) and 18.4% (small colony types) of isolates were tobramycin resistant. *Consistent with these clinical data are the epidemiological studies among CF patients which show that tobramycin resistance in *P. aeruginosa* has nearly doubled since the introduction of tobramycin (TOBI) to the market.*

In [Study 2302](#), TIP treatment patients were observed to have at least 5% of *P. aeruginosa* isolates that were resistant to ceftazadime, ciprofloxacin or meropenem. TOBI treatment patients were observed to have at least 5% of *P. aeruginosa* isolates that were resistant to aztreonam, ceftazadime, or imipenem.

Increased emergence of other pathogens during therapy. There was an observed increase in emergence of other pathogens during therapy for the combined data from [Studies 2301](#) and [2303](#). In the TIP treatment arm, patients had a higher rate of infection with the emergent pathogens: *Haemophilus influenzae*, *Stenotrophomonas maltophilia*, *Penicillium* spp., and *Candida albicans*.

Consequently, my recommendation from the Microbiology perspective to the Division Director was that Tobramycin Inhalation Powder (TIP) for the treatment of *Pseudomonas aeruginosa* in cystic fibrosis patients is NOT APPROVABLE.

However, the Division has decided to approve NDA 201—688 and thus, the Microbiology section of the package insert required modification by this Reviewer. These modifications are found below the proposed PMRs.

Proposed Post-Marketing Requirements

Conduct US surveillance studies for five years from the date of marketing TOBI PODHALER to determine if resistance to tobramycin has developed in *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. These US surveillance studies should also monitor resistance to these additional antibiotics: meropenem, imipenem, ceftazidime, aztreonam and ciprofloxacin.

Protocol Submission: TBD
Study Start: TBD
Final Report Submission: TBD

Conduct US studies to determine the emergence of pathogens other than *Pseudomonas aeruginosa* over the course of treatment (two years from the date of marketing). This should include the monitoring of the emergence during therapy of the following organisms: *Haemophilus influenzae*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, *Candida albicans* and *Penicillium* species.

Protocol Submission: TBD
Study Start: TBD
Final Report Submission: TBD

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
17 October 2012

Microbiology Subsection of the Package Insert

Proposed changes by this Reviewer to the Microbiology section proposed by the Applicant are designated as follows: deletions are indicated by ~~red, strikethrough text~~; additions are indicated by blue, underlined text.

12.4 Microbiology**Mechanism of Action**

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

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

Susceptibility Testing

Interpretive criteria for inhaled antibacterial products are not defined. (b) (4) 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. A single sputum sample from a cystic fibrosis patient may contain multiple morphotypes of *P. aeruginosa* and each morphotype may have a different (b) (4)

Development of Resistance

In clinical studies, (b) (4) from baseline to the end of the treatment period were observed in the (b) (4) tobramycin MIC for each *P. aeruginosa* (b) (4). In general, a higher percentage of patients treated with TOBI Podhaler had increases in tobramycin MIC compared with (b) (4).

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
17 October 2012

(b) (4)



The clinical significance of changes in MICs for *P. aeruginosa* has not been clearly established in the treatment of cystic fibrosis patients.

(b) (4)

**Cross-Resistance**

(b) (4)

**Other**

(b) (4)

REFERENCES

1. [Clinical and Laboratory Standards Institute \(CLSI\). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically Ninth Edition: Approved Standard. CLSI Document M7- A9. CLSI, 950 West Valley Rd., Suite 2500, Wayne, PA 19087, 2012.](#)
2. [CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard – 11th ed. CLSI document M02-A11. CLSI, 2012.](#)
3. [CLSI. Performance Standards for Antimicrobial Susceptibility Testing: 22nd Informational Supplement. CLSI document M100-S22. CLSI, 2012.](#)

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
17 October 2012

Peter Coderre, PhD
Acting Microbiology Team Leader
17 October 2012
FIN

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

/s/

PETER E CODERRE
10/18/2012

Product Quality Microbiology Review

6 September 2012

NDA: 201-688

Drug Product Name

Proprietary: TOBI® Podhaler™

Non-proprietary: tobramycin inhalation powder

Review Number: 1

Dates of Submission(s) Covered by this Review

Submit	Received	Review Request	Assigned to Reviewer
21 December 2011	21 December 2011	31 January 2012	31 January 2012

Submission History (for amendments only): N/A

Applicant/Sponsor

Name: Novartis Pharmaceutical Corporation

Address: One Health Plaza
East Hanover, NJ

Representative: John Noh
Global Regulatory Manager

Telephone: 862-778-1689

Name of Reviewer: Stephen E. Langille, Ph.D.

Conclusion: Recommended for approval

Product Quality Microbiology Data Sheet

- A.**
- 1. TYPE OF SUBMISSION:** Original NDA
 - 2. SUBMISSION PROVIDES FOR:** Manufacturing information to support an inhaled powder drug product.
 - 3. MANUFACTURING SITE:** Novartis Pharmaceutical Corporation
150 Industrial Road
San Carlos, CA
 - 4. DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:**
 - Powder in a hard capsule
 - 28 mg/capsule
 - Oral inhalation
 - 5. METHOD(S) OF STERILIZATION:** Not applicable
 - 6. PHARMACOLOGICAL CATEGORY:** Management of *Pseudomonas aeruginosa* infections in cystic fibrosis patients.
- B. SUPPORTING/RELATED DOCUMENTS:** None
- C. REMARKS:** The submission was provided in eCTD format.

filename: N201688r1

Executive Summary**I. Recommendations**

- A. Recommendation on Approvability -**
NDA 201-688 is recommended for approval from the standpoint of product quality microbiology.
- B. Recommendations on Phase 4 Commitments and/or Agreements, if Approvable -**
Not applicable

II. Summary of Microbiology Assessments

- A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology -**
The drug product is (b) (4) spray dried.
- B. Brief Description of Microbiology Deficiencies -**
No deficiencies were identified based upon the information provided.
- C. Assessment of Risk Due to Microbiology Deficiencies -**
Not applicable

III. Administrative

- A. Reviewer's Signature** _____
Stephen E. Langille, Ph.D.
Senior Microbiology Reviewer
- B. Endorsement Block**
John Metcalfe, Ph.D. – Senior Microbiology Reviewer
- C. CC Block**
N/A

4 Pages have been Withheld in full as b4 (CCI/TS)
immediately following this page

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

/s/

STEPHEN E LANGILLE
09/06/2012

JOHN W METCALFE
09/06/2012
I concur.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
 Novartis Pharmaceuticals Corporation
 NDA 201—688 SN000

Clinical Microbiology Review #9
 Peter Coderre, PhD
 07 August 2012

Applicant:

Novartis Pharmaceuticals Corporation
 Drug Regulatory Affairs
 One Health Plaza
 East Hanover, NJ 07936-1080

Contact:

John Noh, PhD
 Tel: 862-778-1689
 Fax: 973-781-2565
 Email address: john.noh@novartis.com

Submission Reviewed: NDA 201—688 SN000

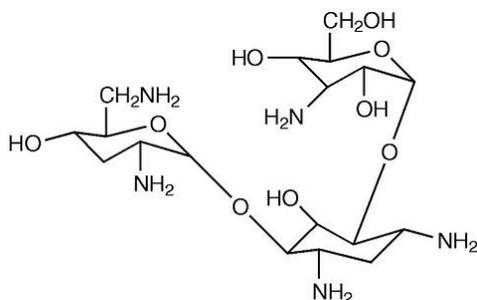
Indication: Management of Cystic Fibrosis (CF) patients infected with *Pseudomonas aeruginosa*

Product Names:

Proprietary: TOBI Podhaler™
 Non-proprietary/USAN: Tobramycin Powder for Inhalation (TPI)

Chemical Name: *O*-3-amino-3-deoxy-(α)-D- glucopyranosyl-(1->4)- *O*-[2,6-diamino-2,3,6-trideoxy-(α)-D- ribo -hexopyranosyl-(1->6)]-2-deoxy-L-streptamine

Molecular Formula: C₁₈ H₃₇ N₅ O₉ ; MW: 467.52

Structural Formula:

Dosage Form and Duration of Treatment: Dry powder for inhalation repeated cycles of 28 days on drug dose of 300 mg twice daily followed by 28 days off drug

Route of Administration: Inhalation by Nektar T-326 Dry Powder Inhaler Device

Dispensed: Rx

Initial Submission Dates:

Received by CDER:	20 December 2011
Received by Reviewer:	21 December 2011
Review Completed:	07 August 2012

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Related Documents: IND 64,409

Remarks:

The Applicant presents an original NDA for TOBI® Podhaler™ for the management of cystic fibrosis patients with *Pseudomonas aeruginosa*. The proposed indication is the same as the approved indication for TOBI® (tobramycin inhalation solution, USP), NDA 50-753.

TOBI Podhaler is a new powder formulation of tobramycin for inhalation to be delivered with the T-326 Inhaler, a dry powder inhaler. TOBI Podhaler was developed as a treatment alternative to TOBI (tobramycin inhalation solution, USP), designed to deliver high concentrations of tobramycin directly to the site of infection in the endobronchial space. The drug-device combination provides comparable efficacy and similar safety as TOBI but with increased ease of use and decreased administration time. TOBI Podhaler provides a more convenient treatment alternative, especially important for the heavily treatment burdened cystic fibrosis patients.

The primary clinical evidence which supports the efficacy and safety of TOBI Podhaler in patients with cystic fibrosis aged ≥ 6 years consists of three Phase III studies:

- [Study C2301](#): Double blind study of tobramycin inhalation powder (TIP) vs placebo (for one cycle, followed by two cycles of open-label TIP treatment) in CF patients aged 6-21 years with no prior exposure to inhaled anti-pseudomonal antibiotics for at least 4 months
- [Study C2302](#): Open-label study of TIP vs TOBI across three cycles of TIP treatment in CF patients aged ≥ 6 years with no prior exposure to inhaled anti-pseudomonals for one month
- [Study C2303](#): Double blind study of TIP vs placebo for one cycle in CF patients aged 6-21 years with no prior exposure to inhaled anti-pseudomonals for at least 4 months

These studies were conducted in a total of 710 randomized cystic fibrosis patients aged ≥ 6 years. A total of 425 patients received at least one dose of TIP in these studies.

Results of the technical, nonclinical, and clinical development programs are included in this application. The NDA presentation complies in structure and content with ICH guidelines and Guidance for Industry pertinent to structure and content of an electronic common technical document.

By agreement at the pre-NDA meeting on 15 December 2009, reference is made to selected information previously submitted to NDA 50-753 (TOBI). Reference is also made to information previously submitted to IND 64,409 (TBM100C, TIP).

This review describes the findings and the recommendations of the Microbiology Reviewer. These recommendations are for evaluation by the Division Director for the determination of a decision whether this NDA application should be approved.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

CONCLUSIONS AND RECOMMENDATION

From the Microbiology perspective, the primary concern regarding the data in this submission is the occurrence of the reduced susceptibility of *Pseudomonas aeruginosa* to tobramycin while on tobramycin inhalation powder therapy and the consequences this may have on successfully reducing manifestations of the disease such as decreased FEV₁. A second safety concern is the potential that these less susceptible *P. aeruginosa* may be transmitted to others in the immediate environment of the cystic fibrosis patient. I believe these to be **safety concerns** based on the following data:

- Low log₁₀ reduction rates of *P. aeruginosa* CFUs in sputum during therapy;
- Large increases in tobramycin MICs for *P. aeruginosa* clinical isolates during therapy;
- Increased resistance of bacteria to tobramycin and other antibiotics during therapy; and
- Increased emergence of other pathogens during therapy.

Low log₁₀ reduction rates of *P. aeruginosa* CFUs in sputum during therapy.

The log₁₀ reduction rates of *P. aeruginosa* CFUs in sputum during therapy in [Studies 2301](#) and [2302](#) are inconsistent. In [Study 2301](#), contrary to expected results, there are *greater* reductions in CFUs in the placebo/TIP/TIP treatment arm than the TIP/TIP/TIP treatment arm. However, in [Study 2302](#), the log₁₀ reduction rate among patients in the TIP treatment arm are greater than the log₁₀ reduction rate among patients in the TOBI treatment arm. Also, log₁₀ reduction rates among patients in both the TIP and TOBI treatment arms of [Study 2302](#) are *lower* than the log₁₀ reduction rates seen in historical data from the TOBI patients from the original clinical trials performed for the approval of TOBI.

Large increases in tobramycin MICs for *P. aeruginosa* clinical isolates during therapy. *Large increases* in tobramycin MICs for *P. aeruginosa* clinical isolates during therapy occurred. In some instances, the MICs increased three or more dilution steps and as high as > 512 mg/ml in the TIP treatment arm. These large increases were not present in either the placebo/TIP/TIP or the TOBI comparator arms. Poor compliance among TIP patients may offer one explanation for the observed data.

Increased resistance to tobramycin and other antibiotics develop during therapy. Increased resistance to tobramycin and other antibiotics was observed during therapy. In [Study 2301](#), between 5.4% (muroid colony types) and 18.6% (small colony types) of isolates were tobramycin resistant; in [Study 2302](#), between 6.7% (muroid colony types) and 18.4% (small colony types) of isolates were tobramycin resistant. *Consistent with these clinical data are the epidemiological studies among CF patients which show that tobramycin resistance in *P. aeruginosa* has nearly doubled since the introduction of tobramycin (TOBI) to the market.*

In [Study 2302](#), TIP treatment patients were observed to have at least 5% of *P. aeruginosa* isolates that were resistant to ceftazadime, ciprofloxacin or meropenem. TOBI treatment patients were observed to have at least 5% of *P. aeruginosa* isolates that were resistant to aztreonam, ceftazadime, or imipenem.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Increased emergence of other pathogens during therapy. There was an observed increase in emergence of other pathogens during therapy for the combined data from [Studies 2301](#) and [2303](#). In the TIP treatment arm, patients had a higher rate of infection with the emergent pathogens: *Haemophilus influenzae*, *Stenotrophomonas maltophilia*, *Penicillium* spp., and *Candida albicans*.

Consequently, my recommendation from the Microbiology perspective to the Division Director is that Tobramycin Inhalation Powder (TIP) for the treatment of *Pseudomonas aeruginosa* in cystic fibrosis patients is NOT APPROVABLE.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

TABLE OF CONTENTS

EXECUTIVE SUMMARY	7
PACKAGE INSERT	22
INTRODUCTION	23
PRECLINICAL EFFICACY—<i>IN VITRO</i>	25
Mechanism of Action	25
Mechanisms of Resistance	25
Spectrum of Activity	28
Surveillance Studies	29
Post Antibiotic Effect	33
Intracellular Effect	34
Bactericidal Activity	34
Antimicrobial Interactions	35
PRECLINICAL EFFICACY—<i>IN VIVO</i>	36
Animal Therapeutic Studies	36
Pharmacodynamics	36
Animal Pharmacokinetics	37
Human Pharmacokinetics	41
CLINICAL SUSCEPTIBILITY TEST METHODS	45
Susceptibility Methods	45
Quality Control Studies	46
Provisional Susceptibility Interpretive Criteria	47
Microbiological Methods	47

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
 Novartis Pharmaceuticals Corporation
 NDA 201—688 SN000

Clinical Microbiology Review #9
 Peter Coderre, PhD
 07 August 2012

CLINICAL EFFICACY	54
Previous Clinical Experience	54
Study 2301	58
<i>P. aeruginosa</i> Density in Sputum	60
Changes in Tobramycin MIC During Therapy	64
Antibiotic Resistance Development on Therapy	67
Shifts in Sputum Density of Baseline Pathogens	68
Treatment-Emergent Organisms	69
Study 2302	70
<i>P. aeruginosa</i> Density in Sputum	72
Changes in Tobramycin MIC During Therapy	76
Antibiotic Resistance Development on Therapy	81
Shifts in Sputum Density of Baseline Pathogens	82
Treatment-Emergent Organisms	84
Study 2303	86
<i>P. aeruginosa</i> Density in Sputum	87
Changes in Tobramycin MIC During Therapy	88
Antibiotic Resistance Development on Therapy	91
Treatment-Emergent Organisms	93
Determination of Interpretive Criteria	95
REVIEW REFERENCES	95

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

EXECUTIVE SUMMARY

The Applicant submits a New Drug Application (NDA 201—688) for Tobramycin Inhalation Powder (TIP), a novel drug-device combination to deliver tobramycin topically to the lungs for management of persons with cystic fibrosis (CF) and *Pseudomonas aeruginosa* (*P. aeruginosa*) infection.

Tobramycin is formulated in TIP by spray-drying (b) (4) droplets of an emulsion of tobramycin, DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), calcium chloride (CaCl₂) and perfluorooctyl bromide (PFOB). Water and PFOB evaporate (b) (4), yielding (b) (4) particles with porous structure (formerly branded PulmoSpheres®). The resulting low-density particles possess favorable aerodynamic characteristics when suspended as particles. Their high surface porosity also reduces particle-to-particle contact, decreasing the energy required for aerosol suspension. TIP formulation is delivered with a T-326 Dry Powder Inhaler (DPI). The TIP drug-device combination represents a substantial improvement in design compared to the TOBI formulation and delivery system, while retaining the delivery of a comparable quantity of tobramycin to the lung per administration.

The Agency gave US marketing authorization for TOBI in 1997 as a chronic intermittent therapy for the management of cystic fibrosis patients with *P. aeruginosa*. Patients inhale TOBI twice daily using an air jet nebulizer coupled to an air compressor, a process which can take 20 minutes per dose with additional time required for set-up and nebulizer cleaning. In addition to inhaled antibiotics such as TOBI, a variety of other chronic therapies are routinely prescribed to reduce the destructive cycles of obstruction, infection, and inflammation in the CF lung. It has been shown that adherence to therapies is a significant problem for persons with CF and that lack of compliance can vary by specific treatment.

Given the treatment burden and adherence challenges associated with preservation of lung function in persons with CF, any improvements in existing therapies that reduce treatment administration time or increase convenience of treatment for patients have the potential to increase patient adherence and thereby increasing therapeutic efficacy. TIP is a new drug-device combination intended to reduce administration time and increase convenience for persons with CF that are prescribed inhaled tobramycin for suppression of *P. aeruginosa* lung infections.

In this review, the microbiology data from both *in vitro* and *in vivo* preclinical efficacy studies as well as data from clinical efficacy studies are reviewed and analyzed. This information will then be available to the Division Director to be used to determine the approvability of this drug product.

PRECLINICAL EFFICACY—IN VITRO**Mechanism of Action**

Tobramycin and other aminoglycosides act on the bacterial cell by inhibiting protein synthesis and increasing cell permeability. Protein synthesis is inhibited at initiation when the antibiotic binds to the 30S ribosome, consequently removing the ribosome

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

from the ribosome pool. The cytoplasmic membrane becomes disorganized, eventually leading to loss of permeability control and ultimately, to cell death.

Mechanisms of Resistance

The main resistance mechanisms utilized by *P. aeruginosa* to overcome tobramycin activity are drug efflux and drug inactivation by modifying enzymes. The unique characteristics of chronic *P. aeruginosa* infections in CF patients, such as biofilm formation and high frequency of genetic mutations, may also be important factors for reduced susceptibility and the difficulties to eradicate *P. aeruginosa* in CF patients. A successful strategy to avoid high resistance rates in patients who need repeated antibiotic therapy courses may be "intermittent administration of tobramycin." This concept, which been widely recommended in CF patients worldwide, has successfully maintained the high efficacy of tobramycin to kill *P. aeruginosa* and other pathogens in the majority of CF patients. It is best explained by the fact that tobramycin-resistant *P. aeruginosa* clones grow more slowly than susceptible clones. Hence, once the antibiotic selective pressure is removed, susceptible clones overgrow resistant clones, making further tobramycin treatment courses clinically effective.

Spectrum of Activity

The spectrum of antibacterial activity of tobramycin primarily encompasses Gram-negative bacteria. The primary pathogen isolated from CF patients is *Pseudomonas aeruginosa*. Over time, the tobramycin susceptibility of this organism has varied; in addition, tobramycin susceptibility can differ by morphotype or colony type i.e. mucoid, dry and small colony variant. Another factor affecting tobramycin susceptibility is whether the organism is planktonic or part of a biofilm, as is the case in cystic fibrosis.

Activity against Gram-positive bacteria is limited; some *Staphylococcus* species are susceptible (*S. aureus* and *S. epidermidis*). Some of the alternative pathogens for CF may be sensitive to tobramycin (*Klebsiella* spp. and *Staphylococcus aureus*) yet other pathogens are very resistant to tobramycin therapy, such as *Burkholderia cepacia*, *Alcaligenes xylosoxidans* and *Stenotrophomonas maltophilia*.

Epidemiological Studies

As tobramycin has been an Agency approved product for many years, few epidemiological studies on *P. aeruginosa* from CF patients have been conducted, particularly in the last three years; thus, recent (within the last three years) susceptibility data is sparse. Only two of the fourteen studies listed in [Table 4-5](#) in the Surveillance Studies Section contain recent tobramycin susceptibility data for *P. aeruginosa* and both of these studies were conducted outside the US (Bulgaria and Germany). In eight of the fourteen studies, the MIC₉₀ value was 16 µg/ml (the resistance breakpoint) or greater with a range of 16 µg/ml to > 1024 µg/ml.

Highlights of some of these studies listed in [Table 4-5](#) are presented below as they are instructive in the epidemiology of tobramycin susceptibility in *P. aeruginosa* from CF patients.

[Pitt et al \(2003\)](#) found that 10% of isolates were shown to be resistant to tobramycin. The number of isolates resistant to colistin was the lowest at 3.1%. However, Cross resistance was extensive with approximately 40% of CF isolates

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

resistant to two or more antibiotics, usually ceftazidime and gentamicin or ceftazidime and piperacillin. The authors concluded "*The level of resistance to front line antipseudomonal agents, with the exception of colistin, is disturbingly high. The prudent use of antimicrobial drugs and closer monitoring of accumulation of resistant strain populations should be actively considered.*"

In a study by [Morosini et al \(2005\)](#), *P. aeruginosa* isolates had tobramycin MIC range, MIC50, and MIC90 values of 0.25 to >1024, 2, and 16 µg/mL, respectively. Thirteen percent of isolates were considered resistant to tobramycin. It is noteworthy that the authors agree with the Spanish Antibiogram Committee (The MENSURA Group) who has tentatively defined specific breakpoints for inhaled tobramycin (susceptible, ≤ 64 µg/ml, and resistant, ≥ 128 µg/ml) when *P. aeruginosa* isolates from CF patients are tested. As in the previous study ([Pitt et al., 2003](#)), colistin had the lowest resistance rate (3%) of all antimicrobials tested.

[Chen et al \(2005\)](#) analyzed 200 mucoid and 200 non-mucoid multi-resistant *P. aeruginosa* isolates from CF patients in the United States that were referred to a reference laboratory between 2001 and 2003. For the mucoid isolates and the non-mucoid isolates, tobramycin MIC90 values were 64 µg/mL and 256 µg/mL, respectively. Of the mucoid and non-mucoid isolates, 35% and 52% were resistant to tobramycin. The prevalence of elevated MICs for tobramycin with mucoid and non-mucoid isolates was comparably high and may be linked to the multi-resistant nature of the test organisms submitted to the reference laboratory, as also reflected in the high percentage resistance observed for all of the test agents.

Isolates collected from 2005 to 2009 from 96 CF patients in Bulgaria were analyzed ([Strateva et al, 2010](#)). Tobramycin activity against non-mucoid *P. aeruginosa* (MIC90=2 µg/ml) was higher than that against mucoid isolates (MIC90=4 µg/ml). The isolates from patients with long-term *P. aeruginosa* colonization (over 5 years) revealed the highest tobramycin MICs (MIC90 >1024 µg/ml). It is apparent from this study that while patients on inhaled tobramycin may exhibit *P. aeruginosa* isolates with tobramycin MICs in the susceptible category initially, with long term usage, *P. aeruginosa* isolates demonstrate very high tobramycin MICs in the resistant category.

[Valenza et al \(2010\)](#) reported on the MIC susceptibility results of 1844 isolates of *P. aeruginosa* that were obtained from the sputum of 22 chronically colonized cystic fibrosis patients over time starting in the 1990s up to the year 2009. The cumulative MIC values of tobramycin (MIC90) of organisms were ≥ 256 µg/ml, from patients after regular exposure to tobramycin. These investigators found that 27.5% of all isolates were resistant to tobramycin; none of the 1844 isolates were resistant to colistin. The MIC90 value for colistin was 0.75 µg/ml. [Valenza et al. \(2010\)](#) concluded that tobramycin resistance in *P. aeruginosa* isolates of chronically colonized CF patients under long-term antimicrobial therapy may be present, even when higher breakpoints suggested for inhaled tobramycin are considered. In contrast, colistin resistance in *P. aeruginosa* remains uncommon even after years of inhaled therapy.

As tobramycin has been an Agency approved product for many years, few epidemiological studies on *P. aeruginosa* from CF patients have been conducted,

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

particularly in the last three years; thus, recent (within the last three years) susceptibility data is sparse. Only two of the fourteen studies listed in [Table 4-5](#) contain recent tobramycin susceptibility data for *P. aeruginosa* and both of these studies were conducted outside the US (Bulgaria and Germany). In eight of the fourteen studies, the MIC₉₀ value was 16 or greater, the resistance breakpoint, with a range of 16 µg/ml to > 1024 µg/ml.

Historically, the initial epidemiological study of tobramycin resistance in *P. aeruginosa* from CF patients was conducted by [Shawar et al \(1999\)](#); this study demonstrated an overall tobramycin resistance rate in *P. aeruginosa* of 5.4% derived from a tobramycin resistance rate of 2.4% among 710 mucoid isolates of *P. aeruginosa* and 9.4% tobramycin resistance rate among 530 non-mucoid isolates of *P. aeruginosa*. Of the fourteen studies, five studies demonstrated tobramycin resistance rates for *P. aeruginosa* of 10% or greater ranging from 10% to 52%; of note is that these studies were conducted in a variety of countries including the US, Spain, Germany, Japan and the UK suggesting that an increase in tobramycin resistance in *P. aeruginosa* from CF patients is worldwide, not just local (US).

Although the data are limited, the tobramycin resistance rate in P. aeruginosa isolates from CF patients since 1999 has nearly doubled, an increase of more than 85%.

Bactericidal Effects

Tobramycin is a bactericidal protein synthesis inhibitor in contrast to bacteriostatic protein synthesis inhibitors such as chloramphenicol. Tobramycin has demonstrated a low MBC/MIC ratio for *P. aeruginosa* of 1-4, suggesting that the agent generally works by killing bacteria rather than by simply inhibiting bacterial growth.

Postantibiotic Effect (PAE)

Treatment with an aminoglycoside antibiotic such as tobramycin may also lead to suppression of bacterial growth that persists after short-term exposure to an antibiotic the so-called postantibiotic effect (PAE). PAE can be measured *in vitro* or in animal models of infection. *In vitro*, the aminoglycosides consistently demonstrate a PAE that varies from 1 to 3 hours in broth and serum for *P. aeruginosa* and from 0.9 to 2.0 hours for the *Enterobacteriaceae*. As the aminoglycoside concentration increases, or the inoculum concentration decreases, the PAE becomes longer. The smaller the inoculum and the higher the oxygen tension, the longer the PAE will be extended. Reduced pH and low oxygen tension may result in the shorter PAE.

Intracellular Effects

P. aeruginosa is not an intracellular pathogen, therefore, studies of intracellular concentrations were neither conducted nor reviewed.

Antibacterial Interactions

There is a general consensus in the scientific literature on the absence of negative interactions of other agents with tobramycin; however, no robust studies have been conducted in the setting of high drug exposure and CF sputum.

Potential for aminoglycoside synergy with β-lactam antibiotics is widely recognized in the medical community based on infection models (e.g. endocarditis) and systemic

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

treatment. Combination treatment (aminoglycoside + β -lactam) in ventilator-associated pneumonia is recommended by the American Thoracic Society (if non-fermenters such as *P. aeruginosa* are suspected as pathogens).

No formal interactions studies for TIP have been performed, and TIP has not been developed for concomitant use with other inhaled antibiotics.

PRECLINICAL EFFICACY—IN VIVO**Animal Efficacy Studies**

No new animal therapeutic or pharmacologic studies were conducted during the TIP development program due to the targeted nature of the drug development program, the prior human experience with inhaled tobramycin, and the prior findings of the Agency which were used to approve TOBI.

Animal Pharmacokinetics

The Applicant examined the pharmacokinetics and product metabolism in animals in which the drug was administered parenterally. The distribution of ^{14}C -labeled tobramycin in rat tissues after subcutaneous administrations of 100mg/kg showed that the greatest concentration of tobramycin was in the kidney. This was expected since the drug is excreted primarily through this organ.

Drug concentration was higher in the lung than in the majority of tissues. In a canine lung model, tobramycin accumulated in bronchial secretions that had concentrations of 31% more than of the concentration in the sera. In guinea pig cochlear lymph, the half-life of the drug was longer than that observed in blood. This was of concern due to the nephrotoxic and ototoxic nature of aminoglycosides.

Serum drug levels in guinea pigs showed more variability than in rats. The observed variability in guinea pig serum levels was likely due to the animal's inherent airway reactivity and spontaneous changes in the ventilatory patterns that led to differences between each animal's inspired aerosol mass.

The systemic distribution of aerosolized drug was not evaluated. However, the Applicant expected that once absorbed, the distribution would be the same as after parenteral administration.

Nonclinical studies were performed in rats and dogs using a dry powder administration of the drug. Absorption was fast in both species. Dose-dependent increases in systemic exposure were observed, as measured by AUC and C_{max} . In both species, tobramycin had a short serum half-life. Higher serum levels were observed in rats compared to dogs. Tobramycin had high persistence in lung tissues, with higher lung exposures observed in rats versus dogs. Drug levels in serum and lung tissues achieved in rats after TPI Powder inhalation were comparable to those observed when tobramycin is administered as a solution for inhalation.

The ADME (absorption, distribution, metabolism and excretion) of tobramycin when administered as a powder for inhalation was determined following single dose administration in dogs and multiple dose administration in rats and dogs.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Beagle dogs were used to determine the pharmacokinetics of tobramycin after a single dose of 5.5 to 41.2 mg/kg aerosol administration of TPI Powder. Tobramycin was rapidly absorbed; all dogs achieved maximum concentrations in the time span of 5-30 min. after the end of dosing. Maximum concentrations after mouth only administration were 0.63 and 2.79 $\mu\text{g/ml}$ at 15 min. and 60 min. respectively. AUC values after mouth only administration were between 0.783 and 26.6 $\mu\text{g h/ml}$ at 15 min. and 60 min. respectively. Both measures of exposure appear to be dependent on dose. Elimination half-lives ranged from 1.21 to 1.63 h and were independent of dose as well.

Evaluation of the pharmacokinetics of the drug following multiple aerosol dose administration (toxicokinetics) was performed in a 28-day toxicology study in rats and in a 7-day study in dogs. In these studies, the total inhaled aerosol dose levels were from 9.9 to 72.9 mg/kg/day in rats and 8.2 to 23.8 mg/kg/day in dogs. Pharmacokinetic evaluations of tobramycin in serum were performed on days 1, 14, and 28 in rats and on days 1 and 7 in dogs. Also, lung samples were obtained from rats on days 1, 14, and 28 of dosing and from recovery animals 28 days after the last dose; lung samples were obtained in dogs at day 8 (24 hours after the last dose) and day 22 (14 days after the last dose).

After multiple dosing in rats, it was found that the drug is rapidly absorbed with maximum concentration in serum ranging between 11.5 and 44.8 $\mu\text{g/ml}$ on day 1 and between 8.5 and 23.4 $\mu\text{g/ml}$ on day 28 in the span of 5 to 30 min. after administration. AUC values were between 23.2 and 126 $\mu\text{g h/ml}$ on day 1 and between 27 and 125 $\mu\text{g h/ml}$ on day 28. The increases in C_{max} and AUC were less than proportional with dose. A low potential for accumulation in serum was indicated by tobramycin concentrations below the limit of quantitation 24 hours after the end of exposure. Upon multiple dosing of the drug in dogs, the drug is rapidly absorbed; maximum concentrations were achieved in the time span of 5 min to 1 h after dosing.

In summary, the multiple-dose pharmacokinetics of tobramycin in serum after administration of TPI was similar in rats and dogs. Dose dependent increases in AUC and C_{max} were observed in both species. Higher systemic exposures were observed in rats. This is most likely related to the nose only administration employed in dosing rats. Tobramycin does not accumulate in serum upon once daily administration.

In rat lung tissue, maximum concentrations of the drug of 55-319 $\mu\text{g/g}$ were observed between 0.083 to 0.5 h after dosing on day 1, and 326 to 1157 $\mu\text{g/g}$ between 0.083 and 6 h after dosing on day 28. In dogs, concentrations in lungs were below the limit of quantitation for animals receiving a low dose of TPI upon necropsy on day 8. In summary, the affinity of tobramycin for lung tissues after administration of TPI was demonstrated in both rats and dogs. Higher lung exposures were observed in rats.

The systemic distribution of aerosolized tobramycin was not evaluated. The Applicant expects that once absorbed, apart from higher persistence in lung tissue, the distribution of the drug would be the same as after parenteral administration in which tobramycin is distributed widely through extracellular fluid.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Tobramycin is not metabolized after intravenous administration and it is renally excreted.

Human Pharmacokinetics

In patients with cystic fibrosis, tobramycin was rapidly absorbed after inhalation of single doses of TIP (28 mg, 56 mg, 84 mg and 112 mg of tobramycin) with maximum serum concentrations observed at approximately 1 hour post dose. Less than dose-proportional increases in systemic exposure were observed after single doses of 28 mg to 112 mg – for a 4- fold increase in dose, mean C_{max} and AUC_{inf} of tobramycin increased by approximately 3- fold. Serum concentrations declined with a terminal half-life of approximately 3 hours [Study TPI001](#).

A single dose of 112 mg TIP (4 capsules of 28 mg each) showed comparable systemic exposure to the approved 300 mg dose of TOBI in CF patients [[Study TPI001](#)].

- C_{max} (mean \pm SD) values were 1.02 ± 0.53 $\mu\text{g/mL}$ and 1.04 ± 0.58 $\mu\text{g/mL}$ after inhalation of single dose of 112 mg TIP and 300 mg TOBI, respectively.
- AUC_{inf} (mean \pm SD) values were 5.08 ± 2.03 $\mu\text{g}\cdot\text{h/mL}$ and 5.30 ± 2.56 $\mu\text{g}\cdot\text{h/mL}$ after inhalation of single dose of 112 mg TIP and 300 mg TOBI, respectively.
- The half-life of tobramycin in serum was also comparable between the 112 mg TIP ($T_{1/2}$ 3.1 ± 0.4 h) and 300 mg TOBI ($T_{1/2}$ 3.0 ± 0.8 h) groups.
- Inter-subject variability in serum C_{max} and AUC_{inf} was 51.9% and 40%, respectively for the 112 mg TIP dose. Inter-subject variability in C_{max} and AUC_{inf} was 55.7% and 48.3% after a single 300 mg dose of TOBI.

Serum tobramycin concentrations after single and multiple b.i.d inhalation of 112 mg of TIP ([Study TPI001](#), [Study C2301](#), [Study C2302](#), [Study C2303](#)) were low relative to the maximum systemic levels recommended for avoidance of the toxicity associated with intravenous tobramycin therapy (peak greater than 12 $\mu\text{g/mL}$). The highest average concentration seen in Phase III studies after twice-daily inhalation of 112 mg TIP for 4 weeks was 1.99 ± 0.59 $\mu\text{g/mL}$ (mean \pm SD, $n=32$) and refers to serum samples taken 60 minutes after inhalation [Study C2301](#), which was about six-fold lower than the toxicity threshold of 12 $\mu\text{g/mL}$. The highest individual serum concentration observed among the phase III studies was 4.94 $\mu\text{g/mL}$ for TIP (from [Study C2301](#)), which was more than two-fold lower than the toxicity threshold of 12 $\mu\text{g/mL}$. In [Study C2302](#) where TIP and TOBI were compared, the highest individual serum concentrations for TIP and TOBI arm were 2.27 $\mu\text{g/mL}$ and 2.08 $\mu\text{g/mL}$, respectively, which were both more than five-fold lower than the toxicity threshold of 12 $\mu\text{g/mL}$. Trough (pre-dose concentrations) also compared favorably with the recommended maximum trough level (2 $\mu\text{g/mL}$); the highest mean trough concentrations after b.i.d TIP administration were 0.38 ± 0.44 $\mu\text{g/mL}$, 0.31 ± 0.19 $\mu\text{g/mL}$, and 0.41 ± 0.51 $\mu\text{g/mL}$ for studies C2301, C2302 and C2303, respectively, and 0.24 ± 0.11 for the TOBI arm in [Study C2302](#). Minimal accumulation of tobramycin in serum, consistent with the short half-life, was observed based on trough concentration levels after multiple b.i.d administration in the Phase III studies.

Sputum concentrations appeared to be generally higher for the 112 mg TIP dose compared with 300 mg TOBI ([\[Study TPI001\]](#), [\[Study C2302\]](#), [\[Study C2303\]](#)). After

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

administration of TIP and TOBI in CF patients, maximum tobramycin concentrations in sputum were achieved on average at 30 minutes, declining thereafter with an average half-life of 0.8 to 2.2 hours [Study TPI001]. Variability in sputum exposure was higher than the variability observed in serum exposure. The mean \pm SD (range) of sputum C_{max} values after single dose of 112 mg TIP and 300 mg TOBI were 1048 \pm 1080 (85.7 to 3609) μ g/g and 737 \pm 1028 (20.4 to 4736.7) μ g/g, respectively. The mean \pm SD (range) sputum AUC_{inf} values after single dose of 112 mg TIP and 300 mg TOBI were 1740 \pm 809 (844 to 3354) μ g/g and 1302 \pm 1127 (343 to 4787) μ g/g, respectively [Study TPI001].

Comparisons between TIP and TOBI based on sputum concentrations and the use of sputum levels as a marker of overall lung deposition may be confounded by the high variability in the sputum concentrations. However, taking the variability into account, the range of sputum levels along with the comparable serum exposure give evidence that 112 mg TIP inhalation resulted in lung exposure that was similar to 300 mg TOBI.

Tobramycin is not metabolized and is primarily excreted unchanged in the urine. Therefore, metabolism assessments have not been performed with TIP.

Tobramycin is eliminated from the systemic circulation principally via the kidney by glomerular filtration with some tubular re-absorption). In CF patients, the apparent elimination half-life (T_{1/2}) of tobramycin from serum was approximately 3 h after inhalation of a single 112 mg dose of TIP, similar to the half-life observed after inhalation of a single 300 mg dose of TOBI (Table 3-2). Tobramycin concentrations in expectorated sputum declined with a half-life of approximately 2 h after both a single 112 mg dose of TIP and a single 300 mg dose of TOBI [Study TPI001].

CLINICAL EFFICACY AND SAFETY

My analyses focused on the following data:

- The absolute change in *P. aeruginosa* density in sputum with the analysis based on changes in log₁₀ colony-forming units [CFU] per gram of sputum.
- The change in tobramycin and other antibiotic MICs from baseline to each post-baseline time point in Cycles 1-3.
- The change in frequency of tobramycin and other antibiotic resistance from baseline to the end of treatment.
- The isolation of treatment-emergent organisms (pooled analysis of Studies C2301 and C2303 only).

P. aeruginosa Density in Sputum

An important analysis in the Phase III studies, given that this is the rationale for use of an antibiotic, was to demonstrate that TIP effectively suppressed *P. aeruginosa* in the lungs of CF patients as demonstrated by reduced numbers of *P. aeruginosa* in sputum specimens. *P. aeruginosa* concentration in sputum was measured at Day 1 and Day 28 for each of the three treatment cycles. *P. aeruginosa* concentration results are summarized using logarithmic scale as log₁₀ CFU per gram of sputum. Absolute changes from baseline to each post-baseline time point in each of the three

Division of Anti-Infective Products

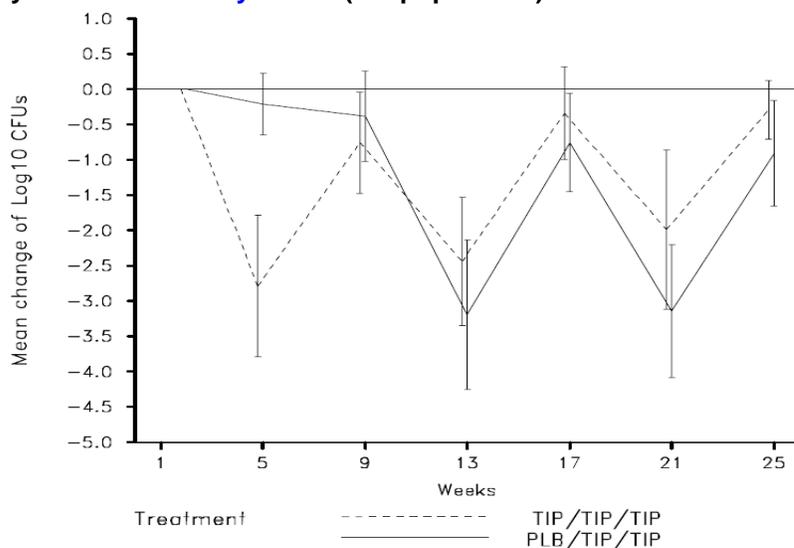
Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

cycles were summarized descriptively by treatment group and colony type (mucoid, dry, and small) of *P. aeruginosa* isolate.

The effect of tobramycin treatments on concentrations of *P. aeruginosa* in sputum in [Study 2301](#) is shown graphically in [Figure A](#).

Figure A. Change from baseline in *P. aeruginosa* sputum concentration (Log₁₀ CFUs) in cycles 1 to 3 - Study C2301 (ITT population)



Note: the vertical bar is 95% confidence interval.

Overall density is used, and it is defined as the sum of bio-types (mucoid, dry and small colony variant).

Source: Figure 4-6, Clinical Pharmacology Summary, this submission.

It is quite apparent that the greatest differences between the TIP treatment group and the placebo groups are seen at Day 28 (week 5) of Cycle 1 (a decrease of 2.79 log₁₀ CFUs in TIP group vs. 0.21 log₁₀ CFUs in placebo group).

After switching from placebo to TIP starting at Cycle 2, the *decreases* in CFU concentration in the comparator arm were *greater* than the test arm of the trial during both Cycles 2 and 3. The mean change of log₁₀ CFUs in the placebo arm were: -3.1 log₁₀ at week 13 and week 21 while the mean change of log₁₀ CFUs in the test arm were only -2.4 and -2.0 log₁₀ CFUs at weeks 13 and 21, respectively. As expected, the viable count of *P. aeruginosa* CFUs in sputum rebounded during the off-treatment cycles. These results are puzzling in light of the fact that both arms of the trial used TIP in at least two treatment cycles.

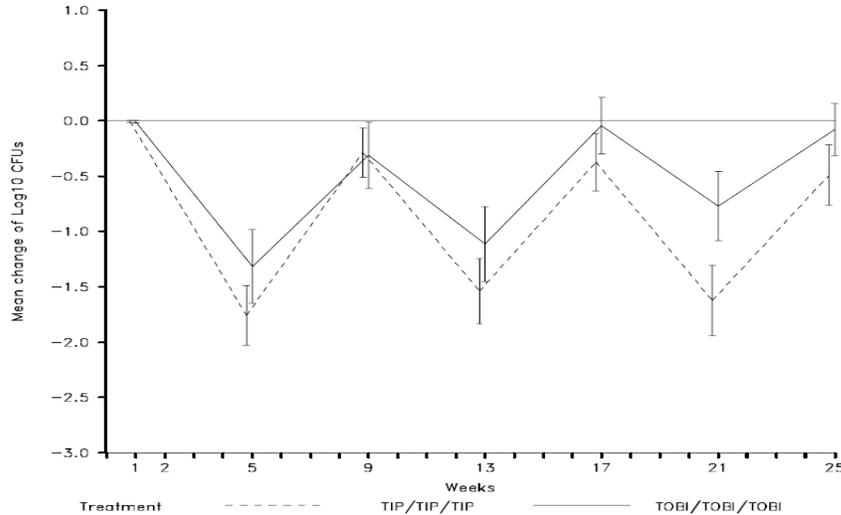
The effect of the treatments on the concentration of *P. aeruginosa* in sputum in [Study 2302](#) is shown graphically in [Figure B](#).

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
 Novartis Pharmaceuticals Corporation
 NDA 201—688 SN000

Clinical Microbiology Review #9
 Peter Coderre, PhD
 07 August 2012

Figure B. Between treatment comparison of change in *P. aeruginosa* sputum concentration (Log10 CFU) – Study C2302 (ITT population)

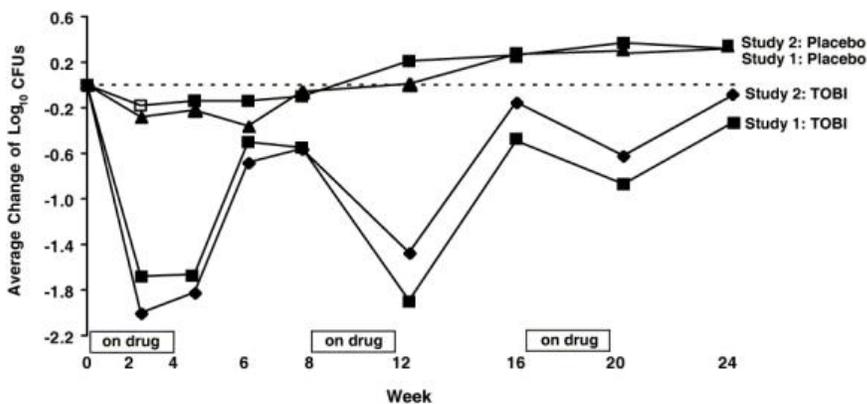


Note: the vertical bar is 95% confidence interval. Overall density is used, and it is defined as the sum of colony types (mucoid, dry and small colony variant).
 Source: Figure 4-7, Clinical Pharmacology Summary, this submission.

Among all colony types combined, the test (TIP) arm reduced the number of *P. aeruginosa* somewhat more than the comparator (TOBI) arm. The log10 reduction at the end of cycles 1, 2 and 3 for the TIP treatment arm was -1.76, -1.54 and 1.61 log10 CFUs, respectively. The log10 reduction at the end of cycles 1, 2 and 3 for the TOBI treatment arm was -1.32, 1.11 and -0.77 log10 CFUs, respectively.

When comparing the TIP and TOBI treatment arms log10 reduction in CFUs with data from the TOBI package insert, neither the reduction in CFUs in either the TIP nor TOBI arms are comparable to the reductions seen in the original clinical trials for TOBI as presented in the package insert for TOBI.

Figure C: Absolute Change from Baseline in Log10 CFUs



Source: package insert for TOBI.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Changes in MIC during Therapy

The MIC summary results are shown as the *maximum value of all colony types* in [Study 2301](#) when more than one colony type was present in a given patient at a given visit in [Table A](#).

Table A. MIC summary for [study C2301](#), ITT Population; maximum of all colony types

Range	Tobramycin MIC (µg/mL)							
	TIP/TIP/TIP				placebo/TIP/TIP			
	N	Range	MIC50	MIC90	N	Range	MIC50	MIC90
Baseline	44	≤0.25->512	0.5	32	48	≤0.25->512	1	8
Week 5	29	≤0.25->512	1	>512	44	≤0.25-8	0.5	2
Week 21	28	≤0.25->512	1	>512	30	≤0.25-256	1	32
Week 25	30	≤0.25->512	1	128	37	≤0.25->512	1	8
Termination	40	≤0.25->512	1	32	48	≤0.25->512	1	8

Color code: yellow=two dilution step increase over baseline; orange=three dilution step increase over baseline; red=four dilution step increase over baseline; blue=two dilution step decrease versus baseline; purple=three or more dilution step decrease versus baseline; green= two or more dilution step difference between MIC90 values for baseline study arms. Source: Table 4-13, Clinical Pharmacology Summary, this submission.

When the MICs from all colony types are pooled and assessed, MICs from isolates in the TIP/TIP/TIP treatment arm were *less susceptible* than those in the placebo/TIP/TIP arm as evidenced by baseline MIC90 values of 32 and 8 µg/mL, respectively. This represents a two-dilution difference in the baseline MIC90 values for the two treatment arms. Thus, the MIC90 of the baseline organisms in the test arm are resistant by the accepted CLSI systemic breakpoints (≥ 16 µg/mL) for tobramycin. This difference in baseline MIC90 is puzzling as it represents a two dilution step difference. A definitive explanation can not be offered for this finding. It may be a result of the original TIP/TIP/TIP treatment eliminating the more susceptible population of *P. aeruginosa* whereas the placebo/TIP/TIP treatment did not do this.

The MIC90 values from all *P. aeruginosa* colony types from the TIP/TIP/TIP treatment arm were identical at termination as the MIC90 values at baseline, 32 µg/mL. However, at weeks 5 and 21, the tobramycin MIC90 had increased to >512 µg/mL, an increase of more than four dilution steps compared to the baseline MIC90.

In the placebo/TIP/TIP arm, the MIC90 value for *P. aeruginosa* colony types were identical at termination as those at baseline, 8 µg/mL. At week 21, the MIC90 increased to 32 µg/mL, a two dilution step increase.

The MIC summary results are shown as the *maximum value of all P. aeruginosa* colony types in [Study 2302](#) when more than one colony type was present in a given patient at a given visit in [Table B](#).

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Table B. MIC summary for Study C2302, ITT population; maximum of all *P. aeruginosa* colony types

Range	Tobramycin MIC ($\mu\text{g/mL}$)							
	TIP				TOBI			
	N	Range	MIC50	MIC90	N	Range	MIC50	MIC90
Baseline	308	$\leq 0.12 \rightarrow 512$	2	64	208	$\leq 0.12 \rightarrow 512$	2	128
Week 5	239	$\leq 0.12 \rightarrow 512$	2	512	173	$\leq 0.12 \rightarrow 512$	4	64
Week 21	199	$\leq 0.12 \rightarrow 512$	4	256	154	$\leq 0.12 \rightarrow 512$	4	256
Week 25	201	$\leq 0.12 \rightarrow 512$	2	256	155	$\leq 0.12 \rightarrow 512$	2	64
Termination	298	$\leq 0.12 \rightarrow 512$	2	512	202	$\leq 0.12 \rightarrow 512$	2	64

Color code: yellow=two dilution step increase over baseline; orange=three dilution step increase over baseline; red=four dilution step increase over baseline; blue=two dilution step decrease versus baseline; purple=three or more dilution step decrease versus baseline; green= two or more dilution step difference between MIC90 values for baseline study arms. Source: Table 4-18, Clinical Pharmacology Summary, this submission.

The MIC90s of the baseline organisms in the TIP arm (64 $\mu\text{g/mL}$) and the TOBI arm (128 $\mu\text{g/mL}$) are resistant by the accepted CLSI systemic breakpoints for tobramycin. It is noteworthy that the Spanish Antibiogram Committee (The MENSURA Group) has tentatively defined specific breakpoints for inhaled tobramycin (susceptible, $\leq 64 \mu\text{g/ml}$, and resistant, $\geq 128 \mu\text{g/ml}$) when *P. aeruginosa* isolates from CF patients are tested. By these standards, the MIC90 values for weeks 5, 21, 25 and at termination all exceed the resistant breakpoint.

The MIC90 values for the colony type-2 isolates in the TIP treatment arm increased such that at both the Week 5 and termination visits, the MIC90 value had increased from 64 to 512 $\mu\text{g/mL}$, an increase of three dilution steps. At weeks 21 and 25, the tobramycin MIC90 had increased to 256 $\mu\text{g/mL}$, an increase of two dilution steps compared to the baseline MIC90.

The single *P. aeruginosa* colony type that appears to be the driver for the increased MICS on therapy appears to be the dry colony as demonstrated for the MIC summary in [Table C](#).

Table C. MIC summary for Study C2302, ITT Population; Colony Type -2, dry colony variant

Range	Tobramycin MIC ($\mu\text{g/mL}$)							
	TIP				TOBI			
	N	Range	MIC50	MIC90	N	Range	MIC50	MIC90
Baseline	214	$\leq 0.12 \rightarrow 512$	2	64	144	$\leq 0.12 \rightarrow 512$	2	128
Week 5	126	$\leq 0.12 \rightarrow 512$	4	512	99	$\leq 0.12 \rightarrow 512$	4	128
Week 21	107	$0.25 \rightarrow 512$	8	512	81	$0.25 \rightarrow 512$	8	256
Week 25	118	$\leq 0.12 \rightarrow 512$	4	512	96	$\leq 0.12 \rightarrow 512$	2	64
Termination	225	$\leq 0.12 \rightarrow 512$	4	512	166	$\leq 0.12 \rightarrow 512$	2	128

Color code: yellow=two dilution step increase over baseline; orange=three dilution step increase over baseline; red=four dilution step increase over baseline; blue=two dilution step decrease versus baseline; purple=three or more dilution step decrease versus baseline; green= two or more dilution step difference between MIC90 values for baseline study arms. Source: Table 4-16, Clinical Pharmacology Summary, this submission.

The MIC90 values for colony type-2 isolates in the TIP treatment arm increased such that by termination, the MIC90 value had increased from 64 to 512 $\mu\text{g/mL}$, an increase of three dilution steps. At weeks 5, 21 and 25, the tobramycin MIC90 had

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

increased to 512 µg/mL as well. Note that the MIC90 of the baseline isolates in the TIP arm (64 µg/mL) and the TOBI arm (128 µg/mL) are resistant by the accepted CLSI systemic breakpoints for tobramycin. Again, by the standards set by the MENSURA Group, the MIC90 values for weeks 5, 21, 25 and at termination all exceed the resistant breakpoint.

Antibiotic Resistance Development on Therapy

Tobramycin

According to the current CLSI systemic interpretive criteria for tobramycin, resistance is defined as a MIC \geq 16 µg/ml for *P. aeruginosa* isolates. The MICs for *P. aeruginosa* isolates from [Study C2301](#) were examined and stratified by colony type i.e. dry, mucoid, small colony and mixed colony types for each treatment arm. The following tables show the percentage of isolates from each colony type demonstrating an increase from Baseline to the Termination visit in MIC to \geq 16 µg/ml, considered a resistant phenotype. Increases in resistance of less than 5% are not shown.

Table D. *P. aeruginosa* tobramycin MIC increase by colony type, [Study C2301](#).

colony type	% increase by treatment arm	
	TIP/TIP/TIP N=46	Placebo/TIP/TIP N=49
dry colony	7.6%	7.2%
mucoid colony	5.4%	4.3%
small colony	18.6%	0%
mixed colony types	8.6%	0%

Source: Table 3.2-1.6, this submission.

Increases of 5% or more in tobramycin resistance while on therapy was observed among all colony types i.e. dry, mucoid, small colony and mixed for patients in the TIP/TIP/TIP treatment arm with the greatest increase occurring among the small colony colony type (18.6%). In contrast, only the dry colony type in the in the Placebo/TIP/TIP treatment arm showed an increase in tobramycin resistance while on therapy (7.2%).

Table E. Tobramycin resistance increase by *P. aeruginosa* colony type, [Study C2302](#).

Colony type	% increase by treatment arm	
	TIP N=308	TOBI N=209
dry colony	6.7%	- 4.3%
mucoid colony	7.3%	- 1.4%
small colony	18.4%	- 6.7%
mixed colony types	7.8%	- 2.3%

Source: Table 14.2-3.3, this submission.

Increases of 5% or more in tobramycin resistance while on therapy was observed among all colony types i.e. dry, mucoid, small colony and mixed for patients in the TIP treatment arm but not the TOBI treatment arm. The greatest increase in resistance occurred among the small colony colony type (18.4%). In contrast, *none*

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

of the colony types in the in the TOBI treatment arm showed an increase in tobramycin resistance while on therapy.

Other Antibiotics

The Applicant examined the increased resistance levels for a variety of antibiotics from baseline to the termination visit for all colony type *P. aeruginosa* isolates. Changes in resistance levels during therapy were examined for the following antibiotics: aztreonam, ciprofloxacin, ceftazidime, imipenem and meropenem (Table 14.2-3.4, this submission).

In Study 2301, no increases in resistance were noted to the following antibiotics: aztreonam, ciprofloxacin, imipenem and meropenem. The only exception was an increase in ceftazidime resistance (6.3%) seen in the placebo arm.

However, in Study 2302, an increase resistance was seen in colony types in both the TIP and TOBI arms.

Increases in antibiotic resistance among isolates from patients on therapy were noted for each antibiotic. In the *TIP treatment arm*, isolates from mucoid colony types showed a 5.9%, 7.3% and 6.5% increase in ceftazidime, ciprofloxacin and meropenem resistance, respectively. In addition, there was 9.1% increase in ciprofloxacin resistance among mixed colony types.

In the *TOBI treatment arm*, isolates from dry colony types showed a 6.3%, 11.2% and 5.5% increase in aztreonam, ceftazidime and ciprofloxacin resistance, respectively. An 8.9% increase in ceftazidime resistance was also seen in small colony variant types. The mixed colony types showed a 5.8% increase in imipenem resistance.

Treatment-Emergent Organisms: Pooled Data for Studies C2301 and C2303

The exclusion criteria for studies C2301 and C2303 did not allow patients that had:

- any use of inhaled anti-pseudomonal antibiotics within four months prior to screening; and
- use of systemic antipseudomonal antibiotics within 28 days prior to study drug administration.

Therefore, organisms isolated at post-baseline visits that were not present at baseline were representative of true treatment-emergent organisms based upon the somewhat tobramycin-naïve nature of the study populations. Given the efforts to exclude patients having recent antibiotic use, it was felt that the analysis of treatment-emergent organisms was most meaningful in these patient populations.

Organisms not present at baseline that appeared at End of Dosing or End of Cycle 1 in Study C2301 and C2303 pooled data are detailed in Table 8. The number of patients in the treatment groups were well balanced (TIP = 78, placebo = 79). A wide variety of species were encountered, though many species were isolated in only one patient. Those organisms present in more than one patient in one of the treatment groups are shown in Table 8. The most prevalent organisms (total isolates = six or more) were *H. parainfluenzae* (14), MSSA (12), *H. influenzae* (9), *A.*

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

fumigatus (7), and *S. maltophilia* (6). For many of the organisms, there were similar small numbers of isolates observed in the TIP and placebo groups. In some instances (*Chryseobacterium indologenes*, *Serratia marcescens*, MRSA, *S. pneumoniae*, *H. parainfluenzae*, and *A. fumigatus*) the number of isolates observed in the placebo group was numerically greater than that observed in the TIP group. Notable exceptions where the number of isolates in the TIP group were greater than that in the placebo group were *H. influenzae* (7 TIP vs. 2 placebo), *S. maltophilia* (4 TIP vs. 2 placebo), and *Penicillium* species (4 TIP vs. 0 placebo).

When the total number of emergent organisms was compared, there were 57 isolates among 78 TIP treatment patients and 45 isolates among 79 patients in the placebo arm. While some patients had more than one emergent organism, roughly more than 50% of patients had emergent organisms. The discrepancy in the number of emergent organisms represents 27% more emergent isolates from patients in the TIP treatment arm than in the placebo arm. This discrepancy in this may partly explained by the increased number of *H. influenzae* isolates in the TIP treatment arm versus the placebo arm, seven versus two isolates, respectively and the number of *Penicillium* spp. isolates in the TIP treatment arm versus the placebo arm, four versus zero isolates, respectively. It should also be noted that twice as many *Stenotrophomonas maltophilia* isolates emerged in the TIP arms (4) versus the placebo arms (2). Also, two patients in the TIP arm developed infections with *Candida albicans*; no patients in the placebo arms developed infections with this organism.

CONCLUSIONS AND RECOMMENDATION

From the Microbiology perspective, the primary concern regarding the data in this submission is the occurrence of the reduced susceptibility of *Pseudomonas aeruginosa* to tobramycin while on tobramycin inhalation powder therapy and the consequences this may have on successfully reducing manifestations of the disease such as decreased FEV₁. A second safety concern the potential that these less susceptible *P. aeruginosa* may be transmitted to others in the immediate environment of the cystic fibrosis patient. I believe this to be **safety concerns** based on the following data:

- Low log₁₀ reduction rates of *P. aeruginosa* CFUs in sputum during therapy;
- Large increases in tobramycin MICs for *P. aeruginosa* clinical isolates during therapy;
- Increased resistance of bacteria to tobramycin and other antibiotics during therapy; and
- Increased emergence of other pathogens during therapy.

Low log₁₀ reduction rates of *P. aeruginosa* CFUs in sputum during therapy. The log₁₀ reduction rates of *P. aeruginosa* CFUs in sputum during therapy in [Studies 2301](#) and [2302](#) are inconsistent. In [Study 2301](#), contrary to expected results, there are *greater* reductions in CFUs in the placebo/TIP/TIP treatment arm than the TIP/TIP/TIP treatment arm. However, in [Study 2302](#), the log₁₀ reduction rate among patients in the TIP treatment arm are greater than the log₁₀ reduction rate among patients in the TOBI treatment arm. Also, log₁₀ reduction rates among patients in both the TIP and TOBI treatment arms of [Study 2302](#) are *lower* than the

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

log₁₀ reduction rates seen in historical data from the TOBI patients from the original clinical trials performed for the approval of TOBI.

Large increases in tobramycin MICs for *P. aeruginosa* clinical isolates during therapy. *Large increases* in tobramycin MICs for *P. aeruginosa* clinical isolates during therapy have occurred. In some instances, the MICs have increased three or more dilution steps and as high as > 512 mg/ml in the TIP treatment arm. These large increases were not present in either the placebo/TIP/TIP or the TOBI comparator arms. Again, poor compliance among TIP patients may offer one explanation for the observed data.

Increased resistance to tobramycin and other antibiotics develop during therapy. Increased resistance to tobramycin and other antibiotics was observed during therapy. In [Study 2301](#), between 5.4% (mucoïd colony types) and 18.6% (small colony types) of isolates were tobramycin resistant; in [Study 2302](#), between 6.7% (mucoïd colony types) and 18.4% (small colony types) of isolates were tobramycin resistant. *Consistent with these clinical data are the epidemiological studies among CF patients which show that tobramycin resistance in *P. aeruginosa* has nearly doubled since the introduction of tobramycin (TOBI) to the market.*

In [Study 2302](#), TIP treatment patients were observed to have at least 5% of *P. aeruginosa* isolates that were resistant to ceftazadime, ciprofloxacin or meropenem. TOBI treatment patients were observed to have at least 5% of *P. aeruginosa* isolates that were resistant to aztreonam, ceftazadime, or imipenem.

Increased emergence of other pathogens during therapy. There was an observed increase in emergence of other pathogens during therapy for the combined data from [Studies 2301](#) and [2303](#). In the TIP treatment arm, patients had a higher rate of infection with the emergent pathogens: *Haemophilus influenzae*, *Stenotrophomonas maltophilia*, *Penicillium* spp., and *Candida albicans*.

Consequently, my recommendation from the Microbiology perspective to the Division Director is that Tobramycin Inhalation Powder (TIP) for the treatment of *Pseudomonas aeruginosa* in cystic fibrosis patients is NOT APPROVABLE.

Microbiology Subsection of the Package Insert

As this application may not be approved, this section has been omitted.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

INTRODUCTION

Cystic fibrosis (CF) is the most common life-shortening mono-genetic disease in the United States and Northern Europe. A mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene affects a chloride-channel protein and chloride ion transport in a variety of tissues, most notably in the lungs and pancreas. This leads to the production of viscous endobronchial and digestive secretions, along with pancreatic insufficiency, obstructive hepatobiliary disease and increased salt loss through the sweat glands.

Respiratory disease is a major cause of morbidity and mortality in CF. The thickened mucus secretion into the airways is associated with impaired mucociliary clearance and results in an increased susceptibility to endobronchial infections. By adolescence, the majority of CF patients have a *Pseudomonas aeruginosa* (*P. aeruginosa*) infection, which, if chronic, can accelerate the progression of obstructive lung disease. *P. aeruginosa* infection is one of the most significant limiting factors in the survival of patients with cystic fibrosis. Patients who acquire *P. aeruginosa* infection have a 2.6 times higher risk of death.

Tobramycin is an aminoglycoside antibiotic active against Gram-negative bacteria including *P. aeruginosa*. Tobramycin administered parenterally has been used since 1975 and its efficacy, safety and pharmacokinetics are now well established. However, to achieve effective concentrations at the site of infection in CF lung disease, high doses are needed when the drug is given parenterally, thereby increasing the risk of oto- and nephrotoxicity. To raise concentrations of tobramycin in the infected airways while avoiding toxicity associated with systemic administration, TOBI® (tobramycin inhalation solution, USP) was developed specifically for the treatment of *P. aeruginosa* infections in cystic fibrosis patients. A dose of 300 mg b.i.d. of TOBI is administered via the PARI LC PLUS nebulizer and the DeVilbiss Pulmo-Aide compressor or suitable alternative. This has become the standard treatment, with a 28 days on, 28 days off chronic treatment regimen.

CF patients inhale TOBI twice daily using a PARI LC PLUS nebulizer coupled to a suitable air compressor, a process which can take up to 20 minutes per dose. For CF patients (and their care-givers in the case of pediatric patients), the overall time required to perform all the required treatments including physiotherapy, exercise and a series of nebulized treatments including bronchodilators, antibiotics and hypertonic saline can be as long as 2 to 3 hours each day. This is a substantial treatment burden and is associated with poor compliance with therapy. Treatment guidelines for other respiratory therapy areas, such as asthma, state that inhalers should be portable and simple to operate (particularly for use in children), should not require an external power source, require minimal cooperation and coordination, and have minimal maintenance requirements.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Tobramycin inhalation powder hard capsules 28 mg (TIP) is a new formulation of tobramycin for inhalation (also referred to as TBM100 28 mg inhalation powder, hard capsules or TBM100C) and is designed for use in the same indication for the treatment of *P. aeruginosa* infections in cystic fibrosis patients, with the same twice daily, 28 day on, 28 off dosing cycle as TOBI. Each capsule contains the TIP formulation corresponding to a nominal dose of 28 mg of tobramycin. Nominal doses are used throughout this document. TIP is delivered with a T-326 Inhaler (DPI) and this drug-device combination represents a substantial improvement in design compared to the TOBI formulation and delivery system. This combination is postulated to reduce the burden of treatment in cystic fibrosis by reducing drug administration time and may increase patient convenience which, in turn, may increase compliance and therapeutic efficacy.

In the TIP development program, two clinical pharmacology studies [Study TPI001] and [Study INH-007] were conducted to compare the systemic exposure, sputum exposure and the lung deposition between TIP and TOBI formulations as the parameters of interest to aid in dose selection. A dose of 112 mg TIP (4 x 28 mg capsules b.i.d) was subsequently selected for Phase III efficacy and safety evaluations [Study C2301], [Study C2302], and [Study C2303], and is the dose intended for registration. During the Phase III program, improvements to the manufacturing process of TIP were made in anticipation of future commercialization of TIP. *In vitro* investigations of the aerosol performance of drug product from the improved process and the batches used in the Phase III studies [Study C2301] and [Study C2302] indicate that they are therapeutically equivalent. Following a request by the Agency, an additional Phase III study, [Study C2303], was performed, which assessed the efficacy, safety, and pharmacokinetics of TIP as manufactured by the improved process.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

PRECLINICAL EFFICACY—*IN VITRO*

Tobramycin has been studied extensively *in vitro* for many years. In this section, the pertinent nonclinical *in vitro* data for tobramycin extracted from the literature are summarized. No new nonclinical *in vitro* microbiology studies have been performed specifically in the context of TIP development due to the body of knowledge currently available for tobramycin, and the Agency's prior experience with tobramycin.

No nonclinical *in vivo* microbiology studies have been performed specifically in the context of TIP development. The Applicant believes that additional animal studies would be of limited relevance given the long history of use of inhaled tobramycin in the management of CF patients with *P. aeruginosa* and the fact that TIP treatment yields similar tobramycin exposure.

MECHANISM OF ACTION

Tobramycin is a highly polar molecule that passes across the outer membrane of Gram-negative bacteria, binds to bacterial ribosomes, and inhibits bacterial protein synthesis. The compound is a potent, irreversible inhibitor of prokaryotic protein synthesis at therapeutic concentrations. Disruption of bacterial protein synthesis leads to altered cell membrane permeability. Differences in antibiotic activity for different species of microorganisms are believed to be due, in part, to differences in cell permeability and not to differences in ribosomal binding. The antibiotic is transported across the inner (or cytoplasmic) membrane by a process that is dependent upon electron transport and is energy-dependent. This process can be blocked or inhibited by anaerobiosis, low pH, hyperosmolality, or divalent cations.

MECHANISMS OF RESISTANCE STUDIES

The most common basic mechanisms used by *P. aeruginosa* to resist the action of tobramycin (Figure 1) are decreased entry or enhanced export of the drug (impermeability resistance and efflux), and drug inactivation. Alteration of the drug target is another common resistance mechanism used by pathogens to overcome antibiotic activity, but this mechanism is rare in *P. aeruginosa*.

The gene sequence for a particular resistance factor is acquired from mobile elements (e.g. plasmids) or by mutations in the chromosomal DNA of *P. aeruginosa*. Chromosomal resistance is the more common route in CF-derived isolates of *P. aeruginosa*.

In isolates from CF patients, impermeability resistance is often the most common aminoglycoside resistance mechanism. Impermeability resistance is characterized by resistance to all aminoglycosides. Such agents usually promote their own uptake by interacting with lipopolysaccharides on the outer face of the cell membrane, which

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

destroys the permeability barrier of the outer membrane allowing the antibiotic to penetrate through the cell wall. However, in impermeability resistance, permeability of the drug is reduced across the cell wall. There have been reports of over-expression of the outer membrane protein, OprH, which protects the lipopolysaccharides from binding to aminoglycosides. However, this form of resistance is rare, and more recent investigations of aminoglycoside-resistant strains have shown that the resistance is more likely to be due to efflux.

Efflux

The characteristic of reduced accumulation of aminoglycosides within *P. aeruginosa* was originally attributed primarily to impermeability resistance, but later attributed to removal of the antibiotic by efflux pumps.

The efflux system involved with aminoglycoside resistance in *P. aeruginosa* is MexXY. It is composed of three protein units: an energy-dependent pump (MexY) located in the cytoplasmic membrane, an outer membrane protein (OM factor), and a linker protein (MexX) that joins the two other components. Aminoglycoside-resistant *P. aeruginosa* strains from CF patients have been reported to overexpress MexY. Indeed, in one study, MexY mRNA overproduction was found in 17/20 isolates collected from CF patients, and it was correlated with decreased susceptibility to aminoglycosides. Furthermore, alterations in the repressor gene of MexXY were found in six out of six aminoglycoside-resistant CF isolates plus one out of four non-CF isolates, indicating the role of the MexXY in the development of resistance to aminoglycosides.

Drug inactivation

Within the cytoplasm of organisms such as *P. aeruginosa*, there may be modifying enzymes which include aminoglycoside phosphoryltransferase (APH), aminoglycoside acetyl transferase (AAC) and aminoglycoside nucleotidyltransferase (ANT). These modifying enzymes use substituents from cytoplasmic cofactors (e.g. acetyl co-enzyme) to acetylate the amino groups or to phosphorylate or adenylate the hydroxyl groups of aminoglycosides (Figure 4-5).

Other Mechanisms

Genetic factors

Isolates from CF patients chronically infected with *P. aeruginosa* have a higher frequency of mutations (hypermutators) than isolates from other sources. Mutator strains are more frequently multidrug resistant than non-mutators. Studies of isolates from CF patients suggest that oxidative stress caused by chronic lung inflammation may be involved in the development of hypermutable *P. aeruginosa* and antibiotic resistance.

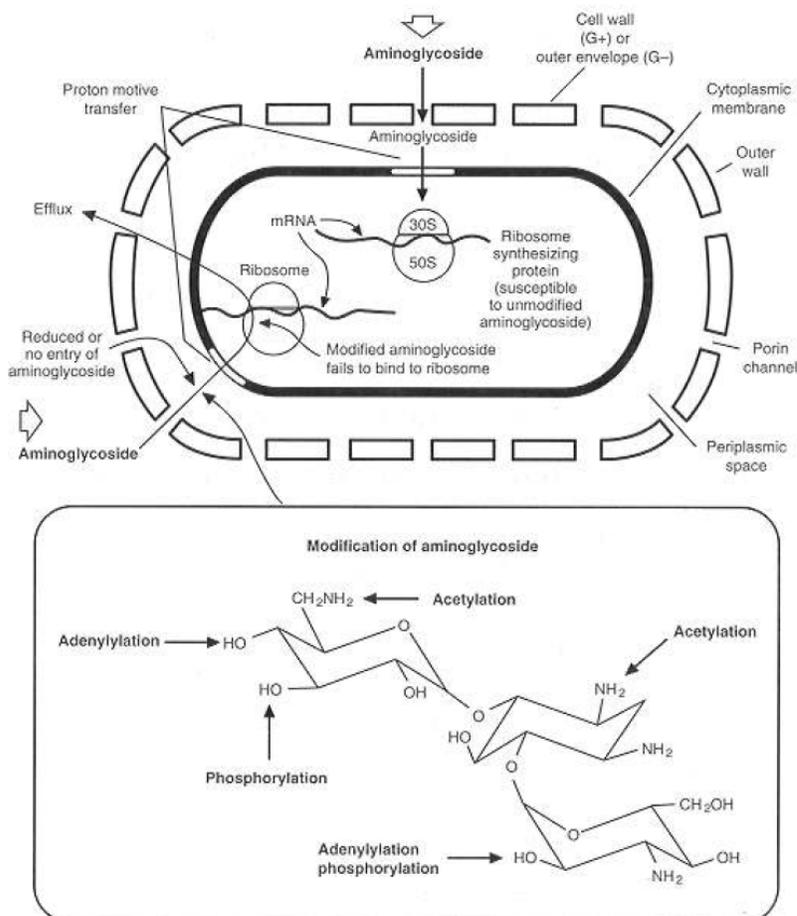
A study investigated potential contributors to low-level aminoglycoside resistance (two-fold or greater increase in tobramycin resistance) that causes a gradual development of resistance over time. A total of 135 novel genes involved in tobramycin resistance were identified. The majority of these genes were involved in energy metabolism.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Figure 1. *P. aeruginosa* resistance mechanisms to aminoglycosides such as tobramycin



Source: Figure 4-5, this submission.

Biofilms and resistance

In CF lung infections, *P. aeruginosa* grows in a biofilm – a microcolony encased in a protective alginate polysaccharide. Bacterial growth within these biofilms exhibits decreased susceptibility to antibiotics, including aminoglycosides. The mechanisms involved in this resistance are not fully elucidated, but they may be linked with anaerobic growth of organisms which lead to defects in aminoglycoside accumulation.

Adaptive resistance

This type of resistance is characterized by the ability to “train” *P. aeruginosa* to grow in the presence of high levels of aminoglycosides. In patients with CF, adaptive resistance of *P. aeruginosa* to tobramycin has been demonstrated 1 – 4 hours post-dosing and full susceptibility to tobramycin returned 24 – 48 hours after dosing.

It is thought that adaptive resistance occurs due to reduced levels of aminoglycoside accumulation, similar to impermeability resistance. It has been proposed that the

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

mechanism leading to adaptive resistance may be the slower growth of resistant colonies compared with susceptible colonies, such that susceptible organisms will overgrow resistant forms after the antibiotic course ends. This mechanism would support the current on-off cycling regimen.

Summary of resistance mechanisms

The main resistance mechanisms utilized by *P. aeruginosa* to overcome tobramycin activity are drug efflux and drug inactivation by modifying enzymes. The unique characteristics of chronic *P. aeruginosa* infections in CF patients, such as biofilm formation and high frequency of genetic mutations, may also be important factors for reduced susceptibility and the difficulties to eradicate *P. aeruginosa* in CF patients. A successful strategy to avoid high resistance rates in patients who need repeated antibiotic therapy courses may be “intermittent administration of tobramycin.” This concept, which has been widely recommended in CF patients worldwide, has successfully maintained the high efficacy of tobramycin to kill *P. aeruginosa* and other pathogens in the majority of CF patients. It is best explained by the fact that tobramycin-resistant *P. aeruginosa* clones grow more slowly than susceptible clones. Hence, once the antibiotic selective pressure is removed, susceptible clones overgrow resistant clones, making further tobramycin treatment courses clinically effective.

SPECTRUM OF ACTIVITY

Tobramycin is an aminoglycoside antibiotic produced by *Streptomyces tenebrarius* that acts primarily by disrupting protein synthesis leading to altered cell membrane permeability, progressive disruption of the cell envelope, and eventual cell death. Tobramycin has *in vitro* activity against a wide range of Gram-negative organisms, including *P. aeruginosa*. Since the proposed indication for TIP includes only *P. aeruginosa*, the discussion that follows will be limited to this organism.

The challenge facing both microbiologists and physicians is establishing a relationship between *in vitro* microbiological results and clinical outcome. The minimal inhibitory concentration (MIC) is defined as the lowest concentration of an antibiotic required to inhibit the growth of a bacterial isolate. Breakpoints are discriminatory antibiotic concentrations that are used to define the resistance or susceptibility of bacterial isolates. The breakpoint is, therefore, a function of the MIC for the pathogen and the achievable, non-toxic serum levels of an antibiotic. For *P. aeruginosa*, the systemic tobramycin resistance breakpoint is a MIC value ≥ 16 $\mu\text{g/mL}$.

It is important to note that the concept of a breakpoint is based on several assumptions: the site of infection is in the bloodstream (or a pharmacokinetically comparable compartment), the bacterial population is homogeneous, and there is a clear clinical endpoint. Interpretive breakpoints published by the CLSI only consider antibiotic levels relevant to systemic administration that can be reached based upon safety considerations (using maximally efficacious but non-toxic bloodstream concentrations) and do not take

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

into account the generally higher tobramycin concentrations achieved in the airways when administered by inhalation. Consequently, while tobramycin systemic interpretive breakpoints can be employed to categorize an isolate, this categorization is not applicable to treatment with inhaled (topical) tobramycin as clinical correlation has not been established.

Also, in the context of chronically-infected CF patients, the aim of therapeutic management is not to eradicate *P. aeruginosa* infection (as is generally the case for an infectious disease) but rather to control the bacterial infection and contain the progressive lung function decline that is characteristic of CF lung disease.

Susceptibility and resistance rates to tobramycin and other antibiotics for *P. aeruginosa* isolates from CF patients have been examined in a number of studies which are summarized below.

SURVEILLANCE STUDIES

[Pitt et al \(2003\)](#) conducted a study that assessed MIC values based on data collected in 2000 from 417 CF patients in 17 hospitals in the United Kingdom. The tobramycin breakpoints used were those recommended by the British Society of Antimicrobial Chemotherapy in 2001 (susceptible: ≤ 1 $\mu\text{g}/\text{mL}$; 2-4 $\mu\text{g}/\text{mL}$ intermediate; resistant: ≥ 8 $\mu\text{g}/\text{mL}$). MIC50 and MIC90 data were not available for this study. The results of the surveillance study are shown in as number (and percentage of isolates) present in each interpretive category ([Table 1](#)).

Table 1 Susceptibility rates of *P. aeruginosa* isolates to antimicrobial agents

Copyright Material



Source: Table 4-3, this submission ([Pitt et al 2003](#)).

Reviewer's comments: Ten percent of isolates were shown to be resistant to tobramycin. The number of isolates resistant to colistin was the lowest at 3.1%. However, Cross resistance was extensive with approximately 40% of CF isolates resistant to two or more antibiotics, usually ceftazidime and gentamicin or ceftazidime and piperacillin. The authors concluded "*The level of resistance to front line antipseudomonal agents, with the exception of colistin, is disturbingly high. The prudent use of antimicrobial drugs and closer monitoring of accumulation of resistant strain populations should be actively considered.*"

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

[Morosini et al \(2005\)](#) conducted a study that evaluated the susceptibility and resistance rates of 206 *P. aeruginosa* isolates from 56 CF patients attending one unit in Madrid, Spain collected during 2003 and 2004.

Table 2. Comparative *in vitro* activity (agar dilution technique) of antimicrobials tested against the 206 CF *P. aeruginosa* isolates

Copyright Material



Source: [Morosini et al 2005](#)

Reviewer's comments: *P. aeruginosa* isolates had tobramycin MIC range, MIC50, and MIC90 values of 0.25 to >1024, 2, and 16 µg/mL, respectively. Thirteen percent of isolates were considered resistant to tobramycin. It is noteworthy that the authors agree with the Spanish Antibiogram Committee (The MENSURA Group) who has tentatively defined specific breakpoints for inhaled tobramycin (susceptible, ≤ 64 µg/ml, and resistant, ≥ 128 µg/ml) when *P. aeruginosa* isolates from CF patients are tested. As in the previous study ([Pitt et al., 2003](#)), colistin had the lowest resistance rate (3%) of all antimicrobials tested.

[Chen et al \(2005\)](#) analyzed 200 mucoid and 200 non-mucoid multi-resistant *P. aeruginosa* isolates and 200 isolates of *Burkholderia cepacia* from CF patients in the United States that were referred to a reference laboratory between 2001 and 2003. For the mucoid isolates, tobramycin had MIC range, MIC50, and MIC90 values of 0.25→512, 8, and 64 µg/mL, respectively. Of the mucoid isolates, 35% were resistant to tobramycin. For the non-mucoid isolates, tobramycin had MIC range, MIC50, and MIC90 values of 0.5 to > 512, 16, and 256 µg/mL, respectively. Of the non-mucoid isolates, 52% were resistant to tobramycin. Isolates of *Burkholderia cepacia* had tobramycin MIC range, MIC50 and MIC90 values of 0.25→512, 256, and >512 µg/mL, respectively. Of these isolates, 96% were resistant to tobramycin. The prevalence of elevated MICs for tobramycin with mucoid and non-mucoid isolates as well as *B. cepacia* was comparably high and may be linked to the multi-resistant nature of the test organisms submitted to the reference laboratory, as also reflected in the high percentage resistance observed for all of the test agents.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

In a study by [Milne & Gould \(2010\)](#), 315 multi-resistant *P. aeruginosa* isolates collected between May 2001 and January 2008 from CF patients in Scotland were subjected to susceptibility testing. Multi-resistance was defined as resistance to agents in two of three classes of antibiotics (quinolones, aminoglycosides and beta-lactam agents [including monobactams and carbapenems]). Isolates were classified as susceptible, intermediate or resistant to tobramycin according to CLSI guidelines (susceptible, ≤ 4 $\mu\text{g}/\text{mL}$; intermediate, 8 $\mu\text{g}/\text{mL}$; and resistant, ≥ 16 $\mu\text{g}/\text{mL}$). The results of the study are shown in [Table 3](#).

Table 3. Susceptibility rates for *P. aeruginosa* isolates collected in Scottish CF patients

Number (%)
Copyright Material

Source: Table 1 ([Milne & Gould 2010](#)).

Tobramycin had MIC range, MIC50, and MIC90 values of 0.38–128, 3, and 8 $\mu\text{g}/\text{mL}$, respectively. A low rate of resistance to tobramycin (4%) was evident; the next most active agent (in terms of percent resistance) was colistin (7%).

Isolates collected from 2005 to 2009 from 96 CF patients in Bulgaria were analyzed ([Strateva et al, 2010](#)). Tobramycin activity against non-mucoid *P. aeruginosa* (MIC50=0.75 and MIC90=2 $\mu\text{g}/\text{ml}$) was higher than that against mucoid isolates (MIC50=1 and MIC90=4 $\mu\text{g}/\text{ml}$). The isolates obtained from patients untreated with inhaled tobramycin (TSI (MIC50=0.75 and MIC90 =1.5 $\mu\text{g}/\text{ml}$) were more susceptible to the drug than those from patients receiving maintenance therapy (MIC50=1.5 and MIC90 =6 $\mu\text{g}/\text{ml}$). The isolates from patients with long-term *P. aeruginosa* colonization (over 5 years) revealed the highest tobramycin MICs (MIC50=1.00 and MIC90 >1024 $\mu\text{g}/\text{ml}$).

Reviewer's comments: It is apparent from the study conducted by [Strateva et al, \(2010\)](#) that while initially, patients on inhaled tobramycin may exhibit *P. aeruginosa* isolates with tobramycin MICs in the susceptible category initially, but with long term usage, *P. aeruginosa* isolates demonstrate very high tobramycin MICs in the resistant category.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

[Valenza et al \(2010\)](#) reported on the MIC susceptibility results of 1844 isolates of *P. aeruginosa* that were obtained from the sputum of 22 chronically colonized cystic fibrosis patients over time starting in the 1990s up to the year 2009. The cumulative MIC values of tobramycin (MIC50) and (MIC90) of organisms were 2 and ≥ 256 $\mu\text{g/ml}$, respectively from patients after regular exposure to tobramycin. These investigators found that 27.5% of all isolates were resistant to tobramycin; none of the 1844 isolates were resistant to colistin. The MIC50 and MIC90 values of colistin were 0.38 and 0.75 $\mu\text{g/ml}$, respectively.

Reviewer's comments: [Valenza et al. \(2010\)](#) concluded that tobramycin resistance in *P. aeruginosa* isolates of chronically colonized CF patients under long-term antimicrobial therapy may be present, even when higher breakpoints suggested for inhaled tobramycin are considered. In contrast, colistin resistance in *P. aeruginosa* remains uncommon even after years of inhaled therapy.

[Table 4](#) is a summary table of MIC susceptibility data for tobramycin from the epidemiological studies surveyed by the Applicant (see below). Several of these studies investigated susceptibility data of tobramycin and other antimicrobial agents against *P. aeruginosa* isolates from patients with CF.

Reviewer's comments: As tobramycin has been an Agency approved product for many years, few epidemiological studies on *P. aeruginosa* from CF patients have been conducted, particularly in the last three years; thus, recent (within the last three years) susceptibility data is sparse. Only two of the fourteen studies listed in [Table 4](#) contain recent tobramycin susceptibility data for *P. aeruginosa* and both of these studies were conducted outside the US (Bulgaria and Germany). In eight of the fourteen studies, the MIC90 value was 16 $\mu\text{g/ml}$ (the resistance breakpoint) or greater with a range of 16 $\mu\text{g/ml}$ to > 1024 $\mu\text{g/ml}$.

Historically, the initial epidemiological study of tobramycin resistance in *P. aeruginosa* from CF patients was conducted by [Shawar et al \(1999\)](#); this study demonstrated an overall tobramycin resistance rate in *P. aeruginosa* of 5.4% derived from a tobramycin resistance rate of 2.4% among 710 mucoid isolates of *P. aeruginosa* and 9.4% tobramycin resistance rate among 530 non-mucoid isolates of *P. aeruginosa*. Of the fourteen studies, five studies demonstrated tobramycin resistance rates for *P. aeruginosa* of 10% or greater ranging from 10% to 52%; of note is that these studies were conducted in a variety of countries including the US, Spain, Germany, Japan and the UK suggesting that an increase in tobramycin resistance in *P. aeruginosa* from CF patients is worldwide, not just local (US).

*Although the data are limited, the tobramycin resistance rate in *P. aeruginosa* isolates from CF patients since 1999 has nearly doubled, an increase of more than 85%.*

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Table 4. A summary of *in vitro* tobramycin susceptibility data for *P. aeruginosa* isolates

Reference	Origin of isolates, study characteristics	type or source and # of isolates	Period of collection	MIC90 (mcg/ml)	%R
(Chen et al 2005)	Copyright Material				
(King et al 2010)					
(MacLeod et al 2009)					
(Morosini et al 2005)					
(Shawar et al 1999)					
(Milne and Gould 2010)					
(Strateva, Petrova and Mitov 2010)					
(Valenza et al 2010)					
(Traczewski and Brown 2006)					
(Tsuji et al 2005)					
(Fujimura et al 2009)					
(Rhomberg and Jones 2009)					
(Eagye et al 2009)					
(Pitt et al 2003)					

POSTANTIBIOTIC EFFECT (PAE)

Treatment with an aminoglycoside antibiotic such as tobramycin may also lead to suppression of bacterial growth that persists after short-term exposure to an antibiotic the

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

so-called postantibiotic effect (PAE). PAE can be measured *in vitro* or in animal models of infection. *In vitro*, the aminoglycosides consistently demonstrate a PAE that varies from 1 to 3 hours in broth and serum for *P. aeruginosa* and from 0.9 to 2.0 hours for the *Enterobacteriaceae*. As the aminoglycoside concentration increases, or the inoculum concentration decreases, the PAE becomes longer. The smaller the inoculum and the higher the oxygen tension, the longer the PAE will be extended. Reduced pH and low oxygen tension may result in the shorter PAE. For the aerobic or facultative Gram-negative rods tested, the combination of a β -lactam and an aminoglycoside resulted in the same PAE as that of the aminoglycoside alone. Rifampin was associated with synergistic enhancement of the PAE induced in *P. aeruginosa* by tobramycin. In contrast, β -lactam antibiotics, other than the carbapenems, have not demonstrated a PAE against aerobic or facultative Gram-negative bacilli.

The effective and safe use of parenteral formulations of aminoglycosides is, to a large extent, hampered and determined by adverse event rates (especially nephro- and ototoxicity) and systemic co-administration of other nephrotoxic substances like vancomycin may further increase this risk.

The described aminoglycoside-induced PAE is part of the rationale for the advocates of intermittent (once-daily) parenteral dosing, since efficacy is mainly dependent on sufficiently high drug exposure (C_{max}). The other part is the attenuated risk of toxicity as a subsequent PAE is thought to allow prolonged antibiotic activity even when systemic drug levels fall and stay as long as possible below critical (renal and otological) toxicity thresholds.

Both factors would apply for TIP and TOBI to an even greater extent, in a way that for b.i.d. inhalation C_{max} (and the associated PAE), is even higher than after systemic dosing, while on the other hand, systemic toxicity thresholds (e.g. 2 mg/L which is considered a clinically relevant trough level) are often not reached even at C_{max} . The duration of PAE for tobramycin against *P. aeruginosa* ATCC 27853 was approximately two hours in a recent study (MacLeod 2009) when tested at concentrations equivalent to 4-fold the MIC. This PAE for *P. aeruginosa* in combination with the extremely high initial topical exposure (C_{max}) is supportive of the 12h dosing schedule which was determined on the basis of the pharmacokinetic parameters established for TOBI and TIP (high C_{max} as PK/PD driver).

INTRACELLULAR EFFECTS

P. aeruginosa is not an intracellular pathogen, therefore, studies of intracellular concentrations were neither conducted nor reviewed.

BACTERICIDAL EFFECTS

Tobramycin is a bactericidal protein synthesis inhibitor in contrast to bacteriostatic protein synthesis inhibitors such as chloramphenicol. Recent work suggests that only bactericidal drugs stimulate hydroxyl radical formation in bacteria as a function of

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

metabolism-related depletion of reduced nicotinamide adenine dinucleotide (NADH), destabilization of iron-sulfur clusters, and stimulation of the Fenton reaction.

The bactericidal effects of an antibiotic can be measured using the minimal bactericidal concentration (MBC) assay, and the subsequent calculation of the MBC to MIC ratio. Tobramycin has demonstrated a low MBC/MIC ratio for *P. aeruginosa* of 1-4, suggesting that the agent generally works by killing bacteria rather than by simply inhibiting bacterial growth.

ANTIMICROBIAL INTERACTIONS

There is a general consensus in the scientific literature on the absence of negative interactions of other agents with tobramycin; however, no robust studies have been conducted in the setting of high drug exposure and CF sputum. The risk of decreased efficacy in critically ill patients is considered to be low.

An expert committee sponsored by The Cystic Fibrosis Foundation has published clinical guidelines regarding key issues in antibiotic therapy. It was recognized that systemic combination therapy in CF patients may offer clinical benefits and that combination therapy was being used by physicians. The guidelines addressed the utility of *in vitro* synergy testing to guide the choice of antibiotic therapy, but the experts concluded that the expense and time required for *in vitro* synergy testing did not justify its use in preference to conventional antibiotic susceptibility testing to guide therapeutic choices. The committee acknowledged the benefit of continuing long-term treatment during acute exacerbation and recommended further studies to resolve other therapeutic questions such as the optimal number of antibiotics to be used or the duration of therapy. No specific recommendations were made regarding the selection of additional antibiotics for combination therapy.

Potential for aminoglycoside synergy with β -lactam antibiotics is widely recognized in the medical community based on infection models (e.g. endocarditis) and systemic treatment. Combination treatment (aminoglycoside + β -lactam) in ventilator-associated pneumonia is recommended by the American Thoracic Society (if non-fermenters such as *P. aeruginosa* are suspected as pathogens).

No formal interactions studies for TIP have been performed, and TIP has not been developed for concomitant use with other inhaled antibiotics. However, concomitant use with systemic antibiotics appears to be safe and effective based upon the data from [Study C2302](#) (to be discussed later).

Other effects of antibacterial drug products

Due to the long human clinical experience with the Agency-approved product TOBI, studies of other potential *in vitro* drug effects were not conducted.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

PRECLINICAL EFFICACY – *IN VIVO*

No new animal therapeutic or pharmacologic studies were conducted during the TIP development program due to the targeted nature of the drug development program, the prior human experience with inhaled tobramycin, and the prior findings of the Agency which were used to approve TOBI.

ANIMAL THERAPEUTIC STUDIES

No new animal therapeutic or pharmacologic studies were conducted on behalf of the TIP development program in light of the long human clinical experience with TOBI.

A recently published article, “Colistin-tobramycin combinations are superior to monotherapy concerning the killing of biofilm *Pseudomonas aeruginosa*” included and *in vitro* and animal experience followed by a clinical pilot study (eight patients) that assessed the tolerability and the efficacy of inhaled colistin followed by inhaled tobramycin. The authors concluded that tobramycin as inhaled monotherapy was more effective than colistin in their animal model. Combined treatment with inhaled tobramycin and inhaled colistin had the strongest effect and was also well tolerated (in a clinical pilot study) and led to a considerable reduction in *P. aeruginosa* colony-forming units in sputum specimens.

PHARMACODYNAMICS

The antibacterial effects of aminoglycosides are known to be concentration dependent. Tobramycin kills bacteria, including *P. aeruginosa*, in a concentration-dependent manner, and the higher the peak concentration of the drug the greater the degree of bacterial killing. For the management of CF patients with *P. aeruginosa*, both the concentration and bioactivity of the tobramycin in the lung are key factors. It has been shown that sputum may counteract the bioactivity of antibiotics in several ways. Components of sputum are known to bind aminoglycosides, thereby potentially reducing their bioactivity. Sputum has a high ionic strength and contains macromolecules and high concentrations of divalent cations, all of which contribute to the *in vitro* inhibition of tobramycin activity. *In vitro* studies demonstrated that in the presence of sputum, a 10-fold excess of tobramycin was required to suppress the growth of *P. aeruginosa*. These findings contributed to the argument that in order to achieve optimal efficacy in sputum, aminoglycosides could be used at considerably higher doses than needed for similar antibacterial effects elsewhere in the body.

Because the *in-vitro* antibiotic activity of tobramycin is inhibited by sputum, the minimum appropriate target concentration has been as argued to be one that exceeds the MIC₉₀ of the clinical isolates by at least 10-fold. In early studies of tobramycin, a sputum concentration of tobramycin greater than 128 µg/g were proposed to be adequate

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

for therapy as it was found to be at least 10-fold higher than the MIC of 90% of patient isolates studied at that time.

TIP treatment achieves high concentrations of tobramycin in sputum with levels comparable to, or greater than, those observed for TOBI. The mean \pm SD (range) sputum C_{max} values after single dose of 112 mg TIP and 300 mg TOBI were 1048 \pm 1080 (85.7 to 3609) μ g/g and 737 \pm 1028 (20.4 to 4736.7) μ g/g, respectively. Comparison of the mean peak sputum level of tobramycin achieved by either TIP or TOBI administration to the MIC value for the isolates in each study demonstrated that a 10-fold multiple of the MIC was achieved for the majority of isolates.

The elevated concentrations of tobramycin achieved in sputum following treatment with TIP (at a lower total dose than TOBI) relative to MIC values for the majority of *P. aeruginosa* isolates in CF patients support the continued application of the agent for the management of CF patients with *P. aeruginosa*.

ANIMAL PHARMACOKINETICS

The pharmacokinetics of TIP in both serum and sputum are summarized below.

Adequate data are available in the literature regarding the absorption, distribution, metabolism and excretion (ADME) of tobramycin and therefore, ADME studies have not been conducted for TIP development. The exposure to tobramycin after aerosol administration of TIP was investigated in rats and dogs as an integral part of the toxicology studies.

Absorption was fast in both species with T_{max} generally occurring within one hour after completion of administration of the dose. Increases in systemic exposure, as measured by AUC and C_{max}, were generally less than dose-proportional. Tobramycin had a short apparent serum half-life in both species (0.7 to 4.4 hours in rats, 1.1 to 3.1 hours in dogs) and did not accumulate in serum with once daily administration.

Tobramycin had a long half life in lung tissues that was highly variable (57 hour to 19 days after 6 month inhalation in rats). Accumulation in rat lung tissues (up to 12-fold in C_{max} and

Assay methods

Tobramycin was analyzed in serum and lung homogenates of rats and dogs using reversed-phase high performance liquid chromatographic (HPLC) methods with ultraviolet detection that involved pre-column derivatization with 2,4-dinitrofluorobenzene. These methods were linear over the range of concentrations analyzed. The lower limits of quantification for tobramycin were 0.2 μ g/mL in rat and dog serum, and 5.0 μ g/g in rat and dog lung homogenates.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Absorption and bioavailability

The systemic exposure to tobramycin after inhalation of TIP is expected to result from pulmonary absorption of the dose fraction delivered to the lungs as tobramycin is not absorbed to any appreciable extent when administered via the oral route. The pharmacokinetics after aerosol administration of TIP were assessed as part of the toxicology studies in rats and dogs. The exposure to tobramycin after TIP inhalation was characterized in serum and lung tissues of these species.

Serum exposure***Rat***

In rats, serum toxicokinetic assessments were performed in the following TIP inhalation toxicity studies:

- the 28-day [Table 2.6.7.7A-Study-MN103741, not shown] – estimated inhaled doses of 9.9 mg/kg to 72.9 mg/kg/day
- the 6-month [Table 2.6.7.7B-Study-N103748, not shown] – estimated inhaled doses of 6.4 mg/kg to 38 mg/kg/day

The results from these studies are presented in [Pharmacokinetics Written Summary, Table 3-3, not shown]. Based on serum measurements, absorption was fast with T_{max} generally occurring within one hour after completion of dosing. In these studies, systemic exposure parameters (C_{max} and AUC) increased in a less than dose-proportional manner. Apparent serum half-life of tobramycin ranged from 0.7 to 4.4 hours in rats and consistent with the short half-life no accumulation was noted after multiple dosing. Gender differences were not observed.

Direct comparisons of the toxicokinetic data for TIP and TOBI are not possible because of the differences in doses used for the two formulations. The exposure data in rats after inhalation of TOBI is summarized in [Pharmacokinetics Written Summary, Section 3.1, not shown]. Based on the cross-study comparison of the TOBI and TIP studies in rats, the tobramycin serum exposure data appears to be consistent between the two formulations.

Dog

The PK of tobramycin after aerosol administration of TIP was evaluated following single doses of 5.4 to 51.1 mg/kg total inhaled tobramycin in a pilot dose-range finding toxicity study in beagle dogs [Table 2.6.5.1-Study-MN103742, not shown]. Single doses of TIP were administered as mouth-only or nose-only inhalation to the non-anesthetized dogs and the pharmacokinetic parameters are provided in [Pharmacokinetics Written Summary, Table 3-2, not shown].

Tobramycin was rapidly absorbed: all dogs achieved maximum serum concentrations in the time span of 5 to 30 minutes after the end of dosing. C_{max} and AUC appeared to be dependent on dose. Systemic exposure after nose-only administration was higher than after mouth-only administration, presumably due to increased absorption via the nasal

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

turbinate vasculature. Apparent elimination half-lives ranged from 1.21 and 1.63 hours, and were independent of dose.

In beagle dogs, toxicokinetic assessments were performed in the following TIP inhalation toxicity studies:

- multiple dose 7-day [Table 2.6.7.7C-Study-MN103743, not shown], estimated inhaled dose of 8.2 mg/kg/day to 23.8 mg/kg/day
- 28-day [Table 2.6.7.7D-Study-N103749, not shown], estimated inhaled dose of 12.0 mg/kg/day to 38.7 mg/kg/day.

Based on serum measurements [Pharmacokinetics Written Summary, Table 3-4, not shown], absorption was fast with T_{max} generally occurring within 1 hour of administration of dose. Increases in the dose of TIP led to less-than-proportional increases in systemic exposure. Serum half-life of tobramycin ranged from 1.1 to 3.1 hours and once-daily inhalation administration of TIP did not result in serum accumulation of tobramycin. Differences in pharmacokinetics based on sex were not observed.

Lung exposure***Rat***

In rats dosed with TIP for 28 days [Table 2.6.7.7A-Study-MN103741, not shown], maximum concentrations of tobramycin in lung tissue were observed between 5 to 30 minutes after dosing on day 1, and between 5 minutes and 6 hours after dosing on day 28 [Pharmacokinetics Written Summary-Table 3-5, not shown]. The increases in C_{max} and AUC were less than dose-proportional. Lung C_{max} values on day 28 was 3 to 7 times higher than those on day 1, indicating accumulation of tobramycin in lung tissue; that is related to the long apparent elimination half-life of tobramycin in lungs (34 to 78 hours).

Similar results were observed in the 6-month toxicity study in rats [Table 2.6.7.7B-Study-N103748, not shown]. Multiple-dose administration of TIP formulation in the study resulted in lung accumulation of tobramycin as indicated by the increase of tobramycin C_{max} (up to 12-fold) and AUC_{0-24h} (up to 29-fold) on Day 176 compared to Day 1. [Pharmacokinetics Written Summary, Table 3-5]. Less than dose-proportional increases in lung exposure were observed and there were no significant differences in exposure based on sex. Based on the half-life estimates on Day 176 (56.9 to 124 hours), steady-state in the lungs would have been achieved by no later than one month. If the lung concentrations at Day 211 (recovery phase) were taken into account, the lung elimination half-life would be approximately 19 days, and steady state in lungs would have been reached at 3 to 4 months after start of dosing.

The lung tissue concentration findings in rats dosed with TIP are consistent with those seen in rats dosed with TOBI. The concentrations of tobramycin in lung tissue of rats had been assessed with TOBI in a 2-year carcinogenicity study [Table 2.6.7.10-TOBI NDA

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

[Carcinogenicity Report 2001](#), not shown]. Average lung tissue concentrations collected at week 52 ranged from 271 to 757 µg/g for the 2.9 mg/kg to 25.7 mg/kg dose groups, and were in the range of the lung concentrations observed in TIP studies [[Pharmacokinetics Written Summary, Table 3-6](#), not shown]. Therefore, distribution into lung tissues appears to be related to the tissue binding affinity of tobramycin and is independent of the formulation used (i.e. TIP or TOBI).

Dog

In dogs receiving a low dose (8.2 mg/kg/day) of inhaled TIP for 7 days [[Table 2.6.7.7C Study- MN103743](#), not shown], tobramycin concentrations in lungs were below the limit of quantitation upon necropsy on day 8 [[Pharmacokinetics Written Summary, Table 3-7](#), not shown]. Dogs that received the high dose (23.8 mg/kg/day) of TIP had tobramycin lung concentrations that ranged from 10.4 to 19.3 µg/g on day 8. On day 22, after a 2-week washout period, only one female in the high-dose group had a quantifiable concentration of tobramycin (23.0 µg/g) in the lung.

In the 28-day inhalation toxicity study in dogs [[Table 2.6.7.7D-Study-N103749](#), not shown], individual lung concentrations ranged from 19.4 to 129.6 µg/g at necropsy on Day 29. On day 57 (i.e. four weeks after the end of administration), tobramycin concentrations were approximately one-half of those observed at day 29, suggesting a lung elimination half-life of about 4 weeks [[Pharmacokinetics Written Summary, Table 3-7](#), not shown]. As in the case of serum, increases in dose of TIP led to less-than proportional increases in the lung exposure of tobramycin, with no observed differences between the sexes.

Distribution (including protein binding)

The distribution of tobramycin has been described in the literature. Following subcutaneous injection (100 mg) of ¹⁴C-labelled tobramycin in rats, all tissues examined contained detectable levels of drug with the highest concentration in the kidneys and the lowest concentration in the brain.

The systemic distribution of aerosolized tobramycin following administration of TIP was not evaluated. However, it is expected that once absorbed, apart from the higher persistence in lung tissues, the distribution would be the same as after parenteral administration where tobramycin is distributed widely through extracellular fluid.

Tobramycin does not bind to serum proteins *in vitro*. Therefore, tobramycin free drug concentrations are not expected to be affected by any changes in serum protein concentrations.

Metabolism

Tobramycin is not metabolized and is primarily excreted unchanged in the urine). Therefore *in vitro* and *in vivo* metabolism studies have not been conducted for TIP.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Excretion

Tobramycin is eliminated from the systemic circulation principally via the kidney by glomerular filtration with some tubular reabsorption. There is no evidence for renal secretion, as probenecid does not alter renal clearance. Serum concentrations of tobramycin are higher and remain more prolonged in individuals with impaired renal function compared with healthy subjects. After intravenous administration, tobramycin is cleared from serum with a half-life of approximately two hours; this half-life is dependent upon renal function and increases with decreasing creatinine clearance.

Systemically absorbed tobramycin following TIP administration is also expected to be eliminated principally by glomerular filtration. In CF patients, the apparent elimination half-life of tobramycin from serum was approximately three hours after inhalation of a single 112 mg dose of TIP, similar to the half-life observed after inhalation of a single 300 mg dose of TOBI.

Tobramycin concentrations in expectorated sputum declined with a half-life of approximately 2 hours, after the 112 mg TIP dose and the 300 mg TOBI dose [[Study TPI001](#)].

A prolonged terminal elimination phase of tobramycin (that begins about 24 h post last dose) with a mean half-life of 146 h has been reported after multiple intravenous dosing of tobramycin. This terminal phase was highly variable in patients, and may be a consequence of tobramycin disposition variability. It was postulated that during this phase, intracellularly bound drug is slowly released and excreted. It is therefore possible that after repeated inhalation of tobramycin a similar prolonged elimination phase occurs, which could explain the low pre-dose tobramycin concentrations observed in some patients (at the start of a new treatment cycle following a 4-week treatment pause) in the phase III study.

Tobramycin can be removed from the body by hemodialysis. Approximately 50% of an administered intravenous dose has been shown to be cleared within 6 hours of hemodialysis.

HUMAN PHARMACOKINETICS

Pharmacokinetic studies

Full details of the clinical pharmacology studies can be found in the [[Summary of Clinical Pharmacology Studies](#)]. Two early studies were performed to evaluate the pharmacokinetic profile of TIP compared with TOBI ([Table 3-1](#)), one study was conducted in healthy subjects using a TIP prototype formulation ([\[Study INH-007\]](#)) and the second study was conducted in CF patients ([\[Study TPI001\]](#)) with TIP. Study INH-007 also assessed the whole lung deposition of tobramycin using scintigraphy after inhalation of TIP and TOBI. A third clinical study ([\[Study TSB-001\]](#)) was not a pharmacokinetic study as no drug product was inhaled.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

This study assessed the ability of CF patients to perform adequate inspiratory maneuvers using simulated DPIs of differing resistance. The results of [Study TSB-001](#) support the appropriateness of the T-326 Inhaler for the intended use in the intended CF patient population. To ensure that patients at the lower end of the typical CF range of inspired volume inhale the full dose, all patients receiving TIP are instructed to inhale twice from each capsule. No specific studies were conducted evaluating the effects of intrinsic or extrinsic factors on the PK of tobramycin.

Table 5. Summary of key pharmacokinetic studies

Study no.	Study objectives, population	No. of subjects randomized	Treatment duration	Medication dose/day	Sampling interval
Healthy volunteers					
INH-007	Single dose lung scintigraphy and pharmacokinetics, males and females	14	5 single dose treatment periods	25 mg TIP 150 mg TIP 300 mg TOBI	pre-dose and up to 48 h
Patients					
TPI001	Pharmacokinetics in males and females	90	Single dose	28 mg, 56 mg, 84 mg, and 112 mg TIP 300 mg TOBI	pre-dose and up to 12 h post dose

Source: [\[Synopses of Individual Studies\]](#), [\[Tabular Listing of all Clinical Studies\]](#)

Sparse PK samples (serum and sputum) were collected in the Phase III trials ([\[Study C2301\]](#), [\[Study C2302\]](#), [\[Study C2303\]](#)). PK results from these studies are included in the following sections. Additionally, the serum concentration data were analyzed as part of a population PK (PopPK) analysis for TIP to determine the influence of various covariates such as age, weight, gender, BMI, creatinine clearance and lung function on the PK of tobramycin after TIP administration.

Absorption, Distribution, Metabolism, Excretion

Absorption and distribution

As tobramycin is not absorbed to any appreciable extent when administered orally, the systemic exposure to tobramycin after inhalation of TIP is expected to result from pulmonary absorption of the dose fraction delivered to the lungs.

Regional lung distribution of a prototype TIP formulation and of the approved TOBI formulation in healthy volunteers was investigated by gamma scintigraphy using ^{99m}Tc labeled formulations of both TIP (nominal dose: 13.3 mg tobramycin) and TOBI (nominal dose: 300 mg tobramycin) ([\[Study INH-007\]](#)). The distribution data showed that 34% of the TIP dose and 5% of the TOBI dose were deposited in the lungs. Lung deposition for TOBI in this study was about half that predicted from published data. It must be noted that inhalation of TOBI was stopped after 15 minutes instead of continuing until the nebulizer began to sputter. The relative distribution of radiolabel within the central, intermediate, and peripheral airways was similar for TIP and TOBI, with a trend

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

for greater deposition in the peripheral (P) vs. central (C) airways, as seen in a P/C ratio of 1.6 ± 0.4 (mean \pm SD) for TIP and 1.5 ± 0.4 for TOBI.

Study TPI001 was performed in CF patients aged between 7 and 50 years. Following a single administration of increasing doses of TIP (28 mg to 112 mg), slightly less than dose proportional increases in systemic exposures (area under curve [AUC] and C_{max}) were observed. For a 4-fold increase in dose, mean C_{max} and area under the curve extrapolated to infinity (AUC_{inf}) of tobramycin increased by approximately 3-fold. Systemic exposure to tobramycin after the 112 mg (4 x 28 mg capsules) TIP dose was comparable to that seen with TOBI 300 mg (**Table 6**).

Table 6. Pharmacokinetic parameters of tobramycin in serum after single dose administration of TIP and TOBI to CF patients in Study TPI001

Parameter	TIP 112 mg	TOBI 300 mg
C _{max} (µg/mL)	1.02 \pm 0.53	1.04 \pm 0.58
T _{max} (h)	1 (0.5 – 2)	1 (0.5 – 2)
AUC _{inf} (µg·h/mL)	5.1 \pm 2.0	5.3 \pm 2.6
T _{1/2} (h)	3.1 \pm 0.4	3.0 \pm 0.8

All parameters except T_{max} are shown as mean \pm SD. T_{max} is shown as median (range)

T_{max} = time of maximum concentration

Source: Table 3-2, Clinical Overview.

Serum tobramycin concentrations after single and multiple b.i.d. inhalation of 112 mg of TIP (**[Study TPI001]**, **[Study C2301]**, **[Study C2302]**, **[Study C2303]**) were low relative to the maximum systemic levels recommended for avoidance of the toxicity associated with intravenous tobramycin therapy (peak greater than 12 µg/mL). The highest average concentration seen in Phase III studies after twice-daily inhalation of 112 mg TIP for 4 weeks was 1.99 ± 0.59 µg/mL (mean \pm SD) and refers to serum samples taken 60 minutes after inhalation (**Study C2301**); the highest individual serum concentration observed in the phase III studies was 4.94 µg/mL (**Study C2301**), which was more than two-fold lower than the toxicity threshold of 12 µg/mL. In **Study C2302** where TIP and TOBI were compared, the highest individual serum concentrations for TIP and TOBI arm were 2.27 µg/mL and 2.08 µg/mL, respectively, which were both more than five-fold lower than the toxicity threshold of 12 µg/mL. Trough concentrations also compared favorably with the recommended maximum trough level (2 µg/mL); the highest mean trough concentrations after b.i.d. TIP administration were 0.38 ± 0.44 µg/mL, 0.31 ± 0.19 µg/mL, and 0.41 ± 0.51 µg/mL for **studies C2301**, **C2302** and **C2303**, respectively, and 0.24 ± 0.11 for the TOBI arm in **Study C2302**. Minimal accumulation of tobramycin in serum, consistent with the short half-life, was observed based on trough concentration levels after multiple b.i.d. administration in the Phase III studies.

Tobramycin sputum concentrations appeared to be generally higher for the 112 mg TIP dose compared with 300 mg TOBI (**[Study TPI001]**, **[Study C2302]**, **[Study C2303]**). Maximum tobramycin sputum concentrations were observed 30 minutes after either TIP or TOBI **[Study TPI001]**. Inter-patient variability in sputum concentrations was greater

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

than in serum. The mean \pm SD (range) sputum C_{max} values after a single dose of 112 mg TIP or 300 mg TOBI were 1048 ± 1080 (85.7 to 3609) $\mu\text{g/g}$ and 737 ± 1028 (20.4 to 4736.7) $\mu\text{g/g}$, respectively. The mean \pm SD (range) sputum AUC_{inf} values after a single dose of 112 mg TIP or 300 mg TOBI were 1740 ± 809 (844 to 3354) $\mu\text{g/g}$ and 1302 ± 1127 (343 to 4787) $\mu\text{g/g}$, respectively [Study TPI001]. Comparisons between TIP and TOBI based on sputum concentrations and the use of sputum levels as a marker of overall lung deposition may be confounded by the high variability in the sputum concentrations. However, taking the variability into account, the range of sputum levels, along with the comparable serum exposure (Table 3-2), demonstrate that 112 mg TIP resulted in lung exposure that was similar to 300 mg TOBI.

Binding of tobramycin to serum proteins is negligible. Therefore, tobramycin free drug concentrations are not expected to be affected by any changes in serum protein concentrations

Metabolism

Tobramycin is not metabolized and is primarily excreted unchanged in the urine. Therefore, metabolism assessments have not been performed with TIP.

Excretion

Tobramycin is eliminated from the systemic circulation principally via the kidney by glomerular filtration with some tubular re-absorption). In CF patients, the apparent elimination half-life ($T_{1/2}$) of tobramycin from serum was approximately 3 h after inhalation of a single 112 mg dose of TIP, similar to the half-life observed after inhalation of a single 300 mg dose of TOBI (Table 3-2). Tobramycin concentrations in expectorated sputum declined with a half-life of approximately 2 h after both a single 112 mg dose of TIP and a single 300 mg dose of TOBI [Study TPI001].

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

SUSCEPTIBILITY TEST METHODS AND DETECTION OF RESISTANT ORGANISMS

Methods for susceptibility testing of tobramycin have been established by CLSI ([CLSI M7- A8, 2009](#); [CLSI M2-A10, 2009](#)) as have interpretive criteria for test results ([CLSI M100- S21, 2011](#)). The interpretive criteria for MIC and disk diffusion tests applicable to parenteral tobramycin are as follows:

Test Result	Interpretation		
	Susceptible	Intermediate	Resistant
MIC (µg/mL)	≤ 4	8	≥ 16
Zone diameter (mm) for 10 µg tobramycin disk	≥ 15	13-14	≤12

Tobramycin-resistant organisms are readily detected using these standard methods and interpretive criteria. However, it must be noted that for inhaled antibiotics, clinical correlates for MIC and zone diameter values have not been established. Therefore, therapy is not selected on the basis of the susceptibility test result relegating it to a position of limited value.

Limitations of susceptibility testing in CF patients

Susceptibility testing is performed on tens of thousands of *P. aeruginosa* per year, yet some investigators question the value of these results from CF patients with chronic infection ([Govan 2006](#)). Studies in CF have shown a poor correlation between antibiotic susceptibility of *P. aeruginosa* isolates and clinical and microbiological efficacy ([Smith et al 2003](#)). Some patients with resistant isolates of *P. aeruginosa* may improve on treatment while, conversely, patients with susceptible isolates may not appear to respond to the same antibiotics. Establishing breakpoints for inhaled therapy in treating airway infection is problematic. A single-compartment pharmacokinetic assumption no longer applies, as the site of infection is a complex compartment, the inflamed lung and associated biofilms ([Govan 2006](#)). In addition, the bacterial population is not homogeneous. In chronically infected patients with CF, more than one strain or clone of *P. aeruginosa* with diverse phenotypes are usually found, and these different strains may have different susceptibilities. Furthermore, there is no clear microbiological endpoint to define successful therapy for pseudomonal lung infections in patients with CF. Hence, clinical interpretation of *in vitro* bacteriological results needs careful evaluation, depending on the route of administration.

One of the problems in testing these isolates is the slow growth of some *P. aeruginosa*, which can lead to false susceptibility in automated testing systems ([Gilligan 2006](#)). To overcome this problem, disk diffusion or E-test methodologies for susceptibility testing have been recommended. To culture, isolate and test a sufficient number of pathogens with these methods is labor intensive, time consuming and the results take at least 48

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

hours, and often several days before becoming available to the treating physician, only 52% of microbiology testing laboratories serving CF centers in the USA were found to use these methods (Zhou 2006).

Another problem with the relevance of susceptibility information of *P. aeruginosa* is that tests are conducted on organisms growing planktonically in aerobic conditions. However, in the CF airway, *P. aeruginosa* grows as part of a biofilm or within mucus plugs that create an anaerobic or microaerophilic environment. Organisms susceptible under aerobic conditions may not be under anaerobic conditions, especially to aminoglycosides which require active transport to enter the cell; active transport uses energy derived from aerobic conditions (Hill et al 2005). Hence, it has been proposed that organisms that are susceptible *in vitro* may not respond *in vivo* (Gilligan 2006). Biofilm-based susceptibility testing may be a more appropriate method, but it is currently not widely available (Gilligan 2006; Hill et al 2005).

These factors do not explain why patients may respond to treatment with antibiotics even though *P. aeruginosa* was found to be resistant *in vitro* (Smith 2003). Various theories have been proposed including the sub-MIC effects of antibiotics and the effect of antibiotics on other species present in CF sputum that may modify the pathogenicity of *P. aeruginosa* (Duan 2003).

Currently, susceptibility test interpretation is based on serum-achievable levels; however, aerosolized agents generally reach levels many times higher than serum levels (Gilligan 2006). Indeed, isolates of *P. aeruginosa* from CF patients have been shown to be resistant using breakpoints based on serum levels of tobramycin, but susceptible using the Spanish proposed breakpoints for aerosolized tobramycin (Morosini et al 2005).

Reviewer's comments: It should also be noted that due to the formation of a biofilm in the infected lungs of CF patients, standard MIC measurements may underestimate the concentration of antibiotic necessary to eradicate the targeted pathogen. This is due to the protective nature of the biofilm to prevent the ability of an antibiotic to access the targeted pathogen. A more accurate and efficient measure of antibiotic MICs from biofilm bacteria is the minimal biofilm eradication concentration (MBEC), which may be more useful to determine true MIC values and perhaps correlate microbial with clinical outcomes. This methodology is currently available and is under consideration by CLSI.

QUALITY CONTROL PARAMETERS

Quality control parameters for MIC and disk diffusion tests with tobramycin have been established by CLSI (CLSI M100-S21, 2011). During the TIP development program, no new studies were conducted to attempt to re-define these quality control criteria. For the testing of isolates derived from the TIP clinical trials, the central laboratory (b) (4) employed CLSI reference methods and quality control guidelines.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

PROVISIONAL INTERPRETIVE CRITERIA

Parenteral tobramycin has been Agency-approved for multiple indications and interpretive breakpoints currently exist ([CLSI M100-S21, 2011](#)). While these interpretive criteria do not apply to inhaled tobramycin, they may be useful for describing the characteristics of bacterial isolates.

Inhaled tobramycin for the management of CF patients with *P. aeruginosa* is currently Agency approved in the form of TOBI. As it is generally understood that interpretive criteria for inhaled antibacterial products are not defined, the Sponsor did not attempt to define interpretive criteria for TIP.

MICROBIOLOGICAL METHODS

Sputum samples were processed centrally by (b) (4), as described in this section. Microbiological assay data were sent electronically to (b) (4). As different colony types of *P. aeruginosa* may have differing susceptibility to antibiotics, the *P. aeruginosa* isolates were sorted as follows: colony type-1 = mucoid colony variant, colony type-2 = dry colony variant, and colony type-3, small colony variant.

For subjects unable to produce a sputum specimen, the study protocol allowed collection of a deep-throat cough swab or an induced sputum, at the investigator's discretion.

Sputum specimens were taken for microbiological analysis on multiple days throughout each of the studies, as shown in [Table 7](#).

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Table 7. Collection Schedule for Sputum Specimens for Microbiological Analysis

Study	Activity	Day of Sputum Specimen (for Microbiology) Collection for Each Study												
		1	1.5	2 Cycle 1	3	4 ^a Interim contact	5	6 ^a Interim contact	7 Cycle 2	8	9 Cycle 3	10	11	
C2301	Visit													
	Treatment	Screen		Start	On	On	Stop	Off	Start	Stop	Start	Stop	FU/ Term	
	Week	-2		1	2	3	5	7	9	13	17	21	25	
	Day ^b	-14 ± 2	-12 to -6	1 ₁	8 ₁ ± 2	15 ₁ ± 2	28 ₁ ± 2	43 ₁ ± 2	1 ₂	28 ₂ ± 2	1 ₃	28 ₃ ± 2	56 ₃ ± 2	
	Sputum specimen ^c	X ^e		X			X		X	X	X	X	X	
C2302	Visit													
	Treatment	Screen		Start	On	On	Stop	Off	Start	Stop	Start	Stop	FU/ Term	
	Week	-4 ^d		1	2	3	5	7	9	13	17	21	25	
	Day ^b	-30 to -12	-12 to -6	1 ₁	8 ₁ ± 2	15 ₁ ± 2	28 ₁ ± 2	43 ₁ ± 2	1 ₂	28 ₂ ± 2	1 ₃	28 ₃ ± 2	56 ₃ ± 2	
	Sputum specimen ^c	X ^e		X			X		X	X	X	X	X	
C2303	Visit													
	Treatment	Screen		Start		TC14	3	4						
	Week	-2/-1		1		3	5	9						
	Day	-14 to -7		1		14 ± 2	29 ± 2	57 ± 2						
C2301	Visit													
	Sputum specimen	X		X			X	X						

a Visits 4 and 6 (weeks 3 and 7) were performed by the investigator or research coordinator via a telephone call or E-mail exchange with the subject (or parent/legal guardian, if applicable).

b Subscripts refer to the cycle.

c Collect pretreatment sputum, preferably first morning specimen; if subject was unable to produce a sputum specimen, collect a deep-throat cough swab or an induced sputum, at the investigator's discretion.

d 4.28 weeks (30 days) is rounded to 4 weeks for simplicity.

e A second sputum sample was allowed within 2 weeks from date of the original sputum sample. If the sample was obtained within this timeframe, the patient did not have to repeat other screening tests (i.e. blood draws, spirometry). If the second sample was obtained after 2 weeks from date of the original sputum sample, then a full rescreening needed to be performed. If the second sample results were negative for the presence of *P. aeruginosa* or the patient failed to provide second sample within the allotted period, then the patient was considered a screen failure.

Source: Table 4-6, this submission.

Sputum sample collection and shipping

Procedures for sputum sample collection and shipment are described in detail in (b) (4) specific to each study. A summary of the methods generally applicable to all three studies is provided below:

Expectorated sputum sample

- Prior to the actual sample collection, a requisition form was filled in, identifying the patient and providing further details such as visit number, date, time, site, etc. The form was completed after the collection with information on the sample time and provided to the central laboratory together with the sample shipment.
- The procurement of the sample was conducted under direct supervision of a nurse or physician.
- The patient rinsed his/her mouth with water and/or gargled in order to remove superficial flora.
- The patient was instructed to cough deeply in order to produce a lower

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

respiratory tract specimen (not a postnasal fluid).

- Upon the cough maneuver, the expectorated sputum sample was collected into a collection system consisting of a graduated 50 mL screw cap tube and a funnel with a hinged lid.

Once the sample was inside the tube, the cap was screwed on tightly. The tube was labeled with a sample type identifier bearing the patient identification number, initials and date of collection. The transport tube and cap were wrapped securely with parafilm, and the wrapped tube was placed into a pocket of a segmented bubble bag provided for shipment of all samples to the central laboratory. Until shipment, specimens were stored refrigerated at 2-8°C.

Induced sputum

- Prior to the actual sample collection, a requisition form was filled in, identifying the patient and providing further details such as visit number, date, time, site, etc. The form was completed after the collection with information on the sample time and provided to the central laboratory together with the sample shipment.
- The procurement of the sample was conducted under direct supervision of a nurse or physician.
- The patient brushed his/her gums and tongue and afterwards rinsed his/her mouth with water to remove superficial flora.
- The patient inhaled approximately 25 mL of normal saline with the aid of the nebulizer.
- Upon a cough maneuver, the induced sputum sample was collected into a collection system consisting of a graduated 50 mL screw cap tube and a funnel with a hinged lid.

Once the sample was inside the tube, the cap was screwed on tightly. The tube was labeled with a sample type identifier bearing the patient identification number, initials and date of collection. The transport tube and cap were wrapped securely with parafilm, and the wrapped tube was placed into a pocket of a segmented bubble bag provided for shipment of all samples to the central laboratory. Until shipment, specimens were stored refrigerated at 2-8°C.

Oropharyngeal swab sample

- Prior to the actual sample collection, a requisition form was filled in, identifying the patient and providing further details such as visit number, date, time, site, etc. The form was completed after the collection with information on the sample time and provided to the central laboratory together with the sample shipment.
- The physician or nurse depressed the tongue with a tongue blade. The throat was vigorously swabbed with a sterile swab. During this maneuver, particular care was given not to touch the tongue or cheek with the swab.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

- The swab was placed into a transport media sleeve. The extruding portion of the swab was broken off and a cap screwed on to secure the transport media sleeve.
- The transport media sleeve was labeled with a sample type identifier bearing the patient identification number, initials and date of collection, and afterwards placed in a transport plastic bag. The sample was shipped to the central laboratory together with other samples from this visit. Until shipment, specimens were stored refrigerated at 2-8°C.

Shipments

- All samples were stored refrigerated at 2-8°C to maintain sample integrity.
- Gel packs were provided together with the shipping container. These gel pads were frozen for at least 12 hours at -20°C prior to the shipment. Shipping containers were designed and labeled according to IATA regulations for infectious specimens.
- All samples are re-checked for accurate labeling, and all bags and/or bubble bags were placed in a Styrofoam box, together with the gel pads. The Styrofoam box was placed into the shipping container, the requisition form enclosed, sealed, and shipped by courier to the central laboratory.

Specimen receipt at central laboratory

- Sputum samples were received in a conical tube together with the requisition form. The source was noted (expectorated sputum, bloody sputum, salivary sputum, induced sputum, throat swab) as detailed on the requisition form. In case no details were given, expectorated sputum was assumed.
- The volume of the sample was measured to the nearest mL and recorded.
- Samples received containing less than 1.0 mL were diluted, but not plated at the 10-1 dilution due to insufficient quantities of sputum.
- All specimens were to be cultured within 48 hours of collection.

Sputum sample processing

Raw sputum samples

Raw sputum samples were inoculated onto a MacConkey agar plate using a sterile swab before the digestion procedure was performed to ensure that even small quantities of *P. aeruginosa* were recovered.

Sputum digestion with sputolysin reagent

- Contents of one vial Sputolysin reagent were diluted to 100 mL with sterile distilled water and gently swirled to dissolve any crystals. Solution was either used immediately or was stored for up to 48 hours at 2-8°C under an inert gas.
- The sputum sample was overlain with an equal volume of the Sputolysin solution in a screw cap conical tube.
- The sputum sample was suspended in the Sputolysin suspension by vortexing for 30 seconds.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

- The suspension was incubated at room temperature for 15 minutes and afterwards centrifuged for 5 minutes at 1500 rpm. The supernatant was removed and discarded, leaving a residual volume of no less than 1.5 mL.

Sputum serial dilution procedure

From the digested sputum suspension, six serial dilutions were prepared. The sputum sample digested with Sputolysin (see above) was used without further dilution, and from that solution, five further 1:10 serial dilutions were prepared by mixing 150 µL samples from one dilution with 1.35 mL sterile saline.

Treatment of throat swabs

Throat swabs were neither digested nor were serial dilutions prepared from them. They were plated out (see below) and growth was reported semi-quantitatively. Estimates of growth were reported as light, moderate, or heavy.

Microbiological culture

Plating of diluted sputum/swabs

- A) 1:1 to 1:1,000 dilutions and swabs were plated according to the roster shown below:

Roster for plating out 1:1 to 1:1,000 dilutions			
33-35°C 5% CO ₂	33-35°C ambient air	30°C ambient air	33-35°C anaerobic
Blood agar	MacConkey agar	Sabouraud Dextrose agar with gentamicin and chloramphenicol	Anaerobic blood agar
Chocolate agar	OFPBL agar or <i>B. cepacia</i> selective agar		
Columbia CNA agar	DNase agar		
Haemophilus isolation agar	Mannitol salt agar		

- B) 1:10,000 and 1:100,000 dilutions: 33-35°C, ambient air: MacConkey agar (no others)
- For inoculation, plates were labeled and 100 µL from the respective dilutions were dispensed onto the plates and spread with a sterile spreader.
 - The inoculated plates were stored inverted in an incubator for 72 hours and examined daily for pathogen growth. The Sabouraud Dextrose agar with chloramphenicol and gentamicin were held for 96 hours.

Quantitative culture of P. aeruginosa from sputum culture and MIC assay

All sputum samples were examined for quantitative growth of *P. aeruginosa*:

- *P. aeruginosa* was differentiated into colony types (mucoïd, dry, small colony variants)
- *P. aeruginosa* concentration in sample was reported in CFU/mL.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

The approved CLSI susceptibility test methods and quality control parameters used for parenteral tobramycin therapy were used to monitor the activity of TIP against *P. aeruginosa* isolates from CF patients.

- Susceptibility testing was performed on *P. aeruginosa* using a broth microdilution method employing Sensititre custom panels. The panel included the following antibiotics: aztreonam, ceftazidime, ciprofloxacin, meropenem, and tobramycin. Briefly, a 10µL volume of a 0.5 McFarland standard suspension of the organism was added to a 10mL tube of Sensititre cation-adjusted Mueller Hinton Broth with TES buffer. A 50 µL volume was added to each well in the plate using either the Sensititre autoinoculator or a manual pipette. Plates were incubated for 18-24 hours at 34-36°C in a non-CO₂ incubator. Weekly quality control was performed with the following quality control strains

- *Staphylococcus aureus* ATCC 29213
- *Enterococcus faecalis* ATCC 29212
- *Escherichia coli* ATCC 25922, and
- *Pseudomonas aeruginosa* ATCC 27853.

- Imipenem E-tests were also performed on isolates of *P. aeruginosa*. A McFarland suspension of *P. aeruginosa* (0.5 Mc Farland suspension for dry and small colony phenotypes; 1.0 for mucoid phenotypes) was prepared. A swab was dipped into the inoculum suspension, excess fluid was removed from the swab by pressing against the inside wall of the tube, and the entire surface of a Mueller Hinton agar plate was swabbed while rotating the plate to obtain even distribution of inoculum. The plate was allowed to stand for 10-15 minutes (until dry), then an E-test strip was placed onto the agar surface ensuring complete contact between the strip and the agar surface. The plate was incubated in ambient air at 35°C for 16-20 hours before the MIC value was read. CLSI guidelines were used for interpretations of results.

Semi-quantitative culture for P. aeruginosa in swab samples

- Growth was reported as light, moderate or heavy, based on growth in quadrants (quadrant plating technique): growth in first quadrant corresponds to “light”, growth in first and second quadrant to “moderate”, growth in first through third or in all quadrants to “heavy”.
- *P. aeruginosa* phenotypes were distinguished (mucoid, dry, small colony variants).
- Susceptibility testing was performed as described above.

Semi-quantitative culture for organisms other than P. aeruginosa

- All cultures were also examined for the presence of the following organisms per Standard Operating Procedures: (b) (4)
- *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, β-hemolytic *Streptococcus*, *Alcaligenes xylosoxidans*, *Stenotrophomonas maltophilia*, other Gram-negative rods (GNR), *B. cepacia*, *Penicillium* sp., *Aspergillus* sp. *S. aureus* was tested using cefoxitin and oxacillin screen and reported as MSSA or MRSA.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

- Semi-quantitative analysis was based on the highest dilution that showed growth in sputum samples: 1:1 corresponded to “light”, 1:10 corresponded to “moderate”, while 1:100 and higher corresponded to “heavy” growth.
- Growth from swab cultures was reported as light, moderate or heavy, based on growth in quadrants (quadrant plating technique): growth in first quadrant corresponded to “light”, growth in first and second quadrant to “moderate”, growth in first through third or in all quadrants to “heavy”.
- MIC assays were not performed with organisms other than *P. aeruginosa* with the exception of *B. cepacia* on isolates detected during screening. MICs were performed as described above.
- Data were compiled for analysis within the context of the clinical studies. Investigators were only informed about detection/presence of *B. cepacia*.

Sample retention

- All isolates were saved in Trypticase Soy Broth with 15% glycerol and stored at -80°C for further reference.
- Isolates of *B. cepacia* additionally were saved on an Amies swab with charcoal and sent for genotyping.
- Isolation and genotyping of *B. cepacia* lead to an immediate report to the site.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

CLINICAL EFFICACY

PREVIOUS CLINICAL EXPERIENCE

Summary of microbiology results from historical TOBI phase 3 trials and extension studies (PC-TNDS-002, -003, -004, and-007)

Summary of TOBI studies

The TOBI studies PC-TNDS-002 and PC-TNDS-003 were randomized, double-blind, placebo-controlled studies conducted in 1995-1996 as part of the clinical development program in support of the use of TOBI in CF [Integrated report, Protocols PC-TNDS-002, -003, -004, and- 007, 1999]. The primary objectives of the studies were to determine the effects of TOBI on pulmonary function and *P. aeruginosa* colony forming units. In addition, the main secondary objectives of the studies were to evaluate the safety profile of TOBI, assess the effects of TOBI on changes in microbial susceptibility to tobramycin, measure the rates of hospitalization and also assess the use of systemic anti-pseudomonal antibiotics.

The inclusion criteria were the same for both studies. Patients had to be aged 6 years or older and have baseline percent predicted FEV1 of between $\geq 25\%$ and $\leq 75\%$. In Study PC-TNDS- 002, 223 patients were included in the intent-to-treat (ITT) population and 186 provided evaluable efficacy data. In Study PC-TNDS-003, 297 patients were included in the ITT population and 254 provided evaluable efficacy data. In both studies patients were randomized to treatment with either TOBI 300 mg or matching placebo (2.25 mg/mL saline solution with 1.25 mg quinine) b.i.d for three treatment cycles (28 days on-treatment followed by 28 days off-treatment). Treatments were administered by the PARI LC PLUS jet nebulizer with the Pulmo-Aide compressor.

Upon completion of studies PC-TNDS-002 and PC-TNDS-003, patients had the option to continue in the follow-up open-label studies PC-TNDS-004, -004X, and subsequently -007 for an additional three cycles of TOBI in each. Hence, the [Integrated report, Protocols PCTNDS- 002, -003, -004, and-007, 1999] provides supportive information on safety, efficacy and microbiology for up to 12 cycles of exposure to inhaled tobramycin (nine cycles for patient randomized to placebo in studies PC-TNDS-002 and PC-TNDS-003). Microbiological assessments included evaluation of change in tobramycin MIC values against *P. aeruginosa* and incidence of isolation of Gram negative pathogenic organisms other than *P.aeruginosa*. In addition, in studies PC-TNDS-002 and PC-TNDS-003 changes in bacterial load have been measured.

Baseline characteristics of the patients participating in the phase 3 TOBI studies were notably close to those in study TIP C2302 (conducted about a decade later), with the majority of patients being adults with mean FEV1% predicted close to 50%, mean *P.*

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

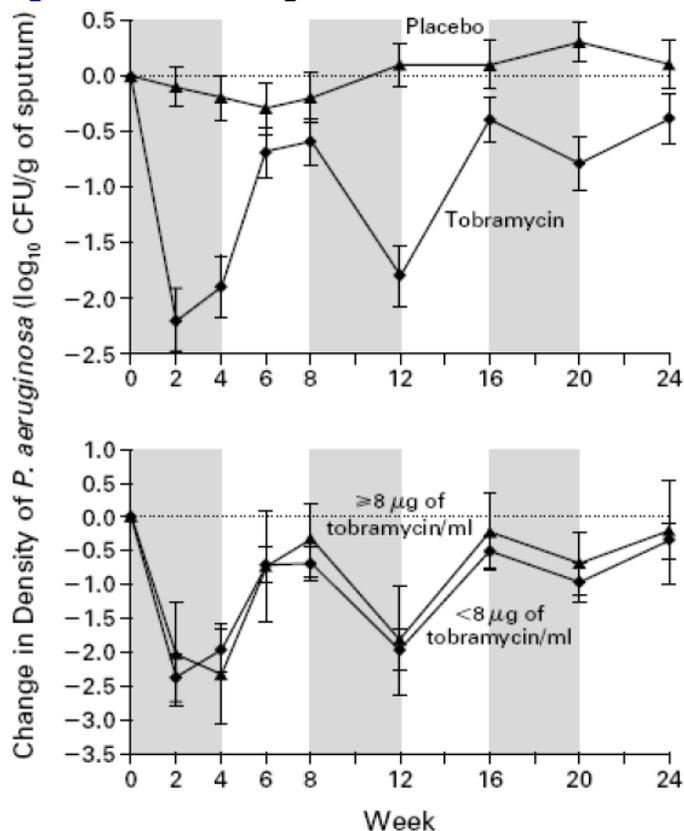
Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

aeruginosa sputum density being above 7 log₁₀ and 22% of patients had tobramycin MIC isolates at baseline ≥ 8 $\mu\text{g}/\text{mL}$ (Ramsey et al 1999).

P. aeruginosa concentration in TOBI studies PC-TNDS-002 and PC-TNDS-003

The concentration of *P. aeruginosa* in expectorated sputum samples from the tobramycin-treated patients decreased during each of the three 28-day on-treatment periods when TOBI was administered and returned towards baseline values during the off-periods as shown in Figure 4-1.

Figure 4-1. Mean change in the concentration of *P. aeruginosa* in sputum samples



The I bars in both panels represent 95 percent confidence intervals.

The upper panel shows the mean change from week 0 at each visit for 249 patients in TOBI group and 241 patients in placebo group.

The lower panel shows the mean change from week 0 at each visit for a subgroup of patients in TOBI group with MIC < 8 $\mu\text{g}/\text{mL}$ at week 0. The diamonds represent 173 patients with MIC < 8 $\mu\text{g}/\text{mL}$ at week 20 and the triangles represent 29 patients with MIC ≥ 8 $\mu\text{g}/\text{mL}$ at week 20.

Source: Figure 4-1, this submission (Ramsey et al 1999).

The *upper panel* shows the mean change from week 0 in the density of *P. aeruginosa* in sputum at each visit for the 249 patients in the tobramycin group and the 241 in the placebo group (data pooled for the two studies). The treatment effects were greatest during the first two treatment cycles, with an average reduction of 2.2 log₁₀ CFU per gram of sputum at week 2, 1.9 log₁₀ CFU per gram at week 4, and 1.8 log₁₀ CFU per gram at week 12. By the end of the third on treatment period of drug administration

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

(week 20), there was an average reduction of 0.8 log₁₀ CFU per gram of sputum, as compared with the value at week 0, whereas the density in the placebo group had increased by 0.3 log₁₀ CFU per gram (P<0.001).

The lower panel in [Figure 4-1](#) shows the mean change from week 0 at each visit for the subgroup of patients in the tobramycin group whose most prevalent isolates had tobramycin MIC < 8 µg/mL at week 0. The diamonds represent the 173 patients whose isolates continued to have a tobramycin MIC < 8 µg/mL at week 20 and the triangles represent the 29 patients whose isolates had tobramycin MIC of 8 µg/mL or more at week 20. Overall the curves and their confidence intervals are overlapping in the lower panel; hence difference in MIC as per established systemic breakpoint of 8 µg/mL does not seem to be associated with diminished microbial reduction in the third cycle.

Tobramycin susceptibility

Distribution of tobramycin MIC values is presented in [Table 4-1](#) below.

Table 4-1. Cumulative frequency distribution of tobramycin MIC values at Visits 3, 10 and 11 (all *P. aeruginosa* isolates –parallel group studies)

MIC (µg/mL)	TOBI			Placebo		
	Visit 3 N=629 n (%)	Visit 10 N=562 n (%)	Visit 11 N=580 n (%)	Visit 3 N=611 n (%)	Visit 10 N=577 n (%)	Visit 11 N=573 n (%)
≤0.25	95 (15.1)	58 (10.3)	51 (8.8)	111 (18.2)	116 (20.1)	128 (22.3)
0.5	128 (35.5)	99 (27.9)	133 (31.7)	153 (43.2)	122 (41.2)	140 (46.8)
1	157 (60.4)	145 (53.7)	138 (55.5)	140 (66.1)	131 (64.0)	130 (69.5)
2	119 (79.3)	83 (68.5)	97 (72.2)	95 (81.7)	86 (78.9)	80 (83.4)
4	58 (88.6)	56 (78.5)	55 (81.7)	48 (89.5)	54 (88.2)	43 (90.9)
8	37 (94.4)	40 (85.6)	37 (88.1)	32 (94.8)	22 (92.0)	26 (95.5)
16	19 (97.5)	34 (91.6)	30 (93.3)	13 (96.9)	25 (96.4)	17 (98.4)
32	6 (98.4)	17 (94.7)	12 (95.3)	12 (98.9)	8 (97.7)	6 (99.5)
64	6 (99.4)	12 (96.8)	8 (96.7)	3 (99.3)	5 (98.6)	3 (100.0)
128	3 (99.8)	7 (98.0)	9 (98.3)	2 (99.7)	4 (99.3)	0 (100.0)
256	0 (99.8)	2 (98.4)	2 (98.6)	0 (99.7)	2 (99.7)	0 (100.0)
512	1 (100.0)	4 (99.1)	2 (99.0)	0 (99.7)	1 (99.8)	0 (100.0)
>512	0 (100.0)	5 (100.0)	6 (100.0)	2 (100.0)	1 (100.0)	0 (100.0)

Note: Percentages are cumulative.

The tobramycin MIC₅₀ is noted by bolding and italics.

The tobramycin MIC₉₀ is noted by bolding.

Source: Table 4-1, [[TOBI NDA Microbiology 1997](#)].

Overall the susceptibility of the majority of *P.aeruginosa* isolates remained unchanged with three cycles of TOBI treatment [[TOBI NDA Microbiology 1997](#)].

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

MIC distribution during follow-up studies **PC-TNDS-004** and **007** and relationship to clinical response to TOBI

Following initiation of TOBI treatment, a shift towards increasing MIC values was observed over time, the data being summarized after each successive three cycles (see [Table 8](#) below). Over 12-cycles of treatment, the tobramycin MIC₅₀ for *P.aeruginosa* increased from 1 to 2 µg/mL and the MIC₉₀ increased from 8 to 32 µg/mL [[Integrated report, Protocols PC-TNDS- 002, -003, -004, and-007, 1999](#)].

Table 8. Frequency distribution of tobramycin MICs: All *P. aeruginosa* colony types

	Initiation	End of 3 rd Cycle	End of 6 th Cycle	End of 9 th Cycle	End of 12 th Cycle
Number of patients	447	384	294	242	106
Number of isolates	1115	958	761	641	291
	n (%)	n (%)	n (%)	n (%)	n (%)
≤0.25	196 (17.6)	105 (11.0)	80 (10.5)	70 (10.9)	27 (9.3)
0.5	247 (39.7)	213 (33.2)	137 (28.5)	104 (27.1)	34 (21.0)
1	275 (64.4)	236 (57.8)	157 (49.1)	146 (49.9)	62 (42.3)
2	192 (81.6)	153 (73.8)	152 (69.1)	95 (64.7)	44 (57.4)
4	90 (89.7)	98 (84.0)	91 (81.1)	82 (77.5)	39 (70.8)
8	60 (95.1)	60 (90.3)	66 (89.8)	41 (83.9)	30 (81.1)
16	33 (98.0)	39 (94.4)	37 (94.6)	36 (89.5)	17 (86.9)
32	10 (98.9)	18 (96.2)	15 (96.6)	27 (93.8)	13 (91.4)
64	8 (99.6)	12 (97.5)	7 (97.5)	14(95.9)	7 (93.8)
128	3 (99.9)	12 (98.7)	8 (98.6)	10 (97.5)	4 (95.2)
256	0 (99.9)	2 (99.0)	0 (98.6)	3 (98.0)	1 (95.5)
512	1 (100.0)	2 (99.2)	7 (99.5)	5 (98.8)	3 (96.6)
>512		8 (100.0)	4 (100.0)	8 (100.0)	10 (100.0)

Note: Percentages are cumulative.

The tobramycin MIC₅₀ is noted by bolding and *italics*.

The tobramycin MIC₉₀ is noted by bolding and underline.

Source: Table 4-2, this submission [[Integrated report, Protocols PC-TNDS-002, -003, -004, and-007, 1999 Table 8.1](#)].

The relationship between tobramycin MIC for *P.aeruginosa* and clinical response to TOBI has been further explored. Two measures of clinical response, the change in FEV₁% predicted and the number of days of i.v.-anti-pseudomonal antibiotic therapy were used to test for a relationship of tobramycin MIC and response to treatment.

At the end of the third on-drug period, patients in the higher MIC categories showed smaller relative changes in FEV₁% predicted than patients included in the ≤ 8 µg/mL category. However a similar percentage of patients with MIC 16-64 µg/mL had improved FEV₁% (58.1%) when compared with the group ≤ 8 µg/mL (61.9%). Mean relative change in FEV₁% predicted after 6-9-12th on-treatment periods appeared not to be dependent on tobramycin MIC. Mean improvement in FEV₁% predicted was in the same range [[Integrated report, Protocols PC-TNDS-002, -003, -004, and-007, 1999](#)]. The publication by [Smith et al \(2003\)](#) explored the association between tobramycin MIC and response to parenteral antibiotic use and shows an absence of a significant trend between MIC and response to treatment.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

[Shawar et al \(1999\)](#) published MIC data for tobramycin and six other antibiotics based on more than 1200 isolates collected between August 1995 and May 1996 from CF patients enrolled in the TOBI phase 3 clinical trials in the United States. The isolates were tested according to National Committee for Clinical Laboratory Standards (NCCLS) 1995 guidelines. Tobramycin had MIC range, MIC₅₀, and MIC₉₀ values of ≤ 0.25 to > 512 , 1, and 8 $\mu\text{g/mL}$, respectively.

Reviewer's comments: In previous clinical trials, treatment with TOBI was shown to have an impact on the tobramycin *in vitro* susceptibility of *P. aeruginosa*, with MIC increasing two dilution steps with the prolonged exposure to TOBI. However, this decrease in susceptibility did not appear to negatively impact the clinical response (improvement in FEV₁% predicted and use of intravenous. anti-pseudomonal antibiotic).

CURRENT CLINICAL TRIALS

The primary clinical evidence used to support the efficacy and safety of TOBI Podhaler in patients with cystic fibrosis aged ≥ 6 years consists of three Phase III studies:

- [Study C2301](#): Double blind study of TIP vs placebo (for one cycle, followed by two cycles of open-label TIP treatment) in CF patients aged 6-21 years with no prior exposure to inhaled anti-psuedomonal antibiotics for at least 4 months
- [Study C2302](#): Open-label study of TIP vs TOBI across three cycles of TIP treatment in CF patients aged ≥ 6 years with no prior exposure to inhaled anti-pseudomonals for one month
- [Study C2303](#): Double blind study of TIP vs placebo for one cycle in CF patients aged 6-21 years with no prior exposure to inhaled anti-pseudomonals for at least 4 months

These studies were conducted in a total of 710 randomized cystic fibrosis patients aged ≥ 6 years. A total of 425 patients received at least one dose of TIP in these studies. What follows are the Clinical Microbiology data and analyses for each individual clinical trial.

CLINICAL STUDY [CTBM100C2301](#)

Data sets analyzed

The number of patients in each analysis population is shown in [Table 4-7](#).

- Randomized population (n=102) included all randomized patients.
- All Randomized Safety population (n=95) included all randomized patients who received at least one capsule of study drug.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

- All ITT and All Safety population (n=69) included patients from Sensitivity Interim Analysis (SIA) population and additional patients from North America and Europe since SIA. The additional Latin American patients since SIA were not included in the All ITT and All Safety population since they did not go through quality review of pulmonary function test by independent external expert review panel.
- SIA Safety and SIA ITT population (n=61) included all patients in the SIA. It included all patients from North America and Europe whose data were available at the interim analysis database lock. In addition, it included 8 patients from Latin America who met the quality review criteria by external review panel.

In all of these populations, the number of patients was generally similar between treatment groups.

Table 9. Analysis populations by treatment group for Study C2301: Number of subjects

	Randomized	All Randomized Safety	All Safety	All ITT	SIA Safety	SIA ITT
TIP	4	46	32	32	29	2
placebo	5	49	37	37	32	32

Source: Table 4-7, Clinical Pharmacology Summary, this submission.

Study Design

[Study C2301](#) was a randomized, three-cycle, two arm trial. Each cycle comprised 28 days on treatment followed by 28 days off treatment. The first cycle was double-blind, placebo-controlled with eligible patients randomized to TIP (4 x 28 mg b.i.d.) or placebo at a 1:1 ratio. Upon completion of the first cycle, all patients received TIP for cycles 2 and 3. The exclusion criteria for [Study C2301](#) did not allow patients that had:

- any use of inhaled antipseudomonal antibiotics within 4 months prior to screening; and
- use of systemic antipseudomonal antibiotics within 28 days prior to study drug administration.

Additional study design details are found in the clinical study report for [\[Study C2301\]](#).

The *primary objective was to demonstrate the efficacy* of a 28 day b.i.d. dosing regimen of TIP versus placebo, as measured by the relative change in FEV1 percent predicted from baseline (Week 1/Cycle 1, Day 1) to the end of Cycle 1 dosing (Week 5/Cycle 1, Day 28).

Secondary microbiology analyses included the following:

- Pathogen identification
- The absolute change in *P. aeruginosa* density in sputum was determined. The analysis was based on logarithmic scale as log₁₀ colony-forming units [CFU] per gram of sputum. Absolute change from baseline to each post-baseline time point in Cycles 1-3 was summarized descriptively by treatment group.
- Semi-quantitative changes in CFU/g sputum for baseline organisms other than *P. aeruginosa*.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

4. The change in tobramycin MIC from baseline to each post-baseline time point in Cycles 1-3 was summarized descriptively by mean changes for each treatment group.
5. The change in frequency by categorical susceptibility description (tobramycin MIC > 8 µg/mL and ≤ 8 µg/mL) was also determined.
6. Isolation of treatment-emergent organisms (as part of pooled analysis with [Study C2303](#)).

CORRELATION OF CLINICAL AND MICROBIOLOGICAL OUTCOME

Efficacy assessments in [Study C2301](#) included measurement of FEV1, measurement of *P. aeruginosa* CFU per gram of sputum, and measurement of *P. aeruginosa* susceptibility as assessed by MIC values. Time to first anti-pseudomonal antibiotic use, first hospitalization due to respiratory events and incidence and length of hospitalization due to respiratory related SAEs were also assessed. In the TIP treatment arm, TIP was administered all three cycles (TIP/TIP/TIP); in the placebo treatment arm, placebo was administered in Cycle 1 followed by TIP for Cycle 2 and 3 (placebo/TIP/TIP).

TIP 112 mg twice daily (4 X 28 mg) bid significantly improved the lung function (FEV1) compared to placebo. Therefore, this pivotal efficacy study successfully met its primary endpoint and achieved its objective. The improvement obtained in the first cycle was maintained over time with additional cycles of TIP treatment.

In this section, the microbiological analyses conducted during this study are summarized.

***P. AERUGINOSA* DENSITY IN SPUTUM**

An important analysis in the Phase III studies, given that this is the rationale for use of an antibiotic, was to demonstrate that TIP effectively suppressed *P. aeruginosa* in the lung as reflected by CFUs cultured from the sputum of CF patients. *P. aeruginosa* density in sputum was measured at Day 1 and Day 28 for each of the three cycles. Results were summarized using logarithmic scale as log₁₀ CFU per gram of sputum. Absolute changes from baseline to each post-baseline time point in each of the three cycles were summarized descriptively by treatment group and cell type (mucoid, dry, small colony).

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Table 10. Change in *P. aeruginosa* density colony forming units (log₁₀ CFUs) from baseline - Study C2301 ITT population by treatment group-- dry colony type

	scheduled week/day		TIP/TIP/TIP N=46				PLB/TIP/TIP N=49			
			N	mean	SD	median	N	mean	SD	median
Baseline		Raw value	27	6.84	1.29	7.00	32	7.03	1.51	7.30
Cycle 1	1	Raw value	27	6.84	1.29	7.00	32	7.03	1.51	7.30
		28	Raw value	15	4.81	2.61	4.64	25	7.13	1.28
		Change	13	-1.99	2.82	-1.99	21	-0.25	1.08	-0.36
Cycle 2	1	Raw value	17	6.42	1.76	6.60	22	7.12	1.28	7.33
		Change	15	-0.4	1.68	-0.70	22	-0.26	1.53	-0.37
	28	Raw value	11	4.62	2.23	4.87	10	5.12	1.97	4.78
		Change	9	-1.83	2.85	-2.38	8	-2.01	2.19	-2.15
Cycle 3	1	Raw value	17	6.44	1.94	7.00	15	5.75	1.65	6.00
		Change	15	-0.54	1.95	-0.41	14	-1.44	2.4	-1.69
	28	Raw value	13	5.52	2.6	5.30	6	4.79	2.99	4.69
		Change	11	-2.67	2.5	-3.12	6	-2.73	2.82	-2.70

Baseline was defined as the latest recorded *P. aeruginosa* density measurement prior to the first dosing

Change = absolute change (post *P. aeruginosa* density – Baseline *P. aeruginosa* density).

The greater value was chosen when subject had more than one measurement at the same visit date.

The later non-missing value was chosen when subject had more than one measurement at the same visit number with different collecting date.

Source: Table 14.2-3.1a.1, this submission.

Reviewer's comments: Among dry colony types, treatment in the test (TIP) arm did not reduce the number of *Pseudomonas* isolates any more so than in the treatment in the comparator (placebo) arm. The log₁₀ reduction from the end of cycle 3 compared to baseline in dry variant *P. aeruginosa* CFUs was similar between the TIP and the placebo arms (-2.67 and -2.73 log₁₀ CFUs, respectively). A similar reduction was seen at the end of cycle 2 (-1.83 and -2.01 log₁₀ CFUs, respectively). At the end of the first cycle of treatment, the TIP arm showed a *greater* reduction in CFUs than the placebo arm (-1.99 and -0.25 log₁₀ CFUs, respectively).

Table 11. Change in *P. aeruginosa* density colony forming units (log₁₀ CFUs) from baseline - Study C2301 ITT population by treatment group--mucoïd colony type

	scheduled week/day		TIP/TIP/TIP N=46				PLB/TIP/TIP N=49			
			N	mean	SD	median	N	mean	SD	median
Baseline		Raw value	30	6.98	1.53	7.45	36	7.14	1.4	7.54
Cycle 1	1	Raw value	30	6.98	1.53	7.45	36	7.14	1.4	7.54
		28	Raw value	23	4.37	2.27	3.66	36	6.98	1.47
		Change	22	-2.83	2.66	-3.30	33	-0.43	1.35	-0.25
Cycle 2	1	Raw value	29	6.66	1.83	7.20	32	7.03	1.56	7.19
		Change	28	-0.55	1.84	-0.22	31	-0.27	1.78	0.35
	28	Raw value	23	4.82	1.97	4.54	22	4.46	2.01	3.76
		Change	23	-2.31	2.11	-2.00	21	-2.7	2.45	-3.02
Cycle 3	1	Raw value	27	6.7	1.55	7.20	27	6.47	1.48	7.00
		Change	26	-0.38	1.69	-0.43	26	-0.69	1.77	-0.69
	28	Raw value	23	5.22	2.29	4.62	23	4.46	1.7	4.00
		Change	22	-1.77	2.63	-1.80	22	-2.65	2.19	-3.11

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Baseline was defined as the latest recorded *P. aeruginosa* density measurement prior to the first dosing.
Change = absolute change (post *P. aeruginosa* density – Baseline *P. aeruginosa* density).
The greater value was chosen when subject had more than one measurement at the same visit date.
The later non-missing value was chosen when subject had more than one measurement at the same visit number with different collecting date.
Source: Table 14.2-3.1a.1, this submission.

Reviewer's comments: Among mucoid colony types, treatment in the test (TIP) arm did not reduce the number of *Pseudomonas* isolates any more so than treatment in the comparator (placebo) arm. The log₁₀ reduction from the end of cycle 3 compared to baseline in mucoid colony type *P. aeruginosa* CFUs was *less* in the TIP compared to the placebo arm (-1.77 and -2.65 log₁₀ CFUs, respectively). A comparable reduction was seen at the end of cycle 2 (-2.31 and -2.7 log₁₀ CFUs, respectively). At the end of the first cycle of treatment, the TIP arm showed a *greater* reduction in CFUs than the placebo arm (-2.83 and -0.43 log₁₀ CFUs, respectively).

The mean change in viable count for all colony types combined (mucoid, dry, small colony variant) for the ITT population is shown in [Table 12](#).

Table 12. Change in *P. aeruginosa* density colony forming units (log₁₀ CFUs) from baseline - Study C2301 ITT population—all colony types

	scheduled week/day		TIP/TIP/TIP				PLB/TIP/TIP			
			N	mean	SD	median	N	mean	SD	median
Baseline		Raw value	44	7.04	(1.70)	7.60	48	7.17	(1.68)	7.59
Cycle 1	1/1	Raw value	37	7.15	(1.49)	7.59	40	7.55	(1.23)	7.77
	5/28	Raw value	28	4.60	(2.33)	4.28	37	7.30	(1.46)	7.53
Cycle 2	9/1	Change	28	-2.79	(2.59)	-3.04	36	-0.21	(1.29)	-0.10
		Raw value	31	6.56	(2.07)	7.16	32	7.29	(1.53)	7.36
	13/28	Change	31	-0.76	(1.96)	-0.49	32	-0.38	(1.79)	-0.22
		Raw value	26	4.75	(2.00)	4.56	23	4.49	(2.12)	3.75
Cycle 3	17/1	Change	26	-2.44	(2.25)	-2.22	23	-3.19	(2.46)	-3.85
		Raw value	30	6.85	(1.74)	7.63	28	6.67	(1.41)	6.93
	21/28	Change	30	-0.34	(1.76)	-0.19	28	-0.76	(1.80)	-0.66
		Raw value	24	5.47	(2.32)	5.01	24	4.42	(1.88)	4.00
Follow-up	25/56	Change	24	-1.99	(2.67)	-1.87	24	-3.14	(2.22)	-3.26
		Raw value	25	7.18	(1.38)	7.49	25	6.88	(1.60)	7.20
Termination visit		Change	25	-0.29	(1.00)	-0.18	25	-0.91	(1.82)	-0.54
		Raw value	40	6.20	(2.04)	6.68	43	6.31	(2.02)	7.00
		Change	40	-1.08	(1.91)	-0.54	42	-1.10	(2.45)	-0.57

Baseline was defined as the latest measurement prior to the first dosing of study medication.

Change = change from baseline.

Overall density is used, and it is defined as the sum of bio-types (mucoid, dry and small colony variant). The log₁₀ is taken on the sum.

Termination visit: last available post-baseline measurement.

The greater value was chosen when subject had more than one measurement at the same visit date.

The later non-missing value was chosen when subject had more than one measurement at the same visit number with different collecting date.

ITT population corresponds to the All Randomized Safety Population in C2301 CSR.

Source: Table 3.2-1.2a, this submission.

Division of Anti-Infective Products

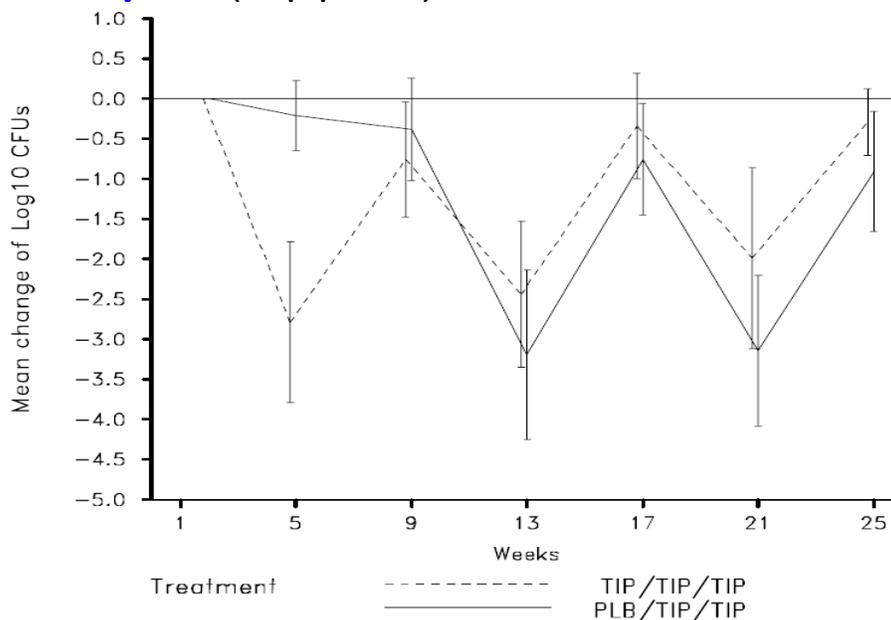
Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Reviewer's comments: Among all colony types combined, the treatment in the test (TIP) arm did not reduce the number of *P. aeruginosa* isolates more than the treatment in the comparator (placebo) arm. At the end of the first cycle of treatment, the TIP arm showed a *greater* reduction in CFUs than the placebo arm (-2.79 and -0.21 log₁₀ CFUs, respectively). At the end of cycle 2, the reduction was *lower* in the TIP arm than the placebo arm (-2.44 and -3.19 log₁₀ CFUs, respectively). The log₁₀ reduction at the end of treatment cycle 3 compared to baseline colony types for *P. aeruginosa* CFUs were *lower* in the TIP than the placebo arm (-1.99 and -3.14 log₁₀ CFUs, respectively). The log₁₀ reduction from the follow-up visit compared to baseline for all *P. aeruginosa* CFUs was similar in the TIP and the placebo arms (-0.29 and -0.91 log₁₀ CFUs, respectively) as was the case in the termination visit (-1.08 and -1.10 log₁₀ CFUs for the TIP and the placebo arms, respectively).

The effect of the two treatments on the concentration of *P. aeruginosa* in sputum is shown graphically in [Figure 2](#).

Figure 2. Change from baseline in *P. aeruginosa* sputum density (Log₁₀ CFUs) in cycles 1 to 3 - Study C2301 (ITT population)



Note: the vertical bar is 95% confidence interval.

Overall density is used, and it is defined as the sum of bio-types (mucoïd, dry and small colony variant).

Source: Figure 4-6, Clinical Pharmacology Summary, this submission.

Reviewer's comments: It is quite apparent that the greatest differences between the TIP treatment group and the placebo groups are seen at Day 28 of Cycle 1 (a decrease of 2.79 log₁₀ CFUs in TIP group vs. 0.21 log₁₀ CFUs in placebo group).

After switching from placebo to TIP starting at Cycle 2, the *decreases* in CFU concentration in the comparator arm were *greater* than the test arm of the trial during both Cycles 2 and 3. The mean change of log₁₀ CFUs in the placebo arm

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

were: -3.1 log₁₀ at week 13 and week 21 while the mean change of log₁₀ CFUs in the test arm were only -2.4 and -2.0 log₁₀ CFUs at weeks 13 and 21, respectively. As expected, the viable count of *P. aeruginosa* CFUs in sputum rebounded during the off-treatment cycles. These results are puzzling in light of the fact that both arms of the trial used TIP in at least two treatment cycles.

CHANGES IN TOBRAMYCIN MIC DURING THERAPY

A distribution table of tobramycin MIC values for *P. aeruginosa* isolates from the ITT population at each study visit was presented for each colony type (colony type-1, mucoid; colony type-2, dry; colony type-3, small colony variant; and maximum of colony types. A summary of these data is presented below.

The tobramycin MIC results for the *mucoid colony variants* are shown in Table 13. At baseline, a wide range of MIC values was evident for both treatment groups. Tobramycin-resistant organisms were included in each treatment group, as evidenced by the range value.

Table 13. MIC Summary for Study C2301, ITT Population; Colony type-1, Mucoid Colony Variants

Range	Tobramycin MIC (µg/mL)							
	TIP/TIP/TIP				placebo/TIP/TIP			
	N	Range	MIC50	MIC90	N	Range	MIC50	MIC90
Baseline	37	≤0.25->512	0.5	16	45	≤0.25-256	0.5	2
Week 5	24	≤0.25->512	0.5	64	42	≤0.25-4	0.5	2
Week 21	27	≤0.25->512	1	32	27	≤0.25-16	0.5	4
Week 25	28	≤0.25->512	0.5	32	34	≤0.25-8	0.5	4
Termination	37	≤0.25->512	0.5	32	47	≤0.25-64	0.5	4

Color code: yellow=two dilution step increase over baseline; orange=three dilution step increase over baseline; red=four dilution step increase over baseline; blue=two dilution step decrease versus baseline; purple=three or more dilution step decrease versus baseline; green= two or more dilution step difference between MIC90 values for baseline study arms. Source: Table 4-10, Clinical Pharmacology Summary, this submission.

Reviewer's comments: Overall, the bacteria in the colony type-1 isolates in the TIP/TIP/TIP treatment arm were *less susceptible* to tobramycin than those in the placebo/TIP/TIP arm as evidenced by baseline MIC90 values of 16 and 2 µg/mL, respectively. Thus, the MIC90 of the baseline organisms in the test arm are resistant by the accepted CLSI breakpoints for tobramycin. This variation in baseline MIC90 is puzzling as it represents a three dilution step difference. This Reviewer cannot offer an explanation for this difference.

The MIC90 values for the colony type-1 isolates in the TIP/TIP/TIP treatment arm increased such that by termination, the MIC90 value had increased by 2-fold from 16 to 32 µg/mL. At one point, at week 5, the tobramycin MIC90 had increased to 64 µg/mL, an increase of 4-fold or two dilution steps compared to the baseline MIC90. In the placebo/TIP/TIP arm, the MIC90 value increased by 2-fold at baseline to 4 µg/mL by the terminal visit.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

It is interesting to note that the high end of the MIC range in the comparator arm decreased for each visit and the high end of the termination visit MIC range was two dilution steps lower than the high end of the of the baseline visit MIC range.

The tobramycin MIC summary results for the *dry colony variants* are shown in [Table 14](#).

Table 14. MIC Summary for Study C2301, ITT Population; colony type-2, Dry Colony Variants

Range	Tobramycin MIC (µg/mL)							
	TIP/TIP/TIP				placebo/TIP/TIP			
	N	Range	MIC50	MIC90	N	Range	MIC50	MIC90
Baseline	32	≤0.25->512	0.5	8	38	≤0.25-256	1	2
Week 5	16	≤0.25->512	1	>512	28	≤0.25-16	0.5	2
Week 21	14	0.5->512	1	>512	10	0.5-256	1	64
Week 25	12	≤0.25-32	2	16	13	≤0.25->512	0.5	16
Termination	36	≤0.25->512	1	32	40	≤0.25->512	0.5	16

Color code: yellow=two dilution step increase over baseline; orange=three dilution step increase over baseline; red=four dilution step increase over baseline; blue=two dilution step decrease versus baseline; purple=three or more dilution step decrease versus baseline; green= two or more dilution step difference between MIC90 values for baseline study arms. Source: Table 4-11, Clinical Pharmacology Summary, this submission.

Reviewer's comments: Overall, the colony type-2 isolates in the TIP/TIP/TIP treatment arm were, again, *less susceptible* to tobramycin than those in the placebo/TIP/TIP arm as evidenced by baseline MIC90 values of 8 and 2 µg/mL, respectively. This represents a two-dilution difference in the baseline MIC90 values for the two treatment arms. Thus, the MIC90 of the baseline organisms in the test arm are intermediate in susceptibility by the accepted CLSI breakpoints for tobramycin. This variation in baseline MIC90 is puzzling as it represents a two dilution step difference. This Reviewer cannot offer an explanation for this difference.

The MIC90 values for the dry colony isolates in the TIP/TIP/TIP treatment arm increased such that by termination, the MIC90 value had increased by 4-fold from 8 to 32 µg/mL, an increase of two dilution steps. At weeks 5 and 21, the tobramycin MIC90 had increased to >512 µg/mL, an increase of more than six dilution steps compared to the baseline MIC90.

In the placebo/TIP/TIP arm, the MIC90 value increased by three dilution steps, 2 µg/mL at baseline to 16 µg/mL by the terminal visit. At week 21, the MIC90 had increased to 64 µg/mL, a five dilution step increase.

Note that the high end of the MIC range in the comparator arm increased for the Week 25 and termination visits by two dilution steps.

It should be noted that the number of dry colony variants decreased on therapy for *both* treatment arms but returned to similar numbers of isolates at termination as those at baseline.

The tobramycin MIC results for the *small colony variants* are shown in [Table 15](#).

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Table 15. MIC Summary for study C2301, ITT Population; colony type-3, Small Colony Variants

Range	Tobramycin MIC (µg/mL)							
	TIP/TIP/TIP				placebo/TIP/TIP			
	N	Range	MIC50	MIC90	N	Range	MIC50	MIC90
Baseline	10	≤0.25-32	0.5	4	12	≤0.25->512	1	2
Week 5	5	≤0.25-32	2	--a	5	≤0.25-2	≤0.25	--
Week 21	4	2--64	-- b	--	2	0.5-1	--	--
Week 25	2	128->512	--b	--	0	--	--	--
Termination	14	≤0.25->512	1	128	12	≤0.25-32	1	4

^a MIC₉₀ value not calculated for any group of <10 isolates

^b MIC₅₀ value not calculated for any group of <5 isolates

Color code: yellow=two dilution step increase over baseline; orange=three dilution step increase over baseline; red=four dilution step increase over baseline; blue=two dilution step decrease versus baseline; purple=three or more dilution step decrease versus baseline; green= two or more dilution step difference between MIC₉₀ values for baseline study arms.

Source: Table 4-12, Clinical Pharmacology Summary, this submission.

Reviewer's comments: Overall, the colony type-3 isolates in the TIP/TIP/TIP treatment arm were, again, *less susceptible* than those in the placebo/TIP/TIP arm as evidenced by baseline MIC₉₀ values of 4 and 2 µg/mL, respectively. This represents a one-dilution difference in the baseline MIC₉₀ values for the two treatment arms.

The MIC₉₀ values for the small colony variant isolates TIP/TIP/TIP treatment arm increased such that by termination, the MIC₉₀ value had increased by five dilution steps from 4 to 128 µg/mL. There were no MIC₉₀ values at weeks 5, 21 and 25.

In the placebo/TIP/TIP arm, the MIC₉₀ value increased by one dilution step, 2 µg/mL at baseline to 4 µg/mL by the terminal visit. There were no MIC₉₀ values at weeks 5, 21 and 25.

Note the high end of the MIC range for the placebo arm decreased dramatically at Week 5, Week 21 and the termination visits such that the high end of the MIC range at the termination visit was five dilution steps lower than at baseline. The high end of the of the MIC range in the test arm increased for the Week 25 and termination visits by two dilution steps over the baseline high end of the MIC range.

Note the very low number of isolates at each visit for either arm of the study. This may be a factor in the variation in the MIC values between baseline and on therapy visits.

The MIC summary results are shown as the *maximum value of all colony types* when more than one colony type was present in a given patient at a given visit in [Table 16](#).

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Table 16. MIC summary for study C2301, ITT Population; maximum of all colony types

Range	Tobramycin MIC (µg/mL)							
	TIP/TIP/TIP				placebo/TIP/TIP			
	N	Range	MIC50	MIC90	N	Range	MIC50	MIC90
Baseline	44	≤0.25->512	0.5	32	48	≤0.25->512	1	8
Week 5	29	≤0.25->512	1	>512	44	≤0.25-8	0.5	2
Week 21	28	≤0.25->512	1	>512	30	≤0.25-256	1	32
Week 25	30	≤0.25->512	1	128	37	≤0.25->512	1	8
Termination	40	≤0.25->512	1	32	48	≤0.25->512	1	8

Color code: yellow=two dilution step increase over baseline; orange=three dilution step increase over baseline; red=four dilution step increase over baseline; blue=two dilution step decrease versus baseline; purple=three or more dilution step decrease versus baseline; green= two or more dilution step difference between MIC90 values for baseline study arms. Source: Table 4-13, Clinical Pharmacology Summary, this submission.

Reviewer's comments: When the MICs from all colony types are pooled and assessed, MICs from isolates in the TIP/TIP/TIP treatment arm were *less susceptible* than those in the placebo/TIP/TIP arm as evidenced by baseline MIC90 values of 32 and 8 µg/mL, respectively. This represents a two-dilution difference in the baseline MIC90 values for the two treatment arms. Thus, the MIC90 of the baseline organisms in the test arm are resistant by the accepted CLSI systemic breakpoints (≥ 16 µg/mL) for tobramycin. This variation in baseline MIC90 is puzzling as it represents a two dilution step difference. This Reviewer cannot offer an explanation for this difference.

The MIC90 values from all *P. aeruginosa* colony types from the TIP/TIP/TIP treatment arm were identical at termination as the MIC90 values at baseline, 32 µg/mL. However, at weeks 5 and 21, the tobramycin MIC90 had increased to >512 µg/mL, an increase of more than four dilution steps compared to the baseline MIC90.

In the placebo/TIP/TIP arm, the MIC90 value for *P. aeruginosa* colony types were identical at termination as those at baseline, 8 µg/mL. At week 21, the MIC90 increased to 32 µg/mL, a two dilution step increase.

ANTIBIOTIC RESISTANCE DEVELOPMENT ON THERAPY

Tobramycin

According to the current CLSI systemic interpretive criteria for tobramycin, resistance is defined as a MIC ≥ 16 µg/ml for *P. aeruginosa* isolates. The MICs for *P. aeruginosa* isolates from Study C2301 were examined and stratified by colony type i.e. dry, mucoid, small colony and mixed colony types for each treatment arm. The following table shows the percentage of isolates from each colony type demonstrating an increase from Baseline to the Termination visit in MIC to ≥ 16 µg/ml, considered a resistant phenotype. Increases in resistance of less than 5% are not shown.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Table 17. *P. aeruginosa* tobramycin MIC increase by colony type, Study C2301.

colony type	% increase by treatment arm	
	TIP/TIP/TIP	Placebo/TIP/TIP
	N=46	N=49
dry colony	7.6%	7.20%
mucoid colony	5.4%	4.3%
small colony	18.6%	0%
mixed colony types	8.6%	0%

Source: Table 3.2-1.6, this submission.

Reviewer's comments: Increases of 5% or more in tobramycin resistance while on therapy was observed among all colony types i.e. dry, mucoid, small colony and mixed for patients in the TIP/TIP/TIP treatment arm with the greatest increase occurring among the small colony colony type (18.6%). In contrast, only the dry colony type in the in the Placebo/TIP/TIP treatment arm showed an increase in tobramycin resistance while on therapy (7.2%).

Other Antibiotics

The Applicant examined the increased resistance levels for a variety of antibiotics from baseline to the termination visit for all colony type *P. aeruginosa* isolates. Changes in resistance levels during therapy were examined for the following antibiotics: aztreonam, ciprofloxacin, ceftazidime, imipenem and meropenem (Table 14.2-3.4, this submission, not shown).

In Study 2301, no increases in resistance were noted to the following antibiotics: aztreonam, ciprofloxacin, imipenem and meropenem. The only exception was an increase in ceftazidime resistance (6.3%) seen in the placebo arm.

SHIFTS IN SPUTUM DENSITY OF BASELINE PATHOGENS OTHER THAN *P. AERUGINOSA*

In Study C2301, a range of pathogens other than *P. aeruginosa* were identified in the baseline sputum of the CF patients as would be expected in this patient population. The majority of pathogens were present in only a small percentage of patients and, therefore, changes in the growth patterns were likely not representative of TIP/TOBI treatment. A complete listing of these organisms is contained in [SCE-Appendix 1-Table 3.2- 1.3; table not shown]. Those organisms which were present in two or more patients in both treatment groups at baseline are discussed further below.

Methicillin-sensitive *Staphylococcus aureus* (MSSA)

At baseline, 11 patients had MSSA isolates (63.6% characterized as high growth) in the TIP treatment group; while in the placebo/TIP treatment group, 11 patients had MSSA isolates (72.7% characterized as high growth). At Week 5, two patients in both the TIP and placebo/TIP groups had shifted to a lesser degree of growth from baseline, compared

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

to two patients in both the TIP and placebo/TIP groups who had a shift to an increased level of growth from baseline. At Week 21, there was a reduction in the number of data pairs for both treatment arms across all growth categories, with similar shifts in both treatment arms. Results at Week 25 demonstrated a shift to a higher category for three patients in the TIP group and 1 patient in the placebo/TIP group, accompanied by shifts to lower categories for patients with high counts at baseline. At the Termination visit, the largest number of data pairs was observed. In the TIP group, 17 patients had MSSA isolates of which 12 were in the high density category. In the placebo/TIP group, an increase in category was observed in two instances, accompanied by a decrease in two instances. Overall, there were shifts to both higher and lower levels in both treatment groups.

Methicillin-resistant *Staphylococcus aureus* (MRSA)

At baseline, two patients in each treatment group had MRSA present in their sputum; both were characterized as a high level of growth in the TIP group while the placebo/TIP group contained one light and one high. At Week 5, two patients in the TIP group were at a high level, while in the placebo/TIP group there was one light and one high. At Week 21, there were one and two entries in the TIP and placebo treatment groups, respectively, with two of three at the high level. A similar pattern to Week 21 was observed at Week 25. At the termination visit, there were three entries in each treatment group. For the TIP group, one of three had decreased from the high category; for the placebo/TIP group, one had increased from light to high while one had decreased from high to moderate. Overall, there were individual instances of both increases and decreases in viable count. However, the small number of observations makes it difficult to render any conclusions regarding MRSA.

TREATMENT-EMERGENT ORGANISMS (EXCLUDING *P. AERUGINOSA*)

Due to the small number of subjects in [Study C2301](#), the issue of treatment-emergent organisms was addressed in an integrated analysis with [Study C2303](#), which will be discussed later under [Study C2303](#).

Of particular interest was *B. cepacia*, an important respiratory pathogen in CF patients due to its multi-drug resistance profile and the need for aggressive antibiotic treatment. A history of sputum culture of throat swab (or BAL) culture yielding *B. cepacia* within two years prior to screening and/or sputum sample yielding *B. cepacia* at screening was an exclusion criterion for [Study C2301](#). Only one enrolled subject (placebo group) had a prior history of *B. cepacia*, contracted more than two years prior to screening, and no new instances of *B. cepacia* were encountered during the trial.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

CLINICAL STUDY CTBM100C2302

Data sets analyzed

The intent to treat (ITT) population was the main efficacy population for Study C2302 and consisted of over 90% of all randomized patients (more than 300 patients in the TIP group and more than 200 in the TOBI group (Table 18). The safety population (All Randomized Safety) was the same as the ITT population. The per protocol (PP) population was used for a sensitivity analysis and was composed of approximately 60% of the randomized population.

Table 18. Analysis populations by treatment group for Study C2302 Analysis population

Analysis population	TIP N=329 n (%)	TOBI N=224 n (%)
Intent-to-treat	308 (93.6)	209 (93.3)
All Randomized Safety	308 (93.6)	209 (93.3)
Per protocol	200 (60.8)	149 (66.5)

N=number of patients

N=percentage of patients

Source: Table 4-8, Clinical Pharmacology Summary, this submission.

The ITT and All Randomized Safety population in the North America region consisted of 195 patients in the TIP group, and 131 in the TOBI group. In the Europe/Rest of World region there were 104 patients in the TIP treatment group, and 71 in the TOBI treatment group in both of the main analysis populations. There were nine patients from Latin America in the TIP ITT and All Randomized Safety populations, and seven in the TOBI ITT and All Randomized Safety populations.

In Study C2302, these groups provided a large, diverse set of *P. aeruginosa* isolates for susceptibility determinations thereby providing a recent survey of drug susceptibility in CF isolates, noting that a majority of patients had prior inhaled tobramycin experience.

Study Design

Study C2302 was a randomized, open-label, active-controlled, parallel-arm trial. Eligible patients were randomized to TIP (4 x 28 mg b.i.d.) or TOBI (300 mg/5 mL b.i.d.) at a 3:2 ratio. Treatment was administered for 28 days, followed by 28 days off therapy (one cycle), for 3 cycles. The enrollment criteria allowed for prior inhaled antipseudomonal antibiotic use within 28 days of study drug administration, and therefore the C2302 represented a “real world” population with regard to prior tobramycin use. Additional study design details are found in the clinical study report for [Study C2302].

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

The *primary objective* was to evaluate the safety of twice-daily (b.i.d.) dosing of TIP delivered with the T-326 inhaler, compared to TOBI delivered with the PARI LC PLUS jet nebulizer and DeVilbiss PulmoAide compressor or suitable alternative.

Secondary microbiology analyses included the following:

- Pathogen identification.
- The absolute change in *P. aeruginosa* density in sputum was determined. Absolute change from (based on CFUs, as described for [Study C2301](#)) to each post-baseline to each post-baseline time point in Cycles 1-3 was summarized descriptively by treatment group.
- Semi-quantitative changes in CFU/g sputum for baseline organisms other than *P. aeruginosa*.
- The change in tobramycin MIC from baseline to each post-baseline time point in Cycles 1-3 was summarized descriptively by mean changes for each treatment group. Additional analyses included analysis of *P. aeruginosa* with elevated MIC values and prevalence of *P. aeruginosa* with MIC values ≥ 128 $\mu\text{g/mL}$.
- The change in frequency by categorical susceptibility description (MIC >8 $\mu\text{g/mL}$ and ≤ 8 $\mu\text{g/mL}$) was also determined.

CORRELATION OF CLINICAL AND MICROBIOLOGICAL OUTCOME

The *primary objective* of [Study C2302](#) was to evaluate the safety of twice-daily (b.i.d.) dosing of TIP delivered with the T-326 inhaler, compared to TOBI delivered with the PARI LC PLUS jet nebulizer and DeVilbiss PulmoAide compressor or suitable alternative. There was no primary efficacy variable for this study.

Secondary efficacy variables were: relative changes in FEV₁ % predicted from baseline at all scheduled post-treatment visits (Weeks 2, 5, 9, 13, 17, 21 and 25), area under the curve (AUC) of relative changes of FEV₁ % predicted from baseline (pre-dose Day 1) to all scheduled post-treatment visits, relative change in FVC and FEV 25-75 % predicted from baseline to all scheduled post-baseline visits, time from start of first inhalation of study drug to first hospitalization due to a respiratory SAE, change in *P. aeruginosa* density (log₁₀ CFU/g sputum) from baseline to all post-baseline visits, and change in *P. aeruginosa* tobramycin MIC values from baseline to all post-baseline visits. An additional secondary endpoint was the report of treatment satisfaction.

Safety results are reported in the study report for [\[Study C2302\]](#). Of the secondary efficacy variables, there was an increase in FEV₁ percent of predicted from baseline to Day 28 of Cycle 3 in both TIP and TOBI treatment groups (relative change of 3.1 and 2.3, respectively). This increase was numerically greater for TIP, and statistically non-inferior.

To make enrollment of the targeted patient numbers feasible and closer to real-life clinical conditions, criteria were more flexible in this study, particularly for pre-study

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

(inhaled) antibiotics (a 28 day exclusion prior to start of study medication) which was expected to have an impact on the pathogen spectrum and density at baseline.

In this section, the microbiological data collected by the Applicant during this study are summarized and analyzed by this Reviewer.

P. AERUGINOSA DENSITY IN SPUTUM

CFU counts for each of the three *P. aeruginosa* colony types as well as all colony types were determined in patient sputum at multiple visits; the data for each colony type, as well as the sum of the three colony types, is shown in Table 19.

Table 19. Change in *P. aeruginosa* density colony forming units (log₁₀ CFUs) from baseline - Study C2302; treatment group—dry colony type

	week/day		TIP N=308				TOBI N=209			
			N	mean	SD	median	N	mean	SD	median
Baseline		Raw value	189	6.61	1.75	7.00	131.00	6.66	1.77	7.00
Cycle 1	1/1	Raw value	143	6.66	1.69	7.00	106.00	6.83	1.75	7.25
	5/28	Raw value	106	5.19	1.92	5.06	88.00	6.03	1.84	6.24
		Change	96	-1.75	2.11	-1.65	78.00	-1.03	2.02	-0.74
Cycle 2	9/1	Raw value	117	6.59	1.82	7.00	84.00	6.83	1.66	7.30
		Change	103	-0.14	1.61	0.00	73.00	-0.21	1.72	0.00
	13/28	Raw value	97	5.16	1.94	4.98	74.00	5.91	1.87	6.21
		Change	89	-1.65	2.05	-1.63	67.00	-1.19	2.06	-0.82
Cycle 3	17/1	Raw value	108	6.45	1.89	6.78	86.00	6.63	1.86	7.01
		Change	90	-0.25	1.85	0.00	72.00	-0.15	1.77	0.00
	21/28	Raw value	81	5.17	1.97	4.95	74.00	6.18	1.91	6.44
		Change	72	-1.77	2.17	-1.59	65.00	-0.73	1.87	-0.92
Follow up	25/56	Raw value	98	6.31	1.88	6.54	88.00	6.72	1.69	7.25
		Change	85	-0.45	1.77	-0.48	72.00	-0.08	1.35	-0.18
Termination		Raw value	195	6.05	2.01	6.18	153.00	6.36	1.78	6.69
		Change	160	-0.53	2.10	-0.40	115.00	-0.38	1.73	-0.30

Baseline was defined as the latest measurement prior to the first dosing of study medication.

Change = change from baseline.

Overall density is used, and it is defined as the sum of bio-types (mucoid, dry and small colony variant). The log₁₀ is taken on the sum.

Termination visit: last available post-baseline measurement.

Source: Table 14.2-2.1, this submission.

Reviewer's comments: Among the *dry* colony type isolates, treatment in the test (TIP) arm reduced the concentration of *P. aeruginosa* slightly more than the treatment in the comparator (TOBI) arm. The log₁₀ reduction at the end of cycles 1, 2 and 3 for the TIP treatment arm was -1.75, -1.65 and -1.77 log₁₀ CFUs, respectively. The log₁₀ reduction at the end of cycles 1, 2 and 3 for the TOBI treatment arm was -1.03, -1.19 and -0.73 log₁₀ CFUs, respectively.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Table 20. Change in *P. aeruginosa* density colony forming units (log₁₀ CFUs) from baseline - Study C2302. treatment group— mucoid colony type

	week/day		TIP N=308				TOBI N=209			
			N	mean	SD	median	N	mean	SD	median
Baseline		Raw value	253	6.93	1.59	7.26	176	7.14	1.53	7.48
Cycle 1	1/1	Raw value	212	7.04	1.50	7.30	152	7.26	1.47	7.60
	5/28	Raw value	182	5.24	1.86	5.18	132	6.02	1.91	6.09
Cycle 2		Change	176	-1.78	1.94	-1.70	129	-1.30	2.13	-1.10
	9/1	Raw value	183	6.75	1.75	7.08	130	6.84	1.64	7.30
		Change	178	-0.26	1.72	0.00	127	-0.49	1.90	-0.30
	13/28	Raw value	153	5.57	1.78	5.60	120	6.15	1.83	6.30
Cycle 3		Change	148	-1.62	2.10	-1.70	115	-1.10	1.99	-0.78
	17/1	Raw value	168	6.70	1.80	6.93	124	7.04	1.65	7.65
		Change	162	-0.28	1.96	-0.15	119	-0.16	1.65	-0.12
	21/28	Raw value	138	5.40	1.87	5.10	115	6.30	1.75	6.38
Follow up		Change	132	-1.60	2.06	-1.40	112	-0.92	2.00	-0.72
	25/56	Raw value	148	6.66	1.73	6.86	120	7.12	1.50	7.61
Termination		Change	140	-0.36	1.80	-0.30	115	-0.01	1.64	-0.15
		Raw value	245	6.55	1.74	6.81	173	6.80	1.62	7.15
		Change	229	-0.40	1.85	-0.30	161	-0.38	1.79	-0.30

Baseline was defined as the latest measurement prior to the first dosing of study medication.

Change = change from baseline.

Overall density is used, and it is defined as the sum of bio-types (mucoid, dry and small colony variant). The log₁₀ is taken on the sum.

Termination visit: last available post-baseline measurement.

Source: Table 14.2-2.1, this submission.

Reviewer's comments: Among the *mucoïd* colony type isolates, treatment in the test (TIP) arm reduced the concentration of *P. aeruginosa* slightly more than the treatment in the comparator (TOBI) arm. The log₁₀ reduction at the end of cycles 1, 2 and 3 for the TIP treatment arm was -1.78, -1.62 and -1.60 log₁₀ CFUs, respectively. The log₁₀ reduction at the end of cycles 1, 2 and 3 for the TOBI treatment arm was -1.30, -1.10 and -0.92 log₁₀ CFUs, respectively.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Table 21. Change in *P. aeruginosa* density colony forming units (log₁₀ CFUs) from baseline - Study C2302. treatment group—small colony variant

week/day		TIP N=308				TOBI N=209			
		N	mean	SD	median	N	mean	SD	median
Baseline	Raw value	50	6.92	1.46	7.35	33	7.45	0.95	7.60
Cycle 1	1/1 Raw value	28	7.01	1.3	7.35	18	7.51	0.80	7.43
	5/28 Raw value	26	5.25	1.77	5.25	14	5.88	1.49	5.94
Cycle 2	Change	12	-1.70	1.83	-1.54	8	-1.35	1.71	-1.05
	9/1 Raw value	34	6.56	1.51	6.74	20	6.94	1.41	7.35
	Change	17	-0.57	1.4	-0.39	9	-0.15	1.00	0.30
	13/28 Raw value	27	6.07	1.49	6.30	16	6.73	1.53	7.08
Cycle 3	Change	12	-0.72	1.22	-0.47	10	-0.71	1.14	-0.40
	17/1 Raw value	23	6.89	1.28	7.28	18	6.74	1.53	7.14
	Change	15	-0.20	0.91	0.06	11	-0.42	1.80	0.17
	21/28 Raw value	20	6.29	1.49	6.50	15	6.33	1.31	6.30
Follow up	25/56 Raw value	23	6.64	1.32	6.90	17	6.72	1.46	7.20
	Change	14	-0.58	1.38	-0.08	8	-0.25	1.19	-0.56
Termination	Raw value	81	6.16	1.63	6.48	53	6.71	1.43	7.08
	Change	31	-0.65	1.58	-0.09	23	-0.59	1.17	-0.55

Baseline was defined as the latest measurement prior to the first dosing of study medication.

Change = change from baseline.

Overall density is used, and it is defined as the sum of bio-types (mucoid, dry and small colony variant). The log₁₀ is taken on the sum.

Termination visit: last available post-baseline measurement.

Source: Table 14.2-2.1, this submission.

Reviewer's comments: Among the *small colony variant* type isolates, treatment in the test (TIP) arm reduced the concentration of *P. aeruginosa* slightly more than the treatment in the comparator (TOBI) arm for cycle 1 only. At the end of cycle 3, the treatment in the comparator arm (TOBI) resulted in a greater reduction in the number of *P. aeruginosa* than the treatment in the test (TIP) arm. Reductions in the number of *P. aeruginosa* were similar for the two treatment arms in cycle 2. The log₁₀ reduction at the end of cycles 1, 2 and 3 for the TIP treatment arm was -1.70, -0.72 and +0.33 log₁₀ CFUs, respectively. The log₁₀ reduction at the end of cycles 1, 2 and 3 for the TOBI treatment arm was -1.35, -0.71 and -1.15 log₁₀ CFUs, respectively.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Table 22. Change in *P. aeruginosa* density colony forming units (log₁₀ CFUs) from baseline - Study C2302. Treatment group—sum of dry, mucoid and small colony variant colony type

week/day		TIP N=308				TOBI N=209			
		N	mean	SD	median	N	mean	SD	median
Baseline	Raw value	279	7.23	1.49	7.64	192	7.35	1.54	7.68
Cycle 1	1/1 Raw value	248	7.24	1.46	7.63	170	7.47	1.50	7.88
	5/28 Raw value	204	5.60	1.84	5.40	148	6.29	1.89	6.36
Cycle 2	Change	202	-1.76	1.96	-1.60	145	-1.32	2.03	-1.00
	9/1 Raw value	204	7.01	1.75	7.42	146	7.14	1.66	7.68
	Change	203	-0.29	1.60	-0.14	144	-0.31	1.82	-0.16
Cycle 3	13/28 Raw value	181	5.83	1.76	5.88	130	6.43	1.77	6.60
	Change	179	-1.54	1.99	-1.46	125	-1.11	1.91	-0.85
	17/1 Raw value	190	6.95	1.78	7.29	136	7.30	1.64	7.92
Cycle 3	Change	187	-0.37	1.80	-0.16	131	-0.04	1.47	-0.01
	21/28 Raw value	158	5.69	1.88	5.40	129	6.59	1.72	6.81
Follow up	Change	157	-1.61	2.03	-1.40	126	-0.77	1.78	-0.67
	25/56 Raw value	167	6.84	1.82	7.24	135	7.33	1.51	7.93
Termination	Change	165	-0.49	1.78	-0.38	130	-0.08	1.36	-0.14
	Raw value	269	6.76	1.85	7.20	189	7.01	1.69	7.58
	Change	263	-0.53	1.92	-0.33	179	-0.33	1.71	-0.24

Baseline was defined as the latest measurement prior to the first dosing of study medication.

Change = change from baseline.

Overall density is used, and it is defined as the sum of bio-types (mucoid, dry and small colony variant). The log₁₀ is taken on the sum.

Termination visit: last available post-baseline measurement.

Source: Table 14.2-2.1, this submission.

Reviewer's comments: Among all colony types combined, treatment in the test (TIP) arm reduced the number of *P. aeruginosa* slightly more than the treatment in the comparator (TOBI) arm. The log₁₀ reduction at the end of cycles 1, 2 and 3 for the TIP treatment arm was -1.76, -1.54 and -1.61 log₁₀ CFUs, respectively. The log₁₀ reduction at the end of cycles 1, 2 and 3 for the TOBI treatment arm was -1.32, -1.11 and -0.77 log₁₀ CFUs, respectively.

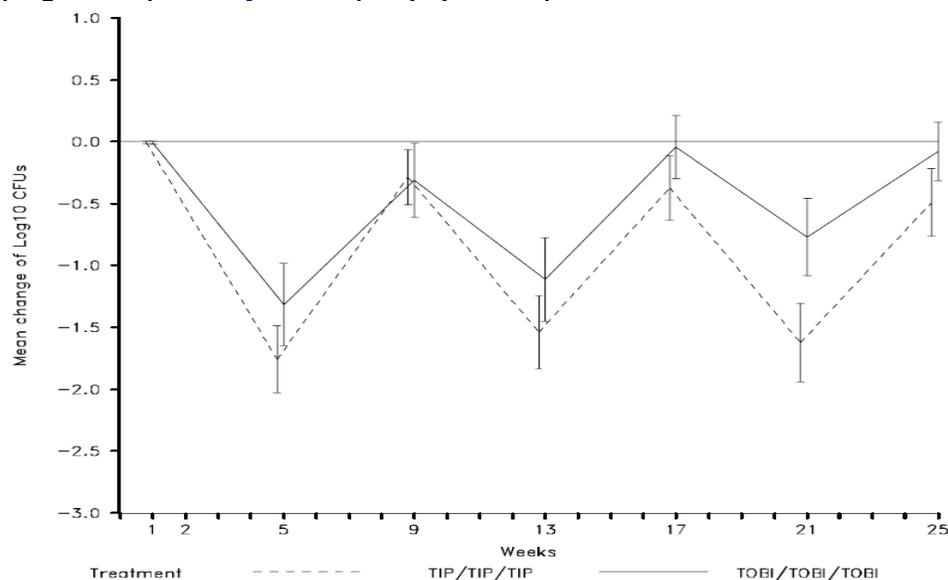
The data for the bacterial reductions of all the colony types is also shown graphically in [Figure 3](#).

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Figure 3. Between treatment comparison of change in *P. aeruginosa* sputum concentration (Log₁₀ CFU) – Study C2302 (ITT population)



Note: the vertical bar is 95% confidence interval. Overall density is used, and it is defined as the sum of colony types (mucoid, dry and small colony variant).

Source: Figure 4-7, Clinical Pharmacology Summary, this submission.

In conclusion, there was a greater decrease in the mean change from total baseline counts in the TIP treatment group compared with the TOBI treatment group at the end of each cycle, especially during Cycle 3. As in [Study C2301](#), the results of [Study C2302](#) showed that in both the TIP and TOBI treatment groups, *P. aeruginosa* concentrations had partially rebounded at the end of the 28-day off-treatment phase but then decreased again during the 28-day on-treatment phase to comparable levels across each cycle of treatment.

CHANGES IN TOBRAMYCIN MIC DURING THERAPY

A distribution table of tobramycin MIC values for *P. aeruginosa* isolates from the ITT population at each study visit was prepared for each colony type (colony type-1, mucoid; colony type-2, dry; colony type-3, small colony variant; and maximum of all colony types present. A summary of these data is presented below.

The tobramycin MIC summary results from patients with the mucoid colony variant isolates are shown in [Table 23](#).

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Table 23. MIC summary for Study C2302, ITT Population; mucoid colony type

Range	Tobramycin MIC (µg/mL)							
	TIP				TOBI			
	N	Range	MIC50	MIC90	N	Range	MIC50	MIC90
Baseline	277	≤0.12->512	1	8	189	≤0.12->512	1	16
Week 5	208	≤0.12->512	1	32	153	≤0.12->512	1	16
Week 21	167	≤0.12->512	1	32	139	≤0.12->512	1	32
Week 25	178	≤0.12->512	1	32	139	≤0.12->512	1	8
Termination	270	≤0.12->512	1	64	185	≤0.12->512	1	16

Color code: yellow=two dilution step increase over baseline; orange=three dilution step increase over baseline; red=four dilution step increase over baseline; blue=two dilution step decrease versus baseline; purple=three or more dilution step decrease versus baseline; green= two or more dilution step difference between MIC90 values for baseline study arms. Source: Table 4-15, Clinical Pharmacology Summary, this submission.

Reviewer's comments: The tobramycin MIC90 values for the mucoid colony type isolates in the TIP treatment arm increased during treatment such that by termination, the MIC90 value had increased by three dilution steps from 8 to 64 µg/mL, an increase of two dilution steps. At weeks 5, 21 and 25, the tobramycin MIC90 had increased to 32 µg/mL, an increase of two dilution steps compared to the baseline MIC90. The tobramycin MIC90 values for the mucoid colony type isolates in the TOBI treatment arm remained within one dilution step throughout the treatment.

The tobramycin MIC summary results from patients with the dry colony type isolates are shown in Table 24.

Table 24. MIC summary for Study C2302, ITT Population; dry colony type

Range	Tobramycin MIC (µg/mL)							
	TIP				TOBI			
	N	Range	MIC50	MIC90	N	Range	MIC50	MIC90
Baseline	214	≤0.12->512	2	64	144	≤0.12->512	2	128
Week 5	126	≤0.12->512	4	512	99	≤0.12->512	4	128
Week 21	107	0.25->512	8	512	81	0.25->512	8	256
Week 25	118	≤0.12->512	4	512	96	≤0.12->512	2	64
Termination	225	≤0.12->512	4	512	166	≤0.12->512	2	128

Color code: yellow=two dilution step increase over baseline; orange=three dilution step increase over baseline; red=four dilution step increase over baseline; blue=two dilution step decrease versus baseline; purple=three or more dilution step decrease versus baseline; green= two or more dilution step difference between MIC90 values for baseline study arms. Source: Table 4-16, Clinical Pharmacology Summary, this submission.

Reviewer's comments: The MIC90 values for the dry colony type isolates in the TIP treatment arm increased such that by termination, the MIC90 value had increased from 64 to 512 µg/mL, an increase of three dilution steps. At weeks 5, 21 and 25, the tobramycin MIC90 had increased to 512 µg/mL as well. Note that the MIC90 of the baseline isolates in the TIP arm (64 µg/mL) and the TOBI arm (128 µg/mL) are resistant resistant by the accepted CLSI systemic breakpoints for tobramycin. The tobramycin MIC90 values for the dry colony type isolates in the TOBI treatment arm remained within one dilution step throughout the treatment.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

The tobramycin MIC summary results from patients with the small colony variant isolates are shown in [Table 25](#).

Table 25. MIC summary for Study C2302, ITT population; small colony variant type

Range	Tobramycin MIC (µg/mL)							
	TIP				TOBI			
	N	Range	MIC50	MIC90	N	Range	MIC50	MIC90
Baseline	58	≤0.12->512	4	256	35	≤0.12->512	4	256
Week 5	28	≤0.12->512	16	>512	15	0.5->512	4	32
Week 21	21	0.25->512	32	512	16	0.25->512	8	512
Week 25	28	≤0.12->512	8	512	19	0.25->512	4	512
Termination	87	≤0.12->512	16	512	56	≤0.12->512	4	512

Color code: yellow=two dilution step increase over baseline; orange=three dilution step increase over baseline; red=four dilution step increase over baseline; blue=two dilution step decrease versus baseline; purple=three or more dilution step decrease versus baseline; green= two or more dilution step difference between MIC90 values for baseline study arms.
Source: Table 4-17, Clinical Pharmacology Summary, this submission.

Reviewer's comments: The MIC90 values for the small colony variant isolates in the TIP treatment arm increased such that by the Week 5 visit, the MIC90 value had increased from 256 to > 512 µg/mL, an increase of two dilution steps. Note that the MIC90 of the baseline isolates (256 µg/mL) in both the TIP arm and the TOBI arm are resistant by the accepted CLSI systemic breakpoints for tobramycin. The tobramycin MIC90 values for the small colony variant type isolates in the TOBI treatment arm remained within one dilution step throughout the treatment except for treatment at week 5 where MIC values decreased by three dilution steps.

An additional analysis using the maximum tobramycin MIC values when more than one colony type was present in a given patient at a given visit is shown in [Table 26](#).

Table 26. MIC summary for Study C2302, ITT population; maximum of all colony types

Range	Tobramycin MIC (µg/mL)							
	TIP				TOBI			
	N	Range	MIC50	MIC90	N	Range	MIC50	MIC90
Baseline	308	≤0.12->512	2	64	208	≤0.12->512	2	128
Week 5	239	≤0.12->512	2	512	173	≤0.12->512	4	64
Week 21	199	≤0.12->512	4	256	154	≤0.12->512	4	256
Week 25	201	≤0.12->512	2	256	155	≤0.12->512	2	64
Termination	298	≤0.12->512	2	512	202	≤0.12->512	2	64

Color code: yellow=two dilution step increase over baseline; orange=three dilution step increase over baseline; red=four dilution step increase over baseline; blue=two dilution step decrease versus baseline; purple=three or more dilution step decrease versus baseline; green= two or more dilution step difference between MIC90 values for baseline study arms.
Source: Table 4-18, Clinical Pharmacology Summary, this submission.

Reviewer's comments: The MIC90s of the baseline organisms in the TIP arm (64 µg/mL) and the TOBI arm (128 µg/mL) are resistant by the accepted CLSI systemic breakpoints for tobramycin.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

The MIC90 values for the all colony type isolates in the TIP treatment arm increased such that at both the Week 5 and termination visits, the MIC90 value had increased from 64 to 512 µg/mL, an increase of three dilution steps. At weeks 21 and 25, the tobramycin MIC90 had increased to 256 µg/mL, an increase of two dilution steps compared to the baseline MIC90.

MIC distribution for other antibiotics in Study C2302

The Applicant examined the increased resistance levels for a variety of antibiotics from baseline to the termination visit for all colony type *P. aeruginosa* isolates. Changes in resistance levels during therapy were examined for the following antibiotics: aztreonam, ciprofloxacin, ceftazidime, imipenem and meropenem (Table 14.2-3.4, this submission, not shown).

In Study 2302, an increase in antibiotic resistance was seen in colony types in both the TIP and TOBI treatment arms. The results are summarized below.

Table 27. Antibiotic resistance increase by *P. aeruginosa* colony type, Study C2302.

antibiotic/colony type	% increase in antibiotic resistance by treatment arm	
	TIP	TOBI
aztreonam		
dry colony	--	6.30%
mucoïd colony	--	--
small colony	--	--
mixed colony types	--	--
ceftazidime		
dry colony	--	11.20%
mucoïd colony	5.90%	--
small colony	--	8.90%
mixed colony types	--	--
ciprofloxacin		
dry colony	--	--
mucoïd colony	7.30%	--
small colony	--	--
mixed colony types	9.10%	--
imipenem		
dry colony	--	--
mucoïd colony	--	--
small colony	--	--
mixed colony types	--	5.80%
meropenem		
dry colony	--	--
mucoïd colony	6.50%	--
small colony	--	--
mixed colony types	--	--

Source: Table 14.2-3.4, this submission.

Reviewer's comments: Increases in resistance on therapy were noted for each antibiotic. In the *TIP treatment arm*, isolates from mucoïd colony types showed a

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

5.9%, 7.3% and 6.5% increase in ceftazadime, ciprofloxacin and meropenem resistance, respectively. In addition, there was 9.1% increase in ciprofloxacin resistance among mixed cell types.

In the *TOBI treatment arm*, isolates from dry colony types showed a 6.3% and 11.2% increase in aztreonam and ceftazidime resistance, respectively. An 8.9% increase in ceftazidime resistance was also seen in isolates from small colony variant colony types. Isolates from the mixed colony types showed a 5.8% increase in imipenem resistance.

Subgroup analysis of lung function change (FEV1% relative change) based on baseline

A search for an association of *in-vitro* susceptibility test results with clinical efficacy had been shown to be inconclusive in the TOBI studies patient groups, when baseline MICs were compared to clinical response; even patients with baseline MIC ≥ 128 $\mu\text{g/mL}$ benefited from TOBI (LiPuma 2001).

The proportion of patients with MICs above the conventional resistance breakpoint for systemic tobramycin treatment was similar in both treatment arms. A comparison of clinical efficacy (FEV1 change) between the MIC groups and between the treatment arms showed that the mean FEV1 relative change was in the same range for both TIP and TOBI, with minimal differences: In the TOBI treatment group, efficacy was numerically (and paradoxically) greater with baseline tobramycin > 8 $\mu\text{g/mL}$ than in patients with Tobramycin ≤ 8 $\mu\text{g/mL}$ at baseline, while the TIP group showed an inverted pattern, see Table 28.

The differences were very small and the confidence intervals encompass 0, indicating that for both subgroup (baseline MIC ≤ 8 $\mu\text{g/mL}$ and MIC > 8 $\mu\text{g/mL}$), TIP had similar efficacy to TOBI, and elevated MICs are still not associated with higher probability of a loss of efficacy.

Table 28. Subgroup analysis of relative change in FEV1 % predicted from baseline to end of dosing in cycle 3 - Study C2302

Subgroup	n	TIP		N	TOBI		Difference (TIP-TOBI)	
		Mean (SD)	LS Mean		Mean (SD)	LS Mean	LS Mean (SE)	CI
Baseline MIC								
> 8 $\mu\text{g/mL}$	48	1.4 (22.56)	1.9	35	3.0 (16.01)	3.8	-1.8 (4.07)	(-9.8, 6.2)
$\leq 8\mu\text{g/mL}$	179	3.6 (19.20)	3.7	135	2.2 (18.04)	1.7	2.0 (2.09)	(-2.2, 6.1)

(Relative change in FEV1 % predicted = treatment + baseline FEV1 % predicted (continuous) + subgroup + subgroup-by-treatment interaction). Note when subgroup is baseline FEV1 % predicted (<50%, $\geq 50\%$), baseline FEV1 % predicted (continuous) won't be included in the model.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

ANTIBIOTIC RESISTANCE DEVELOPMENT ON THERAPY

Tobramycin Resistance

According to the current interpretive criteria for tobramycin given systemically, resistance is defined as a MIC \geq 16 μ g/ml for a *P. aeruginosa* isolate. The MICs for *P. aeruginosa* isolates from [Study C2302](#) were examined and stratified by colony type i.e. dry, mucoid, small colony and mixed colony types for each treatment arm. The following table shows the percentage of isolates from each colony type demonstrating an increase from Baseline to the Termination visit in MIC to \geq 16 μ g/ml, considered a resistant phenotype. Increases in resistance of less than 5% are not shown.

Table 29. Tobramycin resistance increase by *P. aeruginosa* colony type, Study C2302.

Colony type	% increase by treatment arm	
	TIP N=308	TOBI N=209
dry colony	6.7%	- 4.3%
mucoid colony	7.3%	- 1.4%
small colony	18.4%	- 6.7%
mixed colony types	7.8%	- 2.3%

Source: Table 14.2-3.3, this submission.

Reviewer's comments: Increases of 5% or more in tobramycin resistance while on therapy were observed among all colony types i.e. dry, mucoid, small colony and mixed for patients in the TIP treatment arm but not the TOBI treatment arm. The greatest increase in resistance occurred among isolates from the small colony variant (18.4%). In contrast, *no isolates from any colony types* in the in the TOBI treatment arm showed an increase in tobramycin resistance while patients were on therapy.

Resistance to Other Antibiotics

The Applicant examined the increased resistance levels for a variety of antibiotics from baseline to the termination visit for all colony type *P. aeruginosa* isolates. Changes in resistance levels during therapy were examined for the following antibiotics: aztreonam, ciprofloxacin, ceftazodime, imipenem and meropenem ([Table 14.2-3.4](#), this submission, not shown). A summary table is presented here.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Table 30. Antibiotic resistance increase by *P. aeruginosa* colony type, Study C2302.

antibiotic/colony type	% increase in antibiotic resistance by treatment arm	
	TIP	TOBI
aztreonam		
dry colony	1.3%	6.3%
mucoïd colony	3.7%	1.9%
small colony	4.0%	- 2.5%
mixed colony types	4.7%	3.6%
ceftazidime		
dry colony	- 2.3%	11.2%
mucoïd colony	5.9%	-0.2%
small colony	3.5%	8.9%
mixed colony types	3.0%	-2.1%
ciprofloxacin		
dry colony	7.1%	5.5%
mucoïd colony	7.3%	- 0.2%
small colony	3.5%	7.9%
mixed colony types	9.1%	4.4%
imipenem		
dry colony	0.8%	4.7%
mucoïd colony	1.1%	3.2%
small colony	-16.3%	- 0.4
mixed colony types	0.5%	5.8%
meropenem		
dry colony	- 0.9%	1.2%
mucoïd colony	6.5%	0.8%
small colony	- 2.3%	- 2.8%
mixed colony types	3.6%	1.0%

Source: Table 14.2-3.4, this submission.

Reviewer's comments: Increases in antibiotic resistance among isolates from patients on therapy were noted for each antibiotic. In the *TIP treatment arm*, isolates from mucoïd colony types showed a 5.9%, 7.3% and 6.5% increase in ceftazidime, ciprofloxacin and meropenem resistance, respectively. In addition, there was 9.1% increase in ciprofloxacin resistance among mixed colony types.

In the *TOBI treatment arm*, isolates from dry colony types showed a 6.3%, 11.2% and 5.5% increase in aztreonam, ceftazidime and ciprofloxacin resistance, respectively. An 8.9% increase in ceftazidime resistance was also seen in small colony variant types. The mixed colony types showed a 5.8% increase in imipenem resistance.

SHIFTS IN SPUTUM DENSITY OF BASELINE PATHOGENS OTHER THAN *P. AERUGINOSA*

In [Study C2302](#), with over 500 patients enrolled, a range of pathogens other than *P. aeruginosa* were identified in the baseline sputum of the CF patients as would be expected in this patient population ([Table 14.2-2.2](#), this submission, not shown). The

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

majority of pathogens were present in only a small percentage of patients and therefore changes in the growth patterns are likely not representative of TIP/TOBI treatment. Those organisms for which paired (baseline and week 5 or 25) density results were available in more than 10 patients in each treatment group are discussed further below.

Methicillin-sensitive *Staphylococcus aureus* (MSSA)

In the TIP treatment group, of the patients having this pathogen present both at baseline and week 5, a higher proportion had mild and moderate growth at week 5 than at baseline (22.1% vs 14.7% mild, and 23.5% vs 20.6 % moderate,) and fewer patients had heavy growth at week 5 than at baseline (54.4% vs 64.7%). In the patients with this pathogen present both at baseline and week 21 similar trends as at week 5 were noted. These trends were broadly similar for patients with MSSA present both at baseline and week 25 (except moderate growth, where the percentage of patients was similar between baseline and week 25). In the TOBI treatment group, the trends were broadly similar to TIP treatment group.

Methicillin-resistant *Staphylococcus aureus* (MRSA)

In the TIP treatment group, of the patients having this pathogen present both at baseline and week 5, a higher proportion had moderate growth at week 5 than at baseline (23.1% vs 7.7%) and fewer patients had mild and heavy growth at week 5 than at baseline (7.7% vs 11.5% mild, and 69.2% vs 80.8% heavy, respectively). This pattern was broadly similar for changes at week 21 and 25, in particular the proportion of patients with heavy growth were consistently lower post-baseline than at baseline. The proportion of patients with mild and moderate growth of MRSA varied: more mild growth detected at week 21 and 25 than at baseline; similar proportion of moderate growth at week 21 vs baseline and a higher proportion at week 25 than at baseline.

In the TOBI treatment group, the trends were broadly similar to TIP treatment group – mainly the proportion of patients having heavy growth of MRSA was lower at any point tested (week 5, 21, 25) as compared to baseline.

Aspergillus fumigatus

In the TIP treatment group, of the patients having this pathogen present both at baseline and week 5, fewer patients had mild growth at week 5 than at baseline (27.3% vs 36.4%), similar proportion of patients had moderate growth at week 5 than at baseline (40.9%) and a higher proportion of patients had heavy growth at week 5 than at baseline (31.8% vs 22.7%). Changes at week 21 were as follows: fewer patients had mild growth, more patients had a moderate growth and a similar proportion had a heavy growth. Changes at week 25 were as follows: more patients had mild growth, a similar proportion of patients had a moderate growth and a fewer proportion had a heavy growth. It is noteworthy that

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

the proportion of patients with heavy growth decreases progressively over the study duration, from 31.8% at week 5 to 23.5% week 21 and 15.4% at week 25.

In the TOBI treatment group, the proportion of patients varied across visits and density of pathogen, but there was no obvious trend towards increase or decrease. It is noteworthy that all of the patients with heavy growth at baseline had shifts towards lower densities at the subsequent timepoints.

Haemophilus parainfluenzae

Information for more than 10 patients per treatment group is only available for baseline and termination for this pathogen. The percentage of patients with an increased growth was greater than that with decreased growth (by category) at the termination visit in both treatment groups, with a similar proportion of patient having heavy growth between treatment groups (82.9% TIP patients vs. 80.0% TOBI patients).

It is noteworthy that in patients participating in Study C2302, some pathogens of interest in CF patients were detected in less than 10 patients in each treatment group concomitantly at baseline and post-baseline timepoints – week 5, week 21 and week 25. Among them were: *Achromobacter xylosoxidans*, *Alcaligenes xylosoxidans*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia*.

TREATMENT-EMERGENT ORGANISMS

In [study C2302](#) prior inhaled antipseudomonal antibiotic use within 28 days prior to study drug administration was allowed, and therefore representing a “real-world” population with regard to tobramycin use. The majority of patients (97.5%) had a history of antipseudomonal antibiotic (including macrolide) use. The most frequently used compounds (any route of administration) were tobramycin (81.6% of patients, predominantly *i.v.*), azithromycin (59.2% of patients), ciprofloxacin (45.8% of patients, predominantly oral) and ceftazidime (27.9% of patients, mainly *i.v.*). The TIP and TOBI treatment groups showed little difference with regard to prior antibiotic use, although a slightly greater percentage of patients in the TIP treatment group had previously used ceftazidime and levofloxacin than in the TOBI group.

Furthermore, in this population, with extensive prior inhaled antibiotics (78.9% patients on TIP and 77.7% patients on TOBI have used inhaled antibiotics), mainly tobramycin usage, the organisms present at baseline provide a useful proxy for the pattern of pathogens which can be expected to arise from the regular cyclical use of tobramycin for the management of chronic *P.aeruginosa* infection.

Therefore, for [Study C2302](#), an analysis of treatment-emergent organisms in a similar fashion as for the other studies would be confounded by the baseline organisms already present due to prior antibiotic use. In this case, the Sponsor felt that the most meaningful

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

analysis was the shift in sputum density of baseline pathogens other than *P. aeruginosa*, which is described above.

Of particular interest was *B. cepacia*, an important respiratory pathogen in CF patients due its multi-drug resistance profile and the need for aggressive antibiotic treatment. A history of sputum culture of throat swab (or BAL) culture yielding *B. cepacia* within 2 years prior to screening and/or sputum sample yielding *B. cepacia* at screening was an exclusion criterion for Study C2302. Two patients in the TIP treatment arm had reported a history of *B. cepacia*.

During the study, one patient (C2302-0001-00312) had a positive culture for *B. cepacia* while having an SAE lung disorder with lung abscess which required hospitalization. The patient was discontinued from the study due to this event, and the event was not considered by the investigator to be related to the study medication.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

CLINICAL STUDY CTBM100C2303

All randomized patients in [Study C2303](#) were included in both the Safety and the ITT analysis population ([Table 31](#)). Of the 62 patients included in the ITT population, three patients had no valid screening or baseline spirometry measurements, therefore only 59 patients (31 on TIP, 28 on placebo) were included in the analyses. The ITT population included two patients allocated to the TIP group who received placebo due to Investigator error during the drug dispensation process. Three patients in the treatment group randomized to TIP were excluded from the PP population due to protocol violations, of which two were due to mis-dispensed study drug. The audiology subgroup, summarizing patients with an audiology assessment during the study, included 15 patients in the TIP treatment group and 12 placebo patients.

Table 31. Analysis populations by treatment group for [Study C2303](#)

Analysis population	TIP N=32 n (%)	placebo N=30 n (%)	Total N=62 n (%)
Randomized	32 (100.0)	30 (100.0)	62 (100.0)
Safety	30 (93.8)	32 (106.7)	62 (100.0)
Intent-to-treat (ITT)	32 (100.0)	30 (100.0)	62 (100.0)
Per-protocol	29 (90.6)	30 (100.0)	59 (95.2)
Audiology subgroup	15 (46.9)	12 (40.0)	27 (43.5)

Owing to the mis-dispensation of placebo treatment to 2 patients (PID C2303-0231-00001 and PID C2303-0231-00003) randomized to the TIP group, the safety population contained 30 patients who were treated with TIP and 32 patients who were treated with placebo.

Audiology subgroup: All patients in the safety population with at least one audiology testing.

Source: Table 4-9, Clinical Pharmacology Summary, this submission.

As noted in the CSR, further analysis populations were identified post-hoc. This included the modified ITT population (imputing zero for discontinuing patients and setting data to missing for patients with no technically acceptable spirometry on Day 29).

Study Design

[Study C2303](#) was a randomized, double-blind, placebo-controlled study. Eligible patients were randomized to receive TIP (4 x 28 mg b.i.d.) or placebo at a ratio of 1:1 for 28 days on treatment and 28 days off treatment. The exclusion criteria for [Study C2303](#) did not allow patients that had:

- any use of inhaled anti-pseudomonal antibiotics within 4 months prior to screening and
- use of systemic anti-pseudomonal antibiotics within 28 days prior to study drug administration.

Additional study design details are found in the clinical study report for [[Study C2303](#)].

The *primary objective of the study was to evaluate the efficacy* of tobramycin inhalation powder after modifications in the manufacturing process for the treatment of infections with *P. aeruginosa* in CF patients, assessed by relative change from baseline FEV1 percent predicted to day 29, compared to placebo.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

1. Pathogen identification.
2. The absolute change in *P. aeruginosa* density in sputum was determined. Absolute change from baseline, as measured by CFUs, to each post-baseline time point in Cycles 1-3 was summarized descriptively by treatment group.
3. Semi-quantitative changes in CFU/g sputum for baseline organisms other than *P. aeruginosa*.
4. The change in tobramycin MIC from baseline to each post-baseline time point in Cycles 1-3 was summarized descriptively by mean changes for each treatment group.
5. The change in frequency by categorical susceptibility description (MIC >8 µg/mL and ≤ 8 µg/mL) was also determined.
6. Isolation of treatment-emergent organisms (as part of pooled analysis with [Study C2301](#)).

Sputum specimen collection points

Sputum specimens were taken for microbiological analysis on multiple days throughout each of the studies, as shown in [Table 4-6](#) (Microbiology Methods).

CORRELATION OF CLINICAL AND MICROBIOLOGICAL OUTCOME

P. AERUGINOSA DENSITY IN SPUTUM

The Applicant performed tests to examine the change in log₁₀ *P. aeruginosa* density between baseline and the Day 29 visit in the ITT population. The results are found in [Table 32](#).

Table 32. Change of sputum density from baseline to Day 29 (ITT population)

Treatment	-- Treatment --			Comparison	----- Treatment difference -----			
	n	Mean	SE		Mean	SE	95% CI	p-value
<i>P. aeruginosa</i> sputum density (log ₁₀ CFU)								
TIP	29	-1.2	0.25	TIP-placebo	-1.2	0.35	(-1.88, -0.47)	0.002
placebo	26	0.0	0.27					
<i>P. aeruginosa</i> sputum density with NPAI imputed [#] (log ₁₀ CFU)								
TIP	29	-4.2	0.46	TIP-placebo	-4.1	0.63	(-5.32, -2.78)	<0.001
placebo	26	-0.1	0.49					

Mean = least squares mean, SE = standard error of the mean, CI = confidence interval.

P. aeruginosa sputum density refers to overall density, defined as the sum of colony types (mucoïd, dry and small colony variant).

If no *P. aeruginosa* was isolated from a valid post-baseline visit respiratory sample, log₁₀ CFU was imputed with 0 (~maximum decrease).

Source: Table 4-21, this submission. [[Study C2303-PT-Table 14.2-2.6](#)] and [[Study C2303-PT-Table 14.2-2.6i](#)]

Reviewer's comments: The sum of sputum concentration in *P. aeruginosa* colony types showed a mean absolute decrease of 1.2 log₁₀ CFU, while there was no change for the placebo treated group. The exploratory test performed for the difference between treatments indicates a statistically significant improvement for TIP treatment (p=0.002).

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

The Applicant also performed an analysis that uses the findings for *P. aeruginosa* sputum concentration based on the ITT population and NPAI (no *P. aeruginosa* isolated) imputation. IN this analysis the value was imputed as "0". When this methodology was applied, TIP versus placebo treatment achieved a higher level of statistical significance ($p < 0.001$). This analysis is misleading since the lack of cultured *P. aeruginosa* CFUs does not necessarily indicate there is a lack of isolates as the laboratory technique may be flawed or some other problem may be responsible. Also, it is well known that antibiotics such as tobramycin do not eradicate *P. aeruginosa*, the antimicrobial only decreases the CFU count, thus the likelihood of the antibiotic eradicating the organism is low to none.

The Sponsor also enumerated the sputum concentration of *P. aeruginosa* by each colony type [Table 14.2- 2.3, not shown]; for all colony types (mucoïd, dry, small colony variants and sum of all colony types), the greatest decrease in sputum density of *P. aeruginosa* relative to baseline occurred at Day 29. In the TIP treatment arm, isolates from these patients exhibited between -2.2 log₁₀ CFUs and -2.4 log₁₀ CFUs change from baseline. In the placebo treatment arm, isolates from these patients exhibited between -0.2 log₁₀ CFUs and 0.1 log₁₀ CFUs change from baseline.

CHANGES IN ANTIBIOTIC MIC DURING THERAPY

Tobramycin

A distribution table of tobramycin MIC values for *P. aeruginosa* isolates from the ITT population at each study visit was prepared for each colony type (colony type-1, mucoïd; colony type-2, dry; colony type-3, small colony variant; and maximum of colony types; a summary of these data is presented below. MIC results for the comparator agents are summarized by treatment group.

The tobramycin MIC results for the *mucoïd, dry, small colony variants* and all colony types are shown in Tables 33 to 36, respectively.

Table 33. MIC Summary for Study C2303, ITT Population; Colony type-1, Mucoïd Colony Variants

Range	Tobramycin MIC (µg/mL)							
	TIP				Placebo			
	N	Range	MIC50	MIC90	N	Range	MIC50	MIC90
Baseline	28	≤0.25-8	≤0.25	2	29	≤0.25-64	0.5	1
Day 29	14	≤0.25-8	0.5	2	27	≤0.25-256	0.5	8
Day 57	21	≤0.25->512	0.5	8	24	≤0.25-64	0.5	4
Termination	21	≤0.25->512	0.5	8	27	≤0.25-64	0.5	8

Color code: yellow=two dilution step increase over baseline; orange=three dilution step increase over baseline; red=four dilution step increase over baseline; blue=two dilution step decrease versus baseline; purple=three or more dilution step decrease versus baseline; green= two or more dilution step difference between MIC90 values for baseline study arms. Source: Table 4-23, Clinical Pharmacology Summary, this submission.

Reviewer's comments: Overall, the colony type-1 (mucoïd) isolates in the TIP treatment arm were as susceptible as isolates in the placebo arm as evidenced by baseline tobramycin MIC90 values of 2 and 1 µg/mL, respectively. This

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

represents a one-dilution difference in the baseline tobramycin MIC90 values for the two treatment arms.

The tobramycin MIC90 values for the mucoid colony isolates in the *TIP treatment arm* increased such that by both the day 57 and termination visits, the tobramycin MIC90 value had increased by 4-fold from 2 to 8 µg/mL, an increase of two dilution steps. At day 29, the tobramycin MIC90 had remained the same. It is interesting to note that the upper end of the tobramycin MIC range increased from 8 µg/mL at baseline to > 512 µg/mL, an increase of more than six dilution steps.

In the *placebo arm*, the tobramycin MIC90 value increased by three dilution steps, 1 µg/mL at baseline to 8 µg/mL at both the Day 29 and the termination visits. Also at the Day 29 visit, the upper end of the tobramycin MIC range increased from 64 µg/mL at baseline to 256 µg/mL, an increase of more than two dilution steps. It should be noted that the number of mucoid colony variants decreased on therapy for *both* treatment arms.

Table 34. MIC Summary for Study C2303, ITT Population; Colony type-2, Dry Colony Variants

Range	Tobramycin MIC (µg/mL)							
	TIP				Placebo			
	N	Range	MIC50	MIC90	N	Range	MIC50	MIC90
Baseline	23	≤0.25-1	0.5	1	24	≤0.25-32	0.5	32
Day 29	12	≤0.25-8	0.5	2	23	≤0.25->512	1	64
Day 57	15	≤0.25->512	0.5	2	19	≤0.25-4	0.5	1
Termination	17	≤0.25->512	0.5	2	25	≤0.25-128	0.5	4

Color code: yellow=two dilution step increase over baseline; orange=three dilution step increase over baseline; red=four dilution step increase over baseline; blue=two dilution step decrease versus baseline; purple=three or more dilution step decrease versus baseline; green= two or more dilution step difference between MIC90 values for baseline study arms. Source: Table 4-24, Clinical Pharmacology Summary, this submission.

Reviewer's comments: Overall, the dry colony isolates in the placebo treatment arm were *less susceptible* than those in the TIP arm as evidenced by baseline tobramycin MIC90 values of 32 and 1 µg/mL, respectively. This represents a five-dilution step difference in the baseline tobramycin MIC90 values for the two treatment arms. The tobramycin MIC90 of the baseline organisms in the placebo arm are resistant in susceptibility by the accepted CLSI breakpoints for tobramycin. This variation in baseline tobramycin MIC90 is puzzling and this Reviewer cannot offer an explanation for this difference.

The tobramycin MIC90 values for the dry colony isolates in the TIP treatment arm increased slightly such that by termination, the MIC90 value had increased by 2-fold from 1 to 2 µg/mL, an increase of one dilution step. However, at the Day 57 and the termination visits, the upper end of the tobramycin MIC range for tobramycin had increased to >512 µg/mL, an increase of more than ten dilution steps compared to the upper end of the tobramycin MIC range.

In the placebo arm, the tobramycin MIC90 value actually decreased by three dilution steps, 32 µg/mL at baseline to 4 µg/mL by the terminal visit. However,

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

the upper end of the tobramycin MIC range increased from 32 µg/mL at baseline to 128 µg/mL at the terminal visit, an increase of two dilution steps.

Table 35. MIC Summary for Study C2303, ITT Population; Colony type-3, Small Colony Variants

Range	Tobramycin MIC (µg/mL)							
	TIP				Placebo			
	N	Range	MIC50	MIC90	N	Range	MIC50	MIC90
Baseline	7	≤0.25-64	0.5	-- ^a	9	≤0.25-16	1	--
Day 29	2	1--2	-- ^b	--	1	0.5	--	--
Day 57	1	1	--	--	1	>512	--	--
Termination	2	1--2	--	--	2	0.5->512	--	--

^a MIC₉₀ value not calculated for any group of < 10 isolates

^b MIC₅₀ value not calculated for any group of < 5 isolates

Color code: yellow=two dilution step increase over baseline; orange=three dilution step increase over baseline; red=four dilution step increase over baseline; blue=two dilution step decrease versus baseline; purple=three or more dilution step decrease versus baseline; green= two or more dilution step difference between MIC90 values for baseline study arms.

Source: Table 4-25, Clinical Pharmacology Summary, this submission.

Reviewer's comments: Due to the low number of colony type-3 isolates, no tobramycin MIC50 or MIC90 values were available for either treatment arm.

In the TIP treatment arm, the upper limit of the tobramycin MIC range *decreased* from 64 µg/mL at baseline to 2 µg/mL at the termination visit, a decrease of six dilution steps.

In the placebo arm, the upper limit of the tobramycin MIC range increased from 16 µg/mL at baseline to > 512 µg/mL at the termination visit, an increase of five dilution steps.

Note the very low number of isolates at each visit for either arm of the study. This may be a factor in the variation in the tobramycin MIC values between baseline and on therapy visits. Due to the low number of isolates, it is difficult to draw conclusions.

Table 36. MIC Summary for Study C2303, ITT Population; Maximum of All Colony Types

Range	Tobramycin MIC (µg/mL)							
	TIP				Placebo			
	N	Range	MIC50	MIC90	N	Range	MIC50	MIC90
Baseline	32	≤0.25-64	0.5	2	30	≤0.25-64	0.5	4
Day 29	17	≤0.25-8	1	8	30	≤0.25->512	1	64
Day 57	24	≤0.25->512	0.5	8	27	≤0.25->512	0.5	32
Termination	24	≤0.25->512	1	8	30	≤0.25->512	0.5	8

Color code: yellow=two dilution step increase over baseline; orange=three dilution step increase over baseline; red=four dilution step increase over baseline; blue=two dilution step decrease versus baseline; purple=three or more dilution step decrease versus baseline; green= two or more dilution step difference between MIC90 values for baseline study arms.

Source: Table 4-26, Clinical Pharmacology Summary, this submission.

Reviewer's comments: Overall, all colony type isolates in the placebo treatment arm were *less susceptible* than those in the TIP treatment arm as evidenced by baseline tobramycin MIC90 values of 4 and 2 µg/mL, respectively.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

This represents a one-dilution difference in the baseline tobramycin MIC90 values for the two treatment arms.

The tobramycin MIC90 values for all colony type isolates in the TIP treatment arm increased such that by termination, the tobramycin MIC90 value had increased by 4-fold from 2 µg/mL to 8 µg/mL, an increase of two dilution steps. The same increases were seen at the Day 29 and Day 57 visits as well.

In the placebo treatment arm, the tobramycin MIC90 value increased by three and four dilution steps, at the Day 57 and Day 29 visits, respectively. By the termination visit, the tobramycin MIC90 had increased by only one dilution step.

Note that the high end of the tobramycin MIC range in the placebo treatment arm increased for the Day 29, Day 57 and termination visits by four dilution steps from the baseline visit.

ANTIBIOTIC RESISTANCE DEVELOPMENT ON THERAPY

Tobramycin

According to the current CLSI systemic interpretive criteria for tobramycin, resistance is defined as a MIC \geq 16 µg/ml for *P. aeruginosa* isolates. The MICs for *P. aeruginosa* isolates from [Study C2303](#) were examined and stratified by colony type i.e. dry, mucoid, small colony and mixed colony types for each treatment arm. The following table shows the percentage of isolates from each colony type demonstrating an increase from Baseline to the Termination visit in MIC to \geq 16 µg/ml, considered a resistant phenotype. Increases in resistance of less than 5% are not shown.

Table 37. *P. aeruginosa* tobramycin MIC increase by colony type, [Study C2303](#).

colony type	% increase by treatment arm	
	TIP (N=32)	Placebo (N=30)
dry colony	5.9%	- 8.5%
mucoid colony	4.8%	4.0%
small colony	-14.3%*	50%*
mixed colony types	4.2%	0%

*=too few samples

Source: Table 14.2-3.3, this submission.

Reviewer's comments: Increases of 5% or more in tobramycin resistance while on therapy was only observed among the dry colony types for patients in the TIP treatment arm (5.9%). Among patients treated in the placebo arm, only the small colony variant type showed an increase in tobramycin resistance while on therapy (50%) which has questionable reliability considering the small sample number.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Other Antibiotics

The Applicant examined the increased resistance levels for a variety of antibiotics from baseline to the termination visit for all colony type *P. aeruginosa* isolates. Changes in resistance levels during therapy were examined for the following antibiotics: aztreonam, ciprofloxacin, ceftazidime, imipenem and meropenem (Table 14.2-3.3, this submission, not shown). The data are summarized here in Table 38.

Table 38. Antibiotic resistance increase by *P. aeruginosa* colony type, Study C2303.

antibiotic/colony type	% increase by treatment arm	
	TIP (N=32)	Placebo (N=30)
aztreonam		
dry colony	7.5%	- 0.7%
mucoïd colony	9.5%	-13.4%
small colony	- 28.6%*	- 44.4%*
mixed colony types	9.3%	- 20.0%
ceftazidime		
dry colony	- 1.1%	3.2%
mucoïd colony	6.0%	- 2.8%
small colony	35.7%*	5.6%*
mixed colony types	3.1%	- 6.7%
ciprofloxacin		
dry colony	7.5%	- 0.7%
mucoïd colony	9.7%	1.6%
small colony	- 14.3%*	- 22.2%*
mixed colony types	4.2%	3.0%
imipenem		
dry colony	- 2.5%	3.0%
mucoïd colony	3.6%	6.0%
small colony	- 28.6%*	5.6%*
mixed colony types	- 6.2%	6.7%
meropenem		
dry colony	0.4%	7.80%
mucoïd colony	13.00%	4.0%
small colony	- 28.6%*	48.9%*
mixed colony types	3.1%	3.3%

*=too few samples

Source: Table 14.2-3.4, this submission.

Reviewer's comments: Increases in resistance on therapy were noted for each antibiotic between the two treatment arms.

In the *TIP treatment arm*, aztreonam resistance was commonplace. Dry colony, mucoïd colony and mixed colony types showed aztreonam resistance in 7.5%, 9.5%, and 9.3% of isolates, respectively. Isolates from small colony variants showed no aztreonam resistance. Ceftazidime resistance was seen in isolates from the mucoïd colony type (6.0%) and the small colony type (35.7%) but the data from the latter is questionable due to a low sample number. Isolates from dry and mixed colony types showed no ceftazidime resistance. Ciprofloxacin

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

resistance was seen in isolates from the dry colony type (7.5%) and the mucoid colony type (9.7%) but not the small colony or the mixed colony types. No isolates from any of the colony types showed imipenem resistance. Only isolates from the mucoid colony type (13.0%) showed meropenem resistance; there was no meropenem resistance among isolates from the other colony types.

In the *placebo treatment arm*, there was less antibiotic resistance than the TIP treatment arm. There was *no aztreonam or ciprofloxacin resistance* among isolates from any colony types. There was ceftazidime and imipenem resistance (5.6% for either) only in the small variant colony type isolates, however, this is questionable considering the low sample numbers. Meropenem resistance was seen in the dry colony type isolates (7.8%) and the small variant colony type isolates (38.9%) however, the data from the latter is questionable due to the low sample number.

TREATMENT-EMERGENT ORGANISMS: STUDIES C2301 AND C2303 POOLED DATA

The exclusion criteria for studies C2301 and C2303 did not allow patients that had:

- any use of inhaled anti-pseudomonal antibiotics within four months prior to screening; and
- use of systemic antipseudomonal antibiotics within 28 days prior to study drug administration.

Therefore, organisms isolated at post-baseline visits that were not present at baseline were representative of true treatment-emergent organisms based upon the somewhat tobramycin-naïve nature of the study populations. Given the efforts to exclude patients having recent antibiotic use, it was felt that the analysis of treatment-emergent organisms was most meaningful in these patient populations.

Organisms not present at baseline that appeared at End of Dosing or End of Cycle 1 in Study C2301 and C2303 pooled data are detailed in Table 39. The number of patients in the treatment groups were well balanced (TIP = 78, placebo = 79). A wide variety of species were encountered, though many species were isolated in only one patient. Those organisms present in more than one patient in one of the treatment groups are shown in Table 39. The most prevalent organisms (total isolates = six or more) were *H. parainfluenzae* (14), MSSA (12), *H. influenzae* (9), *A. fumigatus* (7), and *S. maltophilia* (6). For many of the organisms, there were similar small numbers of isolates observed in the TIP and placebo groups. In some instances (*Chryseobacterium indologenes*, *Serratia marcescens*, MRSA, *S. pneumoniae*, *H. parainfluenzae*, and *A. fumigatus*) the number of isolates observed in the placebo group was numerically greater than that observed in the TIP group. Notable exceptions where the number of isolates in the TIP group were greater than that in the placebo group were *H. influenzae* (7 TIP vs. 2 placebo), *S. maltophilia* (4 TIP vs. 2 placebo), and *Penicillium* species (4 TIP vs. 0 placebo).

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

Table 39. Treatment-emergent organisms present in more than one patient in pooled data-Study C2301 and C2303

Organism	No. Isolates ^a	
	TIP ^b	Placebo ^c
Bacteria		
<i>Achromobacter xylosoxidans</i>	2	0
<i>Alcaligenes faecalis</i>	2	0
<i>Chryseobacterium indologenes</i>	0	2
<i>Haemophilus influenzae</i>	7	2
<i>Haemophilus parainfluenzae</i>	6	8
<i>Serratia marcescens</i>	1	4
<i>Staphylococcus aureus</i> (methicillin-resistant)	1	2
<i>Staphylococcus aureus</i> (methicillin-sensitive)	6	6
<i>Stenotrophomonas maltophilia</i>	4	2
β -hemolytic <i>Streptococcus</i> , Group A	2	1
β -hemolytic <i>Streptococcus</i> , Group B	2	1
β -hemolytic <i>Streptococcus</i> , Group C	2	1
β -hemolytic <i>Streptococcus</i> , Group G	2	0
<i>Streptococcus pneumoniae</i>	1	3
Yeasts and Fungi		
<i>Candida albicans</i>	2	0
<i>Aspergillus fumigatus</i>	3	4
<i>Penicillium</i> species	4	0
Filamentous mold other than <i>Penicillium/Aspergillus</i>	2	2

a Total number of isolates for End of Dosing and End of Cycle 1

b No. patients = 78

c No. patients = 79

Source: Table 4-37 [SCE-Appendix 1-Table 3.4-1.10], this submission.

Reviewer's comments: When the total number of emergent organisms was compared, there were 57 isolates among 78 TIP treatment patients and 45 isolates among 79 patients in the placebo arm. While some patients had more than one emergent organism, roughly more than 50% of patients had emergent organisms. The discrepancy in the number of emergent organisms represents 27% more emergent isolates from patients in the TIP treatment arm than in the placebo arm. This discrepancy in this may partly explained by the increased number of *H. influenzae* isolates in the TIP treatment arm versus the placebo arm, seven versus two isolates, respectively and the number of *Penicillium* spp. isolates in the TIP treatment arm versus the placebo arm, four versus zero isolates, respectively. Also, there were twice as many isolates of *S. maltophilia* in the test treatment arms (4) than in the control treatment arms (2). *S. maltophilia* is a commensal organism that can arise in CF patients. [Sader and Jones \(2005\)](#) found that among 2076 isolates collected between 1997 and 2003, this organism had a tobramycin MIC90 value of > 16 $\mu\text{g/ml}$; thus, 78.4% of the isolates were tobramycin resistant which alerts one to the necessity of monitoring CF patients for infection by this organism.

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

DETERMINATION OF INTERPRETIVE CRITERIA

Parenteral tobramycin has been Agency-approved for multiple indications and interpretive breakpoints currently exist ([CLSI M100-S21, 2011](#)). While these interpretive criteria do not apply to inhaled tobramycin, they may be useful for describing the characteristics of bacterial isolates.

Inhaled tobramycin for the management of CF patients with *P. aeruginosa* is currently Agency approved in the form of TOBI. As it is generally understood that interpretive criteria for inhaled antibacterial products are not defined, the Sponsor did not attempt to define interpretive criteria for TIP.

REFERENCES

- [[Barclay ML, Begg EJ, Chambers ST, et al \(1996\)](#)] Adaptive resistance to tobramycin in *Pseudomonas aeruginosa* lung infection in cystic fibrosis. *J Antimicrob Chemother*; 37:1155-64.
- [[Chen Y, Garber E, Zhao Q, et al \(2005\)](#)] In vitro activity of doripenem (S-4661) against multidrug-resistant gram-negative bacilli isolated from patients with cystic fibrosis. *Antimicrob Agents Chemother*; 49:2510–11.
- [[Duan K, Dammel C, Stein J, et al \(2003\)](#)] Modulation of *Pseudomonas aeruginosa* gene expression by host microflora through interspecies communication. *Mol Microbiol*; 50:5:1477-91.
- [[Eagye KJ, Kuti JL, Sutherland CA, et al \(2009\)](#)] *In vitro* activity and pharmacodynamics of commonly used antibiotics against adult systemic isolates of *Escherichia coli* and *Pseudomonas aeruginosa* at Forty US Hospitals. *Clinical Therapeutics*; 31(11):2678-88.
- [[Fujimura T, Anan N, Sugimori G, et al \(2009\)](#)] Susceptibility of *Pseudomonas aeruginosa* clinical isolates in Japan to doripenem and other antipseudomonal agents. *International Journal of Antimicrobial Agents*; 34 (6):523-528.
- [[Gilligan PH \(2006\)](#)] Is there value in susceptibility testing of *Pseudomonas aeruginosa* causing chronic infection in patients with cystic fibrosis? *Expert Rev Anti Infect Ther* 4:711-5.
- [[Govan JR \(2006\)](#)] Multidrug-resistant pulmonary infection in cystic fibrosis--what does 'resistant' mean? *J Med Microbiol*; 55(Pt 12):1615–17.
- [[Hill D, Rose B, Pajkos A, et al \(2005\)](#)] Antibiotic susceptibilities of *Pseudomonas aeruginosa* isolates derived from patients with cystic fibrosis under aerobic, anaerobic, and biofilm conditions. *J Clin Microbiol*; 43:5085–90.
- [[MacLeod DL, Nelson LE, Shawar RM, et al \(2000\)](#)] Aminoglycoside-resistance mechanisms for cystic fibrosis *Pseudomonas aeruginosa* isolates are unchanged by long-term, intermittent, inhaled tobramycin treatment. *J Infect Dis*; 181(3):1180-4.
- [[Milne KEN, Gould IM \(2010\)](#)] Combination testing of multidrug-resistant cystic fibrosis

Division of Anti-Infective Products

Tobramycin Powder for Inhalation
Novartis Pharmaceuticals Corporation
NDA 201—688 SN000

Clinical Microbiology Review #9
Peter Coderre, PhD
07 August 2012

isolates of *Pseudomonas aeruginosa*: use of a new parameter, the susceptible breakpoint index. *J. Antimicrob. Chemother.*; 65 (1):82-90.

[Morosini MI, Garcia-Castillo M, Loza E, et al (2005)] Breakpoints for Predicting *Pseudomonas aeruginosa* Susceptibility to Inhaled Tobramycin in Cystic Fibrosis Patients: Use of High-Range Etest Strips. *J. Clin. Microbiol*; 43(9):4480-85.

[Pitt TL, Sparrow M, Warner M, et al (2003)] Survey of resistance of *Pseudomonas aeruginosa* from UK patients with cystic fibrosis to six commonly prescribed antimicrobial agents. *Thorax*; 58:794–6

[Poole K (2005)] Aminoglycoside Resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*; 49(2):479-87.

[Rhomberg PR, Jones RN (2009)] Summary trends for the Meropenem Yearly Susceptibility Test Information Collection Program: a 10-year experience in the United States (1999-- 2008). *Diagnostic Microbiol Infect Dis*; 65 (4):414-26.

[Shawar RM, MacLeod DL, Garber RL, et al (1999)] Activities of Tobramycin and Six Other Antibiotics against *Pseudomonas aeruginosa* Isolates from Patients with Cystic Fibrosis. *Antimicrob. Agents Chemother.*; 43(12):2877-80.

[Strateva T, Petrova G, Mitov I (2010)] Antimicrobial activity of tobramycin against respiratory cystic fibrosis *Pseudomonas aeruginosa* isolates from Bulgaria *J Chemother*; 22 (6):378-383.

[Traczewski MM, Brown SD (2006)] *In Vitro* Activity of Doripenem against *Pseudomonas aeruginosa* and *Burkholderia cepacia* Isolates from both Cystic Fibrosis and Non-Cystic Fibrosis Patients. *Antimicrob Agents Chemother*; 50(2):819-821.

[Tsuji A, Kobayashi I, Oguri T, et al (2005)] An epidemiological study of the susceptibility and frequency of multiple-drug-resistant strains of *Pseudomonas aeruginosa* isolated at medical institutes nationwide in Japan. *J Infect Chemother.*; 11(2):64-70.

[Valenza G, Radike K, Schoen C, et al (2010)] Resistance to tobramycin and colistin in isolates of *Pseudomonas aeruginosa* from chronically colonized patients with cystic fibrosis under antimicrobial treatment. *Scand J Infect Dis*. 42 (11-12):885-9.

[Zhou J, Garber E, Desai M, et al (2006)] Compliance of Clinical Microbiology Laboratories in the United States with Current Recommendations for Processing Respiratory Tract Specimens from Patients with Cystic Fibrosis. *J. Clinical Microbiol*; 44:1547–49.

Peter Coderre, Ph.D., M.B.A.
Microbiology Reviewer

For concurrence only,
Microbiology Team Leader, HFD-520
Frederic Marsik, Ph.D.
13 Aug 12 FIN FJM

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

/s/

PETER E CODERRE
08/13/2012

FREDERIC J MARSIK
08/13/2012

CLINICAL MICROBIOLOGY: 45-Day Meeting Checklist

NDA 201—688 SN000
TOBI Podhaler
Novartis

Peter Coderre, PhD
30 January 2012

On **initial** overview of the NDA application for RTF:

No.	Item	Yes	No	Comments
1	Is the clinical microbiology information (preclinical/nonclinical and clinical) described in different sections of the NDA organized in a manner to allow substantive review to begin?	√		
2	Is the clinical microbiology information (preclinical/nonclinical and clinical) described in different sections of the NDA indexed, paginated, and/or linked in a manner to allow substantive review to begin?	√		
3	Is the clinical microbiology information (preclinical/nonclinical and clinical) in different sections of the NDA legible so that substantive review can begin?	√		
4	On its face, has the applicant <u>submitted</u> <i>in vitro</i> data in necessary quantity, using necessary clinical and non-clinical strains/ isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?	√		
5	Has the applicant <u>submitted</u> draft provisional breakpoint and interpretive criteria, along with quality control (QC) parameters, if applicable, in a manner consistent with contemporary standards, which attempt to correlate criteria with clinical results of NDA studies, and in a manner to allow substantive review to begin?			NA
6	Has the applicant <u>submitted</u> any required animal model studies necessary for approvability of the product based on the submitted draft labeling?			NA
7	Has the applicant <u>submitted</u> all special/critical studies/data requested by the Division during pre-submission discussions?			NA
8	Has the applicant <u>submitted</u> the clinical microbiology datasets in a format which intends to correlate baseline pathogen with clinical and microbiologic outcomes exhibited by relevant pathogens isolated from test of cure or end of treatment?	√		
9	Has the applicant <u>submitted</u> a clinical microbiology dataset in a format which intends to determine resistance development by correlating changes in the phenotype (such as <i>in vitro</i> susceptibility) and/or genotype (such as mutations) of the baseline relevant pathogen with clinical and microbiologic outcome as	√		

CLINICAL MICROBIOLOGY: 45-Day Meeting Checklist

NDA 201—688 SN000
TOBI Podhaler
Novartis

Peter Coderre, PhD
30 January 2012

	exhibited by relevant pathogens isolated from test of cure or end of treatment?			
10	Has the applicant used standardized or nonstandardized methods for measuring microbiologic outcome? If nonstandardized methods were used has the applicant included full details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?	√		
11	Is the clinical microbiology draft labeling consistent with 201.56 and 201.57 of the CFR, current Divisional policy.	√		
12	FROM A CLINICAL MICROBIOLOGY PERSPECTIVE, IS THIS NDA FILEABLE? IF NO, GIVE REASONS BELOW.	√		

Any Additional Clinical Microbiology Comments:

Peter Coderre, PhD
Reviewing Clinical Microbiologist

Frederic Marsik, PhD
Team Leader Clinical Microbiology
3 Feb 12 FIN FJM

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

/s/

PETER E CODERRE
02/09/2012

FREDERIC J MARSIK
02/09/2012