CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

201820Orig1s000

MICROBIOLOGY REVIEW(S)

REV-QUALITYMICRO-02 (Review Noted (NAI))
NDA-201820
ORIG-1
Supporting Document 38
Resubmission/Class 2
Submit Date: 04/12/2012 - FDA Received Date: 04/12/2012

The April 12, 2012 Re-submission contains no new microbiology product quality information. Therefore, the initial review (submitted into DARRTS on 08 July 2011) remains valid. That review recommended approval of the submission.

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/s/

ROBERT J MELLO
06/20/2012

Product Quality Microbiology Review

07 July 2011

NDA: **201-820/N-000**

Drug Product Name
Proprietary: (Pending approval)
Non-proprietary: Tobramycin, USP, 300mg/4ml, Inhalation Solution

Review Number: 1

Dates of Submission(s) Covered by this Review

Submit	Received	Review Request	Assigned to Reviewer
22 October 2010	25 October 2010	03 November 2010	04 November 2010
21 January 2011	24 January 2011	-	-
25 February 2011	28 February 2011	-	-

Submission History (for amendments only): N/A

Applicant/Sponsor
Name: Chiesi Pharmaceuticals Inc.
Address: 9605 Medical Center Drive
Suite 380
Rockville, Maryland 20850
Representative: Erika Panico, VP, Managing Director
Telephone: 301-424-2661

Name of Reviewer: Robert J. Mello, Ph.D.

Conclusion: Recommend Approval

Product Quality Microbiology Data Sheet

A. 1. TYPE OF SUBMISSION: 505(b)(2)

2. SUBMISSION PROVIDES FOR: Marketing authorization

3. MANUFACTURING SITE: Catalent Pharma Solutions, LLC
2200 Lake Shore Drive
Woodstock, IL 60098
(FEI 1419377)

4. DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY: Sterile solution; Inhalation; [redacted (b) (4)], packaged as 300mg/4ml in a clear, LDPE, 4.5 ml, [redacted (b) (4)], [redacted (b) (4)] unit dose ampule.

5. METHOD(S) OF STERILIZATION: [redacted (b) (4)]

6. PHARMACOLOGICAL CATEGORY: Antibiotic: Management of *P. aeruginosa* infections in cystic fibrosis patients

B. SUPPORTING/RELATED DOCUMENTS: None

C. REMARKS:

- An ONDQA Initial Quality Assessment by the Chemist was completed on 20 January 2011. [redacted (b) (4)] were identified as key manufacturing operations.
- The application was submitted in electronic eCTD format.

Filename: N201820N000R1.doc

Executive Summary

I. Recommendations

A. Recommendation on Approvability – Recommend approval

B. Recommendations on Phase 4 Commitments and/or Agreements, if Approvable – N/A

II. Summary of Microbiology Assessments

A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology - Tobramycin 300 mg/4 mL Inhalation Solution is a clear, sterile, aqueous inhalation solution to be used with a nebulizer. It is formulated in [redacted] (b) (4)

B. Brief Description of Microbiology Deficiencies - None

C. Assessment of Risk Due to Microbiology Deficiencies – N/A

III. Administrative

A. Reviewer's Signature: _____

Robert J. Mello, Ph.D.
Senior Microbiology Reviewer

B. Endorsement Block: _____

John W. Metcalfe, Ph.D.
Senior Microbiology Reviewer

C. CC Block
NDA 201-820

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/s/

ROBERT J MELLO
07/08/2011

JOHN W METCALFE
07/08/2011
I concur.

NDA 201-820 Date review completed: 31 May 11
Revision1 July 2011

NOTE: There was a resubmission on 4/12/12 due to a non-approval of the original application. The non-approval had no relation to clinical microbiology. The clinical microbiology data in the re-submission was the same as in the previous submission dated 22 Oct 10. Therefore there are no clinical microbiology changes in this document.

Date company submitted: 22 Oct 10 Date received by CDER: 23 Oct 10
Reviewer: Fred Marsik, Ph.D. Date assigned: 23 Oct 10

Revision: 1 Jul 11 – In the original review a Phase 2 study was called a pivotal Phase 3 study. This is corrected in this review.

NAME AND ADDRESS OF APPLICANT

Chiesi Pharmaceuticals, Inc.
9605 Medical Center Dr.
Suite 380
Rockville, MD 20850

CONTACT PERSON

Erika Panico, RAC (US)
Vice President and Managing Director
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DRUG PRODUCT NAME

Proprietary: None at this time
Established name: Tobramycin
Code Name/Number: CHF 1538, Torineb®, Actitob®, Bramitob®
Chemical name: o-3amino-3-deoxy-α-D-glucopyranosyl-(1→6)-O-[2,6-diamino-2,3,6-trideoxy-α-D-ribohexopyranosyl-(1→4)]-2-deoxy-D-stretamine
Chemical formula: $C_{18}H_{37}N_5O_9$
Molecular weight: 467.5

PROPOSED INDICATION

Management of *Pseudomonas aeruginosa* infections in cystic fibrosis patients

PROPOSED DOSAGE FORM, ROUTE OF ADMINISTRATION, DOSAGE STRENGTH, DOSING INTERVAL, AND DURATION OF TREATMENT

Dosage form: Liquid

NDA 201-820 Date review completed: 31 May 11
Revision1 July 2011

Route of administration: Inhalation –PARI LC Plus® with the (b)(

Strength and dosing interval: 300 mg twice daily
Duration of treatment: 4 weeks

DISPENSED

Rx

RELATED DOCUMENTS

IND 72,068

REMARKS

This is a 505(b)(2) submission for the use of inhaled tobramycin to manage *Pseudomonas aeruginosa* infections in cystic fibrosis patients.

OVERALL CLINICAL MICROBIOLOGY CONCLUSION

The primary objective of the pivotal studies (CT01, CT02) were to demonstrate the non-inferiority of inhaled aerosolized CHF 1538 compared to TOBI in patients with CF with chronic infection of the lungs with *P. aeruginosa*. The primary efficacy variable was FEV_1% predicted normal at the end of the 4 week treatment phase. Consult the medical officer and statistician reviews for primary outcome results of the studies the pivotal studies as well as the additional study CT03.

Secondary microbiological efficacy variables were:

- Microbiological cultures (eradication, persistence, or re-infection with respect to *P. aeruginosa* or super-infection with respect to other bacteria) at Visits 4 and 5.
- Tobramycin MIC_{50} at Visits 4 and 5
- Tobramycin MIC_{90} at Visits 4 and 5
- Tobramycin MIC and Visits 4 and 5; and
- *P. aeruginosa* bacterial load expressed in CFUs at Visits 4 and 5

Microbiology data from all studies showed that many of the *P. aeruginosa* isolates present in the study patients had elevated MIC values to numerous anti-pseudomonas agents, and the minimal inhibitory concentration (MIC) values spanned the range of susceptible to tobramycin to resistant to tobramycin as determined by using tobramycin susceptibility test interpretive criteria for systemically administered tobramycin. Overall the susceptibility profiles of the baseline *P. aeruginosa* isolates were similar in both treatment groups.

Both treatments (CHF 1538 and TOBI) during studies pivotal CT01, CT02 and study CT03 were able to reduce baseline bacterial load in the sputum samples obtained from patients. However, bacterial load increased once tobramycin treatment of the groups stopped. There was no significant difference in the bacterial load between treatment groups after cessation of treatment. In the treatment groups there were increases in the tobramycin MIC for a portion of the *P. aeruginosa* population. There were a low percentage of microbiological eradications in both treatment groups with rates of superinfection and re-infection being similar between treatment groups. The rates of positive and negative outcomes observed for CHF 1538 and TOBI were similar.

From a clinical microbiology perspective there is no evidence in the data from the treatment study groups (CHF1538 and TOBI) that suggest that CHF 1538 is inferior to TOBI for the treatment of *Pseudomonas aeruginosa* infection in the lungs of cystic fibrosis patients.

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EXECUTIVE SUMMARY

Introduction

Cystic fibrosis (CF) is the most common inherited lethal disease of the white population. It occurs primarily in individuals of central and western European origin and affects more than 30,000 Americans. The estimated incidence in the United States is 1 in 2000 to 2600 live white births, 1 in 19,000 live African American births, 1 in 11,500 live Hispanic births, and 1 in 25,000 live Asian American births. CF has an autosomal recessive mode of inheritance. Affected individuals are phenotypic homozygotes and both parents usually are heterozygotes or carriers. The carrier frequency in white individuals in the United States is approximately 1 in 25, with full siblings or children with CF having a 1 in 4 chance of being affected. In cystic fibrosis, the defect in the cyclic adenosine monophosphate-regulated chloride ion channels in the epithelial lining of the respiratory system favors the colonization of gram-negative bacteria (mainly *P. aeruginosa*) resulting in inflammation, compromised pulmonary function and mortality in CF patients.

The microbial species that are clearly associated with lung disease in CF patients (CF lung disease) are relatively few, including *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex. *Pseudomonas aeruginosa* is the most common pathogen of CF lung disease, infecting approximately 60% of the entire CF population and close to 80% of adolescents and adults. *Staphylococcus aureus* is recovered from approximately 50% of the CF population. *Burkholderia cepacia* is recovered from ~3% of American CF patients and 15% of Canadian CF patients.

Studies have shown that *P. aeruginosa* infections in CF patients can be seen as early as infancy. The initial isolates of *P. aeruginosa* that infect CF patients are described as "rough" or "planktonic". These isolates tend to be susceptible to a variety of antimicrobials, are motile and prototrophic and have smooth lipopolysaccharide. It is at this stage that some investigators believe that aggressive antimicrobial therapy can eradicate this organism. However, most patients develop chronic infection with an unusual phenotype of *P. aeruginosa* referred to as mucoid. Mucoid isolates are nonmotile, are frequently auxotrophic have rough lipopolysaccharide, and are frequently resistant to a wide variety of antimicrobial agents. The mucoid material is a polysaccharide polymer referred to as alginate, which forms the biofilm matrix and renders the embedded *P. aeruginosa* refractory to clearance by the immune system. (**21**). Isolation of *P. aeruginosa* from the respiratory secretions of CF patients is easily accomplished.

IN VITRO

IN VITRO SPECTRUM OF ACTIVITY

The Applicant provided information on the minimal inhibitory concentrations (MICs) of tobramycin needed to inhibit the growth of *Pseudomonas aeruginosa* isolates recovered from cystic fibrosis patients in the United States. The susceptibility testing of *P. aeruginosa* isolates was done by the method of the Clinical and Laboratory Standards Institute (CLSI). Along with the susceptibility test results for the isolates the Applicant provided the quality control (QC) results for each batch of tests. All of the QC values are within the acceptable ranges as recommended by CLSI. All isolates were identified as *P. aeruginosa* by standard identification methods.

Tables 4 & 5 show tobramycin as well as other antibacterial susceptibility results over the past 3 years for *P. aeruginosa* isolates obtained from cystic fibrosis patients in the United States. This background information was obtained for isolates from the United States to compare to the tobramycin susceptibility of *P. aeruginosa* isolates obtained during clinical studies to assure that the antibiograms of US isolates were similar to those obtained during clinical studies done in other countries. The percent resistant to the various antibacterials was determined based on interpretive criteria for parenteral forms of antibacterials since there are no interpretive criteria for inhaled antibiotics. Tobramycin resistance by these criteria ($\geq$16 mcg/mL) were identified in 20.4% of *P. aeruginosa* isolated between July 2007 and June 2008 and 17.4% between January 2008 and June 2009. A comparison of the susceptibility profiles of the *P. aeruginosa* isolates from the United States and from those obtained from clinical study sites suggests that the US isolates are similar to those obtained from clinical study sites outside the US (Table 68).

Table 4 Prevalence of Resistant *P. aeruginosa* Isolates for Period

Drug	July 2007 - June 2008 (N=357)	January 2008 - June 2008 (N=178)
	N (%)	N (%)
Tobramycin	73 (20.4%)	31 (17.4%)
Amikacin	106 (29.7%)	47 (26.4%)
Aztreonam	69 (19.3%)	36 (20.2%)
Cefepime	72 (20.2%)	41 (23.0%)
Ceftazidime	70 (19.6%)	37 (20.8%)
Meropenem	51 (14.3%)	33 (18.5%)
Piperacillin/tazobactam	66 (18.5%)	35 (19.7%)
Ticarcillin/clavulanate	85 (23.8%)	44 (24.7%)
Ciprofloxacin	88 (24.6%)	41 (23.0%)
Multidrug Resistance	34 (9.5%)	17 (9.6%)

Table 5 Prevalence of High-Level Resistance Among *P. aeruginosa* Isolates

	Non-Mucoid	Mucoid	Total
	N (%)	N (%)	N (%)
July 2007 - June 2008	174	183	357
P. aeruginosa isolates	14 (8.0%)	8 (4.4%)	22 (6.2%)
Tobramycin MIC > 128 µg/mL			
January 2008 - June 2008	89	89	178
P. aeruginosa isolates	4 (4.5%)	2 (2.2%)	6 (3.4%)
Tobramycin MIC > 128 µg/mL			

Table 68: Comparison of MIC Summary Values for Baseline Isolates from CT01, CT02, CT03, and US Surveillance Isolates

Study	Treatment Arm	No. Isolates	Minimal Inhibitory Concentration (µg/mL)		
			Range	MIC_{50}	MIC_{90}
CT01	CHF1538	29	≤0.25-256	4	64
	Placebo	27	0.5-64	4	16
CT02	CHF1538	160	≤0.25-64	1	64
	Placebo	80	≤0.25-64	1	64
CT03	CHF1538	158	≤0.12->512	1	8
	TOBI	163	≤0.12->512	0.5	4
Surveillance	--	357	≤0.12->512	2	32

Source data: Table 6 , Table 23 , Table 29 , Table 56

MECHANISM OF ACTION

Tobramycin a member of the aminoglycoside family of antimicrobials interferes with the first steps of protein synthesis by causing a misreading and premature termination of the translation or the genetic code of the mRNA template. The aberrant proteins produced maybe inserted into the cell membrane leading to altered permeability and further stimulation of aminoglycoside transport. This disruption eventually leads to death of the bacterial cell.

MECHANISM OF RESISTANCE

As with other antimicrobials, resistance to aminoglycosides may be intrinsic or acquired. Intrinsic resistance may be enzymatic or nonenzymatic. Mutations at the 16S ribosomal RNA (rRNA) can result in resistance. The methylating enzymes that modify the 16S rRNA exemplify enzymatic intrinsic resistance.

Acquired resistance to aminoglycosides results from the combination of decreased drug uptake, efflux pump activity or enzymatic modification of the drug.

The predominant resistance mechanisms to tobramycin in *P. aeruginosa* isolated from CF patients is impermeability and to a lesser extend enzymatic modification. Other mechanisms which cumulatively lead to decreased susceptibility of *P. aeruginosa* to tobramycin.

POST-ANTIBIOTIC EFFECT (PAE)

The PAE is persistent suppression of bacterial growth after short antimicrobial exposure. PAE can be measured in vitro or in animal models of infection. In vivo, the

aminoglycosides consistently demonstrate a PAE that varies from 1 to 3 hours in broth and serum for *P. aeruginosa*.

TOBRAMYCIN INTERACTION WITH OTHER ANTIMICROBIALS

The mechanism of aminoglycoside synergistic activity may not be the same for all target organisms. Enhanced aminoglycoside uptake in the presence of cell-wall-active drugs (e.g. penicillins) has been demonstrated with *P. aeruginosa*.

PHARMACODYNAMICS OF TOBRAMYCIN

Tobramycin exerts its killing effect against bacteria in a concentration-dependent manner, so the higher the peak concentration of the drug (and therefore the higher C_{max}/MIC is reached), the greater is the degree of killing.

IN VITRO SUSCEPTIBILITY TEST METHODS

Susceptibility testing of *P. aeruginosa* can be done by the standardized method of the Clinical and Laboratory Standards Institute (CLSI).

DEVELOPMENT OF QC PARAMETERS FOR IN VITRO SUSCEPTIBILITY TESTING

In vitro susceptibility test QC parameters for the in vitro susceptibility testing of tobramycin are established.

INTERPRETATION OF IN VITRO SUSCEPTIBILITY TEST RESULTS

There are no interpretive criteria for topical agents such as inhaled tobramycin because the concentration of tobramycin achieved at the infected site is many fold higher then the concentration achieved when tobramycin is given systemically and there tends to be great variability in the concentration of the drug at the infected site when it is given by inhalation. While there are no clinical interpretive criteria for tobramycin related to isolates from cystic fibrosis patients that can be used to guide therapy the results of in vitro susceptibility testing of *P. aeruginosa* isolates from individual patients can be used to determine if there is an decrease in the tobramycin susceptibility of the *P. aeruginosa* which may be used indirectly to guide therapy.

IN VIVO

CLINICAL STUDY

Clinical Study CT03

This is the pivotal study for the approval of this tobramycin product. CT03 is an open-label, multinational, multicenter, randomized, parallel group study designed to compare the efficacy and tolerability of aerosolized CHF 1538 and TOBI, both administered via a nebulizer (PARI LC Plus® with the PARI Boy N® compressor, Pari, Germany), over a 4-week treatment in a twice-daily regimen in patients with CF and *P. aeruginosa* chronic infection.

> Study CMA-0631-CSR-0025: A multicentre, multinational, open label, randomized, parallel group clinical trial of Torineb™/Actitob®/Bramitob® (tobramycin solution for nebulization, 300 mg twice daily in 4 mL unit dose ampoules) compared to TOBI in the treatment of patients with cystic fibrosis and chronic infection with *Pseudomonas aeruginosa* (Module 5.3.5.1, CT03 CSR body).

The study was designed to demonstrate the non-inferiority of CHF 1538 compared to TOBI in the primary efficacy variable forced expiratory volume in one second (FEV_1) predicted normal at the end of the treatment phase in patients with CF and chronic *P. aeruginosa* infection of the lungs. Table 44 provides a summary of clinical study CT03. Among the secondary efficacy variables, microbiological tests included quantitative cell counts (CFUs) for *P. aeruginosa* isolated from sputum, and antibiotic susceptibility testing of *P. aeruginosa.*

Sputum Collection and Microbiological Culture Methods

Sputum specimens were collected at the study site and sent under refrigeration to a central laboratory (b) (4)).

Antibiotic Susceptibility Testing

Minimal inhibitory concentrations (MIC) were determined using the CLSI-recommended methods and quality controls for tobramycin and the seven other antibiotics (amikacin, aztreonam, ciprofloxacin, colistin, imipenem, levofloxacin, and piperacillin/tazobactam) were run.

Table 44: Summary of Clinical Study CT03

Study	CT03
Design	Randomized (1:1), open-label, reference product controlled, parallel group multinational, multicenter, study.
Primary Objective	To demonstrate the non-inferiority of CHF 1538 compared to TOBI in the primary efficacy variable forced expiratory volume in one second (FEV_1) % predicted normal at the end of the treatment phase in patients with CF and chronic infection of the lungs with *P. aeruginosa*.
Number of Randomized Patients	324
Age Range (yrs)	6 – 47
FEV_1 (% pred.)	≥ 40% and ≤ 80%
Comparator Daily Dose	TOBI (tobramycin 300 mg/5 mL inhalation solution for nebulization) 600 mg in two divided doses
Tobramycin Daily Dose	CHF 1538 (tobramycin 300 mg/4 mL inhalation solution for nebulization) 600 mg in two divided doses
Duration of Treatment	4 weeks treatment followed by 4-week phase-out period without treatment
Efficacy Assessment	**Primary**: final FEV_1 % predicted normal (week 4) **Secondary**: other pulmonary function tests; *in-vitro* microbiological tests including microbiological outcomes (eradication, persistence, re-infection with respect to *P.aeruginosa*, or superinfection with micro-organisms other than *P. aeruginosa*), tobramycin MIC range, MIC_{50} and MIC_{90}, and *P. aeruginosa* bacterial load (CFUs at Visit 4 and Visit 5).
Safety Assessment	Adverse events (AEs) and adverse drug reactions (ADRs), audiometric tests, laboratory parameters (hematology and blood chemistry), vital signs (heart rate and blood pressure) and physical examination.

Source: Module 5.3.5.1, CT03 Study Report Body, Section 9.1

CT03 - Microbiological Outcome

Microbiological outcome is presented by tobramycin baseline (Visit 1) MIC values, at Visit 4 ("ON" treatment), and Visit 5 ("OFF" treatment) for all *P. aeruginosa* morphotypes combined. Microbiological outcomes by baseline MIC values are compared between the two treatment groups at Visits 4 and 5 for the ITT population.

Microbiological outcome: At "ON" treatment (Visit 4), the following definitions were given on the basis of the results of the microbiologic test:

- Eradication (elimination of *P. aeruginosa*)
- Persistence (persistence of *P. aeruginosa* detected at Visit 1

• Superinfection (appearance of a pathogen other than *P. aeruginosa* not detected at Visit 1

At "OFF" treatment (Visit 5), the following definitions were given on the basis of the results of the microbiologic test:

• Eradication (elimination of *P. aeruginosa*)
• Persistence (persistence of *P. aeruginosa* detected at Visit 4 or at Visit 1 if Visit 4 is missing
• Superinfection (appearance of a pathogen other than *P. aeruginosa* not detected at Visit 4 or at Visit 1, and
• Re-infection (appearance of *P. aeruginosa* detected at Visit 1 and eradicated at Visit 4

Outcomes were analyzed according to the following hierarchy: Superinfection supersedes eradication; Persistence for *P. aeruginosa* supersedes Superinfection; and Re-infection for *P. aeruginosa* supersedes Superinfection. Overall outcomes could be designated as either a "positive outcome" (Eradication) or as a "Negative outcome" if the microbiological outcome was Persistence, Superinfection or Re-infection.

Efficacy Analysis

MIC Distributions

The distributions and percent range of tobramycin MIC results for *P. aeruginosa* clinical trial isolates at Visits 1, 4. and 5 are shown in Table 51. The range of MIC values observed for the CHF 1538 and TOBI study arms show the variability of MIC results independent of tobramycin drug exposure.

At Baseline (Visit 1) the distribution of *P. aeruginosa* with MICs $\leq$4 mcg/mL in the CHF1538 and TOBI arms was 85.4% and 85% respectively. These percentages decreased slightly in each treatment group at Visit 4 (end of "ON" cycle) as the susceptibility populations for CHF 1538 5.3% while TOBI susceptible [populations declined 7.9%. Additional change in the percentage of susceptible *P. aeruginosa* isolates was observed at the end of the first "OFF" cycle (Visit 5) compared to baseline (Visit 1) in both treatment arms; the change in percentage of susceptible isolates for CHF 1538 and TOBI was 7.9% and 9.6% respectively. These results suggest that, throughout the study, the majority of *P. aeruginosa* isolates remained susceptible to tobramycin in both treatment arms and that both tobramycin formulations produced equivalent microbiological treatment effects in the bacterial populations of *P. aeruginosa*.

Table 51: Distribution of Tobramycin MIC values (µg/mL) Overall; Summary by Visit, ITT Population

MIC (µg/mL)	CHF 1538 (n/N (%))			
	Visit 1	Visit 4	Visit 5	% Range
≤0.12	6/158 (3.8%)	8/126 (6.3%)	8/129 (6.2%)	3.8-6.3
0.25	20/158 (12.7%)	13/126 (10.3%)	17/129 (13.2%)	10.3-13.2
0.5	46/158 (29.1%)	29/126 (23.0%)	31/129 (24.0%)	23.0-29.1
1	34/158 (21.5%)	26/126 (20.6%)	25/129 (19.4%)	19.4-21.5
2	22/158 (13.9%)	18/126 (14.3%)	11/129 (8.5%)	8.5-14.3
4	7/158 (4.4%)	7/126 (5.6%)	8/129 (6.2%)	4.4-6.2
8	10/158 (6.3%)	9/126 (7.1%)	5/129 (3.9%)	3.9-7.1
16	1/158 (0.6%)	2/126 (1.6%)	9/129 (7.0%)	0.6-7.0
32	4/158 (2.5%)	2/126 (1.6%)	5/129 (3.9%)	1.6-3.9
64	3/158 (1.9%)	4/126 (3.2%)	2/129 (1.6%)	1.6-3.2
128	2/158 (1.3%)	3/126 (2.4%)	2/129 (1.6%)	1.3-2.4
256	0/158 (0%)	2/126 (1.6%)	1/129 (0.8%)	0-1.6
512	0/158 (0%)	0/126 (0%)	5/129 (3.9%)	0-5.0
>512	3/158 (1.9%)	3/126 (2.4%)	0/129 (0%)	0-1.9
Missing	0	32	29	

Table 51: Distribution of Tobramycin MIC values (µg/mL) Overall; Summary by Visit, ITT Population (Continued)

MIC (µg/mL)	TOBI (n/N (%))			
	Visit 1	Visit 4	Visit 5	% Range
≤ 0.12	11/162 (6.8%)	9/134 (6.7%)	8/128 (6.3%)	6.3-6.8
0.25	26/162 (16.0%)	15/134 (11.2%)	16/128 (12.5%)	11.2-16.0
0.5	51/162 (31.5%)	46/134 (34.3%)	39/128 (30.5%)	30.5-34.3
1	32/162 (19.8%)	19/134 (14.2%)	24/128 (18.8%)	14.2-19.8
2	16/162 (9.9%)	13/134 (9.7%)	8/128 (6.3%)	6.3-9.9
4	10/162 (6.2%)	4/134 (3.0%)	12/128 (9.4%)	3.0-9.4
8	8/162 (4.9%)	6/134 (4.5%)	4/128 (3.1%)	3.1-4.9
16	4/162 (2.5%)	5/134 (3.7%)	3/128 (2.3%)	2.3-3.7
32	2/162 (1.2%)	5/134 (3.7%)	6/128 (4.7%)	1.2-4.7
64	1/162 (0.6%)	2/134 (1.5%)	1/128 (0.8%)	0.6-1.5
128	0/162 (0%)	4/134 (3.0%)	2/128 (1.6%)	0-3.0
256	0/162 (0%)	1/134 (0.7%)	0/128 (0%)	0-0.7
512	0/162 (0%)	1/134 (0.7%)	2/128 (1.6%)	0-1.6
>512	1/162 (0.6%)	4/134 (3.0%)	3/128 (2.3%)	0.6-3.0
Missing	1	29	35	

If a patient has more than one *P. aeruginosa* morphotype at a given visit, then the highest tobramycin MIC value was used, regardless of *P. aeruginosa* morphotype.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.
Source data: Module 5.3.5.1, CT03Appendix 16.2.6.2 and 16.2.6.3

Distribution of Isolates Based Upon Tobramycin Susceptibility

Table 57 shows the distribution of tobramycin MIC values for *P. aeruginosa* for both the CHF 1538 and TOBI treatment arms using the tobramycin susceptibility interpretive criteria for parenteral tobramycin. The susceptibility profiles at the various days in both the CHF 1538 and TOBI study groups are very similar overall. A substantial proportion of isolates in both the CHF 1538 and TOBI treatment groups remained susceptible at the end of the "ON" drug period (Visit 4) with 80.2% and 79.1% susceptible respectively. At the end of Visit 4, there was an increase in the percentage of resistant isolates in both treatment groups with the greatest increase seen in the TOBI treatment group. At visit 5 (end of the "OFF" drug cycle), there was a slight decrease in the percent resistant isolates for both treatment groups relative to visit 4. The percentage of susceptible isolates was similar in both treatment groups at Visit 4 and 5. Overall, the results demonstrate an elevation in MIC value for a small portion of the isolates in both treatment groups during the course of the study.

Table 57: Tobramycin MIC Values (µg/mL) by Susceptibility Class; Summary by Visit, ITT Population

Tobramycin Susceptibility Category at Visit	CHF 1538 (N=158)	TOBI (N=163)
Visit 1		
≤4	135 (85.4%)	146 (90.1%)
8	10 (6.3%)	8 (4.9%)
≥16	13 (8.2%)	8 (4.9%)
Missing	0	1
Visit 4		
≤4	101 (80.2%)	106 (79.1%)
8	9 (7.1%)	6 (4.5%)
≥16	16 (12.7%)	22 (16.4%)
Missing	32	29
Visit 5		
≤4	100 (77.5%)	107 (83.6%)
8	5 (3.9%)	4 (3.1%)
≥16	24 (8.6%)	17 (13.3%)
Missing	29	35

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3

"Missing" includes cases where *P. aeruginosa* has been eradicated and therefore no MIC was available and instances where no specimen was collected or it was not analyzable.
If a patient has more than one *P. aeruginosa* morphotype at a given visit, then the highest tobramycin MIC value was used, regardless of *P. aeruginosa* morphotype.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.
Tobramycin systemic interpretive criteria (Susceptible, ≤ 4 µg/mL; Intermediate, 8 µg/mL; Resistant, ≥16 µg/mL)

Viable Counts - Mean Changes from Baseline

The *P. aeruginosa* bacterial density in $\log_{10}$ CFU/gram of sputum for each treatment group by study is shown in Table 58. This is a summary of the individual values for each patient. Similar bacterial load values were observed for the two treatment groups at each study visit suggesting these are typical population densities per gram of sputum colonizing the lungs of CF patients. The mean change in bacterial density from baseline levels is presented for the end of the "ON" cycle (Visit 4) and the end of the "OFF" cycle (Visit 5) in Table 59. The bacterial load showed a mean reduction of 2.14 and 2.07 $\log_{10}$ CFU/gram was observed at the end of the "ON" cycle for CHF 1538 and TOBI respectively. The bacterial load at the end of the "OFF" cycle was 0.72 and 0.87 $\log_{10}$ CFU/gram for CHF 1538 and TOBI respectively, indicating an increase in bacterial load relative to the end of the "ON" cycle. The ANCOVA model analysis results are shown in Table 60. No significant difference was evident with respect to the treatment or country while a significant difference was observed ($p<0.001$) for change from baseline $\log_{10}$ bacterial load (CFU/gram)

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Table 58: *P. aeruginosa* $\log_{10}$ Bacterial Load (CFUs/gram) Summary by Visit: ITT Population

	CHF 1538 (N=158)	TOBI (N=163)
Visit 1		
N	158	162
Mean (SD)	6.56 (1.70)	6.64 (1.57)
95% CI	[6.30; 6.83]	[6.40; 6.89]
Median	6.90	7.00
Min / Max	1.30 / 8.78	1.30 / 8.78
Missing	0	1
Visit 4		
N	152	157
Mean (SD)	4.41 (2.22)	4.58 (2.25)
95% CI	[4.06; 4.77]	[4.23; 4.93]
Median	4.62	4.90
Min / Max	1.30 / 8.75	1.30 / 8.60
Missing	6	6
Visit 5		
N	147	147
Mean (SD)	5.78 (2.20)	5.81 (2.37)
95% CI	[5.42; 6.14]	[5.42; 6.20]
Median	6.58	6.64
Min / Max	1.30 / 8.78	1.30 / 8.78
Missing	11	16

Source data: Module 5.3.5.1, CT03 Appendices 16.2.6.2 and 16.2.6.3
CFU colony forming units
If a patient has more than one *P. aeruginosa* morphotype at a given visit, and therefore more than one bacterial load value, then the bacterial load value corresponding to the highest tobramycin MIC value regardless of the *P. aeruginosa* morphotype was used.
If the tobramycin MIC value was the same for different *P. aeruginosa* morphotypes, then the bacterial load value corresponding to morphotype 1 (mucoid colony) was used. If morphotype 1 was not available, bacterial load value corresponding to morphotype 2 (dry colony) was used.
In instances of *P. aeruginosa* eradication (i.e. < 20 CFU/g, based upon the dilution factor in the plating and counting procedure), the numeric value of 20 CFU/g was used for bacterial load.
Where the bacterial load was recorded as '>600000000 CFU/g', the numeric value used for calculation of bacterial load was 600000000.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

Table 59: *P. aeruginosa* log_{10} Bacterial Load (CFUs/gram) Change from Baseline (Visit 1): ITT Population

	CHF 1538 (N=158)	TOBI (N=163)
Visit 4		
N	152	156
Mean (SD)	-2.14 (2.41)	-2.07 (2.20)
95% CI	[-2.52; -1.75]	[-2.42; -1.72]
Median	-2.09	-1.79
Min / Max	-7.48 / 4.00	-7.48 / 1.72
Missing	6	7
Visit 5		
N	147	147
Mean (SD)	-0.72 (2.17)	-0.87 (2.23)
95% CI	[-1.07; -0.36]	[-1.24; -0.51]
Median	-0.40	-0.48
Min / Max	-6.54 / 4.90	-7.48 / 6.08
Missing	11	16

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
CFU colony forming units
If a patient has more than one *P. aeruginosa* morphotype at a given visit, and therefore more than one bacterial load value, then the bacterial load value corresponding to the highest tobramycin MIC value regardless of the *P. aeruginosa* morphotype was used.
If the tobramycin MIC value was the same for different *P. aeruginosa* morphotypes, then the bacterial load value corresponding to morphotype 1 (mucoid colony) was used. If morphotype 1 was not available, bacterial load value corresponding to morphotype 2 (dry colony) was used.
In instances of *P. aeruginosa* eradication (i.e. <20 CFU/g, based upon the dilution factor in the plating and counting procedure), the numeric value of 20 CFU/g was used for bacterial load.
Where the bacterial load was recorded as '>600000000 CFU/g', the numeric value used for calculation of bacterial load was 600000000.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

Table 60: *P. aeruginosa* log_{10} Bacterial Load (CFUs/gram) ANCOVA: ITT Population

	CHF 1538 (N=158)		TOBI (N=163)
ANCOVA			
N (missing)	152 (6)		156 (7)
LSMEANS(SEM)	-1.81 (0.21)		-1.85 (0.20)
Fixed effects/Covariate: p-value			
Treatment		0.820	
Country		0.310	
Baseline log_{10} bacterial load (CFU/g) value		< 0.001	
CHF 1538 minus TOBI			
LSMEANS(SEM)		0.04 (0.18)	
95% CI		[-0.31; 0.39]	

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
ANCOVA model: Change from baseline (V1) to V4 in log_{10} bacterial load (CFU/g) value = treatment and country as fixed effects and baseline log_{10} bacterial load (CFU/g) value as covariate
All p-values are two-sided

Overall - Microbiology Outcome

Table 61 provides information on the overall analysis of microbiology outcomes for both study groups. As can be seen for study Visit 4 (end of the "ON" treatment period, there was a low percentage of microbiological eradication in both treatment groups (9.2% for CFH 1538-treated and 7.1% for TOBI-treated). This type of result is not uncommon in cystic fibrosis studies for inhaled, systemically administered and orally administered antibacterials. Similar rates of persistence and superinfection were noted in the two treatment groups. At Visit 5 (end of the "OFF" treatment period), the rates of eradication were lower than at Visit 4 in both treatment groups. While the percentage of re-infection in the CH 1538 treatment group was higher then seen in the TOBI-treatment group the numbers were small. The Cochran-Mantel-Haenzel test controlling for country revealed no statistically significant differences between the CH 1538 and TOBI with respect to percentages in outcome categories. From the data in Table 61 it appears from a microbiology perspective that CH 1538 produces equivalent results to the approved TOBI product.

Table 61: Microbiological Outcomes (Eradication, Persistence, Superinfection, Re-infection); Summary by Visit, ITT Population

	CHF 1538 (N=158)	TOBI (N=163)	CMH Test[1]
Visit 1			
Presence of *P. aeruginosa*	158 (100%)	162 (100%)	
Absence of *P. aeruginosa*	0	0	
Missing	0	1	
Visit 4[2]			
Eradication	14 (9.2%)	11 (7.1%)	p=0.692
Persistence	126 (82.9%)	133 (85.3%)	
Superinfection	12 (7.9%)	12 (7.7%)	
Missing	6	7	
Visit 5[3]			
Eradication	4 (2.7%)	5 (3.4%)	p=0.128
Persistence	116 (78.9%)	122 (83.0%)	
Superinfection	14 (9.5%)	14 (9.5%)	
Re-infection	13 (8.8%)	6 (4.1%)	
Missing	11	16	

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
[1] Cochran-Mantel-Haenszel test controlling for country
Microbiological outcomes are derived considering all *P. aeruginosa* morphotypes together.
[2] At Visit 4:
Eradication=elimination of *P. aeruginosa*
Persistence=persistence of *P. aeruginosa* detected at V1
Superinfection=appearance of a pathogen (other than *P. aeruginosa*) not detected at V1
[3] At Visit 5:
Eradication=elimination of *P. aeruginosa*
Persistence=persistence of *P. aeruginosa* detected at V4 (or at V1 if V4 missing)
Superinfection=appearance of a pathogen (other than *P. aeruginosa*) not detected at V4 or at V1
Re-infection=re-appearance of *P. aeruginosa* detected at V1 and eradicated at V4
Superinfection supercedes eradication. Persistence for *P. aeruginosa* supercedes superinfection.
Re-infection for *P. aeruginosa* supercedes superinfection.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

The analysis of microbiological outcomes is analyzed further at all study visits by baseline MIC values in Table 62. This data is further differentiated into baseline tobramycin susceptibility categories (Susceptible, Intermediate, or Resistant) in Table 63, and summarized in an overall positive or negative outcome in Table 64.

As seen in Table 62 at Visit 4, in both treatment groups, persistence occurred across a broad range of MIC values with no apparent correlation with MIC value. This type of results has been observed in other cystic fibrosis antibacterial treatment studies. At visit 5, a pattern similar to that seen in Visit 4 was observed with regard to MIC versus

eradication, persistence, and superinfection with the exception of eradication which was observed less frequently. Re-infections at Visit 5 were distributed across a wide range of MIC values in both treatment groups. There is an increase in the incidence of reinfection for both treatment arms in Visit 5 compared to Visit 4. This increase, the Applicant hypotheses, is likely do to emergence of *P. aeruginosa* in patients previously categorized as eradication.

The microbiological outcome data shows that there were no uncommon instances of microbiological eradication in either the CHF 1538 or TOBI-treatment groups at either visit 4 or 5. A high percentage of persistors were observed in the treatment groups at Visit 4 and 5, with a modest incidence of superinfection and reinfection (Visit 5). The persistence of *P. aeruginosa* during and after antibacterial treatment is not unexpected and the numbers seen in these studies are consistent with other data.

Table 62: Microbiological Outcomes (Eradication, Persistence, Superinfection, Re-infection) by Baseline MIC Value; Summary by Visit, ITT Population

	CHF 1538 (N=158)				TOBI (N=163)			
	ERAD[1]	PERS	SUPER	REINF	ERAD	PERS	SUPER	REINF
Visit 4[2]								
≤0.12	1/6 (16.7%)	5/6 (83.3%)	0	.	3/11 (27.3%)	6/11 (54.5%)	2/1 (18.2%)	.
0.25	2/19 (10.5%)	15/19 (78.9%)	2/19 (10.5%)	.	2/24 (8.3%)	19/24 (79.2%)	3/24 (12.5%)	.
0.5	6/45 (13.3%)	34/45 (75.6%)	5/45 (11.1%)	.	3/49 (6.1%)	44/49 (89.8%)	2/49 (4.1%)	.
1	2/33 (6.1%)	29/33 (87.9%)	2/33 (6.1%)	.	1/32 (3.1%)	28/32 (87.5%)	3/32 (9.4%)	.
2	2/20 (10.0%)	16/20 (80.0%)	2 /20 (10.0%)	.	0	15/15 (100.0%)	0	.
4	1/7 (14.3%)	6/7 (85.7%)	0	.	0	10/10 (100.0%)	0	.
8	0	9/10 (90.0%)	1/10 (10.0%)	.	1/7 (14.3%)	4/7 (57.1%)	2/7 (28.6%)	.
16	0	1/1 (100.0%)	0	.	0	4/4 (100.0%)	0	.
32	0	4/4 (100.0%)	0	.	1/2 (50.0%)	1/2 (50.0%)	0	.
64	0	3/3 (100.0%)	0	.	0	1/1 (100.0%)	0	.
128	0	2/2 (100.0%)	0	.	0	0	0	.
>512	0	2/2 (100.0%)	0	.	0	1/1 (100.0%)	0	.
Total	14/152 (9.2%)	126/152 (82.9%)	12/152 (7.9%)	.	11/156 (7.1%)	133/156 (85.3%)	12/156 (7.7%)	.

The microbiological outcome data analyzed above was assessed according to systemic tobramycin interpretive criteria as seen in Table 63. For both treatment groups, persistence was reported for isolates that were susceptible, intermediate or resistance to tobramycin. For both Visits 4 and 5, and in both treatment groups, more than 76% of the *P. aeruginosa* isolates were susceptible to tobramycin and yet were persistent. This suggests a lack of correlation between MICs and microbiological outcome a result that is seen in other studies.

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Table 62: Microbiological Outcomes (Eradication, Persistence, Superinfection, Re-infection) by Baseline MIC Value; Summary by Visit, ITT Population (Continued)

	CHF 1538 (N=158)				TOBI (N=163)			
	ERAD	PERS	SUPER	REINF	ERAD	PERS	SUPER	REINF
Visit 5[3]								
≤0.12	0	5/6 (83.3%)	0	1/6 (16.7%)	1/10 (10.0%)	5/10 (50.0%)	4/10 (40.0%)	0
0.25	1/19 (5.3%)	13/19 (68.4%)	2/19 (10.5%)	3/19 (15.8%)	1/23 (4.3%)	18/23 (78.3%)	3/23 (13.0%)	1/23 (4.3%)
0.5	1/39 (2.6%)	29/39 (74.4%)	6/39 (15.4%)	3/39 (7.7%)	0	39/46 (84.8%)	4/46 (8.7%)	3/46 (6.5%)
1	2/34 (5.9%)	26/34 (76.5%)	4/34 (11.8%)	2/34 (5.9%)	3/27 (11.1%)	23/27 (85.2%)	0	1/27 (3.7%)
2	0	17/21 (81.0%)	2/21 (9.5%)	2/21 (9.5%)	0	16/16 (100.0%)	0	0
4	0	5/6 (83.3%)	0	1/6 (16.7%)	0	9/9 (100.0%)	0	0
8	0	9/10 (90.0%)	0	1/10 (10.0%)	0	5/8 (62.5%)	2/8 (25.0%)	1/8 (12.5%)
16	0	1/1 (100.0%)	0	0	0	4/4 (100.0%)	0	0
32	0	4/4 (100.0%)	0	0	0	1/2 (50.0%)	1/2 (50.0%)	0
64	0	3/3 (100.0%)	0	0	0	1/1 (100.0%)	0	0
128	0	2/2 (100.0%)	0	0	0	0	0	0
>512	0	2/2 (100.0%)	0	0	0	1/1 (100.0%)	0	0
Total	4/147 (2.7%)	116/147 (78.9%)	14/147 (9.5%)	13/147 (8.8%)	5/147 (3.4%)	122/147 (83.0%)	14/147 (9.5%)	6/147 (4.1%)

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3

Table populated for patients with an available MIC value at V1. Microbiological outcomes derived considering all *P. aeruginosa* morphotypes together.

[1] Microbiological outcomes are derived considering all *P. aeruginosa* morphotypes together.

[2] At V4: ERAD=elimination of *P. aeruginosa*, PERS=persistence of *P. aeruginosa* detected at V1, SUPER=appearance of a pathogen (other than *P. aeruginosa*) not detected at V1, REINF=reinfection was not an option at Visit 4

[3] At V5: ERAD=elimination of *P. aeruginosa*, PERS=persistence of *P. aeruginosa* detected at V4 (or at V1 if V4 missing), SUPER=appearance of a pathogen (other than *P. aeruginosa*) not detected at V4 or at V1; REINF=re-appearance of *P. aeruginosa* detected at V1 and eradicated at V4. Superinfection supercedes eradication. Persistence for *P. aeruginosa* supercedes superinfection. Re-infection for *P. aeruginosa* supercedes superinfection.

Table 63: Microbiological Outcomes (Eradication, Persistence, Superinfection, Re-infection) by Baseline Tobramycin Susceptibility Designation (Susceptible, Intermediate, or Resistant); Summary by Visit, ITT Population

	CHF 1538 (N=158)			TOBI (N=163)		
	S[1]	I	R	S	I	R
Visit 4[2]						
Eradication	14 (10.8%)	0	0	9 (6.4%)	1 (14.3%)	1 (12.5%)
Persistence	105 (80.8%)	9 (90.0%)	12 (100%)	122 (86.5%)	4 (57.1%)	7 (87.5%)
Superinfection	11 (8.5%)	1 (10.0%)	0	10 (7.1%)	2 (28.6%)	0
Missing	5	0	1	5	1	0
Visit 5[3]						
Eradication	4 (3.2%)	0	0	5 (3.8%)	0	0
Persistence	95 (76.0%)	9 (90.0%)	12 (100%)	110 (84.0%)	5 (62.5%)	7 (87.5%)
Superinfection	14 (11.2%)	0	0	11 (8.4%)	2 (25.0%)	1 (12.5%)
Re-infection	12 (9.6%)	1 (10.0%)	0	5 (3.8%)	1 (12.5%)	0
Missing	10	0	1	15	0	0

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2and 16.2.6.3

[1] Baseline (V1) susceptibility: S: Susceptible (MIC ≤4 µg/mL), I: Intermediate (MIC=8 µg/mL), R: Resistant (MIC ≥ 16 µg/mL)

Table populated for patients with an available susceptibility at V1

Microbiological outcomes are derived considering all *P. aeruginosa* morphotypes together.

[2] At Visit 4: Eradication = elimination of *P. aeruginosa*
Persistence = persistence of *P. aeruginosa* detected at V1
Superinfection = appearance of a pathogen (other than *P. aeruginosa*) not detected at V1

[3] At Visit 5: Eradication = elimination of *P. aeruginosa*
Persistence = persistence of *P. aeruginosa* detected at V4 (or at V1 if V4 missing)
Superinfection = appearance of a pathogen (other than *P. aeruginosa*) not detected at V4 or at V1
Re-infection = re-appearance of *P. aeruginosa* detected at V1 and eradicated at V4

Superinfection supercedes eradication. Persistence for *P. aeruginosa* supercedes superinfection. Re-infection for *P. aeruginosa* supercedes superinfection.

Table 64 provides a summary of microbiological outcomes by visit for the ITT population. The positive and negative outcomes were very similar in the two treatment groups.

Table 64: Microbiological Outcomes (Positive vs Negative Outcomes)-Summary by Visit: ITT Population

	CHF 1538 (N=158)	TOBI (N=163)	CMH Test[1]
Visit 4			
Positive outcome[2]	14 (9.2%)	11 (7.1%)	p=0.465
Negative outcome[3]	138 (90.8%)	145 (92.9%)	
Missing	6	7	
Visit 5			
Positive outcome	4 (2.7%)	5 (3.4%)	p=0.775
Negative outcome	143 (97.3%)	142 (96.6%)	
Missing	11	16	

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
[1] Cochran-Mantel-Haenszel test controlling for country
Microbiological outcomes are derived considering all *P. aeruginosa* morphotypes together.
[2] Positive outcome = eradication
[3] Negative outcome = persistence, superinfection or re-infection
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

MIC Changes during Therapy

Table 66 provides information on the changes in tobramycin MICs in both treatment groups for visits 4 ("ON" tobramycin) and 5 ("OFF" tobramycin). Overall the tendency for MIC values to increase during conduct of the study was similar in the treatment groups.

Table 66: Tobramycin MIC Value (µg/mL) Shift from Baseline (Visit 1) Values: ITT Population

	CHF 1538 (N=158)	TOBI (N=163)
Visit 4		
Decreased[1]	19 (15.1%)	19 (14.3%)
Unchanged[2]	79 (62.7%)	78 (58.6%)
Increased[3]	28 (22.2%)	36 (27.1%)
Missing[4]	32	30
Visit 5		
Decreased	23 (17.8%)	19 (14.8%)
Unchanged	80 (62.0%)	86 (67.2%)
Increased	26 (20.2%)	23 (18.0%)
Missing	29	35

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
[1] MIC decreased: Patients whose *P. aeruginosa* isolate exhibited ≥ 4-fold decrease in the MIC between baseline and end-of-therapy or follow-up visits.
[2] MIC unchanged: Patients whose *P. aeruginosa* isolate exhibited no change or a 2-fold increase or decrease in the MIC between baseline and end-of-therapy or follow-up visits.
[3] MIC increased: Patients whose *P. aeruginosa* isolate exhibited ≥ 4-fold increase in the MIC between baseline and end-of-therapy or follow-up visits.
[4] Missing indicates that for a given patient there was no *P. aeruginosa* isolated at one of the study visits
If a patient has more than one *P. aeruginosa* morphotype at a given visit, then the highest tobramycin MIC value was used, regardless of *P. aeruginosa* morphotype.
When MIC value was recorded as '≤ 0.12 µg/mL', the numeric value used for calculation of shift from baseline was 0.125 µg/mL.
When MIC value was recorded as '> 512 µg/mL', the numeric value used for calculation of shift from baseline was 1024 µg/mL.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

CLINICAL MICROBIOLOGY CONCLUSION

From a clinical microbiology perspective there is no evidence in the data from the treatment study groups (CHF1538 and TOBI) that suggest that CHF 1538 is inferior to TOBI for the treatment of *Pseudomonas aeruginosa* infection in the lungs of cystic fibrosis patients.

INTRODUCTION

Cystic fibrosis (CF) is the most common inherited lethal disease of the white population. It occurs primarily in individuals of central and western European origin and affects more than 30,000 Americans. The estimated incidence in the United States is 1 in 2000 to 2600 live white births, 1 in 19,000 live African American births, 1 in 11,500 live Hispanic births, and 1 in 25,000 live Asian American births (**1**). CF has an autosomal recessive mode of inheritance. Affected individuals are phenotypic homozygotes and both parents usually are heterozygotes or carriers. The carrier frequency in white individuals in the United States is approximately 1 in 25, with full siblings or children with CF having a 1 in 4 chance of being affected (**1**). In cystic fibrosis, the defect in the cyclic adenosine monophosphate-regulated chloride ion channels in the epithelial lining of the respiratory system favors the colonization of gram-negative bacteria (mainly *P. aeruginosa*) resulting in inflammation, compromised pulmonary function and mortality in CF patients (**2,3,4,5**). Upon entry of *P. aeruginosa* in CF lungs, penetration of mucous surface and colonization is followed by a genetic transformation of non-mucoid to mucoid (alginate-producing) phenotype (**6,7**). Although non-mucoid bacteria are more virulent, they are also better recognized by the immune response, while the mucoid phenotype increases bacterial adherence and resistance to phagocytosis (**7,8**). As the infection progresses, bacteria release virulence factors through quorum-sensing (bacterial communication), inducing the inflammatory response (**8**). Unlike normal airway secretions, the inflammatory response increases concentrations of neutrophils-derived DNA and filamentous actin in the CF mucous, which interact and bind to glycoproteins (e.g. mucin) (**9,10,11,12**). The physical interaction of abnormally high concentrations of such factors results in viscous layers (sputum) that cover the epithelia surface and allow for bacterial adherence and biofilm protection (**13,14**).

The microbial species that are clearly associated with lung disease in CF patients (CF lung disease) are relatively few, including *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex (**15**). Organisms having a secondary role in CF lung disease include respiratory viruses, such as respiratory syncytial virus and influenza virus; *Haemophilus influenzae*; and *Aspergillus fumigatus*. *Mycobacterium* spp. but not *Mycobacterium tuberculosis*, *Stenotrophomonas maltophilia* and *Alcaligenes xylosoxidans* are being seen with increasing frequency in CF patients, in part because of the increasing life span of CF patients and the relentless use of antimicrobial agents in this population (**15**). However, the role of the latter organisms in CF lung disease has not been clearly determined. In addition, organisms that phenotypically resemble *B. cepacia* complex organisms (i.e. *Burkholderia gladioli*, *Ralstonia* spp., and *Pandoraea* spp.) are also being seen with increasing frequencies due to the use of selective media that improve the rates of recovery of *B. cepacia* complex isolates (**15**).

Pseudomonas aeruginosa is the most important pathogen of CF lung disease, infecting approximately 60% of the entire CF population and close to 80% of adolescents and adults (**16**). *Staphylococcus aureus* is recovered from approximately 50% of the CF

population. *Burkholderia cepacia* is recovered from ~3% of American CF patients and 15% of Canadian CF patients. The repercussions of infection with *B. cepacia* are extensive. In CF patients, infection with *B. cepacia* complex is associated with increased rates of mortality and morbidity. Roughly 20% of CF patients colonized with *B. cepacia* complex develop the "cepacia syndrome" (**17**). These patients experience a rapid decline in pulmonary function, frequent bacteremia, and ultimately death due to lung failure. Patient to patient transfer of *B. cepacia* has been documented (**17**). The review will concentrate on *P. aeruginosa* since the Sponsor is targeting this organism with nebulized amikacin.

Studies have shown that *P. aeruginosa* infections in CF patients can be seen as early as infancy (**18**). The initial strains of *P. aeruginosa* that infect CF patients are described as "rough" or "planktonic" strains. These strains tend to be susceptible to a variety of antimicrobials, are motile and prototrophic and have smooth lipopolysaccharide. It is at this stage that some investigators believe that aggressive antimicrobial therapy can eradicate this organism (**19)**. However, most patients develop chronic infection with an unusual phenotype of *P. aeruginosa* referred to as mucoid. Mucoid isolates are nonmotile, are frequently auxotrophic have rough lipopolysaccharide, and are frequently resistant to a wide variety of antimicrobial agents (**20**). The mucoid material is a polysaccharide polymer referred to as alginate, which forms the biofilm matrix and renders the embedded *P. aeruginosa* refractory to clearance by the immune system. (**21**). Isolation of *P. aeruginosa* from the respiratory secretions of CF patients is easily accomplished (**21**).

Susceptibility testing of *P. aeruginosa* isolates recovered from CF patients is an area of some controversy. Multiple morphotypes may be recovered from patient sputum. Studies that compared the performance of susceptibility testing of a mixture of different morphotypes versus the performance of testing each morphotypes showed the testing a mixture of morphotypes may underestimate resistance (**22**). Two areas of controversy exist in the area of susceptibility testing of *P. aeruginosa* isolates. The first is that *P. aeruginosa* grows anaerobically within the airways of CF patients (**23**). If this is true then aminoglycosides which have not activity anaerobically should not be active against *P. aeruginosa*. This directly conflicts with the results of studies that show aminoglycosides given intravenously and aerosolized have a positive impact on the lung functions and life expectancies of CF patients (**24**). However, anaerobically growing *P. aeruginosa* may contribute to this organism's ability to persist in the face of high concentrations of aminoglycosides (**23**). A second area of controversy is the microbial form of *P. aeruginosa* that should be used for susceptibility testing of *P. aeruginosa*. The two forms are the biofilm form and the planktonic form. The susceptibilities of mucoid *P. aeruginosa* isolates growing as biofilm have been compared to those strains growing planktonically, which are used in clinical laboratory susceptibility testing. Recently, equipment called the "Calgary device" has been developed that allows susceptibility testing to be performed with mucoid *P. aeruginosa* isolates growing as biofilms.

P. aeruginosa grown in a biofilm were significantly more resistant to antipseudomonal drugs. This data suggests that susceptibility tests obtained by testing planktonically growing isolates may underestimate the drug resistance of mucoid *P. aeruginosa* (**24**).

IN VITRO

IN VITRO SPECTRUM OF ACTIVITY

The Applicant provided information on the minimal inhibitory concentrations (MICs) of tobramycin to inhibit *Pseudomonas aeruginosa* isolates recovered from cystic fibrosis patients in the United States. The susceptibility testing of *P. aeruginosa* isolates was done by the method of the Clinical and Laboratory Standards Institute (CLSI) (**25,26**). Along with the susceptibility test results for the isolates the Applicant provided the quality control (QC) results for each batch of tests. All of the QC values are within the acceptable ranges as recommended by CLSI (**27**) [data not shown (**28**)]. All isolates were identified as *P. aeruginosa* by standard identification methods.

Tables 1 to 5 show the results over the past 3 years for *P. aeruginosa* isolates obtained from cystic fibrosis from the United States for tobramycin and a variety of other antimicrobials. This background information was obtained for isolates from the United States to compare to the tobramycin susceptibility of *P. aeruginosa* isolates obtained during clinical studies to assure that the antibiograms of these US isolates were similar to those obtained during clinical studies done in other countries. The percent resistant to the various antibacterials was determined based on interpretive criteria for parenteral forms of antibacterials (**2**) since there are no interpretive criteria for inhaled antibiotics. Tobramycin resistance by these criteria ($\geq$16 mcg/mL) were identified in 20.4% of *P. aeruginosa* isolated between July 2007 and June 2008 and 17.4% between January 2008 and June 2009 (Table 4). While the *P. aeruginosa* isolates with MICs of $\geq$16 mcg/mL were considered resistant to tobramycin given by the parenteral route the amount of tobramycin reaching the lungs is many time more then 16 mcg/mL therefore the growth of *P. aeruginosa* in the lung may be inhibited by the higher concentrations of tobramycin.

TABLES AND FIGURES

Table 1 Study Enrollment and *P. aeruginosa* Isolates from Sputum

	July 2007 - June 2008	January 2008 - June 2008
Number of unique subjects	137	68
Number of *P. aeruginosa* isolates	357	178

Table 2 Prevalence of Mucoid and Non-Mucoid Phenotypes

	July 2007 - June 2008	January 2008 - June 2008
P. aeruginosa isolates identified from study subjects	357	178
Non-mucoid phenotype	174 (48.7%)	89 (50%)
Mucoid phenotype	183 (51.3%)	89 (50%)

Table 3 MIC_{50} and MIC_{90} Results for *P. aeruginosa* Isolates[1]

Drug	July 2007- June 2008 (N=357)			January 2008- June 2008 (N=178)		
	MIC Range	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	MIC Range	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Tobramycin	≤ 0.12 - > 512	2	32	≤ 0.12 - > 512	2	32
Amikacin	≤ 0.5 - > 128	16	128	≤ 0.5 - > 128	16	128
Aztreonam	≤ 1 - > 128	2	64	≤ 1 - > 128	4	128
Cefepime	≤ 0.5 - > 64	8	64	≤ 0.5 - > 64	8	64
Ceftazidime	≤ 0.5 - > 64	2	64	≤ 0.5 - > 64	2	> 64
Meropenem	≤ 0.12 - > 32	0.5	16	≤ 0.12 - > 32	0.5	16
Piperacillin/tazobactam	≤ 0.5 -> 256	4	256	≤ 0.5 - > 256	4	256
Ticarcillin/clavulanate	≤ 4 - > 256	16	› 256	≤ 4 - > 256	16	> 256
Ciprofloxacin	≤ 0.12 - > 8	1	8	≤ 0.12 - > 8	1	8

[1] MIC_{50} = MIC value at which 50% of isolates were inhibited
MIC_{90} = MIC value at which 90% of isolates were inhibited

Table 4 Prevalence of Resistant *P. aeruginosa* Isolates for Period

Drug	July 2007 - June 2008 (N=357)	January 2008 - June 2008 (N=178)
	N (%)	N (%)
Tobramycin	73 (20.4%)	31 (17.4%)
Amikacin	106 (29.7%)	47 (26.4%)
Aztreonam	69 (19.3%)	36 (20.2%)
Cefepime	72 (20.2%)	41 (23.0%)
Ceftazidime	70 (19.6%)	37 (20.8%)
Meropenem	51 (14.3%)	33 (18.5%)
Piperacillin/tazobactam	66 (18.5%)	35 (19.7%)
Ticarcillin/clavulanate	85 (23.8%)	44 (24.7%)
Ciprofloxacin	88 (24.6%)	41 (23.0%)
Multidrug Resistance	34 (9.5%)	17 (9.6%)

Table 5 Prevalence of High-Level Resistance Among *P. aeruginosa* Isolates

	Non-Mucoid	Mucoid	Total
	N (%)	N (%)	N (%)
July 2007 - June 2008	174	183	357
P. aeruginosa isolates	14 (8.0%)	8 (4.4%)	22 (6.2%)
Tobramycin MIC > 128 µg/mL			
January 2008 - June 2008	89	89	178
P. aeruginosa isolates	4 (4.5%)	2 (2.2%)	6 (3.4%)
Tobramycin MIC > 128 µg/mL			

The tobramycin and other antimicrobial susceptibilities of isolates obtained during the Phase 3 clinical trials are shown in Table 67. Table 68 shows a comparison of the *P. aeruginosa* isolates at baseline from the various studies and the US surveillance isolates. As seen the susceptibilities of the *P. aeruginosa* obtained during the Phase 3 trials were quite similar to the susceptibility profiles of the *P. aeruginosa* from US patients.

Chiesi 2.7.2 Summary of Clinical Pharmacology Studies

Table 67: Comparison of Susceptibility of *P. aeruginosa* from Clinical Study CT03 to US Surveillance Isolates

Antibiotic	Isolate Source	No.	MIC Range	MIC_{50}	MIC_{90}
Tobramycin	CHF 1538 Arm	158	≤0.12- > 512	1	8
	TOBI Arm	162	≤0.12- > 512	0.5	4
	Surveillance	357	≤0.12- > 512	2	32
Amikacin	CHF 1538 Arm	158	≤0.25- > 512	8	64
	TOBI Arm	162	≤0.25-512	4	32
	Surveillance	357	≤0.5- > 128	16	128
Aztreonam	CHF 1538 Arm	158	≤0.25- > 512	4	128
	TOBI Arm	162	≤0.25- > 512	4	128
	Surveillance	357	≤1- >128	2	64
Ciprofloxacin	CHF 1538 Arm	158	≤0.12- > 256	1	4
	TOBI Arm	162	≤0.12-64	0.5	4
	Surveillance	357	≤0.12- > 8	1	8
Piperacillin/tazobactam	CHF 1538 Arm	158	≤0.25- > 512	4	512
	TOBI Arm	162	≤0.25- > 512	4	128
	Surveillance	357	≤0.5- > 256	4	256
Levofloxacin	CHF 1538 Arm	158	≤0.25- > 512	2	16
	TOBI Arm	162	≤0.25-128	1	8
Colistin	CHF 1538 Arm	158	0.25- > 128	1	4
	TOBI Arm	162	≤0.12- > 128	1	4
Imipenem	CHF 1538 Arm	158	≤0.25- >256	1	32
	TOBI Arm	162	≤0.25-256	1	16
Meropenem	Surveillance	357	≤0.12- > 32	0.5	16
Ticarcillin/clavulanate	Surveillance	357	≤4- > 256	16	> 256
Cefepime	Surveillance	357	≤0.5- > 64	8	64
Ceftazidime	Surveillance	357	≤0.5- > 64	2	64

Source data: Table 6 and Table 56

Table 68: Comparison of MIC Summary Values for Baseline Isolates from CT01, CT02, CT03, and US Surveillance Isolates

Study	Treatment Arm	No. Isolates	Minimal Inhibitory Concentration (µg/mL)		
			Range	MIC_{50}	MIC_{90}
CT01	CHF1538	29	≤0.25-256	4	64
	Placebo	27	0.5-64	4	16
CT02	CHF1538	160	≤0.25-64	1	64
	Placebo	80	≤0.25-64	1	64
CT03	CHF1538	158	≤0.12- > 512	1	8
	TOBI	163	≤0.12- > 512	0.5	4
Surveillance	--	357	≤0.12- > 512	2	32

Source data: Table 6 , Table 23 , Table 29 , Table 56

MECHANISM OF ACTION

Tobramycin a member of the aminogylcoside family of antimicrobials interferes with the first steps of protein synthesis by causing a misreading and premature termination of the translation or the genetic code of the mRNA template. The aberrant proteins produced maybe inserted into the cell membrane leading to altered permeability and further stimulation of aminoglycoside transport. This disruption eventually leads to death of the bacterial cell.

PHARMACODYNAMICS OF TOBRAMYCIN

Tobramycin exerts its killing effect against bacteria in a concentration-dependent manner, so the higher the peak concentration of the drug (and therefore the higher C_{max}/MIC is reached), the greater is the degree of killing (**31**).

MECHANISM OF RESISTANCE

As with other antimicrobials, resistance to aminoglycosides may be intrinsic or acquired. Intrinsic resistance may be enzymatic or nonenzymatic. Mutations at the 16S ribosomal RNA (rRNA) can result in resistance. The methylating enzymes that modify the 16S rRNA exemplify enzymatic intrinsic resistance.

Acquired resistance to aminoglycosides results from the combination of decreased drug uptake, efflux pump activity or enzymatic modification of the drug.

All enterococci have intrinsic resistance to aminoglycosides with MICs ranging from 4 to 256 mcg/mL. The resistance in this case is attributed to the facultative anaerobic metabolism of enterococci which in turn reduces the transmembrane potential and hence

limits uptake of the aminoglycoside. Acquisition of genes that encode aminoglycoside-modifying enzymes leads to high-level aminoglycoside resistance with loss of synergistic activity with penicillins or vancomycin.

The predominant mechanism of resistance to tobramycin in *P. aeruginosa* isolated from CF patients is impermeability and to a lesser extend enzymatic modification and other mechanisms which cumulatively lead to decreased susceptibility of *P. aeruginosa* to tobramycin.

POST-ANTIBIOTIC EFFECT (PAE)

The PAE is persistent suppression of bacterial growth after short antimicrobial exposure. PAE can be measured in vitro or in animal models of infection. In vivo, the aminoglycosides consistently demonstrate a PAE that varies from 1 to 3 hours in broth and serum for *P. aeruginosa* (**29**).

TOBRAMYCIN INTERACTION WITH OTHER ANTIMICROBIALS

The mechanism of aminoglycoside synergistic activity may not be the same for all target organisms. Enhanced aminoglycoside uptake in the presence of cell-wall-active drugs (e.g. penicillins) has been demonstrated with *P. aeruginosa* (**30**).

IN VITRO SUSCEPTIBILITY TEST METHODS

Susceptibility testing of *P. aeruginosa* can be done by the standardized method of the Clinical and Laboratory Standards Institute (CLSI) (**25**).

DEVELOPMENT OF QC PARAMETERS FOR IN VITRO SUSCEPTIBILITY TESTING

In vitro susceptibility test QC parameters for the in vitro susceptibility testing of tobramycin have been established (**27**).

INTERPRETATION OF IN VITRO SUSCEPTIBILITY TEST RESULTS

There are no interpretive criteria for topical agents such as inhaled tobramycin because the concentration of tobramycin achieved at the infected site is many fold higher then the concentration achieved when tobramycin is given systemically.

IN VIVO

CLINICAL STUDIES

Studies CT01 and CT02

CT01 – Study DM/RS/10000/001/04 – Double blind, multicenter, randomized, placebo-controlled, parallel groups clinical trial of CHF 1538 tobramycin 300 mg/4 mL inhalation solution (300 mg BID) in the 4-week treatment (plus 4 weeks or run-out) of patients with CF and a positive culture of *P. aeruginosa* (See Module 5.3.5.1, CT01 CSR Body)

CT02 – Study DM/RS/10000/002/04 – Double-blind, multicenter, randomized, placebo-controlled, parallel groups clinical trial of intermittent CHF 1538 (tobramycin 300 mg/mL inhalation solution) or placebo in three 4-week cycles of treatment, given in addition to other anti-pseudomonal treatments, in patients with CF and a positive culture for *P. aeruginosa* (see Module 5.3.5.1, CT02 CSR Body)

In addition, the clinical development program of CHF 1538 also included two additional studies, a clinical pharmacokinetic study comparing bioavailability of CHF-1538 versus TOBI (Study CP01, and an active-comparator-controlled clinical study of CHF 1538 versus TOBI (Study CT03).

Studies CT01 (Phase II) and CT02 (Phase III) were designed to demonstrate the superior efficacy of CHF 1538 (300 mg administered BID) versus inhaled aerosolized placebo in terms of improved lung function and microbiological outcomes. In Study CT02, patients were randomized 2:1 to treatment with either CHF 1538 or placebo. The study designs for both trials are summarized in Table 16 to 19.

The clinical studies with CHF 1538 were performed in Europe. Study CT01 was conducted at 13 active centers located in four countries: Ukraine (seven), Italy (three), France (two) and Moldavia (one). Study Ct02 was conducted in 21 active centers located in three countries: Poland (nine), Hungary (eight, Russia (four).

Microbiology

Bacterial culture of sputum samples was performed at the local study site laboratory in both studies. Antibiotic susceptibility testing included testing of *P. aeruginosa* isolates was done at the local site laboratory in study CT01. In Study CT02, the MIC assessment of tobramycin was also performed by a central analytical laboratory in (b) (4)). The in vitro sputum samples were sent from the investigational sites in all countries using a refrigerated courier with temperature monitoring.

Efficacy variables

The primary efficacy variable of study CT01 and CT02

Chiesi 2.7.2 Summary of Clinical Pharmacology Studies

Table 16: Summary of Clinical Studies CT01 and CT02

Study	CT01	CT02
Design	Randomized (1:1), Double-Blind, placebo-controlled, parallel groups, multicenter study	Randomized (2:1), Double-Blind, placebo-controlled, parallel groups, multicenter study
Primary Objective	Efficacy of inhaled aerosolized tobramycin and placebo in the 4-week treatment of patients with CF and *P. aeruginosa* infection	To demonstrate the superior efficacy of aerosolized intermittent administration of CHF 1538 (300 mg BID) compared to aerosolized Placebo saline solutions given following three 4-week "ON"/4-week "OFF" treatment cycles in CF patients infected with *P. aeruginosa* infection. Four-week treatment periods ("ON" cycles) were followed by 4-week periods without treatment ("OFF" cycles).
Number of Randomized Patients	59	247
Age Range (yrs)	6-30	6-45
FEV_1 (% pred.)	≥ 40% and ≤ 80%	≥ 40% and ≤ 80%
Comparator(s)	Inhaled aerosolized placebo	Inhaled aerosolized placebo
Tobramycin Daily Dose	600 mg in two divided doses	600 mg in two divided doses
Duration of Treatment	4 weeks treatment followed by 4-week phase-out period without treatment	4-week treatment periods ("ON" cycles) followed by 4-week periods without treatment ("OFF" cycles; three "ON" cycles and three "OFF" cycles in total
Efficacy Assessment	**Primary**: final FEV_1 (week 4) **Secondary**: other pulmonary function tests; *in vitro* microbiological tests (tobramycin MIC range, MIC_{90} and MIC_{50}); microbiological outcome (eradication, persistence, superinfection, re-infection)	**Primary**: final FEV_1 (week 20 or end of the last "ON" cycle if premature withdrawal) **Secondary**: other pulmonary function tests; *in vitro* microbiological tests (tobramycin MIC range, MIC_{50} and MIC_{90}); microbiological outcome (eradication, persistence, superinfection, re-infection); number of exacerbations, hospitalizations, number of missed school or work days
Safety Assessment	Adverse events, laboratory parameters (hematology and blood chemistry), vital signs (heart rate and blood pressure), audiometric tests	Serum creatinine, adverse events, other laboratory parameters (hematology and blood chemistry), vital signs (heart rate and blood pressure), audiometric tests

Source: Module 5.3.5.1 CT01 Study Report Body, Section 8.1, 9.1, 9.5 and Module 5.3.5.1 CT02 Study Report Body, Section 8., 9.1, 9.5

Table 17: Inclusion and Exclusion Criteria for Studies CT01 and CT02

Patients were enrolled into the treatment period if they met the following criteria:	
Inclusion Criteria:	Patients of either sex aged ≥ six years. Clinical diagnosis of CF defined as follows: • patients registered in the National Registry of CF (or other documents depending on country legislation); • evidence of one or more features in pulmonary abnormalities (persistent colonization/infection with typical CF pathogens, chronic cough and sputum production, persistent chest radiography abnormalities, airways obstruction, nasal polyps and/or digital clubbing), gastrointestinal and nutritional abnormalities (intestinal, pancreatic, hepatic, nutritional); • positive response (sweat chloride concentration ≥ 60 mmol/L) in the standard sweat test documented in the clinical records and/or gene mutation documented in the clinical records. Sputum containing *P. aeruginosa*; in Study CT01, *P. aeruginosa* must be susceptible to tobramycin by an MIC value based on microdilution testing system used by the local laboratory or by a zone diameter ≥16 mm with 10 µg tobramycin disk. Forced expiratory volume in one second (FEV_1) ≥ 40% and ≤ 80% of the predicted normal value. A co-operative attitude and ability to be trained to correctly use the nebulizer and the provided drug. Written informed consent obtained.
Patients were <u>not</u> enrolled into the treatment period if they met any of the following criteria:	
Exclusion Criteria:	Administration of antipseudomonal antibiotic therapy by any route and (in Study CT02 only, nebulized antibiotic therapy) in the previous 4 weeks. Signs of impaired renal function (serum creatinine level ≥ 1.5 mg/dL). Signs of impaired auditory function (auditory threshold in either ear above 20 dB at frequencies between 250 and 8000 Hz). Sputum culture containing *Burkholderia cepacia*. Patients with end-stage lung disease, candidates for heart-lung transplantation. History of other clinically significant cardiac, renal, neurologic, hepatic or endocrine disease to CF, whose sequelae and/or treatment could interfere with (in Study CT01, the conduct of and) the results of the present study. Pregnant or lactating females or females at risk of pregnancy, i.e. those not demonstrating adequate contraception. A pregnancy test was to be done in fertile aged women. Known hypersensitivity to aminoglycosides; Concomitant participation in another trial.

Source: Module 5.3.5.1 CT01 Study Report Body, Section 9.3.1 and Module 5.3.5.1 CT02 Study Report Body, Section 9.3.2

Table 18: Schematic Design for Study CT01

	Run-in Period (1-8 days)	"ON" Cycle	"ON" Cycle	"ON" Cycle	"OFF" Cycle
Weeks	-1	0	2	4	8
Visit[1]	1	2	3	4	5

[1] Visits took place at the clinics before and after the run-in period (baseline, Visit 1), and after 2 weeks (Visit 3) and four weeks (Visit 4) with a follow-up visit at eight weeks (Visit 5) with an acceptable variation of a maximum three days at each visit.

Table 19: Schematic Design for Study CT02

	Run-in Period (1-8 days)	"ON" Cycle		"OFF" Cycle	"ON" Cycle	"OFF" Cycle	"ON" Cycle	"OFF" Cycle
Weeks	-1 (Approx.) to 0	0 to 2	2 to 4	4 to 8	8 to 12	12 to 16	16 to 20	20 to 24
Visit[1]	1	2 to 3	3 to 4	4 to 5	5 to 6	6 to 7	7 to 8	8 to 9

[1] The study plan included a screening visit (Visit 1, study entry), a run-in period (minimum one, maximum eight days), and three 4-week treatment periods ("ON" cycles) with the assigned drug treatment, each followed by a 4-week run-out period ("OFF" cycle)

Pharmacokinetics of tobramycin

Table 1 shows a summary of the clinical pharmacology studies.

Chiesi 2.7.2 Summary of Clinical Pharmacology Studies

Table 1: Summary Table of the Clinical Pharmacology Studies

Study	Aim of the Study	Study Design/Population	Study Medications	Primary Endpoints
CP01	To evaluate the PK of tobramycin in plasma and sputum of CF patients after a single administration by nebulization of CHF1538 in comparison to the marketed formulation TOBI®	Single dose, randomized, double-blind, two-way crossover study in CF patients	CHF 1538, 300 mg/4 mL unit-dose ampule TOBI, 300 mg/5 mL unit-dose ampule	CHF 1538 concentration and PK parameters in plasma and sputum
CT01 PK Substudy	To measure the local sputum concentration of tobramycin in a subset of patients treated with CHF 1538	Multiple dose, randomized, double-blind, placebo-controlled, parallel groups, multicenter study in CF patients	CHF 1538, 300 mg/4 mL unit-dose ampule Placebo	CHF 1538 concentration in sputum ten minutes after the first and the last dose (Day 28 of treatment) and after 4-week wash-out (Day 56)

Definition of Data Sets

Intent-to Treat – Includes all randomized patients who received at least one dose of study medication and had post-baseline data
Per-Protocol (PP) – Includes all ITT patients who met all inclusion/exclusion criteria and who did not have any major protocol deviation. The primary efficacy endpoint was analyzed on this population.
Safety-Population – Includes all randomized patients who took at least one dose of study medication.

Definition of the data Sets Analyzed

- The Intent-to Treat (ITT) population included all randomized patients who received at least one dose of study medication and had post baseline data;
- The Per Protocol (PP) population include all ITT patients who met all inclusion/exclusion criteria and who did not have any major protocol deviation. The primary efficacy endpoint was analyzed on this population.

Table 22 shows the study analysis populations for studies CT01 and CT02.

Table 22: Study Analysis Populations for Studies CT01 and CT02

Study	CT01		CT02	
	CHF 1538	Placebo	CHF 1538	Placebo
Total	67		312	
Randomized	59		247	
	29	30	161	86
Intent to Treat	59		245	
	29	30	161	84
Per Protocol	56		215	
	28	28	144	71
Safety	59		246	
	29	30	161	85

Source: Module 5.3.5.1, CT01 Table 5 and 38; CT01 Appendix 16.2.3; Module 5.3.5.1, CT02 Study Report Body, Table 41

Efficacy Analyses

Standard microbiological tests for assessment of bacteriological effects were used in these studies. An in vitro assessment (MIC, MIC_{90}) of the susceptibility of *P. aeruginosa* to tobramycin was performed by using the conventional procedures practiced in the countries where the study was conducted. The MIC_{50} value was also calculated later to conform to conventional microbiological reporting practices in the U.S.

Summary MIC values (MIC_{50}, MIC_{90}, and MIC range) were determined for the ITT population. Descriptive values were based on the highest MIC value at each study visit provided for all *P. aeruginosa* morphotypes combined and in CT02, as subgroups by pigment production. Resistance to tobramycin was determined according to CLSI standards for parenteral tobramycin as an MIC $\geq$ 16 mcg/mL. The development of resistance to tobramycin before, during and after treatment was analyzed.

The mean change from baseline bacterial load of *P. aeruginosa* (log_{10} CFU/mL) was analyzed at selected study visits for each treatment group. Categorical results for microbiological outcome (eradication, persistence, superinfection, or re-infection) are summarized (standard descriptive statistics).

2.1. Study CP01 (Module 5.3.1.2)

The objective of Study CP01 was to evaluate the PK of tobramycin in plasma and sputum of CF patients after a single administration by nebulization of CHF 1538 (Chiesi Farmaceutici S.p.A.) in comparison to the marketed formulation TOBI (PathoGenesis).

This was a double-blind, single center, randomized, two-way crossover study. Figure 1 shows the flow chart of the study design. All patients were treated with a single dose of 300 mg tobramycin, both as CHF 1538 and as TOBI. A washout of at least five days and not more than nine days separated the two periods of drug administration.

This study used a double-blind design in which the Investigator and the patients were not aware of the treatment allocated. Since single 300 mg doses of CHF 1538 and TOBI are supplied in different volumes (4 mL for CHF 1538 and 5 mL for TOBI), the study medication was prepared for administration by a nurse or pharmacist in order to maintain the blind for both the patient and the Investigator.

At each study session, patients attended the clinic in a fasting state and blood and sputum samples were taken at the following times to assess tobramycin concentrations:

- Venous blood samples were collected at pre-dose, immediately before nebulization start, immediately after end of nebulization (approximately 0.25 hour), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours post-dose;
- Sputum samples (expectorated sputum) were collected at pre-dose, 15 minutes after the end of nebulization (approximately 0.5 hour), 3, 6, 12 and 24 hours post-dose.

Time 0 was defined as the moment when nebulization began.

Table 3 shows a comparison and analyses of results across studies. As can be seen from the CPO1 and CTO1 studies the concentration of tobramycin in the sputum is extremely variable. In some cases the concentration could be less then the MIC_{90} range (16 to 64 mcg/mL) for *P aeruginosa* isolated from the sputum of cystic fibrosis patients.

3. COMPARISON AND ANALYSES OF RESULTS ACROSS STUDIES

Results of the clinical pharmacology Study CP01 support the safety of CHF 1538 and demonstrate similar systemic exposure of marketed, inhaled TOBI.

CP01 sputum concentrations were consistent with those obtained in Study CT01 (see Module 5.3.5.1) and also with those reported in literature [14,15].

Plasma peak concentrations, derived from Chiesi's pharmacokinetic evaluation of CHF 1538 were consistent with published data [14, 15 and 21] obtained using tobramycin formulations with different concentrations and different types of nebulizers (Table 3). This consistency of data further supports the validity of the clinical pharmacology program for CHF 1538.

Table 3: Peak Sputum and Plasma Concentration (Mean and SD)

Formulation	Nebulizer	Dose (mg)	SPUTUM PEAK CONCENTRATION Mean (SD)	PLASMA PEAK CONCENTRATION Mean (SD)
			C_{max} (µg/g)	C_{max} (µg/mL)
CHF 1538, 75 mg/mL CP01	PARI LC Plus	300 Single dose	1289 ± 851	0.758 ± o.546
CHF 1538, 75 mg/mL CT01	PARI LC Plus	300	696 ± 817 Day 1[1] 717 ± 799 Day 28[1]	-[2]
Tobramycin 60 mg/mL [15]	PARI LC	300 Single dose	687 ± 663	0.570 ± 0.380
Tobramycin 20 mg/mL [15]	UltraNEb	300 Single dose	1498 ± 1331	-
Tobramycin 60 mg/mL [15]	Sidestream	300 Single dose	489 ± 402	0.740 ± 0.430
TOBI, 60 mg/mL [14]	PARI LC Plus	300	754 ± 927 Day 1 769 ± 823 Day 15	0.9 ± 0.5 Day 1 1.3 ± 0.7 Day 15
TOBI, 60 mg/mL [14]	PARI eFlow rapid	300	981 ± 1191 Day 1 1572 ± 2182 Day 15	0.7 ± 0.6 Day 1 1.2 ± 1.0 Day 15

Table 3: Peak Sputum and Plasma Concentration (Mean and SD) (Continued)

Formulation	Nebulizer	Dose (mg)	SPUTUM PEAK CONCENTRATION Mean (SD)	PLASMA PEAK CONCENTRATION Mean (SD)
			C_{max} (µg/g)	C_{max} (ng/mL)
TOBI, 60 mg/mL [21]	PARI LC PLUS®–CR60®	300	-	700
TOBI, 60 mg/mL [21]	PARI LC PLUS®–PortaNeb®	300	-	540

[1] expressed as µg/mL
[2] data not available

Although sputum concentrations may not be homogenous, they are high compared to the very low levels found in serum. Since the known risks of nephrotoxicity and ototoxicity associated with aminoglycosides have been shown to correlate with serum levels, the negligible amount of tobramycin found in serum suggests a potential clinical advantage of aerosol versus intravenous administration. From a safety perspective, the peak plasma concentrations of inhaled tobramycin were at least ten times lower than the usual threshold of 12000 ng/mL commonly used to guide intravenous tobramycin use [14]. Moreover, peak tobramycin levels were also lower than the threshold that should not be exceeded at trough (2000 ng/mL) following parenteral administration [19]. Routine monitoring of tobramycin plasma levels is not required for inhaled tobramycin, but should be considered at the discretion of the treating physician, particularly for patients with renal dysfunction.

Study CT01 (Pivotal Trail)

Study CT01

Tobramycin Susceptibility Profiles

The ITT population contained 29 patients in the CHF 1538-treatment arm and 30 patients in the placebo-treatment arm. Tobramycin susceptibility data are presented as MIC values for *P. aeruginosa* clinical trial isolates. The distributions of MIC results for *P. aeruginosa* are shown in Table 23. As can be seen the MIC range in the CHF 1538 study group was broader then in the placebo arm and the MIC_{90} was higher on visits 1 and 5 in the CH 1538 group then in the placebo group. The significance of this is not known.

Table 23: Tobramycin MIC[1] Summary for Study CT01

	Tobramycin MIC (µg/mL)							
	CHF 1538				Placebo			
Visit[1]	N	Range	MIC_{50}	MIC_{90}	N	Range	MIC_{50}	MIC_{90}
1	29	≤0.25-256	4	64	27	0.5-64	4	16
4	19	≤0.25-256	4	32	17	0.5-32	4	32
5	23	1-256	4	32	17	0.5-32	4	16

[1] Phases of Study: Visit 1= baseline, Visit 4=sample obtained after completion of "ON" cycle; Visit 5=sample obtained at end of "OFF" cycle.
Source: Module 5.3.5.1, CT01 Study Report Body, Table 15 and CT01 Appendix 16.2.6.4

Table 24 shows the distribution of tobramycin MIC values in study CT01 using systemic interpretive breakpoints.

Table 24: Distribution of Tobramycin MIC Values in Study CT01 Using Systemic Interpretive Breakpoints

Tobramycin MIC (µg/mL)	CHF 1538			Placebo		
	Baseline n/N (%)	Visit 4 n/N (%)	Visit 5 n/N (%)	Baseline n/N (%)	Visit 4 n/N (%)	Visit 5 n/N (%)
≤4.0	19/29 (65.5%)	11/19 (57.9%)	16/23 (69.6%)	21/27 (77.8%)	12/17 (70.6%)	14/17 (82.4%)
8.0	3/29 (10.3%)	3/19 (15.8%)	2/23 (8.7%)	2/27 (7.4%)	3/17 (17.6%)	1/17 (5.9%)
≥16.0	7/29 (24.1%)	5/19 (26.3%)	5/23 (21.7%)	4/27 (14.8%)	2/17 (11.8%)	2/17 (11.8%)

Source: Module 5.3.5.1, CT01 Study Report Body, Table 16 and CT01 Appendix 16.2.6.4

Mean Change from Baseline Viable Counts

The mean change in bacterial density from baseline levels is presented after treatment (visit 4) and at the end of the "OFF" cycle (Visit 5) in Table 26. At visit one the baseline bacterial load was similar between groups. BT visit 4, CHF 1538 treatment resulted in a significantly greater reduction in bacterial count than placebo (-2.16 versus -0.89 CFU/gram, p=0.008, 95% CI [-2.18, -0.34]. However, this difference between groups in bacterial load was no longer apparent at the end of the "OFF" cycle (Visit 5).

Table 26: Log_{10} Mean Bacterial Load (CFUs/g) at Baseline and Mean Change in Bacterial Load from Baseline: ITT Population, Study CT01

Visit	Week		CHF 1538	Placebo	P-Value
1	Baseline	N	27	26	
		Mean	5.79	5.84	0.907
4	4 "ON" Drug	N	27	26	
		Mean Change from Baseline[1]	-2.16	-0.89	
		Difference (95% CI)	-1.26 (-2.18, -0.34)		0.008
5	8 "OFF" Drug	N	25	23	
		Mean Change from Baseline	-0.55	-0.72	
		Difference (95% CI)	0.17 (-0.85, 1.20)		0.738

Note: Ns were lower for this analysis compared with other microbiological analyses because of missing data. A value of 20 was used for all instances where the *P. aeruginosa* pathogen was eradicated.
[1] Adjusted for baseline value
Source: Module 5.3.5.1, CT01 Study Report Body, Table 18, Table 105, Table 106, Table 107, Table 108

Microbiological Outcome

The analysis of microbiological outcome at all visits by baseline MIC value is presented in Table 27. At visit 4 (sample obtained after completion of the "ON" cycle treatment), CHF 1538 treatment resulted in a higher rate of eradication than the placebo (34.5% versus 20.8%) as well as a lower percentage of persistence (37.9% versus 75%). The rate of superinfection was substantially higher in the CHF 1538 group (27.6%) than the Placebo group (4.2%). Re-infection was not observed in either treatment arm at Visit 4.

Table 27: Microbiological Outcome at All Visits by Baseline MIC in Study CT01

		CHF 1538				Placebo			
Visit	Baseline MIC (µg/mL)	Erad[1] n/N (%)	Pers[2] n/N (%)	Sup[3] n/N (%)	Reinf[4] n/N (%)	Erad n/N (%)	Pers n/N (%)	Sup n/N (%)	Reinf n/N (%)
4	≤ 0.25	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
	0.5	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	1/2 (50.0%)	1/2 (50.0%)	0/2 (0.0%)	0/2 (0.0%)
	1	1/4 (25.0%)	2/4 (50.0%)	1/4 (25.0%)	0/4 (0.0%)	0/4 (0.0%)	4/4 (100.0%)	0/4 (0.0%)	0/4 (0.0%)
	2	2/6 (33.3%)	1/6 (16.7%)	3/6 (50.0%)	0/6 (0.0%)	2/5 (40.0%)	3/5 (60.0%)	0/5 (0.0%)	0/5 (0.0%)
	4	3/7 (42.9%)	4/7 (57.1%)	0/7 (0.0%)	0/7 (0.0%)	2/9 (22.2%)	7/9 (77.8%)	0/9 (0.0%)	0/9 (0.0%)
	8	1/3 (33.3%)	2/3 (66.7%)	0/3 (0.0%)	0/3 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
	16	0/3 (0.0%)	1/3 (33.3%)	2/3 (66.7%)	0/3 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)
	32	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
	64	1/2 (50.0%)	0/2 (0.0%)	1/2 (50.0%)	0/2 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
	128	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
	256	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
	Total	10/29 (34.5%)	11/29 (37.9%)	8/29 (27.6%)	0/20 (0.0%)	5/24 (20.8%)	18/24 (75.0%)	1/24 (4.2%)	0/24 (0.0%)

Table 27: Microbiological Outcome at All Visits by Baseline MIC (Continued)

		CHF 1538				Placebo			
Visit	Baseline MIC (µg/mL)	Erad[1] n/N (%)	Pers[2] n/N (%)	Sup[3] n/N (%)	Reinf[4] n/N (%)	Erad n/N (%)	Pers n/N (%)	Sup n/N (%)	Reinf n/N (%)
5	≤ 0.25	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
	0.5	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	1/2 (50.0%)	0/2 (0.0%)	0/2 (0.0%)	1/2 (50.0%)
	1	0/4 (0.0%)	2/4 (50.0%)	1/4 (25.0%)	1/4 (25.0%)	0/4 (0.0%)	2/4 (50.0%)	2/4 (50.0%)	0/4 (0.0%)
	2	0/6 (0.0%)	3/6 (50.0%)	2/6 (33.3%)	1/6 (16.7%)	1/4 (25.0%)	2/4 (50.0%)	0/4 (0.0%)	1/4 (25.0%)
	4	1/6 (16.7%)	2/6 (33.3%)	3/6 (50.0%)	0/6 (0.0%)	1/9 (11.1%)	6/9 (66.7%)	2/9 (22.2%)	0/9 (0.0%)
	8	0/3 (0.0%)	1/3 (33.3%)	2/3 (66.7%)	0/3 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
	16	0/3 (0.0%)	2/3 (66.7%)	1/3 (33.3%)	0/3 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)
	32	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
	64	1/2 (50.0%)	1/2 (50.0%)	0/2 (0.0%)	0/2 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
	128	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
	256	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
	Total	3/28 (10.7%)	12/28 (42.9%)	10/28 (35.7%)	3/28 (10.7%)	3/22 (13.6%)	12/22 (54.5%)	5/22 (22.7%)	2/22 (9.1%)

[1] Eradication (elimination of *P. aeruginosa* detected at Visit 1)
[2] Persistent (presence of *P. aeruginosa* detected at Visit 1)
[3] Superinfection (appearance of a pathogen not detected at Visit 1 in presence of persistence of *P. aeruginosa*)
[4] Reinfection (appearance of *P. aeruginosa* detected at Visit 1 and eradicated at Visit 4)
Source: Module 5.3.5.1, CT01 Study Report Body, Table 19; CT01 Appendix 16.2.6.4 and Appendix 16.2.6.5

At Visit 5 (sample obtained at end of the "OFF" cycle), the percentage eradication was substantially lower than Visit 4 in both treatment groups (10.7% for CHF 1538 versus 13.6% for placebo). Similar rates of persistors (42.9% versus 54.5%) were observed in the two treatment groups. The rate of superinfection was higher for the CHF 1538 treated patients (37.5%) than the placebo treated patients (22.7%). A similar rate of re-infection was observed in both groups.

MIC Changes During Therapy

An assessment of *P. aeruginosa* MIC shifts for individual patients from the baseline value to those values observed during Visit 4 or 5 are summarized in Table 28. At visit 4, there were no isolates in either treatment group whose MIC had changed by ≥4-fold increase from the baseline value.

A comparison of baseline to Visit 5 values shows that the MIC value increased in only one CHF 1538 patient and no placebo-treated patients. There are some instances were there were decreases ion the MIC for both the CHF 1538 and placebo treated groups.

The data in Table 28 suggests that the use of tobramycin per the treatment regimen employed in this trial did not significantly alter the susceptibility of the clinical trial isolates.

Table 28: Assessment of MIC Shifts From Observed Baseline Values and Values Observed at Visit 4 and Visit 5 in Study CT01

	Baseline vs. Visit 4		Baseline vs. Visit 5	
Evaluability Criteria	**CHF 1538**	**Placebo**	**CHF 1538**	**Placebo**
MIC increased[1]	0 (0.0%)	0 (0.0%)	1 (4.3%)	0 (0.0%)
MIC unchanged[2]	16 (84.2%)	15 (88.2%)	20 (87.0%)	17 (100%)
MIC decreased[3]	3 (15.8%)	2 (11.8%)	2 (8.7%)	0 (0.0%)

[1] Patients whose *P. aeruginosa* isolate exhibited ≥ 4 fold increase in the MIC between baseline and end of therapy or follow-up visits.
[2] Patients whose *P. aeruginosa* isolate exhibited ± 2 fold change in the MIC between baseline and end of therapy or follow-up visits.
[3] Patients whose *P. aeruginosa* isolate exhibited ≥ 4 fold decrease in the MIC between baseline and end of therapy or follow-up visits.
Source: Module 5.3.5.1, CT01 Study Report Body, Table 20 and CT01 Appendix 16.2.6.4

Study CT02 (Pivotal Study)

Baseline Results

Inclusion criteria for this study required a patient to have *P. aeruginosa* isolated from the sputum sample collected at baseline. The ITT population contained a total of 161

patients in the CHF 1538 treatment arm and 84 patients in the placebo arm. Bacteria in addition to *P. aeruginosa* that were most commonly isolated in both treatment groups at baseline visit included *Staphylococcus aureus* and less often *Candida albicans*, *Haemophilus* species, and streptococci.

The Applicant categorized the isolates from the CHF 1538 and placebo treatment arms by color of the *P. aeruginosa* colonies. *P. aeruginosa* producing green pigment was the predominant type at Visit 1 (`64%) in both treatment groups and remained the predominant type throughout the study in both treatment groups.

MIC Data

MIC values at baseline were similar for *P. aeruginosa* (all morphotypes combined) in both treatment groups. MIC values ranged from ≤0.25 to 64 mcg/mL. These results are in Table 29.

The distribution and percent range of tobramycin MIC results for *P. aeruginosa* clinical trial isolates at Visits 1, 4, 5, 8, and 9 are in Table 29. The range of MICs observed for the CHF 1538 and placebo study arms describe the variability of the MIC results independent of CHF 1538 exposure. This type of result has been seen for other cystic fibrosis studies for not only tobramycin but other antibacterials. Table 29 also shows the comparability of the *P. aeruginosa* tobramycin susceptibility in the treatment and placebo arms and the consistency between visits in the two treatment arms.

Table 29: Tobramycin MIC Summary for Study CT02 (All Morphotypes Combined)

Tobramycin Susceptibility (µg/mL)								
Visit[1]	CHF 1538				Placebo			
	N	Range	MIC_{50}	MIC_{90}	N	Range	MIC_{50}	MIC_{90}
1	160	≤ 0.25-64	1	64	80	≤ 0.25-64	1	64
4	110	≤ 0.25-64	2	64	72	≤ 0.25-64	1	64
5	134	≤ 0.25-64	2	64	72	≤ 0.25-64	1	64
8	104	0.5-64	2	64	65	≤ 0.25-64	1	64
9	114	≤ 0.25-64	2	64	61	≤ 0.25-64	1	64

[1] Phase of Study: Visit 1=baseline, Visit 4 and 8 = sample obtained after completion of "ON" cycle treatment; Visit 5 and 9 = sample obtained at end of "OFF" cycle.
Source: Module 5.3.5.1, CT02 Study Report Body, Tables 15, 173 and 176; CT02 Appendix 16.2.6.17

Table 31 shows the distribution of isolates based on tobramycin susceptibility. The criteria for grouping the isolates was the parenteral susceptibility MIC interpretive criteria (mcg/mL) (≤4 = susceptible, 8 = Intermediate and ≥16 = Resistant.

Table 31: Distribution of Tobramycin MIC Values at Baseline, End of the Last Treatment Cycle, and at Follow-up: ITT Population, Study CT02

Tobramycin MIC (µg/mL)	CHF 1538			Placebo		
	Baseline n/N (%)	EOT[1] n/N (%)	FU[2] n/N (%)	Baseline n/N (%)	EOT n/N (%)	FU n/N (%)
≤ 4.0	136/160 (85.0%)	81/127 (63.8%)	91/143 (63.6%)	63/80 (78.8%)	54/76 (71.1%)	57/77 (74.0%)
8.0	4/160 (2.5%)	10/127 (7.9%)	8/143 (5.6%)	3/80 (3.8%)	3/76 (3.9%)	3/77 (3.9%)
≥ 16.0	20/160 (12.5%)	36/127 (28.3%)	44/143 (30.8%)	14/80 (17.5%)	19/76 (25.0%)	17/77 (22.1%)

[1] Last available MIC value after completion of an "ON" cycle
[2] Last available MIC after completion of an "OFF" cycle
Source: Module 5.3.5.1, CT02 Study Report Body, Table 16

The data were further examined to determine where the isolates with high MIC values (MIC ≥16 mcg/mL) were distributed by study visit. Results of this analysis are shown in Table 32. The data show the percentage of CHF 1538 patients with high MIC values increased after treatment and at follow-up compared to baseline value. The percent of placebo patients with MIC ≥16 mcg/mL also increased, but to a lesser extent, over the course of the study.

Table 32: Percentages of Patients with *P. aeruginosa* Isolates Exhibiting Tobramycin MIC ≥ 16 µg/mL: ITT Population, Study CT02

Visit	CHF 1538		Placebo	
	n/N	%	n/N	%
Baseline	20/160	12.5	14/80	17.5
End of Last Treatment[1]	36/127	28.3	19/76	25.0
Follow-up[2]	44/143	30.8	17/77	22.1

[1] Last available MIC value after completion of an "ON" cycle.
[2] Last available MIC value after completion of an "OFF" cycle.
Source: Module 5.3.5.1, CT02 Study Report Body, Table 17

Mean Change from Baseline Viable Counts

The mean change in bacterial density from baseline levels is presented for at the end of "ON" cycles and at the end of the "OFF" cycles in Table 33. The baseline bacterial load was very similar between groups. By the end of the first "ON" cycle, CHF 1538 treatment resulted in a significantly greater mean reduction in bacterial count than placebo (-1.67 versus -0.57 $\log_{10}$ CFU/gram, p,0.001, 95% CI [-1.59,-0.61]) that was maintained through the end of the first "OFF" cycle. Following additional CHD 1538 treatment, a significantly greater mean reduction in bacterial count than placebo resulted

(-1.73 versus -0.62 log_{10} CFU/gram, p<0.001, 95% CI [-1.65,-0.56]) at the end of the "ON" cycle. The significant difference in bacterial load between treatment groups was not apparent at the end of the last "OFF' cycle.

Table 33: Log_{10} Bacterial Load (CFUs/g) Mean Baseline and Mean Change from Baseline: ITT Dataset, Study CT02

Visit	Week		CHF 1538	Placebo	P-Value[1]
1	Baseline	N	160	84	
		Mean	5.71	5.72	0.954
4	4 "ON" Drug	N	159	84	
		Mean Change from Baseline[2]	-1.67	-0.57	
		Difference (95% CI)	-1.10 (-1.59, -0.61)		< 0.001
5	8 "OFF" Drug	N	155	82	
		Mean Change from Baseline[2]	-0.97	-0.48	
		Difference (95% CI)	-0.50 (-0.97, -0.02)		0.040
8	20 "ON" Drug	N	155	78	
		Mean Change from Baseline[2]	-1.73	-0.62	
		Difference (95% CI)	-1.10 (-1.65, -0.56)		< 0.001
9	24 "OFF" Drug	N	150	77	
		Mean Change from Baseline[2]	-1.03	-0.72	
		Difference (95% CI)	-0.32 (-0.88, 0.25)		0.272

Note: a value of 20 was used for all instances where the *P. aeruginosa* pathogen was eradicated
[1] significance level=0.05
[2] adjusted for baseline value
Source: Module 5.3.5.1, CT02 Study Report Body, Table 13

Microbiological Outcome

The analysis by baseline MIC (Table 34) shows that there were rare instances of microbiological eradication in either the CHF 1538 or placebo groups at any visit. There were a high percentage of persistors in the CHF 1538 group at Visits 4 and 8 with somewhat lower percentage at Visits 5 and 9. A high percentage of persistors were also present in the placebo group at all visits.

Table 34: Microbiological Outcome at All Study Visits by Baseline MIC: ITT Patients, Study CT02

Visit	Baseline MIC (µg/mL)	CHF 1538 (N=161)				Placebo (N=84)			
		Erad1 n/N(%)	Pers2 n/N(%)	Sup3 n/N(%)	Reinf4 n/N(%)	Erad n/N(%)	Pers n/N(%)	Sup n/N(%)	Reinf n/N(%)
4	≤ 0.25	0/4 (0.0%)	3/4 (75.0%)	1/4 (25.0%)	0/4 (0.0%)	0/13 (0.0%)	9/13 (69.2%)	4/13 (30.8%)	0/13 (0.0%)
	0.5	0/23 (0.0%)	16/23 (69.6%)	7/23 (30.4%)	0/23 (0.0%)	0/17 (0.0%)	13/17 (76.5%)	4/17 (23.5%)	0/17 (0.0%)
	1	0/42 (0.0%)	34/42 (81.0%)	8/42 (19.1%)	0/42 (0.0%)	0/22 (0.0%)	18/22 (81.8%)	4/22 (18.2%)	0/22 (0.0%)
	2	0/21 (0.0%)	20/21 (95.2%)	1/21 (4.8%)	0/21 (0.0%)	0/14 (0.0%)	12/14 (85.7%)	2/14 (14.3%)	0/14 (0.0%)
	4	0/8 (0.0%)	8/8 (100.0%)	0/8 (0.0%)	0/8 (0.0%)	0/2 (0.0%)	2/2 (100.0%)	0/2 (0.0%)	0/2 (0.0%)
	8	0/4 (0.0%)	4/4 (100.0%)	0/4 (0.0%)	0/4 (0.0%)	0/3 (0.0%)	3/3 (100.0%)	0/3 (0.0%)	0/3 (0.0%)
	16	0/6 (0.0%)	5/6 (83.3%)	1/6 (16.7%)	0/6 (0.0%)	0/2 (0.0%)	1/2 (50.0%)	1/2 (50.0%)	0/2 (0.0%)
	32	0/4 (0.0%)	2/4 (50.0%)	2/4 (50.0%)	0/4 (0.0%)	0/7 (0.0%)	4/7 (57.1%)	3/7 (42.9%)	0/7 (0.0%)
	64	0/22 (0.0%)	16/22 (72.7%)	6/22 (27.3%)	0/22 (0.0%)	0/10 (0.0%)	8/10 (80.0%)	2/10 (20.0%)	0/10 (0.0%)
	Total	0/134 (0.0%)	108/134 (80.6%)	26/134 (19.4%)	0/134 (0.0%)	0/90 (0.0%)	70/90 (77.8%)	20/90 (22.2%)	0/90 (0.0%)
5	≤ 0.25	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	0/4 (0.0%)	4/4 (100.0%)	0/4 (0.0%)	0/4 (0.0%)
	0.5	0/27 (0.0%)	15/27 (55.6%)	6/27 (22.2%)	6/27 (22.2%)	0/25 (0.0%)	18/25 (72.0%)	6/25 (24.0%)	1/25 (4.0%)
	1	0/56 (0.0%)	32/56 (57.1%)	11/56 (19.6%)	13/56 (23.2%)	0/23 (0.0%)	18/23 (78.3%)	3/23 (13.0%)	2/23 (8.7%)
	2	0/31 (0.0%)	23/31 (74.2%)	5/31 (16.1%)	3/31 (9.7%)	0/13 (0.0%)	7/13 (53.9%)	5/13 (38.5%)	1/13 (7.7%)
	4	0/14 (0.0%)	9/14 (64.3%)	2/14 (14.3%)	3/14 (21.4%)	0/7 (0.0%)	7/7 (100.0%)	0/7 (0.0%)	0/7 (0.0%)
	8	0/6 (0.0%)	3/6 (50.0%)	3/6 (50.0%)	0/6 (0.0%)	0/4 (0.0%)	2/4 (50.0%)	1/4 (25.0%)	1/4 (25.0%)
	16	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
	32	0/7 (0.0%)	5/7 (71.4%)	1/7 (14.3%)	1/7 (14.3%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)

Table 34: Microbiological Outcome at All Study Visits by Baseline MIC: ITT Patients, Study CT02 (Continued)

Visit	Baseline MIC (µg/mL)	CHF 1538 (N=161)				Placebo (N=84)			
		Erad n/N(%)	Pers n/N(%)	Sup n/N(%)	Reinf n/N(%)	Erad n/N(%)	Pers n/N(%)	Sup n/N(%)	Reinf n/N(%)
5 (cont)	64	0/22 (0.0%)	16/22 (72.7%)	2/22 (9.1%)	4/22 (18.2%)	0/16 (0.0%)	11/16 (68.8%)	3/16 (18.8%)	2/16 (12.5%)
	Total	0/164 (0.0%)	104/164 (63.4%)	30/164 (18.3%)	30/164 (18.3%)	0/92 (0.0%)	67/92 (72.8%)	18/92 (19.6%)	7/92 (7.6%)
8	≤ 0.25	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/9 (0.0%)	6/9 (66.7%)	3/9 (33.3%)	0/9 (0.0%)
	0.5	0/13 (0.0%)	12/13 (92.3%)	0/13 (0.0%)	1/13 (7.7%)	0/14 (0.0%)	10/14 (71.4%)	3/14 (21.4%)	1/14 (7.1%)
	1	1/27 (3.7%)	20/27 (74.1%)	4/27 (14.8%)	2/27 (7.4%)	0/25 (0.0%)	17/25 (68.0%)	6/25 (24.0%)	2/25 (8.0%)
	2	0/30 (0.0%)	19/30 (63.3%)	9/30 (30.0%)	2/30 (6.7%)	0/12 (0.0%)	10/12 (83.3%)	2/12 (16.7%)	0/12 (0.0%)
	4	0/12 (0.0%)	11/12 (91.7%)	1/12 (8.3%)	0/12 (0.0%)	0/5 (0.0%)	4/5 (80.0%)	1/5 (20.0%)	0/5 (0.0%)
	8	0/9 (0.0%)	6/9 (66.7%)	3/9 (33.3%)	0/9 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
	16	0/4 (0.0%)	3/4 (75.0%)	1/4 (25.0%)	0/4 (0.0%)	0/2 (0.0%)	1/2 (50.0%)	1/2 (50.0%)	0/2 (0.0%)
	32	0/3 (0.0%)	3/3 (100.0%)	0/3 (0.0%)	0/3 (0.0%)	0/6 (0.0%)	4/6 (66.7%)	1/6 (16.7%)	1/6 (16.7%)
	64	0/34 (0.0%)	22/34 (64.7%)	9/34 (26.5%)	3/34 (8.8%)	0/11 (0.0%)	9/11 (81.8%)	2/11 (18.2%)	0/11 (0.0%)
	Total	1/132 (0.8%)	96/132 (72.7%)	27/132 (20.5%)	8/132 (6.1%)	0/85 (0.0%)	62/85 (72.9%)	19/85 (22.4%)	4/85 (4.7%)
9	≤ 0.25	0/2 (0.0%)	1/2 (50.0%)	0/2 (0.0%)	1/2 (50.0%)	0/6 (0.0%)	6/6 (100.0%)	0/6 (0.0%)	0/6 (0.0%)
	0.5	1/23 (4.4%)	13/23 (56.5%)	6/23 (26.1%)	3/23 (13.0%)	0/19 (0.0%)	15/19 (79.0%)	3/19 (15.8%)	1/19 (5.3%)
	1	0/33 (0.0%)	22/33 (66.7%)	3/33 (9.1%)	8/33 (24.2%)	0/17 (0.0%)	14/17 (82.4%)	3/17 (17.7%)	0/17 (0.0%)
	2	0/20 (0.0%)	12/20 (60.0%)	5/20 (25.0%)	3/20 (15.0%)	0/13 (0.0%)	10/13 (76.9%)	2/13 (15.4%)	1/13 (7.7%)
	4	0/14 (0.0%)	12/14 (85.7%)	1/14 (7.1%)	1/14 (7.1%)	0/4 (0.0%)	3/4 (75.0%)	1/4 (25.0%)	0/4 (0.0%)
	8	0/10 (0.0%)	6/10 (60.0%)	2/10 (20.0%)	2/10 (20.0%)	0/3 (0.0%)	2/3 (66.7%)	1/3 (33.3%)	0/3 (0.0%)

Table 34: Microbiological Outcome at All Study Visits by Baseline MIC: ITT Patients, Study CT02 (Continued)

Visit	Baseline MIC (µg/mL)	CHF 1538 (N=161)				Placebo (N=84)			
		Erad n/N(%)	Pers n/N(%)	Sup n/N(%)	Reinf n/N(%)	Erad n/N(%)	Pers n/N(%)	Sup n/N(%)	Reinf n/N(%)
9 (cont)	16	0/4 (0.0%)	1/4 (25.0%)	0/4 (0.0%)	3/4 (75.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
	32	0/4 (0.0%)	4/4 (100.0%)	0/4 (0.0%)	0/4 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
	64	0/28 (0.0%)	22/28 (78.6%)	3/28 (10.7%)	3/28 (10.7%)	0/10 (0.0%)	7/10 (70.0%)	2/10 (20.0%)	1/10 (10.0%)
	Total	1/138 (0.7%)	93/138 (67.4%)	20/138 (14.5%)	24/138 (17.4%)	0/74 (0.0%)	59/74 (79.7%)	12/74 (16.2%)	3/74 (4.1%)

[1] Eradication (elimination of *P. aeruginosa* detected at baseline)
[2] Persistent (presence of *P. aeruginosa* detected at baseline)
[3] Superinfection (addition of a new pathogen to those reported at baseline, in presence of persistence of *P. aeruginosa*)
[4] Re-infection (return of *P. aeruginosa* without appearance of new species)
Source: Module 5.3.5.1, CT02 Study Report Body, Table 179 and CT02 Appendix 16.2.6.17

MIC Changes During Therapy

Table 35 shows the tobramycin MIC shifts from baseline to follow-up. MIC values were unchanged (+/-2 fold) for 34.9% and 37.5% in the CHF 1538 groups at the end of treatment and the follow-up visits and for 27.1% and 23.4% in the placebo group, respectively. However, at the end of therapy and the follow-up visits, a greater percentage of strains in the CHF 1538 group showed a $\geq$ 4 fold in crease in tobramycin MIC than isolates in the placebo group. MIC values decreased $\geq$ 4 fold for approximately 4% of isolates in each treatment group.

Table 35: Assessment of MIC Shifts from Observed Baseline Values and MIC Values Observed at End of the Last Treatment Cycle and at Follow-up: ITT Population, Study CT02

Evaluability Criteria	Baseline vs. End of Last Treatment Cycle[1]		Baseline vs. Follow-up[2]	
	CHF 1538	Placebo	CHF 1538	Placebo
MIC increased[3]	41 (24.7%)	9 (5.4%)	51 (27.7%)	5 (2.7%)
MIC unchanged[4]	58 (34.9%)	45 (27.1%)	69 (37.5%)	43 (23.4%)
MIC decreased[5]	6 (3.6%)	7 (4.2%)	8 (4.3%)	8 (4.3%)

[1] Last available MIC value after completion of an "ON" cycle.
[2] Last available MIC value after completion of an "OFF" cycle.
[3] Patients whose PA isolate exhibited ≥ 4-fold increase in the MIC between baseline and end of therapy or follow-up visits.
[4] Patients whose PA isolate exhibited ± 2-fold change in the MIC between baseline and end of therapy or follow-up visits.
[5] Patients whose PA isolate exhibited ≥ 4-fold decrease in the MIC between baseline and end of therapy or follow-up visits
Source: Module 5.3.5.1, CT02 Study Report Body, Table 18

Integration of Pivotal CT01 and CT02 Study Results

Inclusion criteria for both studies required a patient to have *P. aeruginosa* isolated from the sputum sample collected at Visit 1 and in Study CT01 only; the *P. aeruginosa* isolate was requested to be susceptible to tobramycin. Study CT01 enrolled patients in a 1:1 ratio, while patients in Study CT02 were randomized 2:1 in the CHF 1538: Placebo treatment groups. *P. aeruginosa* was isolated from a total of 190 CHF 1538 patients and 114 Placebo patients (ITT population) in the 2 studies. Baseline *P. aeruginosa* MIC values were available for a total of 189 CHF 1538 patients and 107 placebo patients (ITT population) in the 2 studies. Organisms in addition to *P. aeruginosa* that were most commonly isolated in both treatment groups at Visit 1 included *Staphylococcus aureus* and less often, *Candida albicans*, *Haemophilus* species, and streptococci.

MIC Distributions

Table 36 shows the integrated results of the distribution of tobramycin MIC results for *P. aeruginosa* at Visits 1, 4, and 5. The distribution of MIC values for isolates at baseline (Visit 1) was comparable between the 2 treatment groups, with the majority of isolates having MIC values ≤8 mcg/mL (CHD 1538 85.7%, Placebo group 83.2%). The distribution of MIC values remained comparable between treatment groups at Visits 4 and 5. Throughout the study,, the majority of *P. aeruginosa* isolates remained susceptible to tobramycin (MIC ≤8 mcg/mL) in both treatment groups, although the susceptible percentage in CHF 1538 group declined slightly compared to Visit 1 at the subsequent visits. While percentages in the placebo group showed less variation.

Table 36: Integrated MIC Distribution for Clinical Isolates: ITT population, Studies CT01 and CT02

		CHF 1538		Placebo	
Visit	MIC (µg/mL)	n/N (%)	Cumulative %	n/N (%)	Cumulative %
1	≤ 0.25	7/189 (3.7%)	3.70	8/107 (7.5%)	7.48
	0.5	37/189 (19.6%)	23.28	17/107 (15.9%)	23.36
	1	53/189 (28.0%)	51.32	23/107 (21.5%)	44.86
	2	40/189 (21.2%)	72.49	19/107 (17.8%)	62.62
	4	18/189 (9.5%)	82.01	17/107 (15.9%)	78.50
	8	7/189 (3.7%)	85.71	5/107 (4.7%)	83.18
	16	5/189 (2.6%)	88.36	5/107 (4.7%)	87.85
	32	2/189 (1.1%)	89.42	4/107 (3.7%)	91.59
	64	19/189 (10.1%)	99.47	9/107 (8.4%)	100.00
	128	0/189 (0.0%)	99.47	0/107 (0.0%)	100.00
	256	1/189 (0.5%)	100.00	0/107 (0.0%)	100.00
4	≤0.25	4/129 (3.1%)	3.10	9/89 (10.1%)	10.11
	0.5	17/129 (13.2%)	16.28	17/89 (19.1%)	29.21
	1	37/129 (28.7%)	44.96	22/89 (24.7%)	53.93
	2	21/129 (16.3%)	61.24	12/89 (13.5%)	67.42
	4	14/129 (10.9%)	72.09	8/89 (9.0%)	76.40
	8	7/129 (5.4%)	77.52	6/89 (6.7%)	83.15
	16	7/129 (5.4%)	82.95	1/89 (1.1%)	84.27
	32	5/129 (3.9%)	86.82	6/89 (6.7%)	91.01
	64	16/129 (12.4%)	99.22	8/89 (9.0%)	100.00
	128	0/129 (0.0%)	99.22	0/89 (0.0%)	100.00
	256	1/129 (0.8%)	100.00	0/89 (0.0%)	100.00

Table 36: Integrated MIC Distribution for Clinical Isolates: ITT population, Studies CT01 and CT02 (Continued)

		CHF 1538		Placebo	
Visit	MIC (µg/mL)	n/N (%)	Cumulative %	n/N (%)	Cumulative %
5	≤ 0.25	1/157 (0.6%)	0.64	4/89 (4.5%)	4.49
	0.5	21/157 (13.4%)	14.01	20/89 (22.5%)	26.97
	1	50/157 (31.8%)	45.86	23/89 (25.8%)	52.81
	2	31/157 (19.7%)	65.61	11/89 (12.4%)	65.17
	4	18/157 (11.5%)	77.07	13/89 (14.6%)	79.78
	8	5/157 (3.2%)	80.25	4/89 (4.5%)	84.27
	16	2/157 (1.3%)	81.53	1/89 (1.1%)	85.39
	32	7/157 (4.5%)	85.99	1/89 (1.1%)	86.52
	64	21/157 (13.4%)	99.36	12/89 (13.5%)	100.00
	128	0/157 (0.0%)	99.36	0/89 (0.0%)	100.00
	256	1/157 (0.6%)	100.00	0/89 (0.0%)	100.00

Source: Module 5.3.5.1, CT01 Appendix 16.2.6.4 and Module 5.3.5.1, CT02 Appendix 16.2.6.17

MIC range, MIC_{50} and MIC_{90} values are summarized for each treatment group by Visit in Table 37.

Table 37: Integrated Tobramycin MIC Summary (Studies CT01 and CT02)

	Tobramycin Susceptibility (µg/mL)							
	CHF 1538				Placebo			
Visit[1]	N	Range	MIC_{50}	MIC_{90}	N	Range	MIC_{50}	MIC_{90}
1	189	≤0.25-256	1	64	107	≤0.25-64	2	32
4	129	≤0.25-256	2	64	89	≤0.25-64	1	32
5	157	≤0.25-256	2	64	89	≤0.25-64	1	64

[1] Phase of Study: Visit 1=baseline, Visit 4 = sample obtained after completion of "ON" cycle' treatment; Visit 5 = sample obtained at end of "OFF" cycle

Source: Module 5.3.5.1, CT01 Appendix 16.2.6.4 and Module 5.3.5.1, CT02 Appendix 16.2.6.17

Distribution of Isolates Based upon Tobramycin Susceptibility

Table 38 shows the distribution of tobramycin MICs based on parenteral MIC interpretive criteria. For the CHF 1538 patients the majority of isolates (82.0%) had MIC values of ≤4 mcg/mL (Susceptible) at baseline. A substantial proportion of isolates in the CHF 1538 treatment group remained Susceptible at the end of therapy (72.1%) and at follow-up (77.1%) visits. For placebo-treated patients, the majority of isolates (78.5%) had MIC values ≤4 mcg/mL at baseline and only varied slightly (76.4% to 79.8%) at later visits in the study.

Only 3.7% if isolates in the CHF 1538 patients were considered intermediate (8mcg/mL) at baseline; the percentage increased slightly at the end of therapy visit but declined to 3.2% by the follow-up visit. The percentage of placebo patients with isolates considered Intermediate showed a similar pattern: 4.7%, slightly higher at the end of therapy, and declining to 4.5% by the follow-up visit.

A higher percentage of isolates considered Resistant (≥16 mcg/mL) was noted in the placebo group (16.8%) than in the CHF 1538 group (14.3%) at baseline. However, the CHF 1538 treatment group had a slightly higher percentage of isolates considered Resistant at the end of therapy (22.5%) in the CHF 1538 group versus 16.9% in the placebo group). The proportion of resistant isolates remained consistent for each treatment group at the follow-up visit.

Table 38: Integrated Distribution of Tobramycin MIC Values at Baseline, End of the Last Treatment (EOT) Cycle, and at Follow-up (FU): ITT Population, Studies CT01 and CT02

Tobramycin MIC (µg/mL)	CHF 1538 (N=190)			Placebo (N=114)		
	Baseline n/N (%)	End of Last Treatment Cycle (Visit 4) n/N (%)	Follow-up (Visit 5) n/N (%)	Baseline n/N (%)	End of Last Treatment Cycle (Visit 4) n/N (%)	Follow-up (Visit 5) n/N (%)
≤ 4.0	155/189 (82.0%)	93/129 (72.1%)	121/157 (77.1%)	84/107 (78.5%)	68/89 (76.4%)	71/89 (79.8%)
8.0	7/189 (3.7%)	7/129 (5.4%)	5/157 (3.2%)	5/107 (4.7%)	6/89 (6.7%)	4/89 (4.5%)
≥ 16.0	27/189 (14.3%)	29/129 (22.5%)	31/157 (19.7%)	18/107 (16.8%)	15/89 (16.9%)	14/89 (15.7%)

Note: Phase of Study: Visit 1=baseline, Visit 4=sample obtained after completion of "ON" cycle treatment; Visit 5=sample obtained at end of "OFF" cycle
Denominator is based upon the number of patients having an MIC value available at a given visit.
Source: Module 5.3.5.1, CT01 Appendix 16.2.6.4 and Module 5.3.5.1, CT02 Appendix 16.2.6.17

Mean Change in Viable *P. aeruginosa* Counts from Baseline

Table 39 provides information on the treatment effect on the *P. aeruginosa* load baseline. Because the data is presented using $\log_{10}$ transformations, a decrease in one $\log_{10}$ as observed for CHF 1538 is equivalent to a reduction of 90% of the bacterial load per gram of sputum. A significant difference in mean change from baseline load occurred after the first "ON" cycle ($p<0.001$; CI=[-1.57, -0.70]). There was no significant difference in the bacterial load for the treatment groups at the end of the "OFF" cycle.

Table 39: Integrated log_{10} Bacterial Load (CFUs/g Sputum) Mean Baseline and Mean Change From Baseline: ITT Population, Studies CT01 and CT02

Visit	Week		CHF 1538	Placebo	P-Value
1	Baseline	N	186	110	
		Mean[1]	5.76	5.77	0.935
End of "ON" Cycle	4	N	186	110	
		Mean Change from Baseline[2]	-1.88	-0.75	< 0.001
		Difference (95% CI)	-1.14 (-1.57, -0.70)		
End of "OFF" Cycle	8	N	180	105	
		Mean Change from Baseline	-0.86	-0.49	0.086
		Difference (95% CI)	-0.38 (-0.80, 0.05)		

[1] A value of 20 CFU/g (based upon the dilution factor in the counting procedure) was used for all instances where the *P. aeruginosa* pathogen was eradicated.
[2] Adjusted for baseline value
N's are based upon the number of patients with a baseline and end of "ON" cycle bacterial load value, or a baseline and end of "OFF" cycle bacterial load value
Source: Module 5.3.5.3, ISE Tables, Tables 1, 2, 3, 4

Microbiological Outcome

The analysis of microbiological outcome is shown at the end of treatment and the follow-up visits at baseline MIC value in Table 40. The analysis by baseline MIC shows that instances of microbiological eradication were rare in either treatment group at any visit. There was a high percentage of persistors in the CHF 1538 group at the end of the "ON" therapy period (Visit 4) with somewhat lower percentages "OFF" therapy at follow-up (Visit 5). A higher percentage of persistors were also present in the placebo group at both the end of "ON" treatment and "OFF" treatment follow-up visits. Persistence of *P. aeruginosa* following tobramycin therapy in CF patient is not unexpected. No correlation was evident between MIC value and microbiological eradication rate. The superinfection rate was similar in both treatment groups. Reinfection was not observed in either treatment group at the end of "ON" treatment period, and the rate of re-infection was slightly higher in the CHF 1538 group at the end of the "OFF" treatment period (Visit 5).

Table 40: Integrated Microbiological Outcome at the End of Treatment (Visit 4) and Follow-up (Visit 5) by Baseline MIC: ITT Population, Studies CT01 and CT02

Baseline MIC (µg/mL)	CHF 1538 (N=190)				Placebo (N=114)			
	Erad1 n/N(%)	Pers2 n/N(%)	Sup3 n/N(%)	Reinf4 n/N(%)	Erad n/N(%)	Pers n/N(%)	Sup n/N(%)	Reinf n/N(%)
	End of Treatment (Visit 4)							
≤ 0.25	1/5 (20.0%)	3/5 (60.0%)	1/5 (20.0%)	0/5 (0.0%)	0/13 (0.0%)	9/13 (69.2%)	4/13 (30.8%)	0/13 (0.0%)
0.5	1/24 (4.2%)	16/24 (66.7%)	7/24 (29.2%)	0/24 (0.0%)	1/19 (5.3%)	14/19 (73.7%)	4/19 (21.1%)	0/19 (0.0%)
1	1/46 (2.2%)	36/46 (78.3%)	9/46 (19.6%)	0/46 (0.0%)	0/26 (0.0%)	22/26 (84.6%)	4/26 (15.4%)	0/26 (0.0%)
2	2/27 (7.4%)	21/27 (77.8%)	4/27 (14.8%)	0/27 (0.0%)	2/19 (10.5%)	15/19 (79.0%)	2/19 (10.5%)	0/19 (0.0%)
4	3/15 (20.0%)	12/15 (80.0%)	0/15 (0.0%)	0/15 (0.0%)	2/11 (18.2%)	9/11 (81.8%)	0/11 (0.0%)	0/11 (0.0%)
8	1/7 (14.3%)	6/7 (85.7%)	0/7 (0.0%)	0/7 (0.0%)	0/4 (0.0%)	4/4 (100.0%)	0/4 (0.0%)	0/4 (0.0%)
16	0/9 (0.0%)	6/9 (66.7%)	3/9 (33.3%)	0/9 (0.0%)	0/3 (0.0%)	1/3 (33.3%)	2/3 (66.7%)	0/3 (0.0%)
32	0/5 (0.0%)	3/5 (60.0%)	2/5 (40.0%)	0/5 (0.0%)	0/8 (0.0%)	5/8 (62.5%)	3/8 (37.5%)	0/8 (0.0%)
64	1/24 (4.2%)	16/24 (66.7%)	7/24 (29.2%)	0/24 (0.0%)	0/11 (0.0%)	9/11 (81.8%)	2/11 (18.2%)	0/11 (0.0%)
128	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
256	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
Total	10/163 (6.1%)	119/163 (73.0%)	34/163 (20.9%)	0/163 (0.0%)	5/114 (4.4%)	88/114 (77.2%)	21/114 (18.4%)	0/114 (0.0%)

CONCLUSION

The data from pivotal studies CT01 and CT02 is presented individually and combined in the preceding pages. The data from CT01 and CT02 individually do not suggest any non-statistical significant microbiological adverse events from use of aerosolized tobramycin for treating CF patients. Combining the data suggests the same.

Treatment-Emergent Microorganisms

In the CHF 1538-treated patients the organism identified most frequently was the Gram-positive bacterium *Staphylococcus aureus* with the yeast *Candida* species (including *C. albicans*) being the nest most prevalent. In the placebo-treated group the same two organisms were the most prevalent. There was a variety of Gram-negative bacteria identified in both treatment groups, though the incidence of any individual species was low. This information is presented in Table 41 (2.7.2 Summary of Clinical Pharmacology Studies pg. 110) which is not shown here.

MIC Changes During Therapy

Table 42 is an assessment of MIC shifts fro individual isolates from the baseline tobramycin MIC to those values observed at end of therapy or at follow-up. MIC values were changed (+/- 2-fold) for 41.7% and 42.5% in the CHF 1538 group at the end of therapy and the follow-up visit, and for 33.2% and 28.5% in the placebo group respectively. However, at both the end of therapy and the follow-up visits, a greater percentage of isolates in the CHF 1538 group showed a ≥4-fold increase in MIC than isolates in the placebo group. MIC values decreased ≥4-fold in a small percent of isolates in both treatment groups.

Table 42: Assessment of MIC Shifts from Observed Baseline Values and MIC Values Observed at End of the First "ON" Cycle and at End of the First "OFF" Cycle: Integrated Results for CT01 and CT02, ITT Population

Evaluability Criteria	Baseline vs. End of ON' Cycle		Baseline vs. End of 'OFF' Cycle	
	CHF 1538	Placebo	CHF 1538	Placebo
MIC increased[1]	22/187 (11.8%)	4/187 (2.1%)	30/207 (14.5%)	9/207 (4.3%)
MIC unchanged[2]	78/187 (41.7%)	62/187 (33.2%)	88/207 (42.5%)	59/207 (28.5%)
MIC decreased[3]	10/187 (5.3%)	11/187 (5.9%)	14/207 (6.8%)	7/207 (3.4%)

[1] Patients whose paired PA isolate exhibited ≥ 4-fold increase in the MIC between baseline and end of therapy or follow-up visits.
[2] Patients whose paired PA isolate exhibited ±2-fold change in the MIC between baseline and end of therapy or follow-up visits.
[3] Patients whose paired PA isolate exhibited ≥ 4-fold decrease in the MIC between baseline and end of therapy or follow-up visits.
Source: Module 5.3.5.1, CT01 Appendix 16.2.6.4 and Module 5.3.5.1, CT02 Appendix 16.2.6.17

CLINICAL STUDY CT03

This is not a pivotal study for the approval of this tobramycin product. CT-03 is an open-label, multinational, multicenter, randomized, parallel group study designed to compare the efficacy and tolerability of aerosolized CHF 1538 and TOBI, both administered via a nebulizer (PARI LC Plus® with the PARI Boy N® compressor, Pari, Germany), over a 4-week treatment in a twice-daily regimen in patients with CF and *P. aeruginosa* chronic infection.

> Study CMA-0631-CSR-0025: A multicentre, multinational, open label, randomized, parallel group clinical trial of Torineb™/Actitob®/Bramitob® (tobramycin solution for nebulization, 300 mg twice daily in 4 mL unit dose ampoules) compared to TOBI in the treatment of patients with cystic fibrosis and chronic infection with *Pseudomonas aeruginosa* (Module 5.3.5.1, CT03 CSR body).

The study was designed to demonstrate the non-inferiority of CHF 1538 compared to TOBI in the primary efficacy variable forced expiratory volume in one second (FEV_1) predicted normal at the end of the treatment phase in patients with CF and chronic *P. aeruginosa* infection of the lungs. Table 44 provides a summary of clinical study CT03. Among the secondary efficacy variables, microbiological tests included quantitative cell counts (CFUs) for *P. aeruginosa* isolated from sputum, antibiotic susceptibility testing (MIC range, MIC_{50}, and MIC_{90} were standard microbiological assessments to evaluate the susceptibility of *P. aeruginosa* to tobramycin.

Sputum Collection and Microbiological Culture Methods

Sputum specimens were collected at the study site and sent under refrigeration to a central laboratory ((b) (4)). The collection and transport of the specimens are described in the Protocol (Module 5.3.5.1, CT03 Appendix 16.1.1). Culture of specimens and identification of isolates were done by recognized methods. Prior to the study the methods for specimen collection, transport, culture of specimens, and identification of isolates were reviewed by this Reviewer and found to be appropriate.

Antibiotic Susceptibility Testing

Minimal inhibitory concentrations (MIC) were determined using the CLSI-recommended methods and quality controls for tobramycin and the seven other antibiotics (amikacin, aztreonam, ciprofloxacin, colistin, imipenem, levofloxacin, piperacillin/tazobactam) were run. The MIC was measured by a broth microdilution method using (b) (4) plates. A positive growth control well was included on every plate. All of these methods had been reviewed prior to the beginning of the clinical study and found to be acceptable by this Reviewer.

Isolates for susceptibility testing were collected at visit 1 (prior to treatment, Visit 4 ("ON"-treatment, and Visit 5 ("OFF"-treatment). MIC values were determined for the ITT population. Susceptibility to tobramycin was interpreted according to Clinical and laboratory standards (CLSI) standards for systemically-administered tobramycin: Susceptible, ≤4 mcg/mL = Susceptible, 8 mcg/mL = Intermediate, ≥ 16 mcg/mL = Resistant.

Table 44: Summary of Clinical Study CT03

Study	CT03
Design	Randomized (1:1), open-label, reference product controlled, parallel group multinational, multicenter, study.
Primary Objective	To demonstrate the non-inferiority of CHF 1538 compared to TOBI in the primary efficacy variable forced expiratory volume in one second (FEV_1) % predicted normal at the end of the treatment phase in patients with CF and chronic infection of the lungs with *P. aeruginosa*.
Number of Randomized Patients	324
Age Range (yrs)	6 – 47
FEV_1 (% pred.)	≥ 40% and ≤ 80%
Comparator Daily Dose	TOBI (tobramycin 300 mg/5 mL inhalation solution for nebulization) 600 mg in two divided doses
Tobramycin Daily Dose	CHF 1538 (tobramycin 300 mg/4 mL inhalation solution for nebulization) 600 mg in two divided doses
Duration of Treatment	4 weeks treatment followed by 4-week phase-out period without treatment
Efficacy Assessment	**Primary**: final FEV_1 % predicted normal (week 4) **Secondary**: other pulmonary function tests; *in-vitro* microbiological tests including microbiological outcomes (eradication, persistence, re-infection with respect to *P.aeruginosa*, or superinfection with micro-organisms other than *P. aeruginosa*), tobramycin MIC range, MIC_{50} and MIC_{90}, and *P. aeruginosa* bacterial load (CFUs at Visit 4 and Visit 5).
Safety Assessment	Adverse events (AEs) and adverse drug reactions (ADRs), audiometric tests, laboratory parameters (hematology and blood chemistry), vital signs (heart rate and blood pressure) and physical examination.

Source: Module 5.3.5.1, CT03 Study Report Body, Section 9.1

Microbiological Outcome by Tobramycin Baseline MIC

Microbiological outcome is presented by tobramycin baseline (Visit 1) MIC values, at Visit 4 ("ON" treatment), and Visit 5 ("OFF" treatment) for all P. aeruginosa

morphotypes combined. Microbiological outcomes by baseline MIC values are compared between the two treatment groups at Visits 4 and 5 for the ITT population.

Microbiological outcome: At "ON" treatment (Visit 4), the following definitions were given on the basis of the results of the microbiologic test:

- Eradication (elimination of *P. aeruginosa*)
- Persistence (persistence of *P. aeruginosa* detected at Visit 1
- Superinfection (appearance of a pathogen other than *P. aeruginosa* not detected at Visit 1

At "OFF" treatment (Visit 5), the following definitions were given on the basis of the results of the microbiologic test:

- Eradication (elimination of *P. aeruginosa*)
- Persistence (persistence of *P. aeruginosa* detected at Visit 4 or at Visit 1 if Visit 4 is missing
- Superinfection (appearance of a pathogen other than *P. aeruginosa* not detected at Visit 4 or at Visit 1, and
- Re-infection (appearance of *P. aeruginosa* detected at Visit 1 and eradicated at Visit 4

Outcomes were analyzed according to the following hierarchy: Superinfection supersedes eradication; Persistence for *P. aeruginosa* supersedes Superinfection; and Re-infection for *P. aeruginosa* supersedes Superinfection. Overall outcomes could be designated as either a "positive outcome" (Eradication) or as a "Negative outcome" if the microbiological outcome was Persistence, Superinfection or Re-infection.

Efficacy Analysis

Categorization by Colony Morphology

The Applicant provided information on the tobramycin MIC as it related to different morphotypes of *P. aeruginosa* isolated from the sputum of patients in each treatment group. Three morphotypes were recognized: mucoid, dry and small colony variant. These morphotypes were equally distributed in the two study groups. Some patients had more then one morphotype at a given visit and some patients had all three morphotypes at a given visit. No attempt was made to correlate morphotype with disease severity. When the tobramycin susceptibility of different morphotypes was determined the morphotype with the highest tobramycin MIC was used for analysis.

The Applicant looked at the tobramycin MICs of the various morphotypes at Visits 1, 4, and 5. A summary of these results is shown in Table 55. There is some suggestion that the mucoid morphotype was more susceptible to tobramycin then either the dry or small

colony variant which has been suggested by other investigators. However, the numbers of isolates and the variability in the MICs seen in these studies makes it impossible to come to any definitive conclusion.

Table 55: Tobramycin MIC (µg/mL) Summary by Morphotype and Overall; Visit 1, ITT Population

	CHF 1538 (N=158)				TOBI (N=163)			
	N (%)	Range	MIC_{50}	MIC_{90}	N (%)	Range	MIC_{50}	MIC_{90}
Visit 1								
Morphotype 1: mucoid	123 (77.8%)	≤ 0.12- > 512	0.5	2	132 (81.0%)	≤ 0.12-16	0.5	2
Morphotype 2: dry	105 (66.5%)	≤ 0.12- > 512	1	8	95 (58.3%)	≤ 0.12-64	0.5	4
Morphotype 3: small colony variant	17 (10.8%)	0.25- > 512	2	32	30 (18.4%)	≤ 0.12- > 512	2	8
Overall	158 (100%)	≤ 0.12- > 512	1	8	162 (99.4%)	≤ 0.12- > 512	0.5	4
Visit 4								
Morphotype 1: mucoid	113 (71.5%)	≤ 0.12- > 512	0.5	8	115 (70.6%)	≤ 0.12- > 512	0.5	4
Morphotype 2: dry	68 (43.0%)	≤ 0.12- > 512	1	64	72 (44.2%)	≤ 0.12- > 512	1	64
Morphotype 3: small colony variant	12 (7.6%)	0.5-256	1	64	13 (8.0%)	0.25-128	1	32
Overall	126 (79.7%)	≤ 0.12- > 512	1	32	134 (82.2%)	≤ 0.12- > 512	0.5	32

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Table 55: Tobramycin MIC (µg/mL) Summary by Morphotype and Overall; Visit 1, ITT Population (Continued)

	CHF 1538 (N=158)					TOBI (N=163)				
	N (%)	Range	MIC_{50}	MIC_{90}		N (%)	Range	MIC_{50}	MIC_{90}	
Visit 5										
Morphotype 1: mucoid	110 (69.6%)	≤ 0.12-512	0.5	4		108 (66.3%)	≤ 0.12- > 512	0.5	4	
Morphotype 2: dry	78 (49.4%)	≤ 0.12-512	0.5	16		71 (43.6%)	≤ 0.12- > 512	0.5	16	
Morphotype 3: small colony variant	24 (15.2%)	≤ 0.12-512	2	128		21 (12.9%)	0.25-128	4	64	
Overall	129 (81.6%)	≤ 0.12-512	1	32		128 (78.5%)	≤ 0.12- > 512	1	32	

Source data: Module 5.3.5.1, CT03 Appendices 16.2.6.2 and 16.2.6.3
% of patients is based on the total number of patients in each treatment group in the ITT population.
One patient can have more than one *P. aeruginosa* morphotype at each visit.
If a patient has more than one *P. aeruginosa* morphotype at a given visit, then the highest tobramycin MIC value was used, regardless of *P. aeruginosa* morphotype.
If a patient has more than one available result for each morphotype then the highest tobramycin MIC value was used. If the tobramycin MIC values are equal then the MIC value for the isolate with the highest bacterial load value was used.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

MIC Distributions

The distributions and percent range of tobramycin MIC results for *P. aeruginosa* clinical trial isolates (all morphotypes) at Visits 1, 4. and 5 are shown in Table 51. The range of MIC values observed for the CHF 1538 and TOBI study arms show the variability of MIC results independent of tobramycin drug exposure.

At Baseline (Visit 1) the distribution of *P. aeruginosa* with MICs ≤4 mcg/mL in the CHF1538 and TOBI arms was 85.4% and 85% respectively. These percentages decreased slightly in each treatment group at Visit 4 (end of "on" cycle) as the susceptibility populations for CHF 1538 5.3% while TOBI susceptible [populations declined 7.9%. Additional change in the percentage of susceptible *P. aeruginosa* isolates was observed at the end of the first "OFF" cycle (Visit 5) compared to baseline (Visit 1) in both treatment arms; the change in percentage of susceptible isolates for CHF 1538 and TOBI was 7.9% and 9.6% respectively. These results suggest that, throughout the study, the majority of *P. aeruginosa* isolates remained susceptible to tobramycin in both treatment arms and that both tobramycin formulations produced equivalent microbiological treatment effects in the bacterial populations of *P. aeruginosa*.

Table 51: Distribution of Tobramycin MIC values (µg/mL) Overall; Summary by Visit, ITT Population

MIC (µg/mL)	CHF 1538 (n/N (%))			
	Visit 1	Visit 4	Visit 5	% Range
≤ 0.12	6/158 (3.8%)	8/126 (6.3%)	8/129 (6.2%)	3.8-6.3
0.25	20/158 (12.7%)	13/126 (10.3%)	17/129 (13.2%)	10.3-13.2
0.5	46/158 (29.1%)	29/126 (23.0%)	31/129 (24.0%)	23.0-29.1
1	34/158 (21.5%)	26/126 (20.6%)	25/129 (19.4%)	19.4-21.5
2	22/158 (13.9%)	18/126 (14.3%)	11/129 (8.5%)	8.5-14.3
4	7/158 (4.4%)	7/126 (5.6%)	8/129 (6.2%)	4.4-6.2
8	10/158 (6.3%)	9/126 (7.1%)	5/129 (3.9%)	3.9-7.1
16	1/158 (0.6%)	2/126 (1.6%)	9/129 (7.0%)	0.6-7.0
32	4/158 (2.5%)	2/126 (1.6%)	5/129 (3.9%)	1.6-3.9
64	3/158 (1.9%)	4/126 (3.2%)	2/129 (1.6%)	1.6-3.2
128	2/158 (1.3%)	3/126 (2.4%)	2/129 (1.6%)	1.3-2.4
256	0/158 (0%)	2/126 (1.6%)	1/129 (0.8%)	0-1.6
512	0/158 (0%)	0/126 (0%)	5/129 (3.9%)	0-5.0
>512	3/158 (1.9%)	3/126 (2.4%)	0/129 (0%)	0-1.9
Missing	0	32	29	

Table 51: Distribution of Tobramycin MIC values (µg/mL) Overall; Summary by Visit, ITT Population (Continued)

MIC (µg/mL)	TOBI (n/N (%))			
	Visit 1	Visit 4	Visit 5	% Range
≤ 0.12	11/162 (6.8%)	9/134 (6.7%)	8/128 (6.3%)	6.3-6.8
0.25	26/162 (16.0%)	15/134 (11.2%)	16/128 (12.5%)	11.2-16.0
0.5	51/162 (31.5%)	46/134 (34.3%)	39/128 (30.5%)	30.5-34.3
1	32/162 (19.8%)	19/134 (14.2%)	24/128 (18.8%)	14.2-19.8
2	16/162 (9.9%)	13/134 (9.7%)	8/128 (6.3%)	6.3-9.9
4	10/162 (6.2%)	4/134 (3.0%)	12/128 (9.4%)	3.0-9.4
8	8/162 (4.9%)	6/134 (4.5%)	4/128 (3.1%)	3.1-4.9
16	4/162 (2.5%)	5/134 (3.7%)	3/128 (2.3%)	2.3-3.7
32	2/162 (1.2%)	5/134 (3.7%)	6/128 (4.7%)	1.2-4.7
64	1/162 (0.6%)	2/134 (1.5%)	1/128 (0.8%)	0.6-1.5
128	0/162 (0%)	4/134 (3.0%)	2/128 (1.6%)	0-3.0
256	0/162 (0%)	1/134 (0.7%)	0/128 (0%)	0-0.7
512	0/162 (0%)	1/134 (0.7%)	2/128 (1.6%)	0-1.6
>512	1/162 (0.6%)	4/134 (3.0%)	3/128 (2.3%)	0.6-3.0
Missing	1	29	35	

If a patient has more than one *P. aeruginosa* morphotype at a given visit, then the highest tobramycin MIC value was used, regardless of *P. aeruginosa* morphotype.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.
Source data: Module 5.3.5.1, CT03Appendix 16.2.6.2 and 16.2.6.3

The activity of seven antibiotics along with tobramycin against the *P. aeruginosa* isolates obtained from patients in both study groups is shown in Table 56. In Table 56 it can be seen that the MICs to the various antibiotics were similar in both the CHF 1538 and TOBI treatment arms.

Table 56: MIC (µg/mL) Summary for Tobramycin, Amikacin, Aztreonam, Ciprofloxacin, Colistin, Imipenem, Levofloxacin, and Piperacillin/tazobactam; Overall at Visit 1, ITT Population

Antibiotic	CHF 1538 (N=158)			TOBI (N=163)		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Tobramycin	≤0.12- > 512	1	8	≤0.12- > 512	0.5	4
Amikacin	≤0.25- > 512	8	64	≤0.25-512	4	32
Aztreonam	≤0.25- > 512	4	128	≤0.25- > 512	4	128
Ciprofloxacin	≤0.12- > 256	1	4	≤0.12- 64	0.5	4
Colistin	0.25- > 128	1	4	≤0.12- > 128	1	4
Imipenem	≤0.25- > 256	1	32	≤0.25-256	1	16
Levofloxacin	≤0.25- > 512	2	16	≤0.25-128	1	8
Piperacillin/tazobactam	≤0.25- > 512	4	512	≤0.25- > 512	4	128

Source data: Module 5.3.5.1, CT03 Study Report Body, Figure 15, Figure 34, Figure 35, Figure 5, Figure 36, Figure 37, Figure 38, Figure 39, and Figure 40
If a patient has more than one *P. aeruginosa* morphotype at a given visit, then the highest MIC value was used, regardless of *P. aeruginosa* morphotype.
Values for piperacillin/tazobactam are reported as the piperacillin value; all concentrations of piperacillin are in combination with 4 µg/mL tazobactam
Patient 707002 is missing at Visit 1 because the sputum was received partially frozen and consequently excluded from the analysis.

Distribution of Strains based Upon Tobramycin Susceptibility

Table 57 shows the distribution of tobramycin MIC values for *P. aeruginosa* for both the CHF 1538 and TOBI treatment arms using the tobramycin susceptibility interpretive criteria for parenteral tobramycin. The susceptibility profiles at the various days in both the CHF 1538 and TOBI study groups are very similar overall. A substantial proportion of isolates in both the CHF 1538 and TOBI treatment groups remained susceptible at the end of the "ON" drug period (Visit 4) with 80.2% and 79.1% susceptible respectively. At the end of Visit 4, there was an increase in the percentage of resistant isolates in both treatment groups with the greatest increase seen in the TOBI treatment group. At visit 5 (end of the "OFF" drug cycle), there was a slight decrease in the percent resistant isolates for both treatment groups relative to visit 4. The percentage of susceptible isolates was similar in both treatment groups at Visit 4 and 5. Overall, the results demonstrate an elevation in MIC value for a small portion of the isolates in both treatment groups during the course of the study.

Table 57: Tobramycin MIC Values (µg/mL) by Susceptibility Class; Summary by Visit, ITT Population

Tobramycin Susceptibility Category at Visit	CHF 1538 (N=158)	TOBI (N=163)
Visit 1		
≤4	135 (85.4%)	146 (90.1%)
8	10 (6.3%)	8 (4.9%)
≥16	13 (8.2%)	8 (4.9%)
Missing	0	1
Visit 4		
≤4	101 (80.2%)	106 (79.1%)
8	9 (7.1%)	6 (4.5%)
≥16	16 (12.7%)	22 (16.4%)
Missing	32	29
Visit 5		
≤4	100 (77.5%)	107 (83.6%)
8	5 (3.9%)	4 (3.1%)
≥16	24 (8.6%)	17 (13.3%)
Missing	29	35

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3

"Missing" includes cases where *P. aeruginosa* has been eradicated and therefore no MIC was available and instances where no specimen was collected or it was not analyzable.
If a patient has more than one *P. aeruginosa* morphotype at a given visit, then the highest tobramycin MIC value was used, regardless of *P. aeruginosa* morphotype.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.
Tobramycin systemic interpretive criteria (Susceptible, ≤ 4 µg/mL; Intermediate, 8 µg/mL; Resistant, ≥16 µg/mL)

Mean Changes from Baseline Viable Counts

The *P. aeruginosa* bacterial density in $\log_{10}$ CFU/gram of sputum for each treatment group by study is shown in Table 58. This is a summary of the individual values for each patient. Similar bacterial load values were observed for the two treatment groups at each study visit suggesting these are typical population densities per gram of sputum colonizing the lungs of CF patients. The mean change in bacterial density from baseline levels is presented for the end of the "ON" cycle (Visit 4) and the end of the "OFF" cycle (Visit 5) in Table 59. The bacterial load showed a mean reduction of 2.14 and 2.07 $\log_{10}$ CFU/gram was observed at the end of the "ON" cycle for CHF 1538 and TOBI respectively. The bacterial load at the end of the "OFF" cycle was 0.72 and 0.87 $\log_{10}$ CFU/gram for CHF 1538 and TOBI respectively, indicating an increase in bacterial load relative to the end of the "ON" cycle. The ANCOVA model analysis results are shown in Table 60. No significant difference was evident with respect to the treatment or country while a significant difference was observed ($p<0.001$) for change from baseline $\log_{10}$ bacterial load (CFU/gram)

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Table 58: *P. aeruginosa* $\log_{10}$ Bacterial Load (CFUs/gram) Summary by Visit: ITT Population

	CHF 1538 (N=158)	TOBI (N=163)
Visit 1		
N	158	162
Mean (SD)	6.56 (1.70)	6.64 (1.57)
95% CI	[6.30; 6.83]	[6.40; 6.89]
Median	6.90	7.00
Min / Max	1.30 / 8.78	1.30 / 8.78
Missing	0	1
Visit 4		
N	152	157
Mean (SD)	4.41 (2.22)	4.58 (2.25)
95% CI	[4.06; 4.77]	[4.23; 4.93]
Median	4.62	4.90
Min / Max	1.30 / 8.75	1.30 / 8.60
Missing	6	6
Visit 5		
N	147	147
Mean (SD)	5.78 (2.20)	5.81 (2.37)
95% CI	[5.42; 6.14]	[5.42; 6.20]
Median	6.58	6.64
Min / Max	1.30 / 8.78	1.30 / 8.78
Missing	11	16

Source data: Module 5.3.5.1, CT03 Appendices 16.2.6.2 and 16.2.6.3
CFU colony forming units
If a patient has more than one *P. aeruginosa* morphotype at a given visit, and therefore more than one bacterial load value, then the bacterial load value corresponding to the highest tobramycin MIC value regardless of the *P. aeruginosa* morphotype was used.
If the tobramycin MIC value was the same for different *P. aeruginosa* morphotypes, then the bacterial load value corresponding to morphotype 1 (mucoid colony) was used. If morphotype 1 was not available, bacterial load value corresponding to morphotype 2 (dry colony) was used.
In instances of *P. aeruginosa* eradication (i.e. < 20 CFU/g, based upon the dilution factor in the plating and counting procedure), the numeric value of 20 CFU/g was used for bacterial load.
Where the bacterial load was recorded as '>600000000 CFU/g', the numeric value used for calculation of bacterial load was 600000000.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

Table 59: *P. aeruginosa* log_{10} Bacterial Load (CFUs/gram) Change from Baseline (Visit 1): ITT Population

	CHF 1538 (N=158)	TOBI (N=163)
Visit 4		
N	152	156
Mean (SD)	-2.14 (2.41)	-2.07 (2.20)
95% CI	[-2.52; -1.75]	[-2.42; -1.72]
Median	-2.09	-1.79
Min / Max	-7.48 / 4.00	-7.48 / 1.72
Missing	6	7
Visit 5		
N	147	147
Mean (SD)	-0.72 (2.17)	-0.87 (2.23)
95% CI	[-1.07; -0.36]	[-1.24; -0.51]
Median	-0.40	-0.48
Min / Max	-6.54 / 4.90	-7.48 / 6.08
Missing	11	16

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
CFU colony forming units
If a patient has more than one *P. aeruginosa* morphotype at a given visit, and therefore more than one bacterial load value, then the bacterial load value corresponding to the highest tobramycin MIC value regardless of the *P. aeruginosa* morphotype was used.
If the tobramycin MIC value was the same for different *P. aeruginosa* morphotypes, then the bacterial load value corresponding to morphotype 1 (mucoid colony) was used. If morphotype 1 was not available, bacterial load value corresponding to morphotype 2 (dry colony) was used.
In instances of *P. aeruginosa* eradication (i.e. <20 CFU/g, based upon the dilution factor in the plating and counting procedure), the numeric value of 20 CFU/g was used for bacterial load.
Where the bacterial load was recorded as '>600000000 CFU/g', the numeric value used for calculation of bacterial load was 600000000.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

Table 60: *P. aeruginosa* log_{10} Bacterial Load (CFUs/gram) ANCOVA: ITT Population

	CHF 1538 (N=158)		TOBI (N=163)
ANCOVA			
N (missing)	152 (6)		156 (7)
LSMEANS(SEM)	-1.81 (0.21)		-1.85 (0.20)
Fixed effects/Covariate: p-value			
Treatment		0.820	
Country		0.310	
Baseline log_{10} bacterial load (CFU/g) value		< 0.001	
CHF 1538 minus TOBI			
LSMEANS(SEM)		0.04 (0.18)	
95% CI		[-0.31; 0.39]	

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
ANCOVA model: Change from baseline (V1) to V4 in log_{10} bacterial load (CFU/g) value = treatment and country as fixed effects and baseline log_{10} bacterial load (CFU/g) value as covariate
All p-values are two-sided

CT03 Microbiology Outcome

Table 61 provides information on the overall analysis of microbiology outcomes for both study groups. As can be seen in study Visit 4 (end of the "ON" treatment period, there was a low percentage of microbiological eradication in both treatment groups (9.2% for CFH 1538-treated and 7.1% for TOBI-treated). This type of result is not uncommon to see in cystic fibrosis studies for both inhaled, systemically administered and orally administered antibacterials. Similar rates of persistence and superinfection were noted in the two treatment groups. At Visit 5 (end of the "OFF" treatment period), the rates of eradication were lower than at Visit 4 in both treatment groups. While the percentage of re-infection in the CH 1538 treatment group was higher then seen in the TOBI-treatment group the numbers were small. The Cochran-Mantel-Haenzel test controlling for country revealed no statistically significant differences between the CH 1538 and TOBI with respect to percentages in outcome categories. From the data in Table 61 it appears from a microbiology perspective that CH 1538 produces equivalent results to the approved TOBI product.

Table 61: Microbiological Outcomes (Eradication, Persistence, Superinfection, Re-infection); Summary by Visit, ITT Population

	CHF 1538 (N=158)	TOBI (N=163)	CMH Test[1]
Visit 1			
Presence of *P. aeruginosa*	158 (100%)	162 (100%)	
Absence of *P. aeruginosa*	0	0	
Missing	0	1	
Visit 4[2]			
Eradication	14 (9.2%)	11 (7.1%)	p=0.692
Persistence	126 (82.9%)	133 (85.3%)	
Superinfection	12 (7.9%)	12 (7.7%)	
Missing	6	7	
Visit 5[3]			
Eradication	4 (2.7%)	5 (3.4%)	p=0.128
Persistence	116 (78.9%)	122 (83.0%)	
Superinfection	14 (9.5%)	14 (9.5%)	
Re-infection	13 (8.8%)	6 (4.1%)	
Missing	11	16	

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
[1] Cochran-Mantel-Haenszel test controlling for country
Microbiological outcomes are derived considering all *P. aeruginosa* morphotypes together.
[2] At Visit 4:
Eradication=elimination of *P. aeruginosa*
Persistence=persistence of *P. aeruginosa* detected at V1
Superinfection=appearance of a pathogen (other than *P. aeruginosa*) not detected at V1
[3] At Visit 5:
Eradication=elimination of *P. aeruginosa*
Persistence=persistence of *P. aeruginosa* detected at V4 (or at V1 if V4 missing)
Superinfection=appearance of a pathogen (other than *P. aeruginosa*) not detected at V4 or at V1
Re-infection=re-appearance of *P. aeruginosa* detected at V1 and eradicated at V4
Superinfection supercedes eradication. Persistence for *P. aeruginosa* supercedes superinfection.
Re-infection for *P. aeruginosa* supercedes superinfection.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

The analysis of microbiological outcomes is analyzed further at all study visits by baseline MIC values in Table 62. This data is further differentiated into baseline tobramycin susceptibility categories (Susceptible, Intermediate, or Resistant) in Table 63, and summarized in an overall positive or negative outcome in Table 64.

As seen in Table 62 at Visit 4, in both treatment groups, persistence occurred across a broad range of MIC values with no apparent correlation with MIC value. This type of results has been observed in other cystic fibrosis antibacterial treatment studies. At visit 5, a pattern similar to that seen in Visit 4 was observed with regard to MIC versus

eradication, persistence, and superinfection with the exception of eradication which was observed less frequently. Re-infections at Visit 5 were distributed across a wide range of MIC values in both treatment groups. There is an increase in the incidence of reinfection for both treatment arms in Visit 5 compared to Visit 4. This increase, the Applicant hypotheses is likely do to emergence of *P. aeruginosa* in patients previously categorized as eradication.

The microbiological outcome data shows that there were no uncommon instances of microbiological eradication in either the CHF 1538 or TOBI-treatment groups at either visit 4 or 5. A high percentage of persistors were observed in the treatment groups at Visit 4 and 5, with a modest incidence of superinfection and reinfection (Visit 5). The persistence of P. aeruginosa during and after antibacterial treatment is not unexpected and the numbers seen in these studies are consistent with other data.

Table 62: Microbiological Outcomes (Eradication, Persistence, Superinfection, Re-infection) by Baseline MIC Value; Summary by Visit, ITT Population

	CHF 1538 (N=158)				TOBI (N=163)			
	ERAD[1]	PERS	SUPER	REINF	ERAD	PERS	SUPER	REINF
Visit 4[2]								
≤0.12	1/6 (16.7%)	5/6 (83.3%)	0	.	3/11 (27.3%)	6/11 (54.5%)	2/1 (18.2%)	.
0.25	2/19 (10.5%)	15/19 (78.9%)	2/19 (10.5%)	.	2/24 (8.3%)	19/24 (79.2%)	3/24 (12.5%)	.
0.5	6/45 (13.3%)	34/45 (75.6%)	5/45 (11.1%)	.	3/49 (6.1%)	44/49 (89.8%)	2/49 (4.1%)	.
1	2/33 (6.1%)	29/33 (87.9%)	2/33 (6.1%)	.	1/32 (3.1%)	28/32 (87.5%)	3/32 (9.4%)	.
2	2/20 (10.0%)	16/20 (80.0%)	2 /20 (10.0%)	.	0	15/15 (100.0%)	0	.
4	1/7 (14.3%)	6/7 (85.7%)	0	.	0	10/10 (100.0%)	0	.
8	0	9/10 (90.0%)	1/10 (10.0%)	.	1/7 (14.3%)	4/7 (57.1%)	2/7 (28.6%)	.
16	0	1/1 (100.0%)	0	.	0	4/4 (100.0%)	0	.
32	0	4/4 (100.0%)	0	.	1/2 (50.0%)	1/2 (50.0%)	0	.
64	0	3/3 (100.0%)	0	.	0	1/1 (100.0%)	0	.
128	0	2/2 (100.0%)	0	.	0	0	0	.
>512	0	2/2 (100.0%)	0	.	0	1/1 (100.0%)	0	.
Total	14/152 (9.2%)	126/152 (82.9%)	12/152 (7.9%)	.	11/156 (7.1%)	133/156 (85.3%)	12/156 (7.7%)	.

The microbiological outcome data analyzed above was assessed according to systemic tobramycin interpretive criteria as seen in Table 63. The general conclusions from this analysis are similar to those stated for outcome versus MIC value analysis (Table 62). For both treatment groups, persistence is reported for isolates that were susceptible, intermediate or resistance to tobramycin. For both Visits 4 and 5, and in both treatment groups, more than 76% of the *P. aeruginosa* isolates were susceptible to tobramycin and yet were persistent. This suggests a lack of correlation between MICs and microbiological outcome a result that has been seen in other studies.

Table 62: Microbiological Outcomes (Eradication, Persistence, Superinfection, Re-infection) by Baseline MIC Value; Summary by Visit, ITT Population (Continued)

	CHF 1538 (N=158)				TOBI (N=163)			
	ERAD	PERS	SUPER	REINF	ERAD	PERS	SUPER	REINF
Visit 5[3]								
≤0.12	0	5/6 (83.3%)	0	1/6 (16.7%)	1/10 (10.0%)	5/10 (50.0%)	4/10 (40.0%)	0
0.25	1/19 (5.3%)	13/19 (68.4%)	2/19 (10.5%)	3/19 (15.8%)	1/23 (4.3%)	18/23 (78.3%)	3/23 (13.0%)	1/23 (4.3%)
0.5	1/39 (2.6%)	29/39 (74.4%)	6/39 (15.4%)	3/39 (7.7%)	0	39/46 (84.8%)	4/46 (8.7%)	3/46 (6.5%)
1	2/34 (5.9%)	26/34 (76.5%)	4/34 (11.8%)	2/34 (5.9%)	3/27 (11.1%)	23/27 (85.2%)	0	1/27 (3.7%)
2	0	17/21 (81.0%)	2/21 (9.5%)	2/21 (9.5%)	0	16/16 (100.0%)	0	0
4	0	5/6 (83.3%)	0	1/6 (16.7%)	0	9/9 (100.0%)	0	0
8	0	9/10 (90.0%)	0	1/10 (10.0%)	0	5/8 (62.5%)	2/8 (25.0%)	1/8 (12.5%)
16	0	1/1 (100.0%)	0	0	0	4/4 (100.0%)	0	0
32	0	4/4 (100.0%)	0	0	0	1/2 (50.0%)	1/2 (50.0%)	0
64	0	3/3 (100.0%)	0	0	0	1/1 (100.0%)	0	0
128	0	2/2 (100.0%)	0	0	0	0	0	0
>512	0	2/2 (100.0%)	0	0	0	1/1 (100.0%)	0	0
Total	4/147 (2.7%)	116/147 (78.9%)	14/147 (9.5%)	13/147 (8.8%)	5/147 (3.4%)	122/147 (83.0%)	14/147 (9.5%)	6/147 (4.1%)

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3

Table populated for patients with an available MIC value at V1. Microbiological outcomes derived considering all *P. aeruginosa* morphotypes together.

[1] Microbiological outcomes are derived considering all *P. aeruginosa* morphotypes together.

[2] At V4: ERAD=elimination of *P. aeruginosa*, PERS=persistence of *P. aeruginosa* detected at V1, SUPER=appearance of a pathogen (other than *P. aeruginosa*) not detected at V1, REINF=reinfection was not an option at Visit 4

[3] At V5: ERAD=elimination of *P. aeruginosa*, PERS=persistence of *P. aeruginosa* detected at V4 (or at V1 if V4 missing), SUPER=appearance of a pathogen (other than *P. aeruginosa*) not detected at V4 or at V1; REINF=re-appearance of *P. aeruginosa* detected at V1 and eradicated at V4. Superinfection supercedes eradication. Persistence for *P. aeruginosa* supercedes superinfection. Re-infection for *P. aeruginosa* supercedes superinfection.

Table 63: Microbiological Outcomes (Eradication, Persistence, Superinfection, Re-infection) by Baseline Tobramycin Susceptibility Designation (Susceptible, Intermediate, or Resistant); Summary by Visit, ITT Population

	CHF 1538 (N=158)			TOBI (N=163)		
	S[1]	I	R	S	I	R
Visit 4[2]						
Eradication	14 (10.8%)	0	0	9 (6.4%)	1 (14.3%)	1 (12.5%)
Persistence	105 (80.8%)	9 (90.0%)	12 (100%)	122 (86.5%)	4 (57.1%)	7 (87.5%)
Superinfection	11 (8.5%)	1 (10.0%)	0	10 (7.1%)	2 (28.6%)	0
Missing	5	0	1	5	1	0
Visit 5[3]						
Eradication	4 (3.2%)	0	0	5 (3.8%)	0	0
Persistence	95 (76.0%)	9 (90.0%)	12 (100%)	110 (84.0%)	5 (62.5%)	7 (87.5%)
Superinfection	14 (11.2%)	0	0	11 (8.4%)	2 (25.0%)	1 (12.5%)
Re-infection	12 (9.6%)	1 (10.0%)	0	5 (3.8%)	1 (12.5%)	0
Missing	10	0	1	15	0	0

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2and 16.2.6.3

[1] Baseline (V1) susceptibility: S: Susceptible (MIC ≤4 µg/mL), I: Intermediate (MIC=8 µg/mL), R: Resistant (MIC ≥ 16 µg/mL)

Table populated for patients with an available susceptibility at V1

Microbiological outcomes are derived considering all *P. aeruginosa* morphotypes together.

[2] At Visit 4: Eradication = elimination of *P. aeruginosa*
Persistence = persistence of *P. aeruginosa* detected at V1
Superinfection = appearance of a pathogen (other than *P. aeruginosa*) not detected at V1

[3] At Visit 5: Eradication = elimination of *P. aeruginosa*
Persistence = persistence of *P. aeruginosa* detected at V4 (or at V1 if V4 missing)
Superinfection = appearance of a pathogen (other than *P. aeruginosa*) not detected at V4 or at V1
Re-infection = re-appearance of *P. aeruginosa* detected at V1 and eradicated at V4

Superinfection supercedes eradication. Persistence for *P. aeruginosa* supercedes superinfection. Re-infection for *P. aeruginosa* supercedes superinfection.

Table 64 provides a summary of microbiological outcomes by visit for the ITT population. The positive and negative outcomes were very similar in the two treatment groups.

Table 64: Microbiological Outcomes (Positive vs Negative Outcomes)-Summary by Visit: ITT Population

	CHF 1538 (N=158)	TOBI (N=163)	CMH Test[1]
Visit 4			
Positive outcome[2]	14 (9.2%)	11 (7.1%)	p=0.465
Negative outcome[3]	138 (90.8%)	145 (92.9%)	
Missing	6	7	
Visit 5			
Positive outcome	4 (2.7%)	5 (3.4%)	p=0.775
Negative outcome	143 (97.3%)	142 (96.6%)	
Missing	11	16	

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
[1] Cochran-Mantel-Haenszel test controlling for country
Microbiological outcomes are derived considering all *P. aeruginosa* morphotypes together.
[2] Positive outcome = eradication
[3] Negative outcome = persistence, superinfection or re-infection
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

Treatment Emergent Microorganisms

Table 65 presents information on the bacteria and yeast isolated from patients with superinfection at Visits 4 and 5. The bacteria and yeast not seen on the first visit are detailed in the table. The bacteria and yeast are similar in both treatment groups. While classified as superinfection none of the patients required treatment for exacerbation of symptoms.

Table 65: Microorganisms Isolated from Patients with Superinfection at Visits 4 or 5; Summary by Treatment Group, ITT Population

	CHF 1538 (N=158) [n (%)]	TOBI (N=163) [n (%)]
Gram-Positive Bacteria		
Staphylococcus aureus	17 (10.8%)	18 (11.0%)
Staphylococcus aureus, methicillin-resistant	2 (1.3%)	1 (0.6%)
β-hemolytic Streptococcus spp. (Group A)	1 (0.6%)	1 (0.6%)
β-hemolytic Streptococcus spp. (Group B)	0	1 (0.6%)
β-hemolytic Streptococcus spp. (Group C)	0	2 (1.2%)
β-hemolytic Streptococcus spp. (Group G)	1 (0.6%)	2 (1.2%)
β-hemolytic Streptococcus spp. (Group F)	2 (1.3%)	2 (1.2%)
Streptococcus pneumoniae	2 (1.3%)	3 (1.8%)
Gram-Negative Bacteria		
Achromobacterium xylosoxidans	2 (1.3%)	7 (4.3%)
Alcaligenes faecalis	2 (1.3%)	0
Burkholderia cepacia	0	1 (0.6%)
Enterobacter cloacae	2 (1.3%)	0
Escherichia coli	2 (1.3%)	1 (0.6%)
Haemophilus influenzae	3 (1.9%)	1 (0.6%)
Haemophilus parainfluenzae	10 (6.3%)	9 (5.5%)
Klebsiella oxytoca	1 (0.6%)	0
Serratia marcescens	1 (0.6%)	1 (0.6%)
Sphingobacterium spiritivorum	0	1 (0.6%)
Stenotrophomonas maltophilia	5 (3.2%)	2 (1.2%)

Table 65: Microorganisms Isolated from Patients with Superinfection at Visits 4 or 5; Summary by Treatment Group, ITT Population (Continued)

	CHF 1538 (N=158) [n (%)]	TOBI (N=163) [n (%)]
Yeasts		
Candida albicans	1 (0.6%)	0
Candida species NOT Candida albicans	1 (0.6%)	0

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
N : total number of patients in each treatment group
n: number of patients with microorganisms other than *P. aeruginosa* at Visit 4 or Visit 5
(%): percentage of patients with microorganisms other than *P. aeruginosa* at Visit 4 or Visit 5
A patient can have more than one microorganism
If a patient has the same microorganism at both Visit 4 and Visit 5 then the patient is counted only once

MIC Changes During Therapy

Table 66 provides information on the changes in tobramycin MICs in both treatment groups for visits 4 ("ON" tobramycin) and 5 ("OFF") tobramycin. Overall the tendency for MIC values to increase during conduct of the study was similar in the treatment groups.

Table 66: Tobramycin MIC Value (µg/mL) Shift from Baseline (Visit 1) Values: ITT Population

	CHF 1538 (N=158)	TOBI (N=163)
Visit 4		
Decreased[1]	19 (15.1%)	19 (14.3%)
Unchanged[2]	79 (62.7%)	78 (58.6%)
Increased[3]	28 (22.2%)	36 (27.1%)
Missing[4]	32	30
Visit 5		
Decreased	23 (17.8%)	19 (14.8%)
Unchanged	80 (62.0%)	86 (67.2%)
Increased	26 (20.2%)	23 (18.0%)
Missing	29	35

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3

[1] MIC decreased: Patients whose *P. aeruginosa* isolate exhibited ≥ 4-fold decrease in the MIC between baseline and end-of-therapy or follow-up visits.

[2] MIC unchanged: Patients whose *P. aeruginosa* isolate exhibited no change or a 2-fold increase or decrease in the MIC between baseline and end-of-therapy or follow-up visits.

[3] MIC increased: Patients whose *P. aeruginosa* isolate exhibited ≥ 4-fold increase in the MIC between baseline and end-of-therapy or follow-up visits.

[4] Missing indicates that for a given patient there was no *P. aeruginosa* isolated at one of the study visits

If a patient has more than one *P. aeruginosa* morphotype at a given visit, then the highest tobramycin MIC value was used, regardless of *P. aeruginosa* morphotype.

When MIC value was recorded as '≤ 0.12 µg/mL', the numeric value used for calculation of shift from baseline was 0.125 µg/mL.

When MIC value was recorded as '> 512 µg/mL', the numeric value used for calculation of shift from baseline was 1024 µg/mL.

Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

OVERALL CONCLUSION

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/s/

FREDERIC J MARSIK
06/21/2012

DIVISION OF ANTIINFECTIVE and OPHTHALMOLOGY PRODUCTS (HFD-520)
CLINICAL MICROBIOLOGY REVIEW

NDA 201-820 Date review completed: 31 May 11

Date company submitted: 22 Oct 10 Date received by CDER: 23 Oct 10
Reviewer: Fred Marsik, Ph.D. Date assigned: 23 Oct 10

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DRUG PRODUCT NAME

Proprietary: None at this time
Established name: Tobramycin
Code Name/Number: CHF 1538, Torineb®, Actitob®, Bramitob®
Chemical name: o-3amino-3-deoxy-α-D-glucopyranosyl-(1→6)-O-[2,6-diamino-2,3,6-trideoxy-α-D-ribohexopyranosyl-(1→4)]-2-deoxy-D-stretamine
Chemical formula: $C_{18}H_{37}N_5O_9$
Molecular weight: 467.5

PROPOSED INDICATION

Management of *Pseudomonas aeruginosa* infections in cystic fibrosis patients

PROPOSED DOSAGE FORM, ROUTE OF ADMINISTRATION, DOSAGE STRENGTH, DOSING INTERVAL, AND DURATION OF TREATMENT

Dosage form: Liquid
Route of administration: Inhalation –PARI LC Plus® with the PARI® Boy N compressor, Pari Germany
Strength and dosing interval: 300 mg twice daily
Duration of treatment: 4 weeks

DISPENSED

Rx

RELATED DOCUMENTS

IND 72,068

REMARKS

This is a 505(b)(2) submission for the use of inhaled tobramycin to manage *Pseudomonas aeruginosa* infections in cystic fibrosis patients.

OVERALL CLINICAL MICROBIOLOGY CONCLUSION

The primary objective of the studies conducted (CT01, CT02 and CT03) were to demonstrate the non-inferiority of inhaled aerosolized CHF 1538 compared to TOBI in patients with CF with chronic infection of the lungs with *P. aeruginosa*. The primary efficacy variable was FEV_1% predicted normal at the end of the 4 week treatment phase. Both the medical officer and statistician reviews should be consulted for the outcome of the primary outcome for study CT03.

Secondary microbiological efficacy variables were:

- Microbiological cultures (eradication, persistence, or re-infection with respect to *P. aeruginosa* or super-infection with respect to other bacteria) at Visits 4 and 5.
- Tobramycin MIC_{50} at Visits 4 and 5
- Tobramycin MIC_{90} at Visits 4 and 5
- Tobramycin MIC and Visits 4 and 5; and
- *P. aeruginosa* bacterial load expressed in CFUs at Visits 4 and 5

Microbiology data from all studies showed that many of the *P. aeruginosa* isolates present in the study patients had elevated MIC values for numerous anti-pseudomonas agents, and the minimal inhibitory concentration (MIC) values spanned the range of susceptible to tobramycin to resistant to tobramycin as determined by using tobramycin susceptibility test interpretive criteria for systemically administered tobramycin. Overall the susceptibility profiles of the baseline *P. aeruginosa* isolates were similar in both treatment groups.

Both treatments (CHF 1538 and TOBI) during the CT03 study were able to significantly reduce baseline bacterial load in the sputum samples obtained from patients. However, bacterial load increased once treatment of both groups stopped. There was no significant difference in the bacterial load between both treatment groups after cessation of treatment. In both treatment groups there were increases in the tobramycin MIC for a portion of the *P. aeruginosa* population. There were a low percentage of microbiological eradications in both treatment groups with rates of superinfection and re-infection being similar between the two treatment groups. The rates of positive and negative outcomes observed for CHF 1538 and TOBI were similar.

From a clinical microbiology perspective there is no evidence in the data from the treatment study groups (CHF1538 and TOBI) that suggest that CHF 1538 is inferior to TOBI for the treatment of *Pseudomonas aeruginosa* infection in the lungs of cystic fibrosis patients.

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EXECUTIVE SUMMARY

Introduction

Cystic fibrosis (CF) is the most common inherited lethal disease of the white population. It occurs primarily in individuals of central and western European origin and affects more than 30,000 Americans. The estimated incidence in the United States is 1 in 2000 to 2600 live white births, 1 in 19,000 live African American births, 1 in 11,500 live Hispanic births, and 1 in 25,000 live Asian American births. CF has an autosomal recessive mode of inheritance. Affected individuals are phenotypic homozygotes and both parents usually are heterozygotes or carriers. The carrier frequency in white individuals in the United States is approximately 1 in 25, with full siblings or children with CF having a 1 in 4 chance of being affected. In cystic fibrosis, the defect in the cyclic adenosine monophosphate-regulated chloride ion channels in the epithelial lining of the respiratory system favors the colonization of gram-negative bacteria (mainly *P. aeruginosa*) resulting in inflammation, compromised pulmonary function and mortality in CF patients.

The microbial species that are clearly associated with lung disease in CF patients (CF lung disease) are relatively few, including *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex. *Pseudomonas aeruginosa* is the most common pathogen of CF lung disease, infecting approximately 60% of the entire CF population and close to 80% of adolescents and adults. *Staphylococcus aureus* is recovered from approximately 50% of the CF population. *Burkholderia cepacia* is recovered from ~3% of American CF patients and 15% of Canadian CF patients.

Studies have shown that *P. aeruginosa* infections in CF patients can be seen as early as infancy. The initial isolates of *P. aeruginosa* that infect CF patients are described as "rough" or "planktonic". These isolates tend to be susceptible to a variety of antimicrobials, are motile and prototrophic and have smooth lipopolysaccharide. It is at this stage that some investigators believe that aggressive antimicrobial therapy can eradicate this organism. However, most patients develop chronic infection with an unusual phenotype of *P. aeruginosa* referred to as mucoid. Mucoid isolates are nonmotile, are frequently auxotrophic have rough lipopolysaccharide, and are frequently resistant to a wide variety of antimicrobial agents. The mucoid material is a polysaccharide polymer referred to as alginate, which forms the biofilm matrix and renders the embedded *P. aeruginosa* refractory to clearance by the immune system. (**21**). Isolation of *P. aeruginosa* from the respiratory secretions of CF patients is easily accomplished.

IN VITRO

IN VITRO SPECTRUM OF ACTIVITY

The Applicant provided information on the minimal inhibitory concentrations (MICs) of tobramycin needed to inhibit the growth of *Pseudomonas aeruginosa* isolates recovered from cystic fibrosis patients in the United States. The susceptibility testing of *P. aeruginosa* isolates was done by the method of the Clinical and Laboratory Standards Institute (CLSI). Along with the susceptibility test results for the isolates the Applicant provided the quality control (QC) results for each batch of tests. All of the QC values are within the acceptable ranges as recommended by CLSI. All isolates were identified as *P. aeruginosa* by standard identification methods.

Tables 4 & 5 show tobramycin as well as other antibacterial susceptibility results over the past 3 years for *P. aeruginosa* isolates obtained from cystic fibrosis patients in the United States. This background information was obtained for isolates from the United States to compare to the tobramycin susceptibility of *P. aeruginosa* isolates obtained during clinical studies to assure that the antibiograms of US isolates were similar to those obtained during clinical studies done in other countries. The percent resistant to the various antibacterials was determined based on interpretive criteria for parenteral forms of antibacterials since there are no interpretive criteria for inhaled antibiotics. Tobramycin resistance by these criteria ($\geq$16 mcg/mL) were identified in 20.4% of *P. aeruginosa* isolated between July 2007 and June 2008 and 17.4% between January 2008 and June 2009. A comparison of the susceptibility profiles of the *P. aeruginosa* isolates from the United States and from those obtained from clinical study sites suggests that the US isolates are similar to those obtained from clinical study sites outside the US (Table 68).

Table 4 Prevalence of Resistant *P. aeruginosa* Isolates for Period

Drug	July 2007 - June 2008 (N=357)	January 2008 - June 2008 (N=178)
	N (%)	N (%)
Tobramycin	73 (20.4%)	31 (17.4%)
Amikacin	106 (29.7%)	47 (26.4%)
Aztreonam	69 (19.3%)	36 (20.2%)
Cefepime	72 (20.2%)	41 (23.0%)
Ceftazidime	70 (19.6%)	37 (20.8%)
Meropenem	51 (14.3%)	33 (18.5%)
Piperacillin/tazobactam	66 (18.5%)	35 (19.7%)
Ticarcillin/clavulanate	85 (23.8%)	44 (24.7%)
Ciprofloxacin	88 (24.6%)	41 (23.0%)
Multidrug Resistance	34 (9.5%)	17 (9.6%)

Table 5 Prevalence of High-Level Resistance Among *P. aeruginosa* Isolates

	Non-Mucoid	Mucoid	Total
	N (%)	N (%)	N (%)
July 2007 - June 2008	174	183	357
P. aeruginosa isolates	14 (8.0%)	8 (4.4%)	22 (6.2%)
Tobramycin MIC > 128 µg/mL			
January 2008 - June 2008	89	89	178
P. aeruginosa isolates	4 (4.5%)	2 (2.2%)	6 (3.4%)
Tobramycin MIC > 128 µg/mL			

Table 68: Comparison of MIC Summary Values for Baseline Isolates from CT01, CT02, CT03, and US Surveillance Isolates

Study	Treatment Arm	No. Isolates	Minimal Inhibitory Concentration (µg/mL)		
			Range	MIC_{50}	MIC_{90}
CT01	CHF1538	29	≤0.25-256	4	64
	Placebo	27	0.5-64	4	16
CT02	CHF1538	160	≤0.25-64	1	64
	Placebo	80	≤0.25-64	1	64
CT03	CHF1538	158	≤0.12->512	1	8
	TOBI	163	≤0.12->512	0.5	4
Surveillance	--	357	≤0.12->512	2	32

Source data: Table 6 , Table 23 , Table 29 , Table 56

MECHANISM OF ACTION

Tobramycin a member of the aminoglycoside family of antimicrobials interferes with the first steps of protein synthesis by causing a misreading and premature termination of the translation or the genetic code of the mRNA template. The aberrant proteins produced maybe inserted into the cell membrane leading to altered permeability and further stimulation of aminoglycoside transport. This disruption eventually leads to death of the bacterial cell.

MECHANISM OF RESISTANCE

As with other antimicrobials, resistance to aminoglycosides may be intrinsic or acquired. Intrinsic resistance may be enzymatic or nonenzymatic. Mutations at the 16S ribosomal RNA (rRNA) can result in resistance. The methylating enzymes that modify the 16S rRNA exemplify enzymatic intrinsic resistance.

Acquired resistance to aminoglycosides results from the combination of decreased drug uptake, efflux pump activity or enzymatic modification of the drug.

The predominant resistance mechanisms to tobramycin in P. aeruginosa isolated from CF patients is impermeability and to a lesser extend enzymatic modification. Other mechanisms which cumulatively lead to decreased susceptibility of *P. aeruginosa* to tobramycin.

POST-ANTIBIOTIC EFFECT (PAE)

The PAE is persistent suppression of bacterial growth after short antimicrobial exposure. PAE can be measured in vitro or in animal models of infection. In vivo, the aminoglycosides consistently demonstrate a PAE that varies from 1 to 3 hours in broth and serum for *P. aeruginosa*.

TOBRAMYCIN INTERACTION WITH OTHER ANTIMICROBIALS

The mechanism of aminoglycoside synergistic activity may not be the same for all target organisms. Enhanced aminoglycoside uptake in the presence of cell-wall-active drugs (e.g. penicillins) has been demonstrated with *P. aeruginosa*.

PHARMACODYNAMICS OF TOBRAMYCIN

Tobramycin exerts its killing effect against bacteria in a concentration-dependent manner, so the higher the peak concentration of the drug (and therefore the higher C_{max}/MIC is reached), the greater is the degree of killing.

IN VITRO SUSCEPTIBILITY TEST METHODS

Susceptibility testing of *P. aeruginosa* can be done by the standardized method of the Clinical and Laboratory Standards Institute (CLSI).

DEVELOPMENT OF QC PARAMETERS FOR IN VITRO SUSCEPTIBILITY TESTING

In vitro susceptibility test QC parameters for the in vitro susceptibility testing of tobramycin are established.

INTERPRETATION OF IN VITRO SUSCEPTIBILITY TEST RESULTS

There are no interpretive criteria for topical agents such as inhaled tobramycin because the concentration of tobramycin achieved at the infected site is many fold higher then the concentration achieved when tobramycin is given systemically and there tends to be great variability in the concentration of the drug at the infected site when it is given by inhalation. While there are no clinical interpretive criteria for tobramycin related to isolates from cystic fibrosis patients that can be used to guide therapy the results of in vitro susceptibility testing of *P. aeruginosa* isolates from individual patients can be used to determine if there is an decrease in the tobramycin susceptibility of the *P. aeruginosa* which may be used indirectly to guide therapy.

IN VIVO

CLINICAL STUDY

Clinical Study CT03

This is the pivotal study for the approval of this tobramycin product. CT03 is an open-label, multinational, multicenter, randomized, parallel group study designed to compare

the efficacy and tolerability of aerosolized CHF 1538 and TOBI, both administered via a nebulizer (PARI LC Plus® with the PARI Boy N® compressor, Pari, Germany), over a 4-week treatment in a twice-daily regimen in patients with CF and *P. aeruginosa* chronic infection.

> Study CMA-0631-CSR-0025: A multicentre, multinational, open label, randomized, parallel group clinical trial of Torineb™/Actitob®/Bramitob® (tobramycin solution for nebulization, 300 mg twice daily in 4 mL unit dose ampoules) compared to TOBI in the treatment of patients with cystic fibrosis and chronic infection with *Pseudomonas aeruginosa* (Module 5.3.5.1, CT03 CSR body).

The study was designed to demonstrate the non-inferiority of CHF 1538 compared to TOBI in the primary efficacy variable forced expiratory volume in one second (FEV_1) predicted normal at the end of the treatment phase in patients with CF and chronic *P. aeruginosa* infection of the lungs. Table 44 provides a summary of clinical study CT03. Among the secondary efficacy variables, microbiological tests included quantitative cell counts (CFUs) for *P. aeruginosa* isolated from sputum, and antibiotic susceptibility testing of *P. aeruginosa.*

Sputum Collection and Microbiological Culture Methods

Sputum specimens were collected at the study site and sent under refrigeration to a central laboratory ((b) (4)).

Antibiotic Susceptibility Testing

Minimal inhibitory concentrations (MIC) were determined using the CLSI-recommended methods and quality controls for tobramycin and the seven other antibiotics (amikacin, aztreonam, ciprofloxacin, colistin, imipenem, levofloxacin, and piperacillin/tazobactam) were run.

Table 44: Summary of Clinical Study CT03

Study	CT03
Design	Randomized (1:1), open-label, reference product controlled, parallel group multinational, multicenter, study.
Primary Objective	To demonstrate the non-inferiority of CHF 1538 compared to TOBI in the primary efficacy variable forced expiratory volume in one second (FEV_1) % predicted normal at the end of the treatment phase in patients with CF and chronic infection of the lungs with *P. aeruginosa.*
Number of Randomized Patients	324
Age Range (yrs)	6 – 47
FEV_1 (% pred.)	≥ 40% and ≤ 80%
Comparator Daily Dose	TOBI (tobramycin 300 mg/5 mL inhalation solution for nebulization) 600 mg in two divided doses
Tobramycin Daily Dose	CHF 1538 (tobramycin 300 mg/4 mL inhalation solution for nebulization) 600 mg in two divided doses
Duration of Treatment	4 weeks treatment followed by 4-week phase-out period without treatment
Efficacy Assessment	**Primary**: final FEV_1 % predicted normal (week 4) **Secondary**: other pulmonary function tests; *in-vitro* microbiological tests including microbiological outcomes (eradication, persistence, re-infection with respect to *P.aeruginosa*, or superinfection with micro-organisms other than *P. aeruginosa*), tobramycin MIC range, MIC_{50} and MIC_{90}, and *P. aeruginosa* bacterial load (CFUs at Visit 4 and Visit 5).
Safety Assessment	Adverse events (AEs) and adverse drug reactions (ADRs), audiometric tests, laboratory parameters (hematology and blood chemistry), vital signs (heart rate and blood pressure) and physical examination.

Source: Module 5.3.5.1, CT03 Study Report Body, Section 9.1

CT03 - Microbiological Outcome

Microbiological outcome is presented by tobramycin baseline (Visit 1) MIC values, at Visit 4 ("ON" treatment), and Visit 5 ("OFF" treatment) for all *P. aeruginosa* morphotypes combined. Microbiological outcomes by baseline MIC values are compared between the two treatment groups at Visits 4 and 5 for the ITT population.

Microbiological outcome: At "ON" treatment (Visit 4), the following definitions were given on the basis of the results of the microbiologic test:

- Eradication (elimination of *P. aeruginosa*)
- Persistence (persistence of *P. aeruginosa* detected at Visit 1
- Superinfection (appearance of a pathogen other than *P. aeruginosa* not detected at Visit 1

At "OFF" treatment (Visit 5), the following definitions were given on the basis of the results of the microbiologic test:

- Eradication (elimination of *P. aeruginosa*)
- Persistence (persistence of *P. aeruginosa* detected at Visit 4 or at Visit 1 if Visit 4 is missing
- Superinfection (appearance of a pathogen other than *P. aeruginosa* not detected at Visit 4 or at Visit 1, and
- Re-infection (appearance of *P. aeruginosa* detected at Visit 1 and eradicated at Visit 4

Outcomes were analyzed according to the following hierarchy: Superinfection supercedes eradication; Persistence for *P. aeruginosa* supersedes Superinfection; and Re-infection for *P. aeruginosa* supersedes Superinfection. Overall outcomes could be designated as either a "positive outcome" (Eradication) or as a "Negative outcome" if the microbiological outcome was Persistence, Superinfection or Re-infection.

Efficacy Analysis

MIC Distributions

The distributions and percent range of tobramycin MIC results for *P. aeruginosa* clinical trial isolates at Visits 1, 4. and 5 are shown in Table 51. The range of MIC values observed for the CHF 1538 and TOBI study arms show the variability of MIC results independent of tobramycin drug exposure.

At Baseline (Visit 1) the distribution of *P. aeruginosa* with MICs $\leq$4 mcg/mL in the CHF1538 and TOBI arms was 85.4% and 85% respectively. These percentages decreased slightly in each treatment group at Visit 4 (end of "ON" cycle) as the susceptibility populations for CHF 1538 5.3% while TOBI susceptible [populations declined 7.9%. Additional change in the percentage of susceptible *P. aeruginosa* isolates was observed at the end of the first "OFF" cycle (Visit 5) compared to baseline (Visit 1) in both treatment arms; the change in percentage of susceptible isolates for CHF 1538 and TOBI was 7.9% and 9.6% respectively. These results suggest that, throughout the study, the majority of *P. aeruginosa* isolates remained susceptible to tobramycin in both treatment arms and that both tobramycin formulations produced equivalent microbiological treatment effects in the bacterial populations of *P. aeruginosa*.

Table 51: Distribution of Tobramycin MIC values (µg/mL) Overall; Summary by Visit, ITT Population

MIC (µg/mL)	CHF 1538 (n/N (%))			
	Visit 1	Visit 4	Visit 5	% Range
≤ 0.12	6/158 (3.8%)	8/126 (6.3%)	8/129 (6.2%)	3.8-6.3
0.25	20/158 (12.7%)	13/126 (10.3%)	17/129 (13.2%)	10.3-13.2
0.5	46/158 (29.1%)	29/126 (23.0%)	31/129 (24.0%)	23.0-29.1
1	34/158 (21.5%)	26/126 (20.6%)	25/129 (19.4%)	19.4-21.5
2	22/158 (13.9%)	18/126 (14.3%)	11/129 (8.5%)	8.5-14.3
4	7/158 (4.4%)	7/126 (5.6%)	8/129 (6.2%)	4.4-6.2
8	10/158 (6.3%)	9/126 (7.1%)	5/129 (3.9%)	3.9-7.1
16	1/158 (0.6%)	2/126 (1.6%)	9/129 (7.0%)	0.6-7.0
32	4/158 (2.5%)	2/126 (1.6%)	5/129 (3.9%)	1.6-3.9
64	3/158 (1.9%)	4/126 (3.2%)	2/129 (1.6%)	1.6-3.2
128	2/158 (1.3%)	3/126 (2.4%)	2/129 (1.6%)	1.3-2.4
256	0/158 (0%)	2/126 (1.6%)	1/129 (0.8%)	0-1.6
512	0/158 (0%)	0/126 (0%)	5/129 (3.9%)	0-5.0
>512	3/158 (1.9%)	3/126 (2.4%)	0/129 (0%)	0-1.9
Missing	0	32	29	

Table 51: Distribution of Tobramycin MIC values (µg/mL) Overall; Summary by Visit, ITT Population (Continued)

MIC (µg/mL)	TOBI (n/N (%))			
	Visit 1	Visit 4	Visit 5	% Range
≤ 0.12	11/162 (6.8%)	9/134 (6.7%)	8/128 (6.3%)	6.3-6.8
0.25	26/162 (16.0%)	15/134 (11.2%)	16/128 (12.5%)	11.2-16.0
0.5	51/162 (31.5%)	46/134 (34.3%)	39/128 (30.5%)	30.5-34.3
1	32/162 (19.8%)	19/134 (14.2%)	24/128 (18.8%)	14.2-19.8
2	16/162 (9.9%)	13/134 (9.7%)	8/128 (6.3%)	6.3-9.9
4	10/162 (6.2%)	4/134 (3.0%)	12/128 (9.4%)	3.0-9.4
8	8/162 (4.9%)	6/134 (4.5%)	4/128 (3.1%)	3.1-4.9
16	4/162 (2.5%)	5/134 (3.7%)	3/128 (2.3%)	2.3-3.7
32	2/162 (1.2%)	5/134 (3.7%)	6/128 (4.7%)	1.2-4.7
64	1/162 (0.6%)	2/134 (1.5%)	1/128 (0.8%)	0.6-1.5
128	0/162 (0%)	4/134 (3.0%)	2/128 (1.6%)	0-3.0
256	0/162 (0%)	1/134 (0.7%)	0/128 (0%)	0-0.7
512	0/162 (0%)	1/134 (0.7%)	2/128 (1.6%)	0-1.6
>512	1/162 (0.6%)	4/134 (3.0%)	3/128 (2.3%)	0.6-3.0
Missing	1	29	35	

If a patient has more than one *P. aeruginosa* morphotype at a given visit, then the highest tobramycin MIC value was used, regardless of *P. aeruginosa* morphotype.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.
Source data: Module 5.3.5.1, CT03Appendix 16.2.6.2 and 16.2.6.3

Distribution of Isolates Based Upon Tobramycin Susceptibility

Table 57 shows the distribution of tobramycin MIC values for *P. aeruginosa* for both the CHF 1538 and TOBI treatment arms using the tobramycin susceptibility interpretive criteria for parenteral tobramycin. The susceptibility profiles at the various days in both the CHF 1538 and TOBI study groups are very similar overall. A substantial proportion of isolates in both the CHF 1538 and TOBI treatment groups remained susceptible at the end of the "ON" drug period (Visit 4) with 80.2% and 79.1% susceptible respectively. At the end of Visit 4, there was an increase in the percentage of resistant isolates in both treatment groups with the greatest increase seen in the TOBI treatment group. At visit 5 (end of the "OFF" drug cycle), there was a slight decrease in the percent resistant isolates for both treatment groups relative to visit 4. The percentage of susceptible isolates was similar in both treatment groups at Visit 4 and 5. Overall, the results demonstrate an elevation in MIC value for a small portion of the isolates in both treatment groups during the course of the study.

Table 57: Tobramycin MIC Values (µg/mL) by Susceptibility Class; Summary by Visit, ITT Population

Tobramycin Susceptibility Category at Visit	CHF 1538 (N=158)	TOBI (N=163)
Visit 1		
≤4	135 (85.4%)	146 (90.1%)
8	10 (6.3%)	8 (4.9%)
≥16	13 (8.2%)	8 (4.9%)
Missing	0	1
Visit 4		
≤4	101 (80.2%)	106 (79.1%)
8	9 (7.1%)	6 (4.5%)
≥16	16 (12.7%)	22 (16.4%)
Missing	32	29
Visit 5		
≤4	100 (77.5%)	107 (83.6%)
8	5 (3.9%)	4 (3.1%)
≥16	24 (8.6%)	17 (13.3%)
Missing	29	35

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3

"Missing" includes cases where *P. aeruginosa* has been eradicated and therefore no MIC was available and instances where no specimen was collected or it was not analyzable.
If a patient has more than one *P. aeruginosa* morphotype at a given visit, then the highest tobramycin MIC value was used, regardless of *P. aeruginosa* morphotype.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.
Tobramycin systemic interpretive criteria (Susceptible, ≤ 4 µg/mL; Intermediate, 8 µg/mL; Resistant, ≥16 µg/mL)

Viable Counts - Mean Changes from Baseline

The *P. aeruginosa* bacterial density in $\log_{10}$ CFU/gram of sputum for each treatment group by study is shown in Table 58. This is a summary of the individual values for each patient. Similar bacterial load values were observed for the two treatment groups at each study visit suggesting these are typical population densities per gram of sputum colonizing the lungs of CF patients. The mean change in bacterial density from baseline levels is presented for the end of the "ON" cycle (Visit 4) and the end of the "OFF" cycle (Visit 5) in Table 59. The bacterial load showed a mean reduction of 2.14 and 2.07 $\log_{10}$ CFU/gram was observed at the end of the "ON" cycle for CHF 1538 and TOBI respectively. The bacterial load at the end of the "OFF" cycle was 0.72 and 0.87 $\log_{10}$ CFU/gram for CHF 1538 and TOBI respectively, indicating an increase in bacterial load relative to the end of the "ON" cycle. The ANCOVA model analysis results are shown in Table 60. No significant difference was evident with respect to the treatment or country while a significant difference was observed ($p<0.001$) for change from baseline $\log_{10}$ bacterial load (CFU/gram)

Table 58: *P. aeruginosa* $\log_{10}$ Bacterial Load (CFUs/gram) Summary by Visit: ITT Population

	CHF 1538 (N=158)	TOBI (N=163)
Visit 1		
N	158	162
Mean (SD)	6.56 (1.70)	6.64 (1.57)
95% CI	[6.30; 6.83]	[6.40; 6.89]
Median	6.90	7.00
Min / Max	1.30 / 8.78	1.30 / 8.78
Missing	0	1
Visit 4		
N	152	157
Mean (SD)	4.41 (2.22)	4.58 (2.25)
95% CI	[4.06; 4.77]	[4.23; 4.93]
Median	4.62	4.90
Min / Max	1.30 / 8.75	1.30 / 8.60
Missing	6	6
Visit 5		
N	147	147
Mean (SD)	5.78 (2.20)	5.81 (2.37)
95% CI	[5.42; 6.14]	[5.42; 6.20]
Median	6.58	6.64
Min / Max	1.30 / 8.78	1.30 / 8.78
Missing	11	16

Source data: Module 5.3.5.1, CT03 Appendices 16.2.6.2 and 16.2.6.3
CFU colony forming units
If a patient has more than one *P. aeruginosa* morphotype at a given visit, and therefore more than one bacterial load value, then the bacterial load value corresponding to the highest tobramycin MIC value regardless of the *P. aeruginosa* morphotype was used.
If the tobramycin MIC value was the same for different *P. aeruginosa* morphotypes, then the bacterial load value corresponding to morphotype 1 (mucoid colony) was used. If morphotype 1 was not available, bacterial load value corresponding to morphotype 2 (dry colony) was used.
In instances of *P. aeruginosa* eradication (i.e. < 20 CFU/g, based upon the dilution factor in the plating and counting procedure), the numeric value of 20 CFU/g was used for bacterial load.
Where the bacterial load was recorded as '>600000000 CFU/g', the numeric value used for calculation of bacterial load was 600000000.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

Table 59: *P. aeruginosa* $\log_{10}$ Bacterial Load (CFUs/gram) Change from Baseline (Visit 1): ITT Population

	CHF 1538 (N=158)	TOBI (N=163)
Visit 4		
N	152	156
Mean (SD)	-2.14 (2.41)	-2.07 (2.20)
95% CI	[-2.52; -1.75]	[-2.42; -1.72]
Median	-2.09	-1.79
Min / Max	-7.48 / 4.00	-7.48 / 1.72
Missing	6	7
Visit 5		
N	147	147
Mean (SD)	-0.72 (2.17)	-0.87 (2.23)
95% CI	[-1.07; -0.36]	[-1.24; -0.51]
Median	-0.40	-0.48
Min / Max	-6.54 / 4.90	-7.48 / 6.08
Missing	11	16

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
CFU colony forming units
If a patient has more than one *P. aeruginosa* morphotype at a given visit, and therefore more than one bacterial load value, then the bacterial load value corresponding to the highest tobramycin MIC value regardless of the *P. aeruginosa* morphotype was used.
If the tobramycin MIC value was the same for different *P. aeruginosa* morphotypes, then the bacterial load value corresponding to morphotype 1 (mucoid colony) was used. If morphotype 1 was not available, bacterial load value corresponding to morphotype 2 (dry colony) was used.
In instances of *P. aeruginosa* eradication (i.e. <20 CFU/g, based upon the dilution factor in the plating and counting procedure), the numeric value of 20 CFU/g was used for bacterial load.
Where the bacterial load was recorded as '>600000000 CFU/g', the numeric value used for calculation of bacterial load was 600000000.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

Table 60: *P. aeruginosa* log_{10} Bacterial Load (CFUs/gram) ANCOVA: ITT Population

	CHF 1538 (N=158)		TOBI (N=163)
ANCOVA			
N (missing)	152 (6)		156 (7)
LSMEANS(SEM)	-1.81 (0.21)		-1.85 (0.20)
Fixed effects/Covariate: p-value			
Treatment		0.820	
Country		0.310	
Baseline log_{10} bacterial load (CFU/g) value		< 0.001	
CHF 1538 minus TOBI			
LSMEANS(SEM)		0.04 (0.18)	
95% CI		[-0.31; 0.39]	

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
ANCOVA model: Change from baseline (V1) to V4 in log_{10} bacterial load (CFU/g) value = treatment and country as fixed effects and baseline log_{10} bacterial load (CFU/g) value as covariate
All p-values are two-sided

Overall - Microbiology Outcome

Table 61 provides information on the overall analysis of microbiology outcomes for both study groups. As can be seen for study Visit 4 (end of the "ON" treatment period, there was a low percentage of microbiological eradication in both treatment groups (9.2% for CFH 1538-treated and 7.1% for TOBI-treated). This type of result is not uncommon in cystic fibrosis studies for inhaled, systemically administered and orally administered antibacterials. Similar rates of persistence and superinfection were noted in the two treatment groups. At Visit 5 (end of the "OFF" treatment period), the rates of eradication were lower than at Visit 4 in both treatment groups. While the percentage of re-infection in the CH 1538 treatment group was higher then seen in the TOBI-treatment group the numbers were small. The Cochran-Mantel-Haenzel test controlling for country revealed no statistically significant differences between the CH 1538 and TOBI with respect to percentages in outcome categories. From the data in Table 61 it appears from a microbiology perspective that CH 1538 produces equivalent results to the approved TOBI product.

Table 61: Microbiological Outcomes (Eradication, Persistence, Superinfection, Re-infection); Summary by Visit, ITT Population

	CHF 1538 (N=158)	TOBI (N=163)	CMH Test[1]
Visit 1			
Presence of *P. aeruginosa*	158 (100%)	162 (100%)	
Absence of *P. aeruginosa*	0	0	
Missing	0	1	
Visit 4[2]			
Eradication	14 (9.2%)	11 (7.1%)	p=0.692
Persistence	126 (82.9%)	133 (85.3%)	
Superinfection	12 (7.9%)	12 (7.7%)	
Missing	6	7	
Visit 5[3]			
Eradication	4 (2.7%)	5 (3.4%)	p=0.128
Persistence	116 (78.9%)	122 (83.0%)	
Superinfection	14 (9.5%)	14 (9.5%)	
Re-infection	13 (8.8%)	6 (4.1%)	
Missing	11	16	

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
[1] Cochran-Mantel-Haenszel test controlling for country
Microbiological outcomes are derived considering all *P. aeruginosa* morphotypes together.
[2] At Visit 4:
Eradication=elimination of *P. aeruginosa*
Persistence=persistence of *P. aeruginosa* detected at V1
Superinfection=appearance of a pathogen (other than *P. aeruginosa*) not detected at V1
[3] At Visit 5:
Eradication=elimination of *P. aeruginosa*
Persistence=persistence of *P. aeruginosa* detected at V4 (or at V1 if V4 missing)
Superinfection=appearance of a pathogen (other than *P. aeruginosa*) not detected at V4 or at V1
Re-infection=re-appearance of *P. aeruginosa* detected at V1 and eradicated at V4
Superinfection supercedes eradication. Persistence for *P. aeruginosa* supercedes superinfection.
Re-infection for *P. aeruginosa* supercedes superinfection.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

The analysis of microbiological outcomes is analyzed further at all study visits by baseline MIC values in Table 62. This data is further differentiated into baseline tobramycin susceptibility categories (Susceptible, Intermediate, or Resistant) in Table 63, and summarized in an overall positive or negative outcome in Table 64.

As seen in Table 62 at Visit 4, in both treatment groups, persistence occurred across a broad range of MIC values with no apparent correlation with MIC value. This type of results has been observed in other cystic fibrosis antibacterial treatment studies. At visit 5, a pattern similar to that seen in Visit 4 was observed with regard to MIC versus eradication, persistence, and superinfection with the exception of eradication which was

observed less frequently. Re-infections at Visit 5 were distributed across a wide range of MIC values in both treatment groups. There is an increase in the incidence of reinfection for both treatment arms in Visit 5 compared to Visit 4. This increase, the Applicant hypotheses, is likely do to emergence of *P. aeruginosa* in patients previously categorized as eradication.

The microbiological outcome data shows that there were no uncommon instances of microbiological eradication in either the CHF 1538 or TOBI-treatment groups at either visit 4 or 5. A high percentage of persistors were observed in the treatment groups at Visit 4 and 5, with a modest incidence of superinfection and reinfection (Visit 5). The persistence of *P. aeruginosa* during and after antibacterial treatment is not unexpected and the numbers seen in these studies are consistent with other data.

Table 62: Microbiological Outcomes (Eradication, Persistence, Superinfection, Re-infection) by Baseline MIC Value; Summary by Visit, ITT Population

	CHF 1538 (N=158)				TOBI (N=163)			
	ERAD[1]	PERS	SUPER	REINF	ERAD	PERS	SUPER	REINF
Visit 4[2]								
≤0.12	1/6 (16.7%)	5/6 (83.3%)	0	.	3/11 (27.3%)	6/11 (54.5%)	2/1 (18.2%)	.
0.25	2/19 (10.5%)	15/19 (78.9%)	2/19 (10.5%)	.	2/24 (8.3%)	19/24 (79.2%)	3/24 (12.5%)	.
0.5	6/45 (13.3%)	34/45 (75.6%)	5/45 (11.1%)	.	3/49 (6.1%)	44/49 (89.8%)	2/49 (4.1%)	.
1	2/33 (6.1%)	29/33 (87.9%)	2/33 (6.1%)	.	1/32 (3.1%)	28/32 (87.5%)	3/32 (9.4%)	.
2	2/20 (10.0%)	16/20 (80.0%)	2 /20 (10.0%)	.	0	15/15 (100.0%)	0	.
4	1/7 (14.3%)	6/7 (85.7%)	0	.	0	10/10 (100.0%)	0	.
8	0	9/10 (90.0%)	1/10 (10.0%)	.	1/7 (14.3%)	4/7 (57.1%)	2/7 (28.6%)	.
16	0	1/1 (100.0%)	0	.	0	4/4 (100.0%)	0	.
32	0	4/4 (100.0%)	0	.	1/2 (50.0%)	1/2 (50.0%)	0	.
64	0	3/3 (100.0%)	0	.	0	1/1 (100.0%)	0	.
128	0	2/2 (100.0%)	0	.	0	0	0	.
>512	0	2/2 (100.0%)	0	.	0	1/1 (100.0%)	0	.
Total	14/152 (9.2%)	126/152 (82.9%)	12/152 (7.9%)	.	11/156 (7.1%)	133/156 (85.3%)	12/156 (7.7%)	.

The microbiological outcome data analyzed above was assessed according to systemic tobramycin interpretive criteria as seen in Table 63. For both treatment groups, persistence was reported for isolates that were susceptible, intermediate or resistance to tobramycin. For both Visits 4 and 5, and in both treatment groups, more than 76% of the *P. aeruginosa* isolates were susceptible to tobramycin and yet were persistent. This suggests a lack of correlation between MICs and microbiological outcome a result that is seen in other studies.

Table 62: Microbiological Outcomes (Eradication, Persistence, Superinfection, Re-infection) by Baseline MIC Value; Summary by Visit, ITT Population (Continued)

	CHF 1538 (N=158)				TOBI (N=163)			
	ERAD	PERS	SUPER	REINF	ERAD	PERS	SUPER	REINF
Visit 5[3]								
≤0.12	0	5/6 (83.3%)	0	1/6 (16.7%)	1/10 (10.0%)	5/10 (50.0%)	4/10 (40.0%)	0
0.25	1/19 (5.3%)	13/19 (68.4%)	2/19 (10.5%)	3/19 (15.8%)	1/23 (4.3%)	18/23 (78.3%)	3/23 (13.0%)	1/23 (4.3%)
0.5	1/39 (2.6%)	29/39 (74.4%)	6/39 (15.4%)	3/39 (7.7%)	0	39/46 (84.8%)	4/46 (8.7%)	3/46 (6.5%)
1	2/34 (5.9%)	26/34 (76.5%)	4/34 (11.8%)	2/34 (5.9%)	3/27 (11.1%)	23/27 (85.2%)	0	1/27 (3.7%)
2	0	17/21 (81.0%)	2/21 (9.5%)	2/21 (9.5%)	0	16/16 (100.0%)	0	0
4	0	5/6 (83.3%)	0	1/6 (16.7%)	0	9/9 (100.0%)	0	0
8	0	9/10 (90.0%)	0	1/10 (10.0%)	0	5/8 (62.5%)	2/8 (25.0%)	1/8 (12.5%)
16	0	1/1 (100.0%)	0	0	0	4/4 (100.0%)	0	0
32	0	4/4 (100.0%)	0	0	0	1/2 (50.0%)	1/2 (50.0%)	0
64	0	3/3 (100.0%)	0	0	0	1/1 (100.0%)	0	0
128	0	2/2 (100.0%)	0	0	0	0	0	0
>512	0	2/2 (100.0%)	0	0	0	1/1 (100.0%)	0	0
Total	4/147 (2.7%)	116/147 (78.9%)	14/147 (9.5%)	13/147 (8.8%)	5/147 (3.4%)	122/147 (83.0%)	14/147 (9.5%)	6/147 (4.1%)

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3

Table populated for patients with an available MIC value at V1. Microbiological outcomes derived considering all *P. aeruginosa* morphotypes together.

[1] Microbiological outcomes are derived considering all *P. aeruginosa* morphotypes together.

[2] At V4: ERAD=elimination of *P. aeruginosa*, PERS=persistence of *P. aeruginosa* detected at V1, SUPER=appearance of a pathogen (other than *P. aeruginosa*) not detected at V1, REINF=reinfection was not an option at Visit 4

[3] At V5: ERAD=elimination of *P. aeruginosa*, PERS=persistence of *P. aeruginosa* detected at V4 (or at V1 if V4 missing), SUPER=appearance of a pathogen (other than *P. aeruginosa*) not detected at V4 or at V1; REINF=re-appearance of *P. aeruginosa* detected at V1 and eradicated at V4. Superinfection supercedes eradication. Persistence for *P. aeruginosa* supercedes superinfection. Re-infection for *P. aeruginosa* supercedes superinfection.

Table 63: Microbiological Outcomes (Eradication, Persistence, Superinfection, Re-infection) by Baseline Tobramycin Susceptibility Designation (Susceptible, Intermediate, or Resistant); Summary by Visit, ITT Population

	CHF 1538 (N=158)			TOBI (N=163)		
	S[1]	I	R	S	I	R
Visit 4[2]						
Eradication	14 (10.8%)	0	0	9 (6.4%)	1 (14.3%)	1 (12.5%)
Persistence	105 (80.8%)	9 (90.0%)	12 (100%)	122 (86.5%)	4 (57.1%)	7 (87.5%)
Superinfection	11 (8.5%)	1 (10.0%)	0	10 (7.1%)	2 (28.6%)	0
Missing	5	0	1	5	1	0
Visit 5[3]						
Eradication	4 (3.2%)	0	0	5 (3.8%)	0	0
Persistence	95 (76.0%)	9 (90.0%)	12 (100%)	110 (84.0%)	5 (62.5%)	7 (87.5%)
Superinfection	14 (11.2%)	0	0	11 (8.4%)	2 (25.0%)	1 (12.5%)
Re-infection	12 (9.6%)	1 (10.0%)	0	5 (3.8%)	1 (12.5%)	0
Missing	10	0	1	15	0	0

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2and 16.2.6.3

[1] Baseline (V1) susceptibility: S: Susceptible (MIC ≤4 µg/mL), I: Intermediate (MIC=8 µg/mL), R: Resistant (MIC ≥ 16 µg/mL)

Table populated for patients with an available susceptibility at V1

Microbiological outcomes are derived considering all *P. aeruginosa* morphotypes together.

[2] At Visit 4: Eradication = elimination of *P. aeruginosa*
Persistence = persistence of *P. aeruginosa* detected at V1
Superinfection = appearance of a pathogen (other than *P. aeruginosa*) not detected at V1

[3] At Visit 5: Eradication = elimination of *P. aeruginosa*
Persistence = persistence of *P. aeruginosa* detected at V4 (or at V1 if V4 missing)
Superinfection = appearance of a pathogen (other than *P. aeruginosa*) not detected at V4 or at V1
Re-infection = re-appearance of *P. aeruginosa* detected at V1 and eradicated at V4

Superinfection supercedes eradication. Persistence for *P. aeruginosa* supercedes superinfection. Re-infection for *P. aeruginosa* supercedes superinfection.

Table 64 provides a summary of microbiological outcomes by visit for the ITT population. The positive and negative outcomes were very similar in the two treatment groups.

Table 64: Microbiological Outcomes (Positive vs Negative Outcomes)-Summary by Visit: ITT Population

	CHF 1538 (N=158)	TOBI (N=163)	CMH Test[1]
Visit 4			
Positive outcome[2]	14 (9.2%)	11 (7.1%)	p=0.465
Negative outcome[3]	138 (90.8%)	145 (92.9%)	
Missing	6	7	
Visit 5			
Positive outcome	4 (2.7%)	5 (3.4%)	p=0.775
Negative outcome	143 (97.3%)	142 (96.6%)	
Missing	11	16	

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
[1] Cochran-Mantel-Haenszel test controlling for country
Microbiological outcomes are derived considering all *P. aeruginosa* morphotypes together.
[2] Positive outcome = eradication
[3] Negative outcome = persistence, superinfection or re-infection
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

MIC Changes during Therapy

Table 66 provides information on the changes in tobramycin MICs in both treatment groups for visits 4 ("ON" tobramycin) and 5 ("OFF" tobramycin). Overall the tendency for MIC values to increase during conduct of the study was similar in the treatment groups.

Table 66: Tobramycin MIC Value (µg/mL) Shift from Baseline (Visit 1) Values: ITT Population

	CHF 1538 (N=158)	TOBI (N=163)
Visit 4		
Decreased[1]	19 (15.1%)	19 (14.3%)
Unchanged[2]	79 (62.7%)	78 (58.6%)
Increased[3]	28 (22.2%)	36 (27.1%)
Missing[4]	32	30
Visit 5		
Decreased	23 (17.8%)	19 (14.8%)
Unchanged	80 (62.0%)	86 (67.2%)
Increased	26 (20.2%)	23 (18.0%)
Missing	29	35

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3

[1] MIC decreased: Patients whose *P. aeruginosa* isolate exhibited ≥ 4-fold decrease in the MIC between baseline and end-of-therapy or follow-up visits.

[2] MIC unchanged: Patients whose *P. aeruginosa* isolate exhibited no change or a 2-fold increase or decrease in the MIC between baseline and end-of-therapy or follow-up visits.

[3] MIC increased: Patients whose *P. aeruginosa* isolate exhibited ≥ 4-fold increase in the MIC between baseline and end-of-therapy or follow-up visits.

[4] Missing indicates that for a given patient there was no *P. aeruginosa* isolated at one of the study visits

If a patient has more than one *P. aeruginosa* morphotype at a given visit, then the highest tobramycin MIC value was used, regardless of *P. aeruginosa* morphotype.

When MIC value was recorded as '≤ 0.12 µg/mL', the numeric value used for calculation of shift from baseline was 0.125 µg/mL.

When MIC value was recorded as '> 512 µg/mL', the numeric value used for calculation of shift from baseline was 1024 µg/mL.

Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

CLINICAL MICROBIOLOGY CONCLUSION

From a clinical microbiology perspective there is no evidence in the data from the treatment study groups (CHF1538 and TOBI) that suggest that CHF 1538 is inferior to TOBI for the treatment of *Pseudomonas aeruginosa* infection in the lungs of cystic fibrosis patients.

INTRODUCTION

Cystic fibrosis (CF) is the most common inherited lethal disease of the white population. It occurs primarily in individuals of central and western European origin and affects more than 30,000 Americans. The estimated incidence in the United States is 1 in 2000 to 2600 live white births, 1 in 19,000 live African American births, 1 in 11,500 live Hispanic births, and 1 in 25,000 live Asian American births (**1**). CF has an autosomal recessive mode of inheritance. Affected individuals are phenotypic homozygotes and both parents usually are heterozygotes or carriers. The carrier frequency in white individuals in the United States is approximately 1 in 25, with full siblings or children with CF having a 1 in 4 chance of being affected (**1**). In cystic fibrosis, the defect in the cyclic adenosine monophosphate-regulated chloride ion channels in the epithelial lining of the respiratory system favors the colonization of gram-negative bacteria (mainly *P. aeruginosa*) resulting in inflammation, compromised pulmonary function and mortality in CF patients (**2,3,4,5**). Upon entry of *P. aeruginosa* in CF lungs, penetration of mucous surface and colonization is followed by a genetic transformation of non-mucoid to mucoid (alginate-producing) phenotype (**6,7**). Although non-mucoid bacteria are more virulent, they are also better recognized by the immune response, while the mucoid phenotype increases bacterial adherence and resistance to phagocytosis (**7,8**). As the infection progresses, bacteria release virulence factors through quorum-sensing (bacterial communication), inducing the inflammatory response (**8**). Unlike normal airway secretions, the inflammatory response increases concentrations of neutrophils-derived DNA and filamentous actin in the CF mucous, which interact and bind to glycoproteins (e.g. mucin) (**9,10,11,12**). The physical interaction of abnormally high concentrations of such factors results in viscous layers (sputum) that cover the epithelia surface and allow for bacterial adherence and biofilm protection (**13,14**).

The microbial species that are clearly associated with lung disease in CF patients (CF lung disease) are relatively few, including *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex (**15**). Organisms having a secondary role in CF lung disease include respiratory viruses, such as respiratory syncytial virus and influenza virus; *Haemophilus influenzae*; and *Aspergillus fumigatus*. *Mycobacterium* spp. but not *Mycobacterium tuberculosis*, *Stenotrophomonas maltophilia* and *Alcaligenes xylosoxidans* are being seen with increasing frequency in CF patients, in part because of the increasing life span of CF patients and the relentless use of antimicrobial agents in this population (**15**). However, the role of the latter organisms in CF lung disease has not been clearly determined. In addition, organisms that phenotypically resemble *B. cepacia* complex organisms (i.e. *Burkholderia gladioli*, *Ralstonia* spp., and *Pandoraea* spp.) are also being seen with increasing frequencies due to the use of selective media that improve the rates of recovery of *B. cepacia* complex isolates (**15**).

Pseudomonas aeruginosa is the most important pathogen of CF lung disease, infecting approximately 60% of the entire CF population and close to 80% of adolescents and adults (**16**). *Staphylococcus aureus* is recovered from approximately 50% of the CF population. *Burkholderia cepacia* is recovered from ~3% of American CF patients and

15% of Canadian CF patients. The repercussions of infection with *B. cepacia* are extensive. In CF patients, infection with *B. cepacia* complex is associated with increased rates of mortality and morbidity. Roughly 20% of CF patients colonized with *B. cepacia* complex develop the "cepacia syndrome" (**17**). These patients experience a rapid decline in pulmonary function, frequent bacteremia, and ultimately death due to lung failure. Patient to patient transfer of *B. cepacia* has been documented (**17**). The review will concentrate on *P. aeruginosa* since the Sponsor is targeting this organism with nebulized amikacin.

Studies have shown that *P. aeruginosa* infections in CF patients can be seen as early as infancy (**18**). The initial strains of *P. aeruginosa* that infect CF patients are described as "rough" or "planktonic" strains. These strains tend to be susceptible to a variety of antimicrobials, are motile and prototrophic and have smooth lipopolysaccharide. It is at this stage that some investigators believe that aggressive antimicrobial therapy can eradicate this organism (**19)**. However, most patients develop chronic infection with an unusual phenotype of *P. aeruginosa* referred to as mucoid. Mucoid isolates are nonmotile, are frequently auxotrophic have rough lipopolysaccharide, and are frequently resistant to a wide variety of antimicrobial agents (**20**). The mucoid material is a polysaccharide polymer referred to as alginate, which forms the biofilm matrix and renders the embedded *P. aeruginosa* refractory to clearance by the immune system. (**21**). Isolation of *P. aeruginosa* from the respiratory secretions of CF patients is easily accomplished (**21**).

Susceptibility testing of *P. aeruginosa* isolates recovered from CF patients is an area of some controversy. Multiple morphotypes may be recovered from patient sputum. Studies that compared the performance of susceptibility testing of a mixture of different morphotypes versus the performance of testing each morphotypes showed the testing a mixture of morphotypes may underestimate resistance (**22**). Two areas of controversy exist in the area of susceptibility testing of *P. aeruginosa* isolates. The first is that *P. aeruginosa* grows anaerobically within the airways of CF patients (**23**). If this is true then aminoglycosides which have not activity anaerobically should not be active against *P. aeruginosa*. This directly conflicts with the results of studies that show aminoglycosides given intravenously and aerosolized have a positive impact on the lung functions and life expectancies of CF patients (**24**). However, anaerobically growing *P. aeruginosa* may contribute to this organism's ability to persist in the face of high concentrations of aminoglycosides (**23**). A second area of controversy is the microbial form of *P. aeruginosa* that should be used for susceptibility testing of *P. aeruginosa*. The two forms are the biofilm form and the planktonic form. The susceptibilities of mucoid *P. aeruginosa* isolates growing as biofilm have been compared to those strains growing planktonically, which are used in clinical laboratory susceptibility testing. Recently, equipment called the "Calgary device" has been developed that allows susceptibility testing to be performed with mucoid *P. aeruginosa* isolates growing as biofilms. *P. aeruginosa* grown in a biofilm were significantly more resistant to antipseudomonal drugs. This data suggests that susceptibility tests obtained by testing planktonically growing isolates may underestimate the drug resistance of mucoid *P. aeruginosa* (**24**).

IN VITRO

IN VITRO SPECTRUM OF ACTIVITY

The Applicant provided information on the minimal inhibitory concentrations (MICs) of tobramycin to inhibit *Pseudomonas aeruginosa* isolates recovered from cystic fibrosis patients in the United States. The susceptibility testing of *P. aeruginosa* isolates was done by the method of the Clinical and Laboratory Standards Institute (CLSI) (**25,26**). Along with the susceptibility test results for the isolates the Applicant provided the quality control (QC) results for each batch of tests. All of the QC values are within the acceptable ranges as recommended by CLSI (**27**) [data not shown (**28**)]. All isolates were identified as *P. aeruginosa* by standard identification methods.

Tables 1 to 5 show the results over the past 3 years for *P. aeruginosa* isolates obtained from cystic fibrosis from the United States for tobramycin and a variety of other antimicrobials. This background information was obtained for isolates from the United States to compare to the tobramycin susceptibility of *P. aeruginosa* isolates obtained during clinical studies to assure that the antibiograms of these US isolates were similar to those obtained during clinical studies done in other countries. The percent resistant to the various antibacterials was determined based on interpretive criteria for parenteral forms of antibacterials (**2**) since there are no interpretive criteria for inhaled antibiotics. Tobramycin resistance by these criteria ($\geq$16 mcg/mL) were identified in 20.4% of *P. aeruginosa* isolated between July 2007 and June 2008 and 17.4% between January 2008 and June 2009 (Table 4). While the *P. aeruginosa* isolates with MICs of $\geq$16 mcg/mL were considered resistant to tobramycin given by the parenteral route the amount of tobramycin reaching the lungs is many time more then 16 mcg/mL therefore the growth of *P. aeruginosa* in the lung may be inhibited by the higher concentrations of tobramycin.

TABLES AND FIGURES

Table 1 Study Enrollment and *P. aeruginosa* Isolates from Sputum

	July 2007 - June 2008	January 2008 - June 2008
Number of unique subjects	137	68
Number of *P. aeruginosa* isolates	357	178

Table 2 Prevalence of Mucoid and Non-Mucoid Phenotypes

	July 2007 - June 2008	January 2008 - June 2008
P. aeruginosa isolates identified from study subjects	357	178
Non-mucoid phenotype	174 (48.7%)	89 (50%)
Mucoid phenotype	183 (51.3%)	89 (50%)

Table 3 MIC_{50} and MIC_{90} Results for *P. aeruginosa* Isolates[1]

Drug	July 2007- June 2008 (N=357)			January 2008- June 2008 (N=178)		
	MIC Range	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	MIC Range	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Tobramycin	≤ 0.12 - > 512	2	32	≤ 0.12 - > 512	2	32
Amikacin	≤ 0.5 - > 128	16	128	≤ 0.5 - > 128	16	128
Aztreonam	≤ 1 - > 128	2	64	≤ 1 - > 128	4	128
Cefepime	≤ 0.5 - > 64	8	64	≤ 0.5 - > 64	8	64
Ceftazidime	≤ 0.5 - > 64	2	64	≤ 0.5 - > 64	2	> 64
Meropenem	≤ 0.12 - > 32	0.5	16	≤ 0.12 - > 32	0.5	16
Piperacillin/tazobactam	≤ 0.5 -> 256	4	256	≤ 0.5 - > 256	4	256
Ticarcillin/clavulanate	≤ 4 - > 256	16	› 256	≤ 4 - > 256	16	> 256
Ciprofloxacin	≤ 0.12 - > 8	1	8	≤ 0.12 - > 8	1	8

[1] MIC_{50} = MIC value at which 50% of isolates were inhibited
MIC_{90} = MIC value at which 90% of isolates were inhibited

Table 4 Prevalence of Resistant *P. aeruginosa* Isolates for Period

Drug	July 2007 - June 2008 (N=357)	January 2008 - June 2008 (N=178)
	N (%)	N (%)
Tobramycin	73 (20.4%)	31 (17.4%)
Amikacin	106 (29.7%)	47 (26.4%)
Aztreonam	69 (19.3%)	36 (20.2%)
Cefepime	72 (20.2%)	41 (23.0%)
Ceftazidime	70 (19.6%)	37 (20.8%)
Meropenem	51 (14.3%)	33 (18.5%)
Piperacillin/tazobactam	66 (18.5%)	35 (19.7%)
Ticarcillin/clavulanate	85 (23.8%)	44 (24.7%)
Ciprofloxacin	88 (24.6%)	41 (23.0%)
Multidrug Resistance	34 (9.5%)	17 (9.6%)

Table 5 Prevalence of High-Level Resistance Among *P. aeruginosa* Isolates

	Non-Mucoid	Mucoid	Total
	N (%)	N (%)	N (%)
July 2007 - June 2008	174	183	357
P. aeruginosa isolates	14 (8.0%)	8 (4.4%)	22 (6.2%)
Tobramycin MIC > 128 µg/mL			
January 2008 - June 2008	89	89	178
P. aeruginosa isolates	4 (4.5%)	2 (2.2%)	6 (3.4%)
Tobramycin MIC > 128 µg/mL			

The tobramycin and other antimicrobial susceptibilities of isolates obtained during the Phase 3 clinical trials are shown in Table 67. Table 68 shows a comparison of the *P. aeruginosa* isolates at baseline from the various studies and the US surveillance isolates. As seen the susceptibilities of the *P. aeruginosa* obtained during the Phase 3 trials were quite similar to the susceptibility profiles of the *P. aeruginosa* from US patients.

Chiesi 2.7.2 Summary of Clinical Pharmacology Studies

Table 67: Comparison of Susceptibility of *P. aeruginosa* from Clinical Study CT03 to US Surveillance Isolates

Antibiotic	Isolate Source	No.	MIC Range	MIC_{50}	MIC_{90}
Tobramycin	CHF 1538 Arm	158	≤0.12- > 512	1	8
	TOBI Arm	162	≤0.12- > 512	0.5	4
	Surveillance	357	≤0.12- > 512	2	32
Amikacin	CHF 1538 Arm	158	≤0.25- > 512	8	64
	TOBI Arm	162	≤0.25-512	4	32
	Surveillance	357	≤0.5- > 128	16	128
Aztreonam	CHF 1538 Arm	158	≤0.25- > 512	4	128
	TOBI Arm	162	≤0.25- > 512	4	128
	Surveillance	357	≤1- >128	2	64
Ciprofloxacin	CHF 1538 Arm	158	≤0.12- > 256	1	4
	TOBI Arm	162	≤0.12-64	0.5	4
	Surveillance	357	≤0.12- > 8	1	8
Piperacillin/tazobactam	CHF 1538 Arm	158	≤0.25- > 512	4	512
	TOBI Arm	162	≤0.25- > 512	4	128
	Surveillance	357	≤0.5- > 256	4	256
Levofloxacin	CHF 1538 Arm	158	≤0.25- > 512	2	16
	TOBI Arm	162	≤0.25-128	1	8
Colistin	CHF 1538 Arm	158	0.25- > 128	1	4
	TOBI Arm	162	≤0.12- > 128	1	4
Imipenem	CHF 1538 Arm	158	≤0.25- >256	1	32
	TOBI Arm	162	≤0.25-256	1	16
Meropenem	Surveillance	357	≤0.12- > 32	0.5	16
Ticarcillin/clavulanate	Surveillance	357	≤4- > 256	16	> 256
Cefepime	Surveillance	357	≤0.5- > 64	8	64
Ceftazidime	Surveillance	357	≤0.5- > 64	2	64

Source data: Table 6 and Table 56

Table 68: Comparison of MIC Summary Values for Baseline Isolates from CT01, CT02, CT03, and US Surveillance Isolates

Study	Treatment Arm	No. Isolates	Minimal Inhibitory Concentration (µg/mL)		
			Range	MIC_{50}	MIC_{90}
CT01	CHF1538	29	≤0.25-256	4	64
	Placebo	27	0.5-64	4	16
CT02	CHF1538	160	≤0.25-64	1	64
	Placebo	80	≤0.25-64	1	64
CT03	CHF1538	158	≤0.12->512	1	8
	TOBI	163	≤0.12->512	0.5	4
Surveillance	--	357	≤0.12->512	2	32

Source data: Table 6 , Table 23 , Table 29 , Table 56

MECHANISM OF ACTION

Tobramycin a member of the aminogylcoside family of antimicrobials interferes with the first steps of protein synthesis by causing a misreading and premature termination of the translation or the genetic code of the mRNA template. The aberrant proteins produced maybe inserted into the cell membrane leading to altered permeability and further stimulation of aminoglycoside transport. This disruption eventually leads to death of the bacterial cell.

PHARMACODYNAMICS OF TOBRAMYCIN

Tobramycin exerts its killing effect against bacteria in a concentration-dependent manner, so the higher the peak concentration of the drug (and therefore the higher C_{max}/MIC is reached), the greater is the degree of killing (**31**).

MECHANISM OF RESISTANCE

As with other antimicrobials, resistance to aminoglycosides may be intrinsic or acquired. Intrinsic resistance may be enzymatic or nonenzymatic. Mutations at the 16S ribosomal RNA (rRNA) can result in resistance. The methylating enzymes that modify the 16S rRNA exemplify enzymatic intrinsic resistance.

Acquired resistance to aminoglycosides results from the combination of decreased drug uptake, efflux pump activity or enzymatic modification of the drug.

All enterococci have intrinsic resistance to aminoglycosides with MICs ranging from 4 to 256 mcg/mL. The resistance in this case is attributed to the facultative anaerobic metabolism of enterococci which in turn reduces the transmembrane potential and hence limits uptake of the aminoglycoside. Acquisition of genes that encode aminoglycoside-

modifying enzymes leads to high-level aminoglycoside resistance with loss of synergistic activity with penicillins or vancomycin.

The predominant mechanism of resistance to tobramycin in *P. aeruginosa* isolated from CF patients is impermeability and to a lesser extend enzymatic modification and other mechanisms which cumulatively lead to decreased susceptibility of *P. aeruginosa* to tobramycin.

POST-ANTIBIOTIC EFFECT (PAE)

The PAE is persistent suppression of bacterial growth after short antimicrobial exposure. PAE can be measured in vitro or in animal models of infection. In vivo, the aminoglycosides consistently demonstrate a PAE that varies from 1 to 3 hours in broth and serum for *P. aeruginosa* (**29**).

TOBRAMYCIN INTERACTION WITH OTHER ANTIMICROBIALS

The mechanism of aminoglycoside synergistic activity may not be the same for all target organisms. Enhanced aminoglycoside uptake in the presence of cell-wall-active drugs (e.g. penicillins) has been demonstrated with *P. aeruginosa* (**30**).

IN VITRO SUSCEPTIBILITY TEST METHODS

Susceptibility testing of *P. aeruginosa* can be done by the standardized method of the Clinical and Laboratory Standards Institute (CLSI) (**25**).

DEVELOPMENT OF QC PARAMETERS FOR IN VITRO SUSCEPTIBILITY TESTING

In vitro susceptibility test QC parameters for the in vitro susceptibility testing of tobramycin have been established (**27**).

INTERPRETATION OF IN VITRO SUSCEPTIBILITY TEST RESULTS

There are no interpretive criteria for topical agents such as inhaled tobramycin because the concentration of tobramycin achieved at the infected site is many fold higher then the concentration achieved when tobramycin is given systemically.

IN VIVO

CLINICAL STUDIES

Studies CT01 and CT02

CT01 – Study DM/RS/10000/001/04 – Double blind, multicenter, randomized, placebo-controlled, parallel groups clinical trial of CHF 1538 tobramycin 300 mg/4 mL inhalation solution (300 mg BID) in the 4-week treatment (plus 4 weeks or run-out) of patients with CF and a positive culture of *P. aeruginosa* (See Module 5.3.5.1, CT01 CSR Body)

CT02 – Study DM/RS/10000/002/04 – Double-blind, multicenter, randomized, placebo-controlled, parallel groups clinical trial of intermittent CHF 1538 (tobramycin 300 mg/mL inhalation solution) or placebo in three 4-week cycles of treatment, given in addition to other anti-pseudomonal treatments, in patients with CF and a positive culture for *P. aeruginosa* (see Module 5.3.5.1, CT02 CSR Body)

In addition, the clinical development program of CHF 1538 also included two additional studies, a clinical pharmacokinetic study comparing bioavailability of CHF-1538 versus TOBI (Study CP01, and an active-comparator-controlled clinical study of CHF 1538 versus TOBI (Study CT03).

Studies CT01 (Phase II) and CT02 (Phase III) were designed to demonstrate the superior efficacy of CHF 1538 (300 mg administered BID) versus inhaled aerosolized placebo in terms of improved lung function and microbiological outcomes. In Study CT02, patients were randomized 2:1 to treatment with either CHF 1538 or placebo. The study designs for both trials are summarized in Table 16 to 19.

The clinical studies with CHF 1538 were performed in Europe. Study CT01 was conducted at 13 active centers located in four countries: Ukraine (seven), Italy (three), France (two) and Moldavia (one). Study Ct02 was conducted in 21 active centers located in three countries: Poland (nine), Hungary (eight, Russia (four).

Microbiology

Bacterial culture of sputum samples was performed at the local study site laboratory in both studies. Antibiotic susceptibility testing included testing of *P. aeruginosa* isolates was done at the local site laboratory in study CT01. In Study CT02, the MIC assessment of tobramycin was also performed by a central analytical laboratory in (b) (4) . The in vitro sputum samples were sent from the investigational sites in all countries using a refrigerated courier with temperature monitoring.

Efficacy variables

The primary efficacy variable of study CT01 and CT02

Table 16: Summary of Clinical Studies CT01 and CT02

Study	CT01	CT02
Design	Randomized (1:1), Double-Blind, placebo-controlled, parallel groups, multicenter study	Randomized (2:1), Double-Blind, placebo-controlled, parallel groups, multicenter study
Primary Objective	Efficacy of inhaled aerosolized tobramycin and placebo in the 4-week treatment of patients with CF and *P. aeruginosa* infection	To demonstrate the superior efficacy of aerosolized intermittent administration of CHF 1538 (300 mg BID) compared to aerosolized Placebo saline solutions given following three 4-week "ON"/4-week "OFF" treatment cycles in CF patients infected with *P. aeruginosa* infection. Four-week treatment periods ("ON" cycles) were followed by 4-week periods without treatment ("OFF" cycles).
Number of Randomized Patients	59	247
Age Range (yrs)	6-30	6-45
FEV_1 (% pred.)	≥ 40% and ≤ 80%	≥ 40% and ≤ 80%
Comparator(s)	Inhaled aerosolized placebo	Inhaled aerosolized placebo
Tobramycin Daily Dose	600 mg in two divided doses	600 mg in two divided doses
Duration of Treatment	4 weeks treatment followed by 4-week phase-out period without treatment	4-week treatment periods ("ON" cycles) followed by 4-week periods without treatment ("OFF" cycles; three "ON" cycles and three "OFF" cycles in total
Efficacy Assessment	**Primary**: final FEV_1 (week 4) **Secondary**: other pulmonary function tests; *in vitro* microbiological tests (tobramycin MIC range, MIC_{90} and MIC_{50}); microbiological outcome (eradication, persistence, superinfection, re-infection)	**Primary**: final FEV_1 (week 20 or end of the last "ON" cycle if premature withdrawal) **Secondary**: other pulmonary function tests; *in vitro* microbiological tests (tobramycin MIC range, MIC_{50} and MIC_{90}); microbiological outcome (eradication, persistence, superinfection, re-infection); number of exacerbations, hospitalizations, number of missed school or work days
Safety Assessment	Adverse events, laboratory parameters (hematology and blood chemistry), vital signs (heart rate and blood pressure), audiometric tests	Serum creatinine, adverse events, other laboratory parameters (hematology and blood chemistry), vital signs (heart rate and blood pressure), audiometric tests

Source: Module 5.3.5.1 CT01 Study Report Body, Section 8.1, 9.1, 9.5 and Module 5.3.5.1 CT02 Study Report Body, Section 8., 9.1, 9.5

Table 17: Inclusion and Exclusion Criteria for Studies CT01 and CT02

Patients were enrolled into the treatment period if they met the following criteria:	
Inclusion Criteria:	Patients of either sex aged ≥ six years. Clinical diagnosis of CF defined as follows: • patients registered in the National Registry of CF (or other documents depending on country legislation); • evidence of one or more features in pulmonary abnormalities (persistent colonization/infection with typical CF pathogens, chronic cough and sputum production, persistent chest radiography abnormalities, airways obstruction, nasal polyps and/or digital clubbing), gastrointestinal and nutritional abnormalities (intestinal, pancreatic, hepatic, nutritional); • positive response (sweat chloride concentration ≥ 60 mmol/L) in the standard sweat test documented in the clinical records and/or gene mutation documented in the clinical records. Sputum containing *P. aeruginosa*; in Study CT01, *P. aeruginosa* must be susceptible to tobramycin by an MIC value based on microdilution testing system used by the local laboratory or by a zone diameter ≥16 mm with 10 µg tobramycin disk. Forced expiratory volume in one second (FEV_1) ≥ 40% and ≤ 80% of the predicted normal value. A co-operative attitude and ability to be trained to correctly use the nebulizer and the provided drug. Written informed consent obtained.
Patients were <u>not</u> enrolled into the treatment period if they met any of the following criteria:	
Exclusion Criteria:	Administration of antipseudomonal antibiotic therapy by any route and (in Study CT02 only, nebulized antibiotic therapy) in the previous 4 weeks. Signs of impaired renal function (serum creatinine level ≥ 1.5 mg/dL). Signs of impaired auditory function (auditory threshold in either ear above 20 dB at frequencies between 250 and 8000 Hz). Sputum culture containing *Burkholderia cepacia*. Patients with end-stage lung disease, candidates for heart-lung transplantation. History of other clinically significant cardiac, renal, neurologic, hepatic or endocrine disease to CF, whose sequelae and/or treatment could interfere with (in Study CT01, the conduct of and) the results of the present study. Pregnant or lactating females or females at risk of pregnancy, i.e. those not demonstrating adequate contraception. A pregnancy test was to be done in fertile aged women. Known hypersensitivity to aminoglycosides; Concomitant participation in another trial.

Source: Module 5.3.5.1 CT01 Study Report Body, Section 9.3.1 and Module 5.3.5.1 CT02 Study Report Body, Section 9.3.2

Table 18: Schematic Design for Study CT01

	Run-in Period (1-8 days)	"ON" Cycle	"ON" Cycle	"ON" Cycle	"OFF" Cycle
Weeks	-1	0	2	4	8
Visit[1]	1	2	3	4	5

[1] Visits took place at the clinics before and after the run-in period (baseline, Visit 1), and after 2 weeks (Visit 3) and four weeks (Visit 4) with a follow-up visit at eight weeks (Visit 5) with an acceptable variation of a maximum three days at each visit.

Table 19: Schematic Design for Study CT02

	Run-in Period (1-8 days)	"ON" Cycle		"OFF" Cycle	"ON" Cycle	"OFF" Cycle	"ON" Cycle	"OFF" Cycle
Weeks	-1 (Approx.) to 0	0 to 2	2 to 4	4 to 8	8 to 12	12 to 16	16 to 20	20 to 24
Visit[1]	1	2 to 3	3 to 4	4 to 5	5 to 6	6 to 7	7 to 8	8 to 9

[1] The study plan included a screening visit (Visit 1, study entry), a run-in period (minimum one, maximum eight days), and three 4-week treatment periods ("ON" cycles) with the assigned drug treatment, each followed by a 4-week run-out period ("OFF" cycle)

Pharmacokinetics of tobramycin

Table 1 shows a summary of the clinical pharmacology studies.

Chiesi 2.7.2 Summary of Clinical Pharmacology Studies

Table 1: Summary Table of the Clinical Pharmacology Studies

Study	Aim of the Study	Study Design/Population	Study Medications	Primary Endpoints
CP01	To evaluate the PK of tobramycin in plasma and sputum of CF patients after a single administration by nebulization of CHF1538 in comparison to the marketed formulation TOBI®	Single dose, randomized, double-blind, two-way crossover study in CF patients	CHF 1538, 300 mg/4 mL unit-dose ampule TOBI, 300 mg/5 mL unit-dose ampule	CHF 1538 concentration and PK parameters in plasma and sputum
CT01 PK Substudy	To measure the local sputum concentration of tobramycin in a subset of patients treated with CHF 1538	Multiple dose, randomized, double-blind, placebo-controlled, parallel groups, multicenter study in CF patients	CHF 1538, 300 mg/4 mL unit-dose ampule Placebo	CHF 1538 concentration in sputum ten minutes after the first and the last dose (Day 28 of treatment) and after 4-week wash-out (Day 56)

Definition of Data Sets

Intent-to Treat – Includes all randomized patients who received at least one dose of study medication and had post-baseline data
Per-Protocol (PP) – Includes all ITT patients who met all inclusion/exclusion criteria and who did not have any major protocol deviation. The primary efficacy endpoint was analyzed on this population.
Safety-Population – Includes all randomized patients who took at least one dose of study medication.

Definition of the data Sets Analyzed

- The Intent-to Treat (ITT) population included all randomized patients who received at least one dose of study medication and had post baseline data;
- The Per Protocol (PP) population include all ITT patients who met all inclusion/exclusion criteria and who did not have any major protocol deviation. The primary efficacy endpoint was analyzed on this population.

Table 22 shows the study analysis populations for studies CT01 and CT02.

Table 22: Study Analysis Populations for Studies CT01 and CT02

Study	CT01		CT02	
	CHF 1538	Placebo	CHF 1538	Placebo
Total	67		312	
Randomized	59		247	
	29	30	161	86
Intent to Treat	59		245	
	29	30	161	84
Per Protocol	56		215	
	28	28	144	71
Safety	59		246	
	29	30	161	85

Source: Module 5.3.5.1, CT01 Table 5 and 38; CT01 Appendix 16.2.3; Module 5.3.5.1, CT02 Study Report Body, Table 41

Efficacy Analyses

Standard microbiological tests for assessment of bacteriological effects were used in these studies. An in vitro assessment (MIC, MIC_{90}) of the susceptibility of *P. aeruginosa* to tobramycin was performed by using the conventional procedures practiced in the countries where the study was conducted. The MIC_{50} value was also calculated later to conform to conventional microbiological reporting practices in the U.S.

Summary MIC values (MIC_{50}, MIC_{90}, and MIC range) were determined for the ITT population. Descriptive values were based on the highest MIC value at each study visit provided for all *P. aeruginosa* morphotypes combined and in CT02, as subgroups by pigment production. Resistance to tobramycin was determined according to CLSI standards for parenteral tobramycin as an MIC $\geq$ 16 mcg/mL. The development of resistance to tobramycin before, during and after treatment was analyzed.

The mean change from baseline bacterial load of *P. aeruginosa* (log_{10} CFU/mL) was analyzed at selected study visits for each treatment group. Categorical results for microbiological outcome (eradication, persistence, superinfection, or re-infection) are summarized (standard descriptive statistics).

2.1. Study CP01 (Module 5.3.1.2)

The objective of Study CP01 was to evaluate the PK of tobramycin in plasma and sputum of CF patients after a single administration by nebulization of CHF 1538 (Chiesi Farmaceutici S.p.A.) in comparison to the marketed formulation TOBI (PathoGenesis).

This was a double-blind, single center, randomized, two-way crossover study. Figure 1 shows the flow chart of the study design. All patients were treated with a single dose of 300 mg tobramycin, both as CHF 1538 and as TOBI. A washout of at least five days and not more than nine days separated the two periods of drug administration.

This study used a double-blind design in which the Investigator and the patients were not aware of the treatment allocated. Since single 300 mg doses of CHF 1538 and TOBI are supplied in different volumes (4 mL for CHF 1538 and 5 mL for TOBI), the study medication was prepared for administration by a nurse or pharmacist in order to maintain the blind for both the patient and the Investigator.

At each study session, patients attended the clinic in a fasting state and blood and sputum samples were taken at the following times to assess tobramycin concentrations:

- Venous blood samples were collected at pre-dose, immediately before nebulization start, immediately after end of nebulization (approximately 0.25 hour), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours post-dose;
- Sputum samples (expectorated sputum) were collected at pre-dose, 15 minutes after the end of nebulization (approximately 0.5 hour), 3, 6, 12 and 24 hours post-dose.

Time 0 was defined as the moment when nebulization began.

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Table 3 shows a comparison and analyses of results across studies. As can be seen from the CPO1 and CTO1 studies the concentration of tobramycin in the sputum is extremely variable. In some cases the concentration could be less then the MIC_{90} range (16 to 64 mcg/mL) for *P aeruginosa* isolated from the sputum of cystic fibrosis patients.

3. COMPARISON AND ANALYSES OF RESULTS ACROSS STUDIES

Results of the clinical pharmacology Study CP01 support the safety of CHF 1538 and demonstrate similar systemic exposure of marketed, inhaled TOBI.

CP01 sputum concentrations were consistent with those obtained in Study CT01 (see Module 5.3.5.1) and also with those reported in literature [14,15].

Plasma peak concentrations, derived from Chiesi's pharmacokinetic evaluation of CHF 1538 were consistent with published data [14, 15 and 21] obtained using tobramycin formulations with different concentrations and different types of nebulizers (Table 3). This consistency of data further supports the validity of the clinical pharmacology program for CHF 1538.

Table 3: Peak Sputum and Plasma Concentration (Mean and SD)

Formulation	Nebulizer	Dose (mg)	SPUTUM PEAK CONCENTRATION Mean (SD)	PLASMA PEAK CONCENTRATION Mean (SD)
			C_{max} (µg/g)	C_{max} (µg/mL)
CHF 1538, 75 mg/mL CP01	PARI LC Plus	300 Single dose	1289 ± 851	0.758 ± o.546
CHF 1538, 75 mg/mL CT01	PARI LC Plus	300	696 ± 817 Day 1[1] 717 ± 799 Day 28[1]	-[2]
Tobramycin 60 mg/mL [15]	PARI LC	300 Single dose	687 ± 663	0.570 ± 0.380
Tobramycin 20 mg/mL [15]	UltraNEb	300 Single dose	1498 ± 1331	-
Tobramycin 60 mg/mL [15]	Sidestream	300 Single dose	489 ± 402	0.740 ± 0.430
TOBI, 60 mg/mL [14]	PARI LC Plus	300	754 ± 927 Day 1 769 ± 823 Day 15	0.9 ± 0.5 Day 1 1.3 ± 0.7 Day 15
TOBI, 60 mg/mL [14]	PARI eFlow rapid	300	981 ± 1191 Day 1 1572 ± 2182 Day 15	0.7 ± 0.6 Day 1 1.2 ± 1.0 Day 15

Table 3: Peak Sputum and Plasma Concentration (Mean and SD) (Continued)

Formulation	Nebulizer	Dose (mg)	SPUTUM PEAK CONCENTRATION Mean (SD)	PLASMA PEAK CONCENTRATION Mean (SD)
			C_{max} (µg/g)	C_{max} (ng/mL)
TOBI, 60 mg/mL [21]	PARI LC PLUS®–CR60®	300	-	700
TOBI, 60 mg/mL [21]	PARI LC PLUS®–PortaNeb®	300	-	540

[1] expressed as µg/mL
[2] data not available

Although sputum concentrations may not be homogenous, they are high compared to the very low levels found in serum. Since the known risks of nephrotoxicity and ototoxicity associated with aminoglycosides have been shown to correlate with serum levels, the negligible amount of tobramycin found in serum suggests a potential clinical advantage of aerosol versus intravenous administration. From a safety perspective, the peak plasma concentrations of inhaled tobramycin were at least ten times lower than the usual threshold of 12000 ng/mL commonly used to guide intravenous tobramycin use [14]. Moreover, peak tobramycin levels were also lower than the threshold that should not be exceeded at trough (2000 ng/mL) following parenteral administration [19]. Routine monitoring of tobramycin plasma levels is not required for inhaled tobramycin, but should be considered at the discretion of the treating physician, particularly for patients with renal dysfunction.

Study CT01

Study CT01

Tobramycin Susceptibility Profiles

The ITT population contained 29 patients in the CHF 1538-treatment arm and 30 patients in the placebo-treatment arm. Tobramycin susceptibility data are presented as MIC values for *P. aeruginosa* clinical trial isolates. The distributions of MIC results for *P. aeruginosa* are shown in Table 23. As can be seen the MIC range in the CHF 1538 study group was broader then in the placebo arm and the MIC_{90} was higher on visits 1 and 5 in the CH 1538 group then in the placebo group. The significance of this is not known.

Table 23: Tobramycin MIC[1] Summary for Study CT01

	Tobramycin MIC (µg/mL)							
	CHF 1538				Placebo			
Visit[1]	N	Range	MIC_{50}	MIC_{90}	N	Range	MIC_{50}	MIC_{90}
1	29	≤0.25-256	4	64	27	0.5-64	4	16
4	19	≤0.25-256	4	32	17	0.5-32	4	32
5	23	1-256	4	32	17	0.5-32	4	16

[1] Phases of Study: Visit 1= baseline, Visit 4=sample obtained after completion of "ON" cycle; Visit 5=sample obtained at end of "OFF" cycle.
Source: Module 5.3.5.1, CT01 Study Report Body, Table 15 and CT01 Appendix 16.2.6.4

Table 24 shows the distribution of tobramycin MIC values in study CT01 using systemic interpretive breakpoints.

Table 24: Distribution of Tobramycin MIC Values in Study CT01 Using Systemic Interpretive Breakpoints

Tobramycin MIC (µg/mL)	CHF 1538			Placebo		
	Baseline n/N (%)	Visit 4 n/N (%)	Visit 5 n/N (%)	Baseline n/N (%)	Visit 4 n/N (%)	Visit 5 n/N (%)
≤4.0	19/29 (65.5%)	11/19 (57.9%)	16/23 (69.6%)	21/27 (77.8%)	12/17 (70.6%)	14/17 (82.4%)
8.0	3/29 (10.3%)	3/19 (15.8%)	2/23 (8.7%)	2/27 (7.4%)	3/17 (17.6%)	1/17 (5.9%)
≥16.0	7/29 (24.1%)	5/19 (26.3%)	5/23 (21.7%)	4/27 (14.8%)	2/17 (11.8%)	2/17 (11.8%)

Source: Module 5.3.5.1, CT01 Study Report Body, Table 16 and CT01 Appendix 16.2.6.4

Mean Change from Baseline Viable Counts

The mean change in bacterial density from baseline levels is presented after treatment (visit 4) and at the end of the "OFF" cycle (Visit 5) in Table 26. At visit one the baseline bacterial load was similar between groups. BT visit 4, CHF 1538 treatment resulted in a significantly greater reduction in bacterial count than placebo (-2.16 versus -0.89 CFU/gram, p=0.008, 95% CI [-2.18, -0.34]. However, this difference between groups in bacterial load was no longer apparent at the end of the "OFF" cycle (Visit 5).

Table 26: Log_{10} Mean Bacterial Load (CFUs/g) at Baseline and Mean Change in Bacterial Load from Baseline: ITT Population, Study CT01

Visit	Week		CHF 1538	Placebo	P-Value
1	Baseline	N	27	26	
		Mean	5.79	5.84	0.907
4	4 "ON" Drug	N	27	26	
		Mean Change from Baseline[1]	-2.16	-0.89	
		Difference (95% CI)	-1.26 (-2.18, -0.34)		0.008
5	8 "OFF" Drug	N	25	23	
		Mean Change from Baseline	-0.55	-0.72	
		Difference (95% CI)	0.17 (-0.85, 1.20)		0.738

Note: Ns were lower for this analysis compared with other microbiological analyses because of missing data. A value of 20 was used for all instances where the *P. aeruginosa* pathogen was eradicated.
[1] Adjusted for baseline value
Source: Module 5.3.5.1, CT01 Study Report Body, Table 18, Table 105, Table 106, Table 107, Table 108

Microbiological Outcome

The analysis of microbiological outcome at all visits by baseline MIC value is presented in Table 27. At visit 4 (sample obtained after completion of the "ON" cycle treatment), CHF 1538 treatment resulted in a higher rate of eradication than the placebo (34.5% versus 20.8%) as well as a lower percentage of persistence (37.9% versus 75%). The rate of superinfection was substantially higher in the CHF 1538 group (27.6%) than the Placebo group (4.2%). Re-infection was not observed in either treatment arm at Visit 4.

Table 27: Microbiological Outcome at All Visits by Baseline MIC in Study CT01

		CHF 1538				Placebo			
Visit	Baseline MIC (µg/mL)	Erad[1] n/N (%)	Pers[2] n/N (%)	Sup[3] n/N (%)	Reinf[4] n/N (%)	Erad n/N (%)	Pers n/N (%)	Sup n/N (%)	Reinf n/N (%)
4	≤ 0.25	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
	0.5	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	1/2 (50.0%)	1/2 (50.0%)	0/2 (0.0%)	0/2 (0.0%)
	1	1/4 (25.0%)	2/4 (50.0%)	1/4 (25.0%)	0/4 (0.0%)	0/4 (0.0%)	4/4 (100.0%)	0/4 (0.0%)	0/4 (0.0%)
	2	2/6 (33.3%)	1/6 (16.7%)	3/6 (50.0%)	0/6 (0.0%)	2/5 (40.0%)	3/5 (60.0%)	0/5 (0.0%)	0/5 (0.0%)
	4	3/7 (42.9%)	4/7 (57.1%)	0/7 (0.0%)	0/7 (0.0%)	2/9 (22.2%)	7/9 (77.8%)	0/9 (0.0%)	0/9 (0.0%)
	8	1/3 (33.3%)	2/3 (66.7%)	0/3 (0.0%)	0/3 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
	16	0/3 (0.0%)	1/3 (33.3%)	2/3 (66.7%)	0/3 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)
	32	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
	64	1/2 (50.0%)	0/2 (0.0%)	1/2 (50.0%)	0/2 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
	128	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
	256	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
	Total	10/29 (34.5%)	11/29 (37.9%)	8/29 (27.6%)	0/20 (0.0%)	5/24 (20.8%)	18/24 (75.0%)	1/24 (4.2%)	0/24 (0.0%)

Table 27: Microbiological Outcome at All Visits by Baseline MIC (Continued)

Visit	Baseline MIC (µg/mL)	CHF 1538				Placebo			
		Erad[1] n/N (%)	Pers[2] n/N (%)	Sup[3] n/N (%)	Reinf[4] n/N (%)	Erad n/N (%)	Pers n/N (%)	Sup n/N (%)	Reinf n/N (%)
5	≤ 0.25	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
	0.5	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	1/2 (50.0%)	0/2 (0.0%)	0/2 (0.0%)	1/2 (50.0%)
	1	0/4 (0.0%)	2/4 (50.0%)	1/4 (25.0%)	1/4 (25.0%)	0/4 (0.0%)	2/4 (50.0%)	2/4 (50.0%)	0/4 (0.0%)
	2	0/6 (0.0%)	3/6 (50.0%)	2/6 (33.3%)	1/6 (16.7%)	1/4 (25.0%)	2/4 (50.0%)	0/4 (0.0%)	1/4 (25.0%)
	4	1/6 (16.7%)	2/6 (33.3%)	3/6 (50.0%)	0/6 (0.0%)	1/9 (11.1%)	6/9 (66.7%)	2/9 (22.2%)	0/9 (0.0%)
	8	0/3 (0.0%)	1/3 (33.3%)	2/3 (66.7%)	0/3 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
	16	0/3 (0.0%)	2/3 (66.7%)	1/3 (33.3%)	0/3 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)
	32	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
	64	1/2 (50.0%)	1/2 (50.0%)	0/2 (0.0%)	0/2 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
	128	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
	256	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
	Total	3/28 (10.7%)	12/28 (42.9%)	10/28 (35.7%)	3/28 (10.7%)	3/22 (13.6%)	12/22 (54.5%)	5/22 (22.7%)	2/22 (9.1%)

[1] Eradication (elimination of *P. aeruginosa* detected at Visit 1)
[2] Persistent (presence of *P. aeruginosa* detected at Visit 1)
[3] Superinfection (appearance of a pathogen not detected at Visit 1 in presence of persistence of *P. aeruginosa*)
[4] Reinfection (appearance of *P. aeruginosa* detected at Visit 1 and eradicated at Visit 4)
Source: Module 5.3.5.1, CT01 Study Report Body, Table 19; CT01 Appendix 16.2.6.4 and Appendix 16.2.6.5

At Visit 5 (sample obtained at end of the "OFF" cycle), the percentage eradication was substantially lower than Visit 4 in both treatment groups (10.7% for CHF 1538 versus 13.6% for placebo). Similar rates of persistors (42.9% versus 54.5%) were observed in the two treatment groups. The rate of superinfection was higher for the CHF 1538 treated patients (37.5%) than the placebo treated patients (22.7%). A similar rate of re-infection was observed in both groups.

MIC Changes During Therapy

An assessment of *P. aeruginosa* MIC shifts for individual patients from the baseline value to those values observed during Visit 4 or 5 are summarized in Table 28. At visit 4, there were no isolates in either treatment group whose MIC had changed by ≥4-fold increase from the baseline value.

A comparison of baseline to Visit 5 values shows that the MIC value increased in only one CHF 1538 patient and no placebo-treated patients. There are some instances were there were decreases ion the MIC for both the CHF 1538 and placebo treated groups.

The data in Table 28 suggests that the use of tobramycin per the treatment regimen employed in this trial did not significantly alter the susceptibility of the clinical trial isolates.

Table 28: Assessment of MIC Shifts From Observed Baseline Values and Values Observed at Visit 4 and Visit 5 in Study CT01

	Baseline vs. Visit 4		Baseline vs. Visit 5	
Evaluability Criteria	**CHF 1538**	**Placebo**	**CHF 1538**	**Placebo**
MIC increased[1]	0 (0.0%)	0 (0.0%)	1 (4.3%)	0 (0.0%)
MIC unchanged[2]	16 (84.2%)	15 (88.2%)	20 (87.0%)	17 (100%)
MIC decreased[3]	3 (15.8%)	2 (11.8%)	2 (8.7%)	0 (0.0%)

[1] Patients whose *P. aeruginosa* isolate exhibited ≥ 4 fold increase in the MIC between baseline and end of therapy or follow-up visits.
[2] Patients whose *P. aeruginosa* isolate exhibited ± 2 fold change in the MIC between baseline and end of therapy or follow-up visits.
[3] Patients whose *P. aeruginosa* isolate exhibited ≥ 4 fold decrease in the MIC between baseline and end of therapy or follow-up visits.
Source: Module 5.3.5.1, CT01 Study Report Body, Table 20 and CT01 Appendix 16.2.6.4

Study CT02

Baseline Results

Inclusion criteria for this study required a patient to have *P. aeruginosa* isolated from the sputum sample collected at baseline. The ITT population contained a total of 161 patients in the CHF 1538 treatment arm and 84 patients in the placebo arm. Bacteria in

addition to *P. aeruginosa* that were most commonly isolated in both treatment groups at baseline visit included *Staphylococcus aureus* and less often *Candida albicans*, *Haemophilus* species, and streptococci.

The Applicant categorized the isolates from the CHF 1538 and placebo treatment arms by color of the *P. aeruginosa* colonies. *P. aeruginosa* producing green pigment was the predominant type at Visit 1 (`64%) in both treatment groups and remained the predominant type throughout the study in both treatment groups.

MIC Data

MIC values at baseline were similar for *P. aeruginosa* (all morphotypes combined) in both treatment groups. MIC values ranged from ≤0.25 to 64 mcg/mL. These results are seen in Table 29.

The distribution and percent range of tobramycin MIC results for P. aeruginosa clinical trial isolates at Visits 1, 4, 5, 8, and 9 are shown in Table 29. The range of MICs observed for the CHF 1538 and placebo study arms describe the variability of the MIC results independent of CHF 1538 exposure. This type of result has been seen for other cystic fibrosis studies for not only tobramycin but other antibacterials. Table 29 also shows the comparability of the *P. aeruginosa* tobramycin susceptibility in the treatment and placebo arms and the consistency between visits in the two treatment arms.

Table 29: Tobramycin MIC Summary for Study CT02 (All Morphotypes Combined)

Tobramycin Susceptibility (µg/mL)								
Visit[1]	CHF 1538				Placebo			
	N	Range	MIC_{50}	MIC_{90}	N	Range	MIC_{50}	MIC_{90}
1	160	≤0.25-64	1	64	80	≤0.25-64	1	64
4	110	≤0.25-64	2	64	72	≤0.25-64	1	64
5	134	≤0.25-64	2	64	72	≤0.25-64	1	64
8	104	0.5-64	2	64	65	≤0.25-64	1	64
9	114	≤0.25-64	2	64	61	≤0.25-64	1	64

[1] Phase of Study: Visit 1=baseline, Visit 4 and 8 = sample obtained after completion of "ON" cycle treatment; Visit 5 and 9 = sample obtained at end of "OFF" cycle.
Source: Module 5.3.5.1, CT02 Study Report Body, Tables 15, 173 and 176; CT02 Appendix 16.2.6.17

Table 31 shows the distribution of isolates based on tobramycin susceptibility. The criteria for grouping the isolates was the parenteral susceptibility MIC interpretive criteria (mcg/mL) (≤4 = susceptible, 8 = Intermediate and ≥16 = Resistant.

Table 31: Distribution of Tobramycin MIC Values at Baseline, End of the Last Treatment Cycle, and at Follow-up: ITT Population, Study CT02

Tobramycin MIC (µg/mL)	CHF 1538			Placebo		
	Baseline n/N (%)	EOT[1] n/N (%)	FU[2] n/N (%)	Baseline n/N (%)	EOT n/N (%)	FU n/N (%)
≤ 4.0	136/160 (85.0%)	81/127 (63.8%)	91/143 (63.6%)	63/80 (78.8%)	54/76 (71.1%)	57/77 (74.0%)
8.0	4/160 (2.5%)	10/127 (7.9%)	8/143 (5.6%)	3/80 (3.8%)	3/76 (3.9%)	3/77 (3.9%)
≥ 16.0	20/160 (12.5%)	36/127 (28.3%)	44/143 (30.8%)	14/80 (17.5%)	19/76 (25.0%)	17/77 (22.1%)

[1] Last available MIC value after completion of an "ON" cycle
[2] Last available MIC after completion of an "OFF" cycle
Source: Module 5.3.5.1, CT02 Study Report Body, Table 16

The data were further examined to determine where the isolates with high MIC values (MIC ≥16 mcg/mL) were distributed by study visit. Results of this analysis are shown in Table 32. The data show the percentage of CHF 1538 patients with high MIC values increased after treatment and at follow-up compared to baseline value. The percent of placebo patients with MIC ≥16 mcg/mL also increased, but to a lesser extent, over the course of the study.

Table 32: Percentages of Patients with *P. aeruginosa* Isolates Exhibiting Tobramycin MIC ≥ 16 µg/mL: ITT Population, Study CT02

Visit	CHF 1538		Placebo	
	n/N	%	n/N	%
Baseline	20/160	12.5	14/80	17.5
End of Last Treatment[1]	36/127	28.3	19/76	25.0
Follow-up[2]	44/143	30.8	17/77	22.1

[1] Last available MIC value after completion of an "ON" cycle.
[2] Last available MIC value after completion of an "OFF" cycle.
Source: Module 5.3.5.1, CT02 Study Report Body, Table 17

Mean Change from Baseline Viable Counts

The mean change in bacterial density from baseline levels is presented for at the end of "ON" cycles and at the end of the "OFF" cycles in Table 33. The baseline bacterial load was very similar between groups. By the end of the first "ON" cycle, CHF 1538 treatment resulted in a significantly greater mean reduction in bacterial count than placebo (-1.67 versus -0.57 $\log_{10}$ CFU/gram, p,0.001, 95% CI [-1.59,-0.61]) that was maintained through the end of the first "OFF" cycle. Following additional CHD 1538 treatment, a significantly greater mean reduction in bacterial count than placebo resulted (-1.73 versus -0.62 $\log_{10}$ CFU/gram, p<0.001, 95% CI [-1.65,-0.56]) at the end of the

"ON" cycle. The significant difference in bacterial load between treatment groups was not apparent at the end of the last "OFF' cycle.

Table 33: Log_{10} Bacterial Load (CFUs/g) Mean Baseline and Mean Change from Baseline: ITT Dataset, Study CT02

Visit	Week		CHF 1538	Placebo	P-Value[1]
1	Baseline	N	160	84	
		Mean	5.71	5.72	0.954
4	4 "ON" Drug	N	159	84	
		Mean Change from Baseline[2]	-1.67	-0.57	
		Difference (95% CI)	-1.10 (-1.59, -0.61)		< 0.001
5	8 "OFF" Drug	N	155	82	
		Mean Change from Baseline[2]	-0.97	-0.48	
		Difference (95% CI)	-0.50 (-0.97, -0.02)		0.040
8	20 "ON" Drug	N	155	78	
		Mean Change from Baseline[2]	-1.73	-0.62	
		Difference (95% CI)	-1.10 (-1.65, -0.56)		< 0.001
9	24 "OFF" Drug	N	150	77	
		Mean Change from Baseline[2]	-1.03	-0.72	
		Difference (95% CI)	-0.32 (-0.88, 0.25)		0.272

Note: a value of 20 was used for all instances where the *P. aeruginosa* pathogen was eradicated
[1] significance level=0.05
[2] adjusted for baseline value
Source: Module 5.3.5.1, CT02 Study Report Body, Table 13

Microbiological Outcome

The analysis by baseline MIC (Table 34) shows that there were rare instances of microbiological eradication in either the CHF 1538 or placebo groups at any visit. There were a high percentage of persistors in the CHF 1538 group at Visits 4 and 8 with somewhat lower percentage at Visits 5 and 9. A high percentage of persistors were also present in the placebo group at all visits.

Table 34: Microbiological Outcome at All Study Visits by Baseline MIC: ITT Patients, Study CT02

Visit	Baseline MIC (µg/mL)	CHF 1538 (N=161)				Placebo (N=84)			
		Erad1 n/N(%)	Pers2 n/N(%)	Sup3 n/N(%)	Reinf4 n/N(%)	Erad n/N(%)	Pers n/N(%)	Sup n/N(%)	Reinf n/N(%)
4	≤ 0.25	0/4 (0.0%)	3/4 (75.0%)	1/4 (25.0%)	0/4 (0.0%)	0/13 (0.0%)	9/13 (69.2%)	4/13 (30.8%)	0/13 (0.0%)
	0.5	0/23 (0.0%)	16/23 (69.6%)	7/23 (30.4%)	0/23 (0.0%)	0/17 (0.0%)	13/17 (76.5%)	4/17 (23.5%)	0/17 (0.0%)
	1	0/42 (0.0%)	34/42 (81.0%)	8/42 (19.1%)	0/42 (0.0%)	0/22 (0.0%)	18/22 (81.8%)	4/22 (18.2%)	0/22 (0.0%)
	2	0/21 (0.0%)	20/21 (95.2%)	1/21 (4.8%)	0/21 (0.0%)	0/14 (0.0%)	12/14 (85.7%)	2/14 (14.3%)	0/14 (0.0%)
	4	0/8 (0.0%)	8/8 (100.0%)	0/8 (0.0%)	0/8 (0.0%)	0/2 (0.0%)	2/2 (100.0%)	0/2 (0.0%)	0/2 (0.0%)
	8	0/4 (0.0%)	4/4 (100.0%)	0/4 (0.0%)	0/4 (0.0%)	0/3 (0.0%)	3/3 (100.0%)	0/3 (0.0%)	0/3 (0.0%)
	16	0/6 (0.0%)	5/6 (83.3%)	1/6 (16.7%)	0/6 (0.0%)	0/2 (0.0%)	1/2 (50.0%)	1/2 (50.0%)	0/2 (0.0%)
	32	0/4 (0.0%)	2/4 (50.0%)	2/4 (50.0%)	0/4 (0.0%)	0/7 (0.0%)	4/7 (57.1%)	3/7 (42.9%)	0/7 (0.0%)
	64	0/22 (0.0%)	16/22 (72.7%)	6/22 (27.3%)	0/22 (0.0%)	0/10 (0.0%)	8/10 (80.0%)	2/10 (20.0%)	0/10 (0.0%)
	Total	0/134 (0.0%)	108/134 (80.6%)	26/134 (19.4%)	0/134 (0.0%)	0/90 (0.0%)	70/90 (77.8%)	20/90 (22.2%)	0/90 (0.0%)
5	≤ 0.25	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	0/4 (0.0%)	4/4 (100.0%)	0/4 (0.0%)	0/4 (0.0%)
	0.5	0/27 (0.0%)	15/27 (55.6%)	6/27 (22.2%)	6/27 (22.2%)	0/25 (0.0%)	18/25 (72.0%)	6/25 (24.0%)	1/25 (4.0%)
	1	0/56 (0.0%)	32/56 (57.1%)	11/56 (19.6%)	13/56 (23.2%)	0/23 (0.0%)	18/23 (78.3%)	3/23 (13.0%)	2/23 (8.7%)
	2	0/31 (0.0%)	23/31 (74.2%)	5/31 (16.1%)	3/31 (9.7%)	0/13 (0.0%)	7/13 (53.9%)	5/13 (38.5%)	1/13 (7.7%)
	4	0/14 (0.0%)	9/14 (64.3%)	2/14 (14.3%)	3/14 (21.4%)	0/7 (0.0%)	7/7 (100.0%)	0/7 (0.0%)	0/7 (0.0%)
	8	0/6 (0.0%)	3/6 (50.0%)	3/6 (50.0%)	0/6 (0.0%)	0/4 (0.0%)	2/4 (50.0%)	1/4 (25.0%)	1/4 (25.0%)
	16	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
	32	0/7 (0.0%)	5/7 (71.4%)	1/7 (14.3%)	1/7 (14.3%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)

Table 34: Microbiological Outcome at All Study Visits by Baseline MIC: ITT Patients, Study CT02 (Continued)

Visit	Baseline MIC (µg/mL)	CHF 1538 (N=161)				Placebo (N=84)			
		Erad n/N(%)	Pers n/N(%)	Sup n/N(%)	Reinf n/N(%)	Erad n/N(%)	Pers n/N(%)	Sup n/N(%)	Reinf n/N(%)
5 (cont)	64	0/22 (0.0%)	16/22 (72.7%)	2/22 (9.1%)	4/22 (18.2%)	0/16 (0.0%)	11/16 (68.8%)	3/16 (18.8%)	2/16 (12.5%)
	Total	0/164 (0.0%)	104/164 (63.4%)	30/164 (18.3%)	30/164 (18.3%)	0/92 (0.0%)	67/92 (72.8%)	18/92 (19.6%)	7/92 (7.6%)
8	≤ 0.25	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/9 (0.0%)	6/9 (66.7%)	3/9 (33.3%)	0/9 (0.0%)
	0.5	0/13 (0.0%)	12/13 (92.3%)	0/13 (0.0%)	1/13 (7.7%)	0/14 (0.0%)	10/14 (71.4%)	3/14 (21.4%)	1/14 (7.1%)
	1	1/27 (3.7%)	20/27 (74.1%)	4/27 (14.8%)	2/27 (7.4%)	0/25 (0.0%)	17/25 (68.0%)	6/25 (24.0%)	2/25 (8.0%)
	2	0/30 (0.0%)	19/30 (63.3%)	9/30 (30.0%)	2/30 (6.7%)	0/12 (0.0%)	10/12 (83.3%)	2/12 (16.7%)	0/12 (0.0%)
	4	0/12 (0.0%)	11/12 (91.7%)	1/12 (8.3%)	0/12 (0.0%)	0/5 (0.0%)	4/5 (80.0%)	1/5 (20.0%)	0/5 (0.0%)
	8	0/9 (0.0%)	6/9 (66.7%)	3/9 (33.3%)	0/9 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
	16	0/4 (0.0%)	3/4 (75.0%)	1/4 (25.0%)	0/4 (0.0%)	0/2 (0.0%)	1/2 (50.0%)	1/2 (50.0%)	0/2 (0.0%)
	32	0/3 (0.0%)	3/3 (100.0%)	0/3 (0.0%)	0/3 (0.0%)	0/6 (0.0%)	4/6 (66.7%)	1/6 (16.7%)	1/6 (16.7%)
	64	0/34 (0.0%)	22/34 (64.7%)	9/34 (26.5%)	3/34 (8.8%)	0/11 (0.0%)	9/11 (81.8%)	2/11 (18.2%)	0/11 (0.0%)
	Total	1/132 (0.8%)	96/132 (72.7%)	27/132 (20.5%)	8/132 (6.1%)	0/85 (0.0%)	62/85 (72.9%)	19/85 (22.4%)	4/85 (4.7%)
9	≤ 0.25	0/2 (0.0%)	1/2 (50.0%)	0/2 (0.0%)	1/2 (50.0%)	0/6 (0.0%)	6/6 (100.0%)	0/6 (0.0%)	0/6 (0.0%)
	0.5	1/23 (4.4%)	13/23 (56.5%)	6/23 (26.1%)	3/23 (13.0%)	0/19 (0.0%)	15/19 (79.0%)	3/19 (15.8%)	1/19 (5.3%)
	1	0/33 (0.0%)	22/33 (66.7%)	3/33 (9.1%)	8/33 (24.2%)	0/17 (0.0%)	14/17 (82.4%)	3/17 (17.7%)	0/17 (0.0%)
	2	0/20 (0.0%)	12/20 (60.0%)	5/20 (25.0%)	3/20 (15.0%)	0/13 (0.0%)	10/13 (76.9%)	2/13 (15.4%)	1/13 (7.7%)
	4	0/14 (0.0%)	12/14 (85.7%)	1/14 (7.1%)	1/14 (7.1%)	0/4 (0.0%)	3/4 (75.0%)	1/4 (25.0%)	0/4 (0.0%)
	8	0/10 (0.0%)	6/10 (60.0%)	2/10 (20.0%)	2/10 (20.0%)	0/3 (0.0%)	2/3 (66.7%)	1/3 (33.3%)	0/3 (0.0%)

Table 34: Microbiological Outcome at All Study Visits by Baseline MIC: ITT Patients, Study CT02 (Continued)

Visit	Baseline MIC (µg/mL)	CHF 1538 (N=161)				Placebo (N=84)			
		Erad n/N(%)	Pers n/N(%)	Sup n/N(%)	Reinf n/N(%)	Erad n/N(%)	Pers n/N(%)	Sup n/N(%)	Reinf n/N(%)
9 (cont)	16	0/4 (0.0%)	1/4 (25.0%)	0/4 (0.0%)	3/4 (75.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
	32	0/4 (0.0%)	4/4 (100.0%)	0/4 (0.0%)	0/4 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)
	64	0/28 (0.0%)	22/28 (78.6%)	3/28 (10.7%)	3/28 (10.7%)	0/10 (0.0%)	7/10 (70.0%)	2/10 (20.0%)	1/10 (10.0%)
	Total	1/138 (0.7%)	93/138 (67.4%)	20/138 (14.5%)	24/138 (17.4%)	0/74 (0.0%)	59/74 (79.7%)	12/74 (16.2%)	3/74 (4.1%)

[1] Eradication (elimination of *P. aeruginosa* detected at baseline)
[2] Persistent (presence of *P. aeruginosa* detected at baseline)
[3] Superinfection (addition of a new pathogen to those reported at baseline, in presence of persistence of *P. aeruginosa*)
[4] Re-infection (return of *P. aeruginosa* without appearance of new species)
Source: Module 5.3.5.1, CT02 Study Report Body, Table 179 and CT02 Appendix 16.2.6.17

MIC Changes During Therapy

Table 35 shows the tobramycin MIC shifts from baseline to follow-up. MIC values were unchanged (+/-2 fold) for 34.9% and 37.5% in the CHF 1538 groups at the end of treatment and the follow-up visits and for 27.1% and 23.4% in the placebo group, respectively. However, at the end of therapy and the follow-up visits, a greater percentage of strains in the CHF 1538 group showed a $\geq$ 4 fold in crease in tobramycin MIC than isolates in the placebo group. MIC values decreased $\geq$ 4 fold for approximately 4% of isolates in each treatment group.

Table 35: Assessment of MIC Shifts from Observed Baseline Values and MIC Values Observed at End of the Last Treatment Cycle and at Follow-up: ITT Population, Study CT02

Evaluability Criteria	Baseline vs. End of Last Treatment Cycle[1]		Baseline vs. Follow-up[2]	
	CHF 1538	Placebo	CHF 1538	Placebo
MIC increased[3]	41 (24.7%)	9 (5.4%)	51 (27.7%)	5 (2.7%)
MIC unchanged[4]	58 (34.9%)	45 (27.1%)	69 (37.5%)	43 (23.4%)
MIC decreased[5]	6 (3.6%)	7 (4.2%)	8 (4.3%)	8 (4.3%)

[1] Last available MIC value after completion of an "ON" cycle.
[2] Last available MIC value after completion of an "OFF" cycle.
[3] Patients whose PA isolate exhibited ≥ 4-fold increase in the MIC between baseline and end of therapy or follow-up visits.
[4] Patients whose PA isolate exhibited ± 2-fold change in the MIC between baseline and end of therapy or follow-up visits.
[5] Patients whose PA isolate exhibited ≥ 4-fold decrease in the MIC between baseline and end of therapy or follow-up visits
Source: Module 5.3.5.1, CT02 Study Report Body, Table 18

Integration of CT01 and CT02 Results

Inclusion criteria for both studies required a patient to have *P. aeruginosa* isolated from the sputum sample collected at Visit 1 and in Study CT01 only; the *P. aeruginosa* isolate was requested to be susceptible to tobramycin. Study CT01 enrolled patients in a 1:1 ratio, while patients in Study CT02 were randomized 2:1 in the CHF 1538: Placebo treatment groups. *P. aeruginosa* was isolated from a total of 190 CHF 1538 patients and 114 Placebo patients (ITT population) in the 2 studies. Baseline *P. aeruginosa* MIC values were available for a total of 189 CHF 1538 patients and 107 placebo patients (ITT population) in the 2 studies. Organisms in addition to *P. aeruginosa* that were most commonly isolated in both treatment groups at Visit 1 included *Staphylococcus aureus* and less often, *Candida albicans*, *Haemophilus* species, and streptococci.

MIC Distributions

Table 36 shows the integrated results of the distribution of tobramycin MIC results for *P. aeruginosa* at Visits 1, 4, and 5. The distribution of MIC values for isolates at baseline (Visit 1) was comparable between the 2 treatment groups, with the majority of isolates having MIC values ≤8 mcg/mL (CHD 1538 85.7%, Placebo group 83.2%). The distribution of MIC values remained comparable between treatment groups at Visits 4 and 5. Throughout the study,, the majority of *P. aeruginosa* isolates remained susceptible to tobramycin (MIC ≤8 mcg/mL) in both treatment groups, although the susceptible percentage in CHF 1538 group declined slightly compared to Visit 1 at the subsequent visits. While percentages in the placebo group showed less variation.

Table 36: Integrated MIC Distribution for Clinical Isolates: ITT population, Studies CT01 and CT02

		CHF 1538		Placebo	
Visit	MIC (µg/mL)	n/N (%)	Cumulative %	n/N (%)	Cumulative %
1	≤ 0.25	7/189 (3.7%)	3.70	8/107 (7.5%)	7.48
	0.5	37/189 (19.6%)	23.28	17/107 (15.9%)	23.36
	1	53/189 (28.0%)	51.32	23/107 (21.5%)	44.86
	2	40/189 (21.2%)	72.49	19/107 (17.8%)	62.62
	4	18/189 (9.5%)	82.01	17/107 (15.9%)	78.50
	8	7/189 (3.7%)	85.71	5/107 (4.7%)	83.18
	16	5/189 (2.6%)	88.36	5/107 (4.7%)	87.85
	32	2/189 (1.1%)	89.42	4/107 (3.7%)	91.59
	64	19/189 (10.1%)	99.47	9/107 (8.4%)	100.00
	128	0/189 (0.0%)	99.47	0/107 (0.0%)	100.00
	256	1/189 (0.5%)	100.00	0/107 (0.0%)	100.00
4	≤0.25	4/129 (3.1%)	3.10	9/89 (10.1%)	10.11
	0.5	17/129 (13.2%)	16.28	17/89 (19.1%)	29.21
	1	37/129 (28.7%)	44.96	22/89 (24.7%)	53.93
	2	21/129 (16.3%)	61.24	12/89 (13.5%)	67.42
	4	14/129 (10.9%)	72.09	8/89 (9.0%)	76.40
	8	7/129 (5.4%)	77.52	6/89 (6.7%)	83.15
	16	7/129 (5.4%)	82.95	1/89 (1.1%)	84.27
	32	5/129 (3.9%)	86.82	6/89 (6.7%)	91.01
	64	16/129 (12.4%)	99.22	8/89 (9.0%)	100.00
	128	0/129 (0.0%)	99.22	0/89 (0.0%)	100.00
	256	1/129 (0.8%)	100.00	0/89 (0.0%)	100.00

Table 36: Integrated MIC Distribution for Clinical Isolates: ITT population, Studies CT01 and CT02 (Continued)

		CHF 1538		Placebo	
Visit	MIC (µg/mL)	n/N (%)	Cumulative %	n/N (%)	Cumulative %
5	≤ 0.25	1/157 (0.6%)	0.64	4/89 (4.5%)	4.49
	0.5	21/157 (13.4%)	14.01	20/89 (22.5%)	26.97
	1	50/157 (31.8%)	45.86	23/89 (25.8%)	52.81
	2	31/157 (19.7%)	65.61	11/89 (12.4%)	65.17
	4	18/157 (11.5%)	77.07	13/89 (14.6%)	79.78
	8	5/157 (3.2%)	80.25	4/89 (4.5%)	84.27
	16	2/157 (1.3%)	81.53	1/89 (1.1%)	85.39
	32	7/157 (4.5%)	85.99	1/89 (1.1%)	86.52
	64	21/157 (13.4%)	99.36	12/89 (13.5%)	100.00
	128	0/157 (0.0%)	99.36	0/89 (0.0%)	100.00
	256	1/157 (0.6%)	100.00	0/89 (0.0%)	100.00

Source: Module 5.3.5.1, CT01 Appendix 16.2.6.4 and Module 5.3.5.1, CT02 Appendix 16.2.6.17

MIC range, MIC_{50} and MIC_{90} values are summarized for each treatment group by Visit in Table 37.

Table 37: Integrated Tobramycin MIC Summary (Studies CT01 and CT02)

	Tobramycin Susceptibility (µg/mL)							
	CHF 1538				Placebo			
Visit[1]	N	Range	MIC_{50}	MIC_{90}	N	Range	MIC_{50}	MIC_{90}
1	189	≤ 0.25-256	1	64	107	≤ 0.25-64	2	32
4	129	≤ 0.25-256	2	64	89	≤ 0.25-64	1	32
5	157	≤ 0.25-256	2	64	89	≤ 0.25-64	1	64

[1] Phase of Study: Visit 1=baseline, Visit 4 = sample obtained after completion of "ON" cycle' treatment; Visit 5 = sample obtained at end of "OFF" cycle

Source: Module 5.3.5.1, CT01 Appendix 16.2.6.4 and Module 5.3.5.1, CT02 Appendix 16.2.6.17

Distribution of Isolates Based upon Tobramycin Susceptibility

Table 38 shows the distribution of tobramycin MICs based on parenteral MIC interpretive criteria. For the CHF 1538 patients the majority of isolates (82.0%) had MIC values of ≤4 mcg/mL (Susceptible) at baseline. A substantial proportion of isolates in the CHF 1538 treatment group remained Susceptible at the end of therapy (72.1%) and at follow-up (77.1%) visits. For placebo-treated patients, the majority of isolates (78.5%) had MIC values ≤4 mcg/mL at baseline and only varied slightly (76.4% to 79.8%) at later visits in the study.

Only 3.7% if isolates in the CHF 1538 patients were considered intermediate (8mcg/mL) at baseline; the percentage increased slightly at the end of therapy visit but declined to 3.2% by the follow-up visit. The percentage of placebo patients with isolates considered Intermediate showed a similar pattern: 4.7%, slightly higher at the end of therapy, and declining to 4.5% by the follow-up visit.

A higher percentage of isolates considered Resistant (≥16 mcg/mL) was noted in the placebo group (16.8%) than in the CHF 1538 group (14.3%) at baseline. However, the CHF 1538 treatment group had a slightly higher percentage of isolates considered Resistant at the end of therapy (22.5%) in the CHF 1538 group versus 16.9% in the placebo group). The proportion of resistant isolates remained consistent for each treatment group at the follow-up visit.

Table 38: Integrated Distribution of Tobramycin MIC Values at Baseline, End of the Last Treatment (EOT) Cycle, and at Follow-up (FU): ITT Population, Studies CT01 and CT02

Tobramycin MIC (µg/mL)	CHF 1538 (N=190)			Placebo (N=114)		
	Baseline n/N (%)	End of Last Treatment Cycle (Visit 4) n/N (%)	Follow-up (Visit 5) n/N (%)	Baseline n/N (%)	End of Last Treatment Cycle (Visit 4) n/N (%)	Follow-up (Visit 5) n/N (%)
≤ 4.0	155/189 (82.0%)	93/129 (72.1%)	121/157 (77.1%)	84/107 (78.5%)	68/89 (76.4%)	71/89 (79.8%)
8.0	7/189 (3.7%)	7/129 (5.4%)	5/157 (3.2%)	5/107 (4.7%)	6/89 (6.7%)	4/89 (4.5%)
≥ 16.0	27/189 (14.3%)	29/129 (22.5%)	31/157 (19.7%)	18/107 (16.8%)	15/89 (16.9%)	14/89 (15.7%)

Note: Phase of Study: Visit 1=baseline, Visit 4=sample obtained after completion of "ON" cycle treatment; Visit 5=sample obtained at end of "OFF" cycle
Denominator is based upon the number of patients having an MIC value available at a given visit.
Source: Module 5.3.5.1, CT01 Appendix 16.2.6.4 and Module 5.3.5.1, CT02 Appendix 16.2.6.17

Mean Change in Viable *P. aeruginosa* Counts from Baseline

Table 39 provides information on the treatment effect on the *P. aeruginosa* load baseline. Because the data is presented using $\log_{10}$ transformations, a decrease in one $\log_{10}$ as observed for CHF 1538 is equivalent to a reduction of 90% of the bacterial load per gram of sputum. A significant difference in mean change from baseline load occurred after the first "ON" cycle ($p<0.001$; CI=[-1.57, -0.70]). There was no significant difference in the bacterial load for the treatment groups at the end of the "OFF" cycle.

Table 39: Integrated log_{10} Bacterial Load (CFUs/g Sputum) Mean Baseline and Mean Change From Baseline: ITT Population, Studies CT01 and CT02

Visit	Week		CHF 1538	Placebo	P-Value
1	Baseline	N	186	110	
		Mean[1]	5.76	5.77	0.935
End of "ON" Cycle	4	N	186	110	
		Mean Change from Baseline[2]	-1.88	-0.75	< 0.001
		Difference (95% CI)	-1.14 (-1.57, -0.70)		
End of "OFF" Cycle	8	N	180	105	
		Mean Change from Baseline	-0.86	-0.49	0.086
		Difference (95% CI)	-0.38 (-0.80, 0.05)		

[1] A value of 20 CFU/g (based upon the dilution factor in the counting procedure) was used for all instances where the *P. aeruginosa* pathogen was eradicated.
[2] Adjusted for baseline value
N's are based upon the number of patients with a baseline and end of "ON" cycle bacterial load value, or a baseline and end of "OFF" cycle bacterial load value
Source: Module 5.3.5.3, ISE Tables, Tables 1, 2, 3, 4

Microbiological Outcome

The analysis of microbiological outcome is shown at the end of treatment and the follow-up visits at baseline MIC value in Table 40. The analysis by baseline MIC shows that instances of microbiological eradication were rare in either treatment group at any visit. There was a high percentage of persistors in the CHF 1538 group at the end of the "ON" therapy period (Visit 4) with somewhat lower percentages "OFF" therapy at follow-up (Visit 5). A higher percentage of persistors were also present in the placebo group at both the end of "ON" treatment and "OFF" treatment follow-up visits. Persistence of *P. aeruginosa* following tobramycin therapy in CF patient is not unexpected. No correlation was evident between MIC value and microbiological eradication rate. The superinfection rate was similar in both treatment groups. Reinfection was not observed in either treatment group at the end of "ON" treatment period, and the rate of re-infection was slightly higher in the CHF 1538 group at the end of the "OFF" treatment period (Visit 5).

Table 40: Integrated Microbiological Outcome at the End of Treatment (Visit 4) and Follow-up (Visit 5) by Baseline MIC: ITT Population, Studies CT01 and CT02

Baseline MIC (µg/mL)	CHF 1538 (N=190)				Placebo (N=114)			
	Erad1 n/N(%)	Pers2 n/N(%)	Sup3 n/N(%)	Reinf4 n/N(%)	Erad n/N(%)	Pers n/N(%)	Sup n/N(%)	Reinf n/N(%)
	End of Treatment (Visit 4)							
≤ 0.25	1/5 (20.0%)	3/5 (60.0%)	1/5 (20.0%)	0/5 (0.0%)	0/13 (0.0%)	9/13 (69.2%)	4/13 (30.8%)	0/13 (0.0%)
0.5	1/24 (4.2%)	16/24 (66.7%)	7/24 (29.2%)	0/24 (0.0%)	1/19 (5.3%)	14/19 (73.7%)	4/19 (21.1%)	0/19 (0.0%)
1	1/46 (2.2%)	36/46 (78.3%)	9/46 (19.6%)	0/46 (0.0%)	0/26 (0.0%)	22/26 (84.6%)	4/26 (15.4%)	0/26 (0.0%)
2	2/27 (7.4%)	21/27 (77.8%)	4/27 (14.8%)	0/27 (0.0%)	2/19 (10.5%)	15/19 (79.0%)	2/19 (10.5%)	0/19 (0.0%)
4	3/15 (20.0%)	12/15 (80.0%)	0/15 (0.0%)	0/15 (0.0%)	2/11 (18.2%)	9/11 (81.8%)	0/11 (0.0%)	0/11 (0.0%)
8	1/7 (14.3%)	6/7 (85.7%)	0/7 (0.0%)	0/7 (0.0%)	0/4 (0.0%)	4/4 (100.0%)	0/4 (0.0%)	0/4 (0.0%)
16	0/9 (0.0%)	6/9 (66.7%)	3/9 (33.3%)	0/9 (0.0%)	0/3 (0.0%)	1/3 (33.3%)	2/3 (66.7%)	0/3 (0.0%)
32	0/5 (0.0%)	3/5 (60.0%)	2/5 (40.0%)	0/5 (0.0%)	0/8 (0.0%)	5/8 (62.5%)	3/8 (37.5%)	0/8 (0.0%)
64	1/24 (4.2%)	16/24 (66.7%)	7/24 (29.2%)	0/24 (0.0%)	0/11 (0.0%)	9/11 (81.8%)	2/11 (18.2%)	0/11 (0.0%)
128	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
256	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
Total	10/163 (6.1%)	119/163 (73.0%)	34/163 (20.9%)	0/163 (0.0%)	5/114 (4.4%)	88/114 (77.2%)	21/114 (18.4%)	0/114 (0.0%)

CONCLUSION

The data from studies CT01 and CT02 is presented individually and combined in the preceding pages. The data from CT01 and CT02 individually do not suggest any non-statistical significant microbiological adverse events from use of aerosolized tobramycin for treating CF patients. Combining the data suggests the same.

Treatment-Emergent Microorganisms

In the CHF 1538-treated patients the organism identified most frequently was the Gram-positive bacterium *Staphylococcus aureus* with the yeast *Candida* species (including *C. albicans*) being the nest most prevalent. In the placebo-treated group the same two organisms were the most prevalent. There was a variety of Gram-negative bacteria identified in both treatment groups, though the incidence of any individual species was low. This information is presented in Table 41 (2.7.2 Summary of Clinical Pharmacology Studies pg. 110) which is not shown here.

MIC Changes During Therapy

Table 42 is an assessment of MIC shifts fro individual isolates from the baseline tobramycin MIC to those values observed at end of therapy or at follow-up. MIC values were changed (+/- 2-fold) for 41.7% and 42.5% in the CHF 1538 group at the end of therapy and the follow-up visit, and for 33.2% and 28.5% in the placebo group respectively. However, at both the end of therapy and the follow-up visits, a greater percentage of isolates in the CHF 1538 group showed a ≥4-fold increase in MIC than isolates in the placebo group. MIC values decreased ≥4-fold in a small percent of isolates in both treatment groups.

Table 42: Assessment of MIC Shifts from Observed Baseline Values and MIC Values Observed at End of the First "ON" Cycle and at End of the First "OFF" Cycle: Integrated Results for CT01 and CT02, ITT Population

Evaluability Criteria	Baseline vs. End of ON' Cycle		Baseline vs. End of 'OFF' Cycle	
	CHF 1538	Placebo	CHF 1538	Placebo
MIC increased[1]	22/187 (11.8%)	4/187 (2.1%)	30/207 (14.5%)	9/207 (4.3%)
MIC unchanged[2]	78/187 (41.7%)	62/187 (33.2%)	88/207 (42.5%)	59/207 (28.5%)
MIC decreased[3]	10/187 (5.3%)	11/187 (5.9%)	14/207 (6.8%)	7/207 (3.4%)

[1] Patients whose paired PA isolate exhibited ≥ 4-fold increase in the MIC between baseline and end of therapy or follow-up visits.
[2] Patients whose paired PA isolate exhibited ±2-fold change in the MIC between baseline and end of therapy or follow-up visits.
[3] Patients whose paired PA isolate exhibited ≥ 4-fold decrease in the MIC between baseline and end of therapy or follow-up visits.
Source: Module 5.3.5.1, CT01 Appendix 16.2.6.4 and Module 5.3.5.1, CT02 Appendix 16.2.6.17

PHASE 3 - CLINICAL STUDY CT03

This is the pivotal study for the approval of this tobramycin product. CT-3 is an open-label, multinational, multicenter, randomized, parallel group study designed to compare the efficacy and tolerability of aerosolized CHF 1538 and TOBI, both administered via a nebulizer (PARI LC Plus® with the PARI Boy N® compressor, Pari, Germany), over a 4-week treatment in a twice-daily regimen in patients with CF and *P. aeruginosa* chronic infection.

> Study CMA-0631-CSR-0025: A multicentre, multinational, open label, randomized, parallel group clinical trial of Torineb™/Actitob®/Bramitob® (tobramycin solution for nebulization, 300 mg twice daily in 4 mL unit dose ampoules) compared to TOBI in the treatment of patients with cystic fibrosis and chronic infection with *Pseudomonas aeruginosa* (Module 5.3.5.1, CT03 CSR body).

The study was designed to demonstrate the non-inferiority of CHF 1538 compared to TOBI in the primary efficacy variable forced expiratory volume in one second (FEV_1) predicted normal at the end of the treatment phase in patients with CF and chronic *P. aeruginosa* infection of the lungs. Table 44 provides a summary of clinical study CT03. Among the secondary efficacy variables, microbiological tests included quantitative cell counts (CFUs) for *P. aeruginosa* isolated from sputum, antibiotic susceptibility testing (MIC range, MIC_{50}, and MIC_{90} were standard microbiological assessments to evaluate the susceptibility of *P. aeruginosa* to tobramycin.

Sputum Collection and Microbiological Culture Methods

Sputum specimens were collected at the study site and sent under refrigeration to a central laboratory ((b) (4)). The collection and transport of the specimens are described in the Protocol (Module 5.3.5.1, CT03 Appendix 16.1.1). Culture of specimens and identification of isolates were done by recognized methods. Prior to the study the methods for specimen collection, transport, culture of specimens, and identification of isolates were reviewed by this Reviewer and found to be appropriate.

Antibiotic Susceptibility Testing

Minimal inhibitory concentrations (MIC) were determined using the CLSI-recommended methods and quality controls for tobramycin and the seven other antibiotics (amikacin, aztreonam, ciprofloxacin, colistin, imipenem, levofloxacin, piperacillin/tazobactam) were run. The MIC was measured by a broth microdilution method using (b) (4) plates. A positive growth control well was included on every plate. All of these methods had been reviewed prior to the beginning of the clinical study and found to be acceptable by this Reviewer.

Isolates for susceptibility testing were collected at visit 1 (prior to treatment, Visit 4 ("ON"-treatment, and Visit 5 ("OFF"-treatment). MIC values were determined for the ITT population. Susceptibility to tobramycin was interpreted according to Clinical and laboratory standards (CLSI) standards for systemically-administered tobramycin: Susceptible, ≤4 mcg/mL = Susceptible, 8 mcg/mL = Intermediate, ≥ 16 mcg/mL = Resistant.

Table 44: Summary of Clinical Study CT03

Study	CT03
Design	Randomized (1:1), open-label, reference product controlled, parallel group multinational, multicenter, study.
Primary Objective	To demonstrate the non-inferiority of CHF 1538 compared to TOBI in the primary efficacy variable forced expiratory volume in one second (FEV_1) % predicted normal at the end of the treatment phase in patients with CF and chronic infection of the lungs with *P. aeruginosa*.
Number of Randomized Patients	324
Age Range (yrs)	6 – 47
FEV_1 (% pred.)	≥ 40% and ≤ 80%
Comparator Daily Dose	TOBI (tobramycin 300 mg/5 mL inhalation solution for nebulization) 600 mg in two divided doses
Tobramycin Daily Dose	CHF 1538 (tobramycin 300 mg/4 mL inhalation solution for nebulization) 600 mg in two divided doses
Duration of Treatment	4 weeks treatment followed by 4-week phase-out period without treatment
Efficacy Assessment	**Primary**: final FEV_1 % predicted normal (week 4) **Secondary**: other pulmonary function tests; *in-vitro* microbiological tests including microbiological outcomes (eradication, persistence, re-infection with respect to *P.aeruginosa*, or superinfection with micro-organisms other than *P. aeruginosa*), tobramycin MIC range, MIC_{50} and MIC_{90}, and *P. aeruginosa* bacterial load (CFUs at Visit 4 and Visit 5).
Safety Assessment	Adverse events (AEs) and adverse drug reactions (ADRs), audiometric tests, laboratory parameters (hematology and blood chemistry), vital signs (heart rate and blood pressure) and physical examination.

Source: Module 5.3.5.1, CT03 Study Report Body, Section 9.1

Microbiological Outcome by Tobramycin Baseline MIC

Microbiological outcome is presented by tobramycin baseline (Visit 1) MIC values, at Visit 4 ("ON" treatment), and Visit 5 ("OFF" treatment) for all P. aeruginosa morphotypes combined. Microbiological outcomes by baseline MIC values are compared between the two treatment groups at Visits 4 and 5 for the ITT population.

Microbiological outcome: At "ON" treatment (Visit 4), the following definitions were given on the basis of the results of the microbiologic test:

- Eradication (elimination of *P. aeruginosa*)
- Persistence (persistence of *P. aeruginosa* detected at Visit 1
- Superinfection (appearance of a pathogen other than *P. aeruginosa* not detected at Visit 1

At "OFF" treatment (Visit 5), the following definitions were given on the basis of the results of the microbiologic test:

- Eradication (elimination of *P. aeruginosa*)
- Persistence (persistence of *P. aeruginosa* detected at Visit 4 or at Visit 1 if Visit 4 is missing
- Superinfection (appearance of a pathogen other than *P. aeruginosa* not detected at Visit 4 or at Visit 1, and
- Re-infection (appearance of *P. aeruginosa* detected at Visit 1 and eradicated at Visit 4

Outcomes were analyzed according to the following hierarchy: Superinfection supercedes eradication; Persistence for *P. aeruginiosa* supersedes Superinfection; and Re-infection for *P. aeruginosa* supersedes Superinfection. Overall outcomes could be designated as either a "positive outcome" (Eradication) or as a "Negative outcome" if the microbiological outcome was Persistence, Superinfection or Re-infection.

Efficacy Analysis

Categorization by Colony Morphology

The Applicant provided information on the tobramycin MIC as it related to different morphotypes of *P. aeruginosa* isolated from the sputum of patients in each treatment group. Three morphotypes were recognized: mucoid, dry and small colony variant. These morphotypes were equally distributed in the two study groups. Some patients had more then one morphotype at a given visit and some patients had all three morphotypes at a given visit. No attempt was made to correlated morphotype with disease severity. When the tobramycin susceptibility of different morphotypes was determined the morphotype with the highest tobramycin MIC was used for analysis.

The Applicant looked at the tobramycin MICs of the various morphotypes at Visits 1, 4, and 5. A summary of these results is shown in Table 55. There is some suggestion that the mucoid morphotype was more susceptible to tobramycin then either the dry or small colony variant which has been suggested by other investigators. However, the numbers of isolates and the variability in the MICs seen in these studies makes it impossible to come to any definitive conclusion.

Table 55: Tobramycin MIC (µg/mL) Summary by Morphotype and Overall; Visit 1, ITT Population

	CHF 1538 (N=158)				TOBI (N=163)			
	N (%)	Range	MIC_{50}	MIC_{90}	N (%)	Range	MIC_{50}	MIC_{90}
Visit 1								
Morphotype 1: mucoid	123 (77.8%)	≤ 0.12- > 512	0.5	2	132 (81.0%)	≤ 0.12-16	0.5	2
Morphotype 2: dry	105 (66.5%)	≤ 0.12- > 512	1	8	95 (58.3%)	≤ 0.12-64	0.5	4
Morphotype 3: small colony variant	17 (10.8%)	0.25- > 512	2	32	30 (18.4%)	≤ 0.12- > 512	2	8
Overall	158 (100%)	≤ 0.12- > 512	1	8	162 (99.4%)	≤ 0.12- > 512	0.5	4
Visit 4								
Morphotype 1: mucoid	113 (71.5%)	≤ 0.12- > 512	0.5	8	115 (70.6%)	≤ 0.12- > 512	0.5	4
Morphotype 2: dry	68 (43.0%)	≤ 0.12- > 512	1	64	72 (44.2%)	≤ 0.12- > 512	1	64
Morphotype 3: small colony variant	12 (7.6%)	0.5-256	1	64	13 (8.0%)	0.25-128	1	32
Overall	126 (79.7%)	≤ 0.12- > 512	1	32	134 (82.2%)	≤ 0.12- > 512	0.5	32

Table 55: Tobramycin MIC (µg/mL) Summary by Morphotype and Overall; Visit 1, ITT Population (Continued)

	CHF 1538 (N=158)				TOBI (N=163)			
	N (%)	Range	MIC_{50}	MIC_{90}	N (%)	Range	MIC_{50}	MIC_{90}
Visit 5								
Morphotype 1: mucoid	110 (69.6%)	≤ 0.12-512	0.5	4	108 (66.3%)	≤ 0.12- > 512	0.5	4
Morphotype 2: dry	78 (49.4%)	≤ 0.12-512	0.5	16	71 (43.6%)	≤ 0.12- > 512	0.5	16
Morphotype 3: small colony variant	24 (15.2%)	≤ 0.12-512	2	128	21 (12.9%)	0.25-128	4	64
Overall	129 (81.6%)	≤ 0.12-512	1	32	128 (78.5%)	≤ 0.12- > 512	1	32

Source data: Module 5.3.5.1, CT03 Appendices 16.2.6.2 and 16.2.6.3
% of patients is based on the total number of patients in each treatment group in the ITT population.
One patient can have more than one *P. aeruginosa* morphotype at each visit.
If a patient has more than one *P. aeruginosa* morphotype at a given visit, then the highest tobramycin MIC value was used, regardless of *P. aeruginosa* morphotype.
If a patient has more than one available result for each morphotype then the highest tobramycin MIC value was used. If the tobramycin MIC values are equal then the MIC value for the isolate with the highest bacterial load value was used.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

MIC Distributions

The distributions and percent range of tobramycin MIC results for *P. aeruginosa* clinical trial isolates (all morphotypes) at Visits 1, 4. and 5 are shown in Table 51. The range of MIC values observed for the CHF 1538 and TOBI study arms show the variability of MIC results independent of tobramycin drug exposure.

At Baseline (Visit 1) the distribution of *P. aeruginosa* with MICs ≤4 mcg/mL in the CHF1538 and TOBI arms was 85.4% and 85% respectively. These percentages decreased slightly in each treatment group at Visit 4 (end of "on" cycle) as the susceptibility populations for CHF 1538 5.3% while TOBI susceptible [populations declined 7.9%. Additional change in the percentage of susceptible *P. aeruginosa* isolates was observed at the end of the first "OFF" cycle (Visit 5) compared to baseline (Visit 1) in both treatment arms; the change in percentage of susceptible isolates for CHF 1538 and TOBI was 7.9% and 9.6% respectively. These results suggest that, throughout the study, the majority of *P. aeruginosa* isolates remained susceptible to tobramycin in both treatment arms and that both tobramycin formulations produced equivalent microbiological treatment effects in the bacterial populations of *P. aeruginosa*.

Table 51: Distribution of Tobramycin MIC values (µg/mL) Overall; Summary by Visit, ITT Population

MIC (µg/mL)	CHF 1538 (n/N (%))			
	Visit 1	Visit 4	Visit 5	% Range
≤0.12	6/158 (3.8%)	8/126 (6.3%)	8/129 (6.2%)	3.8-6.3
0.25	20/158 (12.7%)	13/126 (10.3%)	17/129 (13.2%)	10.3-13.2
0.5	46/158 (29.1%)	29/126 (23.0%)	31/129 (24.0%)	23.0-29.1
1	34/158 (21.5%)	26/126 (20.6%)	25/129 (19.4%)	19.4-21.5
2	22/158 (13.9%)	18/126 (14.3%)	11/129 (8.5%)	8.5-14.3
4	7/158 (4.4%)	7/126 (5.6%)	8/129 (6.2%)	4.4-6.2
8	10/158 (6.3%)	9/126 (7.1%)	5/129 (3.9%)	3.9-7.1
16	1/158 (0.6%)	2/126 (1.6%)	9/129 (7.0%)	0.6-7.0
32	4/158 (2.5%)	2/126 (1.6%)	5/129 (3.9%)	1.6-3.9
64	3/158 (1.9%)	4/126 (3.2%)	2/129 (1.6%)	1.6-3.2
128	2/158 (1.3%)	3/126 (2.4%)	2/129 (1.6%)	1.3-2.4
256	0/158 (0%)	2/126 (1.6%)	1/129 (0.8%)	0-1.6
512	0/158 (0%)	0/126 (0%)	5/129 (3.9%)	0-5.0
>512	3/158 (1.9%)	3/126 (2.4%)	0/129 (0%)	0-1.9
Missing	0	32	29	

Table 51: Distribution of Tobramycin MIC values (µg/mL) Overall; Summary by Visit, ITT Population (Continued)

MIC (µg/mL)	TOBI (n/N (%))			
	Visit 1	Visit 4	Visit 5	% Range
≤ 0.12	11/162 (6.8%)	9/134 (6.7%)	8/128 (6.3%)	6.3-6.8
0.25	26/162 (16.0%)	15/134 (11.2%)	16/128 (12.5%)	11.2-16.0
0.5	51/162 (31.5%)	46/134 (34.3%)	39/128 (30.5%)	30.5-34.3
1	32/162 (19.8%)	19/134 (14.2%)	24/128 (18.8%)	14.2-19.8
2	16/162 (9.9%)	13/134 (9.7%)	8/128 (6.3%)	6.3-9.9
4	10/162 (6.2%)	4/134 (3.0%)	12/128 (9.4%)	3.0-9.4
8	8/162 (4.9%)	6/134 (4.5%)	4/128 (3.1%)	3.1-4.9
16	4/162 (2.5%)	5/134 (3.7%)	3/128 (2.3%)	2.3-3.7
32	2/162 (1.2%)	5/134 (3.7%)	6/128 (4.7%)	1.2-4.7
64	1/162 (0.6%)	2/134 (1.5%)	1/128 (0.8%)	0.6-1.5
128	0/162 (0%)	4/134 (3.0%)	2/128 (1.6%)	0-3.0
256	0/162 (0%)	1/134 (0.7%)	0/128 (0%)	0-0.7
512	0/162 (0%)	1/134 (0.7%)	2/128 (1.6%)	0-1.6
>512	1/162 (0.6%)	4/134 (3.0%)	3/128 (2.3%)	0.6-3.0
Missing	1	29	35	

If a patient has more than one *P. aeruginosa* morphotype at a given visit, then the highest tobramycin MIC value was used, regardless of *P. aeruginosa* morphotype.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.
Source data: Module 5.3.5.1, CT03Appendix 16.2.6.2 and 16.2.6.3

The activity of seven antibiotics along with tobramycin against the *P. aeruginosa* isolates obtained from patients in both study groups is shown in Table 56. In Table 56 it can be seen that the MICs to the various antibiotics were similar in both the CHF 1538 and TOBI treatment arms.

Table 56: MIC (µg/mL) Summary for Tobramycin, Amikacin, Aztreonam, Ciprofloxacin, Colistin, Imipenem, Levofloxacin, and Piperacillin/tazobactam; Overall at Visit 1, ITT Population

Antibiotic	CHF 1538 (N=158)			TOBI (N=163)		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Tobramycin	≤0.12- > 512	1	8	≤0.12- > 512	0.5	4
Amikacin	≤0.25- > 512	8	64	≤0.25-512	4	32
Aztreonam	≤0.25- > 512	4	128	≤0.25- > 512	4	128
Ciprofloxacin	≤0.12- > 256	1	4	≤0.12- 64	0.5	4
Colistin	0.25- > 128	1	4	≤0.12- > 128	1	4
Imipenem	≤0.25- > 256	1	32	≤0.25-256	1	16
Levofloxacin	≤0.25- > 512	2	16	≤0.25-128	1	8
Piperacillin/tazobactam	≤0.25- > 512	4	512	≤0.25- > 512	4	128

Source data: Module 5.3.5.1, CT03 Study Report Body, Figure 15, Figure 34, Figure 35, Figure 5, Figure 36, Figure 37, Figure 38, Figure 39, and Figure 40
If a patient has more than one *P. aeruginosa* morphotype at a given visit, then the highest MIC value was used, regardless of *P. aeruginosa* morphotype.
Values for piperacillin/tazobactam are reported as the piperacillin value; all concentrations of piperacillin are in combination with 4 µg/mL tazobactam
Patient 707002 is missing at Visit 1 because the sputum was received partially frozen and consequently excluded from the analysis.

Distribution of Strains based Upon Tobramycin Susceptibility

Table 57 shows the distribution of tobramycin MIC values for *P. aeruginosa* for both the CHF 1538 and TOBI treatment arms using the tobramycin susceptibility interpretive criteria for parenteral tobramycin. The susceptibility profiles at the various days in both the CHF 1538 and TOBI study groups are very similar overall. A substantial proportion of isolates in both the CHF 1538 and TOBI treatment groups remained susceptible at the end of the "ON" drug period (Visit 4) with 80.2% and 79.1% susceptible respectively. At the end of Visit 4, there was an increase in the percentage of resistant isolates in both treatment groups with the greatest increase seen in the TOBI treatment group. At visit 5 (end of the "OFF" drug cycle), there was a slight decrease in the percent resistant isolates for both treatment groups relative to visit 4. The percentage of susceptible isolates was similar in both treatment groups at Visit 4 and 5. Overall, the results demonstrate an elevation in MIC value for a small portion of the isolates in both treatment groups during the course of the study.

Table 57: Tobramycin MIC Values (µg/mL) by Susceptibility Class; Summary by Visit, ITT Population

Tobramycin Susceptibility Category at Visit	CHF 1538 (N=158)	TOBI (N=163)
Visit 1		
≤4	135 (85.4%)	146 (90.1%)
8	10 (6.3%)	8 (4.9%)
≥16	13 (8.2%)	8 (4.9%)
Missing	0	1
Visit 4		
≤4	101 (80.2%)	106 (79.1%)
8	9 (7.1%)	6 (4.5%)
≥16	16 (12.7%)	22 (16.4%)
Missing	32	29
Visit 5		
≤4	100 (77.5%)	107 (83.6%)
8	5 (3.9%)	4 (3.1%)
≥16	24 (8.6%)	17 (13.3%)
Missing	29	35

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3

"Missing" includes cases where *P. aeruginosa* has been eradicated and therefore no MIC was available and instances where no specimen was collected or it was not analyzable.
If a patient has more than one *P. aeruginosa* morphotype at a given visit, then the highest tobramycin MIC value was used, regardless of *P. aeruginosa* morphotype.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.
Tobramycin systemic interpretive criteria (Susceptible, ≤ 4 µg/mL; Intermediate, 8 µg/mL; Resistant, ≥16 µg/mL)

Mean Changes from Baseline Viable Counts

The *P. aeruginosa* bacterial density in $\log_{10}$ CFU/gram of sputum for each treatment group by study is shown in Table 58. This is a summary of the individual values for each patient. Similar bacterial load values were observed for the two treatment groups at each study visit suggesting these are typical population densities per gram of sputum colonizing the lungs of CF patients. The mean change in bacterial density from baseline levels is presented for the end of the "ON" cycle (Visit 4) and the end of the "OFF" cycle (Visit 5) in Table 59. The bacterial load showed a mean reduction of 2.14 and 2.07 $\log_{10}$ CFU/gram was observed at the end of the "ON" cycle for CHF 1538 and TOBI respectively. The bacterial load at the end of the "OFF" cycle was 0.72 and 0.87 $\log_{10}$ CFU/gram for CHF 1538 and TOBI respectively, indicating an increase in bacterial load relative to the end of the "ON" cycle. The ANCOVA model analysis results are shown in Table 60. No significant difference was evident with respect to the treatment or country while a significant difference was observed ($p<0.001$) for change from baseline $\log_{10}$ bacterial load (CFU/gram)

Table 58: *P. aeruginosa* $\log_{10}$ Bacterial Load (CFUs/gram) Summary by Visit: ITT Population

	CHF 1538 (N=158)	TOBI (N=163)
Visit 1		
N	158	162
Mean (SD)	6.56 (1.70)	6.64 (1.57)
95% CI	[6.30; 6.83]	[6.40; 6.89]
Median	6.90	7.00
Min / Max	1.30 / 8.78	1.30 / 8.78
Missing	0	1
Visit 4		
N	152	157
Mean (SD)	4.41 (2.22)	4.58 (2.25)
95% CI	[4.06; 4.77]	[4.23; 4.93]
Median	4.62	4.90
Min / Max	1.30 / 8.75	1.30 / 8.60
Missing	6	6
Visit 5		
N	147	147
Mean (SD)	5.78 (2.20)	5.81 (2.37)
95% CI	[5.42; 6.14]	[5.42; 6.20]
Median	6.58	6.64
Min / Max	1.30 / 8.78	1.30 / 8.78
Missing	11	16

Source data: Module 5.3.5.1, CT03 Appendices 16.2.6.2 and 16.2.6.3
CFU colony forming units
If a patient has more than one *P. aeruginosa* morphotype at a given visit, and therefore more than one bacterial load value, then the bacterial load value corresponding to the highest tobramycin MIC value regardless of the *P. aeruginosa* morphotype was used.
If the tobramycin MIC value was the same for different *P. aeruginosa* morphotypes, then the bacterial load value corresponding to morphotype 1 (mucoid colony) was used. If morphotype 1 was not available, bacterial load value corresponding to morphotype 2 (dry colony) was used.
In instances of *P. aeruginosa* eradication (i.e. < 20 CFU/g, based upon the dilution factor in the plating and counting procedure), the numeric value of 20 CFU/g was used for bacterial load.
Where the bacterial load was recorded as '>600000000 CFU/g', the numeric value used for calculation of bacterial load was 600000000.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

Table 59: *P. aeruginosa* $\log_{10}$ Bacterial Load (CFUs/gram) Change from Baseline (Visit 1): ITT Population

	CHF 1538 (N=158)	TOBI (N=163)
Visit 4		
N	152	156
Mean (SD)	-2.14 (2.41)	-2.07 (2.20)
95% CI	[-2.52; -1.75]	[-2.42; -1.72]
Median	-2.09	-1.79
Min / Max	-7.48 / 4.00	-7.48 / 1.72
Missing	6	7
Visit 5		
N	147	147
Mean (SD)	-0.72 (2.17)	-0.87 (2.23)
95% CI	[-1.07; -0.36]	[-1.24; -0.51]
Median	-0.40	-0.48
Min / Max	-6.54 / 4.90	-7.48 / 6.08
Missing	11	16

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
CFU colony forming units
If a patient has more than one *P. aeruginosa* morphotype at a given visit, and therefore more than one bacterial load value, then the bacterial load value corresponding to the highest tobramycin MIC value regardless of the *P. aeruginosa* morphotype was used.
If the tobramycin MIC value was the same for different *P. aeruginosa* morphotypes, then the bacterial load value corresponding to morphotype 1 (mucoid colony) was used. If morphotype 1 was not available, bacterial load value corresponding to morphotype 2 (dry colony) was used.
In instances of *P. aeruginosa* eradication (i.e. <20 CFU/g, based upon the dilution factor in the plating and counting procedure), the numeric value of 20 CFU/g was used for bacterial load.
Where the bacterial load was recorded as '>600000000 CFU/g', the numeric value used for calculation of bacterial load was 600000000.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

Table 60: *P. aeruginosa* log_{10} Bacterial Load (CFUs/gram) ANCOVA: ITT Population

	CHF 1538 (N=158)		TOBI (N=163)
ANCOVA			
N (missing)	152 (6)		156 (7)
LSMEANS(SEM)	-1.81 (0.21)		-1.85 (0.20)
Fixed effects/Covariate: p-value			
Treatment		0.820	
Country		0.310	
Baseline log_{10} bacterial load (CFU/g) value		< 0.001	
CHF 1538 minus TOBI			
LSMEANS(SEM)		0.04 (0.18)	
95% CI		[-0.31; 0.39]	

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
ANCOVA model: Change from baseline (V1) to V4 in log_{10} bacterial load (CFU/g) value = treatment and country as fixed effects and baseline log_{10} bacterial load (CFU/g) value as covariate
All p-values are two-sided

CT03 Microbiology Outcome

Table 61 provides information on the overall analysis of microbiology outcomes for both study groups. As can be seen in study Visit 4 (end of the "ON" treatment period, there was a low percentage of microbiological eradication in both treatment groups (9.2% for CFH 1538-treated and 7.1% for TOBI-treated). This type of result is not uncommon to see in cystic fibrosis studies for both inhaled, systemically administered and orally administered antibacterials. Similar rates of persistence and superinfection were noted in the two treatment groups. At Visit 5 (end of the "OFF" treatment period), the rates of eradication were lower than at Visit 4 in both treatment groups. While the percentage of re-infection in the CH 1538 treatment group was higher then seen in the TOBI-treatment group the numbers were small. The Cochran-Mantel-Haenzel test controlling for country revealed no statistically significant differences between the CH 1538 and TOBI with respect to percentages in outcome categories. From the data in Table 61 it appears from a microbiology perspective that CH 1538 produces equivalent results to the approved TOBI product.

Table 61: Microbiological Outcomes (Eradication, Persistence, Superinfection, Re-infection); Summary by Visit, ITT Population

	CHF 1538 (N=158)	TOBI (N=163)	CMH Test[1]
Visit 1			
Presence of *P. aeruginosa*	158 (100%)	162 (100%)	
Absence of *P. aeruginosa*	0	0	
Missing	0	1	
Visit 4[2]			
Eradication	14 (9.2%)	11 (7.1%)	p=0.692
Persistence	126 (82.9%)	133 (85.3%)	
Superinfection	12 (7.9%)	12 (7.7%)	
Missing	6	7	
Visit 5[3]			
Eradication	4 (2.7%)	5 (3.4%)	p=0.128
Persistence	116 (78.9%)	122 (83.0%)	
Superinfection	14 (9.5%)	14 (9.5%)	
Re-infection	13 (8.8%)	6 (4.1%)	
Missing	11	16	

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
[1] Cochran-Mantel-Haenszel test controlling for country
Microbiological outcomes are derived considering all *P. aeruginosa* morphotypes together.
[2] At Visit 4:
Eradication=elimination of *P. aeruginosa*
Persistence=persistence of *P. aeruginosa* detected at V1
Superinfection=appearance of a pathogen (other than *P. aeruginosa*) not detected at V1
[3] At Visit 5:
Eradication=elimination of *P. aeruginosa*
Persistence=persistence of *P. aeruginosa* detected at V4 (or at V1 if V4 missing)
Superinfection=appearance of a pathogen (other than *P. aeruginosa*) not detected at V4 or at V1
Re-infection=re-appearance of *P. aeruginosa* detected at V1 and eradicated at V4
Superinfection supercedes eradication. Persistence for *P. aeruginosa* supercedes superinfection.
Re-infection for *P. aeruginosa* supercedes superinfection.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

The analysis of microbiological outcomes is analyzed further at all study visits by baseline MIC values in Table 62. This data is further differentiated into baseline tobramycin susceptibility categories (Susceptible, Intermediate, or Resistant) in Table 63, and summarized in an overall positive or negative outcome in Table 64.

As seen in Table 62 at Visit 4, in both treatment groups, persistence occurred across a broad range of MIC values with no apparent correlation with MIC value. This type of results has been observed in other cystic fibrosis antibacterial treatment studies. At visit 5, a pattern similar to that seen in Visit 4 was observed with regard to MIC versus eradication, persistence, and superinfection with the exception of eradication which was

observed less frequently. Re-infections at Visit 5 were distributed across a wide range of MIC values in both treatment groups. There is an increase in the incidence of reinfection for both treatment arms in Visit 5 compared to Visit 4. This increase, the Applicant hypotheses is likely do to emergence of *P. aeruginosa* in patients previously categorized as eradication.

The microbiological outcome data shows that there were no uncommon instances of microbiological eradication in either the CHF 1538 or TOBI-treatment groups at either visit 4 or 5. A high percentage of persistors were observed in the treatment groups at Visit 4 and 5, with a modest incidence of superinfection and reinfection (Visit 5). The persistence of P. aeruginosa during and after antibacterial treatment is not unexpected and the numbers seen in these studies are consistent with other data.

Table 62: Microbiological Outcomes (Eradication, Persistence, Superinfection, Re-infection) by Baseline MIC Value; Summary by Visit, ITT Population

	CHF 1538 (N=158)				TOBI (N=163)			
	ERAD[1]	PERS	SUPER	REINF	ERAD	PERS	SUPER	REINF
Visit 4[2]								
≤0.12	1/6 (16.7%)	5/6 (83.3%)	0	.	3/11 (27.3%)	6/11 (54.5%)	2/1 (18.2%)	.
0.25	2/19 (10.5%)	15/19 (78.9%)	2/19 (10.5%)	.	2/24 (8.3%)	19/24 (79.2%)	3/24 (12.5%)	.
0.5	6/45 (13.3%)	34/45 (75.6%)	5/45 (11.1%)	.	3/49 (6.1%)	44/49 (89.8%)	2/49 (4.1%)	.
1	2/33 (6.1%)	29/33 (87.9%)	2/33 (6.1%)	.	1/32 (3.1%)	28/32 (87.5%)	3/32 (9.4%)	.
2	2/20 (10.0%)	16/20 (80.0%)	2 /20 (10.0%)	.	0	15/15 (100.0%)	0	.
4	1/7 (14.3%)	6/7 (85.7%)	0	.	0	10/10 (100.0%)	0	.
8	0	9/10 (90.0%)	1/10 (10.0%)	.	1/7 (14.3%)	4/7 (57.1%)	2/7 (28.6%)	.
16	0	1/1 (100.0%)	0	.	0	4/4 (100.0%)	0	.
32	0	4/4 (100.0%)	0	.	1/2 (50.0%)	1/2 (50.0%)	0	.
64	0	3/3 (100.0%)	0	.	0	1/1 (100.0%)	0	.
128	0	2/2 (100.0%)	0	.	0	0	0	.
>512	0	2/2 (100.0%)	0	.	0	1/1 (100.0%)	0	.
Total	14/152 (9.2%)	126/152 (82.9%)	12/152 (7.9%)	.	11/156 (7.1%)	133/156 (85.3%)	12/156 (7.7%)	.

The microbiological outcome data analyzed above was assessed according to systemic tobramycin interpretive criteria as seen in Table 63. The general conclusions from this analysis are similar to those stated for outcome versus MIC value analysis (Table 62). For both treatment groups, persistence is reported for isolates that were susceptible, intermediate or resistance to tobramycin. For both Visits 4 and 5, and in both treatment groups, more than 76% of the *P. aeruginosa* isolates were susceptible to tobramycin and yet were persistent. This suggests a lack of correlation between MICs and microbiological outcome a result that has been seen in other studies.

Table 62: Microbiological Outcomes (Eradication, Persistence, Superinfection, Re-infection) by Baseline MIC Value; Summary by Visit, ITT Population (Continued)

	CHF 1538 (N=158)				TOBI (N=163)			
	ERAD	PERS	SUPER	REINF	ERAD	PERS	SUPER	REINF
Visit 5[3]								
≤0.12	0	5/6 (83.3%)	0	1/6 (16.7%)	1/10 (10.0%)	5/10 (50.0%)	4/10 (40.0%)	0
0.25	1/19 (5.3%)	13/19 (68.4%)	2/19 (10.5%)	3/19 (15.8%)	1/23 (4.3%)	18/23 (78.3%)	3/23 (13.0%)	1/23 (4.3%)
0.5	1/39 (2.6%)	29/39 (74.4%)	6/39 (15.4%)	3/39 (7.7%)	0	39/46 (84.8%)	4/46 (8.7%)	3/46 (6.5%)
1	2/34 (5.9%)	26/34 (76.5%)	4/34 (11.8%)	2/34 (5.9%)	3/27 (11.1%)	23/27 (85.2%)	0	1/27 (3.7%)
2	0	17/21 (81.0%)	2/21 (9.5%)	2/21 (9.5%)	0	16/16 (100.0%)	0	0
4	0	5/6 (83.3%)	0	1/6 (16.7%)	0	9/9 (100.0%)	0	0
8	0	9/10 (90.0%)	0	1/10 (10.0%)	0	5/8 (62.5%)	2/8 (25.0%)	1/8 (12.5%)
16	0	1/1 (100.0%)	0	0	0	4/4 (100.0%)	0	0
32	0	4/4 (100.0%)	0	0	0	1/2 (50.0%)	1/2 (50.0%)	0
64	0	3/3 (100.0%)	0	0	0	1/1 (100.0%)	0	0
128	0	2/2 (100.0%)	0	0	0	0	0	0
>512	0	2/2 (100.0%)	0	0	0	1/1 (100.0%)	0	0
Total	4/147 (2.7%)	116/147 (78.9%)	14/147 (9.5%)	13/147 (8.8%)	5/147 (3.4%)	122/147 (83.0%)	14/147 (9.5%)	6/147 (4.1%)

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3

Table populated for patients with an available MIC value at V1. Microbiological outcomes derived considering all *P. aeruginosa* morphotypes together.

[1] Microbiological outcomes are derived considering all *P. aeruginosa* morphotypes together.

[2] At V4: ERAD=elimination of *P. aeruginosa*, PERS=persistence of *P. aeruginosa* detected at V1, SUPER=appearance of a pathogen (other than *P. aeruginosa*) not detected at V1, REINF=reinfection was not an option at Visit 4

[3] At V5: ERAD=elimination of *P. aeruginosa*, PERS=persistence of *P. aeruginosa* detected at V4 (or at V1 if V4 missing), SUPER=appearance of a pathogen (other than *P. aeruginosa*) not detected at V4 or at V1; REINF=re-appearance of *P. aeruginosa* detected at V1 and eradicated at V4. Superinfection supercedes eradication. Persistence for *P. aeruginosa* supercedes superinfection. Re-infection for *P. aeruginosa* supercedes superinfection.

Table 63: Microbiological Outcomes (Eradication, Persistence, Superinfection, Re-infection) by Baseline Tobramycin Susceptibility Designation (Susceptible, Intermediate, or Resistant); Summary by Visit, ITT Population

	CHF 1538 (N=158)			TOBI (N=163)		
	S[1]	I	R	S	I	R
Visit 4[2]						
Eradication	14 (10.8%)	0	0	9 (6.4%)	1 (14.3%)	1 (12.5%)
Persistence	105 (80.8%)	9 (90.0%)	12 (100%)	122 (86.5%)	4 (57.1%)	7 (87.5%)
Superinfection	11 (8.5%)	1 (10.0%)	0	10 (7.1%)	2 (28.6%)	0
Missing	5	0	1	5	1	0
Visit 5[3]						
Eradication	4 (3.2%)	0	0	5 (3.8%)	0	0
Persistence	95 (76.0%)	9 (90.0%)	12 (100%)	110 (84.0%)	5 (62.5%)	7 (87.5%)
Superinfection	14 (11.2%)	0	0	11 (8.4%)	2 (25.0%)	1 (12.5%)
Re-infection	12 (9.6%)	1 (10.0%)	0	5 (3.8%)	1 (12.5%)	0
Missing	10	0	1	15	0	0

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2and 16.2.6.3

[1] Baseline (V1) susceptibility: S: Susceptible (MIC ≤4 µg/mL), I: Intermediate (MIC=8 µg/mL), R: Resistant (MIC ≥ 16 µg/mL)

Table populated for patients with an available susceptibility at V1

Microbiological outcomes are derived considering all *P. aeruginosa* morphotypes together.

[2] At Visit 4: Eradication = elimination of *P. aeruginosa*
Persistence = persistence of *P. aeruginosa* detected at V1
Superinfection = appearance of a pathogen (other than *P. aeruginosa*) not detected at V1

[3] At Visit 5: Eradication = elimination of *P. aeruginosa*
Persistence = persistence of *P. aeruginosa* detected at V4 (or at V1 if V4 missing)
Superinfection = appearance of a pathogen (other than *P. aeruginosa*) not detected at V4 or at V1
Re-infection = re-appearance of *P. aeruginosa* detected at V1 and eradicated at V4

Superinfection supercedes eradication. Persistence for *P. aeruginosa* supercedes superinfection. Re-infection for *P. aeruginosa* supercedes superinfection.

Table 64 provides a summary of microbiological outcomes by visit for the ITT population. The positive and negative outcomes were very similar in the two treatment groups.

Table 64: Microbiological Outcomes (Positive vs Negative Outcomes)-Summary by Visit: ITT Population

	CHF 1538 (N=158)	TOBI (N=163)	CMH Test[1]
Visit 4			
Positive outcome[2]	14 (9.2%)	11 (7.1%)	p=0.465
Negative outcome[3]	138 (90.8%)	145 (92.9%)	
Missing	6	7	
Visit 5			
Positive outcome	4 (2.7%)	5 (3.4%)	p=0.775
Negative outcome	143 (97.3%)	142 (96.6%)	
Missing	11	16	

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
[1] Cochran-Mantel-Haenszel test controlling for country
Microbiological outcomes are derived considering all *P. aeruginosa* morphotypes together.
[2] Positive outcome = eradication
[3] Negative outcome = persistence, superinfection or re-infection
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

Treatment Emergent Microorganisms

Table 65 presents information on the bacteria and yeast isolated from patients with superinfection at Visits 4 and 5. The bacteria and yeast not seen on the first visit are detailed in the table. The bacteria and yeast are similar in both treatment groups. While classified as superinfection none of the patients required treatment for exacerbation of symptoms.

Table 65: Microorganisms Isolated from Patients with Superinfection at Visits 4 or 5; Summary by Treatment Group, ITT Population

	CHF 1538 (N=158) [n (%)]	TOBI (N=163) [n (%)]
Gram-Positive Bacteria		
Staphylococcus aureus	17 (10.8%)	18 (11.0%)
Staphylococcus aureus, methicillin-resistant	2 (1.3%)	1 (0.6%)
β-hemolytic Streptococcus spp. (Group A)	1 (0.6%)	1 (0.6%)
β-hemolytic Streptococcus spp. (Group B)	0	1 (0.6%)
β-hemolytic Streptococcus spp. (Group C)	0	2 (1.2%)
β-hemolytic Streptococcus spp. (Group G)	1 (0.6%)	2 (1.2%)
β-hemolytic Streptococcus spp. (Group F)	2 (1.3%)	2 (1.2%)
Streptococcus pneumoniae	2 (1.3%)	3 (1.8%)
Gram-Negative Bacteria		
Achromobacterium xylosoxidans	2 (1.3%)	7 (4.3%)
Alcaligenes faecalis	2 (1.3%)	0
Burkholderia cepacia	0	1 (0.6%)
Enterobacter cloacae	2 (1.3%)	0
Escherichia coli	2 (1.3%)	1 (0.6%)
Haemophilus influenzae	3 (1.9%)	1 (0.6%)
Haemophilus parainfluenzae	10 (6.3%)	9 (5.5%)
Klebsiella oxytoca	1 (0.6%)	0
Serratia marcescens	1 (0.6%)	1 (0.6%)
Sphingobacterium spiritivorum	0	1 (0.6%)
Stenotrophomonas maltophilia	5 (3.2%)	2 (1.2%)

Table 65: Microorganisms Isolated from Patients with Superinfection at Visits 4 or 5; Summary by Treatment Group, ITT Population (Continued)

	CHF 1538 (N=158) [n (%)]	TOBI (N=163) [n (%)]
Yeasts		
Candida albicans	1 (0.6%)	0
Candida species NOT Candida albicans	1 (0.6%)	0

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
N : total number of patients in each treatment group
n: number of patients with microorganisms other than *P. aeruginosa* at Visit 4 or Visit 5
(%): percentage of patients with microorganisms other than *P. aeruginosa* at Visit 4 or Visit 5
A patient can have more than one microorganism
If a patient has the same microorganism at both Visit 4 and Visit 5 then the patient is counted only once

MIC Changes During Therapy

Table 66 provides information on the changes in tobramycin MICs in both treatment groups for visits 4 ("ON" tobramycin) and 5 ("OFF") tobramycin. Overall the tendency for MIC values to increase during conduct of the study was similar in the treatment groups.

Table 66: Tobramycin MIC Value (µg/mL) Shift from Baseline (Visit 1) Values: ITT Population

	CHF 1538 (N=158)	TOBI (N=163)
Visit 4		
Decreased[1]	19 (15.1%)	19 (14.3%)
Unchanged[2]	79 (62.7%)	78 (58.6%)
Increased[3]	28 (22.2%)	36 (27.1%)
Missing[4]	32	30
Visit 5		
Decreased	23 (17.8%)	19 (14.8%)
Unchanged	80 (62.0%)	86 (67.2%)
Increased	26 (20.2%)	23 (18.0%)
Missing	29	35

Source data: Module 5.3.5.1, CT03 Appendix 16.2.6.2 and 16.2.6.3
[1] MIC decreased: Patients whose *P. aeruginosa* isolate exhibited ≥ 4-fold decrease in the MIC between baseline and end-of-therapy or follow-up visits.
[2] MIC unchanged: Patients whose *P. aeruginosa* isolate exhibited no change or a 2-fold increase or decrease in the MIC between baseline and end-of-therapy or follow-up visits.
[3] MIC increased: Patients whose *P. aeruginosa* isolate exhibited ≥ 4-fold increase in the MIC between baseline and end-of-therapy or follow-up visits.
[4] Missing indicates that for a given patient there was no *P. aeruginosa* isolated at one of the study visits
If a patient has more than one *P. aeruginosa* morphotype at a given visit, then the highest tobramycin MIC value was used, regardless of *P. aeruginosa* morphotype.
When MIC value was recorded as '≤ 0.12 µg/mL', the numeric value used for calculation of shift from baseline was 0.125 µg/mL.
When MIC value was recorded as '> 512 µg/mL', the numeric value used for calculation of shift from baseline was 1024 µg/mL.
Patient 707002 is missing at V1 because the sputum was received partially frozen and consequently excluded from the analysis.

OVERALL CONCLUSION

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