CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

204384Orig1s000

MICROBIOLOGY REVIEW(S)

Date Company Submitted: 06/28/2012
Date Received by CDER: 06/29/2012
Date Assigned: 07/02/2012
Reviewer: Lynette Y. Berkeley

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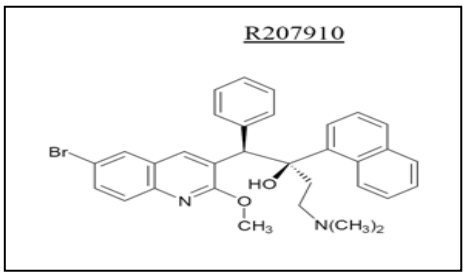
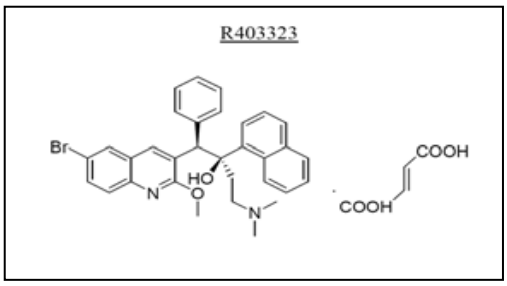
DRUG PRODUCT NAME

Proprietary: Sirturo
Established: Bedaquiline. BEDAQUILINE, R207910
Chemical name: R207910 (Free base form) :(1R,2S)-6-bromo-alpha-[2-(dimethylamino)-ethyl]-2-methoxy-alpha-1 naphthalenyl -beta-phenyl-3-quinolineethanol.
R403323 (fumarate salt) :(1R,2S)-6-bromo-alpha [2-(dimethylamino)ethyl]-2-methoxy-alpha-(1 naphthyl)-beta-phenyl-3-quinolineethanol (2E)-2-butenedioate(1:1) (salt)
(cyclohexylcarbonyl) 1,2,3,6,7,11b-hexahydro-4H-pyrazino [2, 1-a] isoquinolin-4-one

Chemical Structure





Molecular Formula: $C_{32}H_{31}BrN_2O_2$
Molecular weight: 555.50 Da.

Molecular Formula: $C_{32}H_{31}BrN_2.O_2C_4H_4O_4$
Molecular weight: 671.58 (555.50 ++116.07) Da.

PROPOSED INDICATION
Multi- Drug Resistant Pulmonary Tuberculosis

PROPOSED DOSAGE FORM, STRENGTH, ROUTE OF ADMINISTRATION AND DURATION OF TREATMENT
Dosage form: Tablets
Route of Administration: Oral
Dosage: Dosing regimen (400 mg once daily [q.d.] for the first 2 weeks and 200 mg 3 times weekly [t.i.w.] for the following 22 weeks).
Strength: 100 mg
Duration of treatment: 24 weeks

DISPENSED
Rx.

RELATED DOCUMENTS
IND #: 69600

REMARKS
The applicant has performed indepth studies to elucidate the mechanism of action. The applicant has investigated and confirmed the proposed mechanism with supporting studies. The antibiotic ATP synthase target is also present in eukaryotic cells therefore the applicant tested the effect of bedaquiline on the human mitochondria. The results showed that bedaquiline had a > 20,000-fold lower affinity for human tissue than it had on bacterial cells.

One of the major problems in the treatment of tuberculosis is poor compliance because of the long duration. Studies have shown that bedaquiline shortens the treatment period and will thereby help to alleviate one of the big drawbacks to successful treatment in more individuals. Therapy for tuberculosis is performed using combinations of drugs. The applicant tested bedaquiline with currently used regimens as well as new drugs such as PA-824 which may be included in treatment regimens in the future. No cross-resistance was found with other drugs

The mechanism of resistance was investigated and the primary mechanism of resistance was found to be mutation in the *atpE* gene. Additionally, the applicant also investigated other putative mechanisms that are likely to be responsible for an increase in the minimum inhibitory concentration.

The identification of isolates was systematic. The applicant used more than one method to isolate and identify the bacterial isolate. The tests were accompanied by appropriate quality control which provided a significant level of assurance of accuracy. The applicant utilized a variety of methods both classical and molecular that achieved a high level of identification. Identification tests also cover the types of tests that will be employed in both the industrialized and developing countries. This is very important since most MDR-TB is found in the poorer

Reference ID: 3225917

countries. The methods employed for susceptibility testing offered flexibility as well as accuracy.
Tests also showed that bedaquiline was active against strains resistant to many important anti-mycobacterium drugs such as rifampin and isoniazid.
Overall the microbiology aspects of this study appeared scientific, thorough, methodological and accurate. From the clinical microbiology perspective, this NDA submission may be approved provided the applicant makes the changes in the microbiology subsection of the proposed label recommended by the Agency and included in section 10.

EXECUTIVE SUMMARY

Bedaquiline (TMC207, R207190), is a new anti-tuberculosis drug for the treatment of multi-drug resistant tuberculosis. This section summarizes the microbiological findings from human and animal studies.

Mechanism of Action

The mechanism of action of bedaquiline, a diarylquinoline, is different from that of other anti-mycobacterium agents. The applicant has submitted a number of studies that support the mechanism of action which show that bedaquiline binds to ATP (adenosine 5'-triphosphate)-synthase 3 and inhibits the production of energy in mycobacterium cells, as a result the bacterial cells die. Bedaquiline, *in vitro*, inhibits both actively replicating and non-replicating sensitive and resistant *Mycobacterium tuberculosis.* One study showed that bedaquiline killed dormant cells found in latent tuberculosis even though there is low cellular ATP level during dormancy.

N-monodesmethylmetabolite (M2) is the main circulating metabolite of bedaquiline. This metabolite is at least 4 to 6 times less active against *M. tuberculosis* than the parent compound. Studies have shown that bedaquiline may have inhibitory activity against other species in the genus *Mycobacterium*. However, the drug appears to have a narrow spectrum of activity and appears to be specific for *Mycobacterium* species. Tests of efficacy involving other selected gram-positive and gram-negative bacteria showed a lack of inhibition even to large doses of this drug.

The safety of bedaquiline for humans was demonstrated by Haagsma et al., (2009) in ovarian cancer cells using *M. smegmatis* mc^2 155. The authors showed that bedaquiline has > 20,000-fold lower affinity for human mitochondrial ATP synthase than it has for the mycobacterium ATP synthase. The IC_{50} for mycobacterium strains was 0.01 μM and the IC_{50} for the human cancer cells was >200 μM.

Mechanism of Resistance

The results of studies suggest a potential for development of resistance and particularly at low concentrations of bedaquiline. The main resistance mechanism is due to mutations in the *atpE* gene. Recent studies have shown that major resistance is caused by substitutions in at least six

Reference ID: 3225917

different amino acids. At a concentration of 0.3 mcg/ml (10 X the MIC), the mutation rates ranged from 4.7 x10^{-7} to 8.9 x 10^{-9} mutations per cell division. At a concentration of 1.0 mcg/mL (30 X the MIC), the mutation rates ranged from 3.9 x 10^{-8} to 2.4 x 10^{-9} mutations per cell division. No resistant mutants were obtained at 3 mcg/mL (100 X the MIC). This suggests that the rate of mutation against bedaquiline decreases with the increase in the concentration of the antibiotic. One explanation for this occurrence is that at low concentrations the antibiotic tended to be more bacteriostatic allowing the bacilli to live long enough to mutate whereas higher concentrations killed the bacteria. Sixteen percent of the *M. tuberculosis H37Rv* mutants demonstrated mutations in amino acid residues alanine 63 to proline (Ala63→ Pro). The substitution of Ala63 → Pro results in the greatest effect of resistance in the mycobacterium subunit c. This mutation caused an increase in the MIC value for *M. tuberculosis* by 133-fold. It was found that there was a dose dependent decrease in ATP. A second putative mechanism of resistance to bedaquiline is the efflux pump.

Cross resistance was tested against other TB drugs (isoniazid, rifampin, streptomycin, ethambutol, pyrazinamide, PA-824, amikacin, moxifloxacin). The resistant mutant BK12 and susceptible strains of H37Rv were used in determining cross-resistance. It was found that there was no cross-resistance between bedaquiline and the anti-mycobacterium drugs listed above.

Pharmacokinetic studies in Swiss mice indicated that the AUC correlated better with a decrease in CFU than any other PK parameter. The MIC_{50} and MIC_{90} of bedaquiline against *M. tuberculosis* H37Rv infected female Swiss mice were 0.03 mcg/mL and 0.06 mcg/mL respectively. One of the objectives of Study 209 was to determine the MIC of bedaquiline against isolates resistant and susceptible to other anti-mycobacterium drugs. The results showed that there was no difference in the susceptibility to bedaquiline of isolates resistant and susceptible to other anti-mycobacterium drugs. The MIC of both groups ranged from 0.03 to 0.06 mcg/mL.

One of the innate defense mechanisms against *M. tuberculosis* in the human body is the engulfment and encasement of the tubercle bacilli by macrophages, CD4+ T lymphocytes and other cells. It is therefore important that a mycobacterium drug be tested for intracellular activity. Mouse peritoneal macrophages and J774A.1 cells were used to investigate the intracellular and extracellular activity of bedaquiline. The bactericidal effect of bedaquiline was slow extra-cellularly whereas it was rapid intra-cellularly and growth inhibition was concentration dependent (Table 1).

Table 1: Effect of Bedaquiline Extra-cellularly and Intra-cellularly

Bedaquiline (mcg/mL)	Log_{10} Colony Forming Units	
	Extracellular	J774 cells (intracellular)
0.12	-0.07	-0.13
0.25	-0.08	-0.21
0.50	-0.09	-0.28
1.00	-0.11	-0.35

Source: NDA 204384

Methods for Isolation, Identification and Susceptibility Testing
Several methods were used for the isolation, identification and susceptibility testing of *M. tuberculosis* from sputum samples. These included:

- Two methods for microscopy
- Two methods for culture
- A number of identification tests
- Three primary methods for susceptibility testing. Susceptibility test screening for MDR-TB was performed using the MTBDR plus test (Hain).

The primary methods of susceptibility testing employed in this study were the agar method using Middlebrook 7H11, the BACTEC 460 radiometric and BACTEC MGIT 960 methods and the Reasurin Microtiter Assay (REMA). Growth was obtained faster in liquid media than on solid agar medium. The sensitivity and specificity of the majority of the methods for isolation, identification and susceptibility testing ranged from 84 to 100%. Quality control was included for all testing.. The quality control bacteria were *M. tuberculosis* H37Rv a pan susceptible strain and the mutated BK12 that is resistant to bedaquiline.

The accepted treatment protocol for mycobacterium infections is the use of multiple drugs. It is therefore important to examine the interactions between the drugs for efficacy. There was indifference in MIC for *M. tuberculosis* H37Rv when bedaquiline was combined with rifampin alone. However, in a three drug combination with bedaquiline, SQ109 and rifampin at Day 1 a decrease of > 90% in metabolic activity was achieved by 4 X and 8 X bedaquiline MIC for the same bacterium. The bacterial count was 0.003% of the initial inoculum by the end of Day 2. Killing occurred in Day 1 even in the combination with 2 X MIC of bedaquiline. Overall as shown in Table 2 most combinations containing bedaquiline were significantly ($p = 0.05$) more effective than those without this drug. In this study regimens with INH and RIF that included bedaquiline resulted in lung and spleen negativity within 2 months. There was no antagonistic effect with any of the drugs. The combination of bedaquiline with moxifloxacin and isoniazid appears less effective than other combinations.

Reference ID: 3225917

Table 2: Treatment of *M. tuberculosis* from Lung Cultures of infected Swiss Mice with Anti-mycobacterium Antibiotics including Bedaquiline

Groups	Mean Colony Forming Units in the Lungs		Percentage culture-negative
	1 month	2 months	At 2 months
Untreated		Untreated	
JZ	1.6±1.6	0	100
JMZ	1.4±1.2	0.03±0.1	78
JRZ	2.3±1.5	0.07± 0.2	70
JHZ	1.7± 1.4	0.18 ± 0.5	78
JHR	4.4 ±1.1	1.2± 1.1	20
JMR	4.4 ±0.3	1.4 ± 0.8	11
RMZ	4.6 ±0.8	1.4± 0.4	20
RHZ	3.9 ±0.7	2.2± 0.6	0
J=bedaquiline, Z=parazinamide, M=moxifloxacin, R= rifampin, H = isoniazid			

Source: Table NDA 204384

Clinical Studies

The primary expected outcome of Study 208 was the determination of the time to sputum culture conversion. Sputum conversion was defined as the number of days between the initiation of treatment for MDR-TB and the first of the two (≥25 days apart) consecutive negative sputum cultures before the end of the double-blind treatment period. The secondary objective was to determine the number of CFU in the sputum samples. Thirty-four patients from 2 sites were studied to determine the CFU in the sputum. When bedaquiline was added to the five drug treatment regimen 10/21 subjects in the Stage 1 treatment period became culture negative as compared to 2/23 treated with the background regimen only. The difference was statistically significant (p=0.034). The patients in the bedaquiline treatment group also converted to negative faster than the background regimen group. In study C208, three isolates from the non-bedaquiline treatment group had greater than 4-fold increase in MIC over the baseline MIC. One subject in the bedaquiline treatment group had an increase in titer of greater than four-fold. The mean time to negativity was 8 days for both bedaquiline and placebo treated cultures. The MIC_{90} for susceptibility tests performed on 7H11 was 0.12 mcg/mL and for the REMA test the MIC_{90} was 0.125 mcg/mL.

In study C209 bedaquiline was tested against isolates that were fully susceptible to *M. tuberculosis* clinical isolates and strains that were resistant to isoniazid, rifampin, both isoniazid and rifampin, isoniazid and streptomycin, ethambutol, pyrazinamide and a fluoroquinolone. The MICs for susceptible isolates ranged from 0.03 to 0.06 mcg/mL which was the same for isolates that were resistant to isoniazid and rifampin. The MIC of isolates that were resistant to the other anti-mycobacterium agents ranged from 0.01 mcg/mL to 0.12 mcg/mL.

Interpretive Criteria and Clinical Correlation

The determination of the breakpoint for bedaquiline included the MIC results of pre-clinical isolates. The results of the MIC for bedaquiline found no preclinical isolate with an MIC that was > 0.5 mcg/mL therefore the 7H11 agar breakpoint was set at ≤ 0.5 mcg/mL. When the REMA method was used only one isolate resulted in an MIC of > 0.25 mcg/ leading to the REMA breakpoint of ≤ 0.25 mcg/mL. The breakpoints are shown in Table 3.

Table 3: Minimum Inhibitory Concentration of Bedaquiline proposed by the Applicant

Pathogen	Minimum Inhibitory Concentration (mcg/mL)	
	Agar (7H11) method	REMA (7H9 broth) method
M. tuberculosis	≤ 0.5	≤ 0.25

Source: NDA 204384

Results of both agar and REMA susceptibility testing indicated that there was no correlation between the MIC values and proposed breakpoints and the clinical outcome. Therefore resistance as defined by values that are greater than the breakpoints did not play a role in treatment failures. Insufficient data with respect to treatment of tuberculosis with bedaquiline is very likely a significant contributor to the inability to determine a resistant breakpoint.

Using the rationale and the data provided these values seem adequate. The breakpoints will have to be revisited when more clinical data are available. Table 4 shows the combined correlation between MIC values and clinical outcomes for C208 stage 2 and C209.

Table 4: Correlation between Provisional Interpretive Criteria and Clinical Outcome in Studies C208 Stage 2 and C209 at Week 24 Evaluation compared to Baseline Values.

Clinical Outcome	Agar Method		REMA Method	
	No (%)	MIC range (mcg/mL)	No (%)	MIC range (mcg/mL)
Success	188/193 (79%)	≤0.008 – 0.5	188/141 (79%)	≤0.008- 0.5
Failure	45 (19%)	0.015- >1	44 (19%)	≤0.008 – 0.25

When resistant strains were genotyped there appeared to be no consistent change in the *atpE* gene between baseline and resistant isolates. This indicated that for these isolates mutation in this gene did not contribute to resistance.

TABLE OF CONTENTS

EXECUTIVE SUMMARY **3**
1.0 INTRODUCTION **9**
2.0 NON-CLINICAL INFORMATION **10**
2.1 ANTIMICROBIAL SPECTRUM OF ACTIVITY 10
2.1.1 Activity against other genera of bacteria **11**
2.1.2 Activity of metabolites **12**
2.2 MECHANISM(S) OF ACTION 12
2.2.1 Latent tuberculosis **15**
2.2.2 In vitro activity of bedaquiline with mycobacterium strains **17**
2.2.3 Effect of bedaquiline on ATP production in human cells **20**
2.3 MECHANISM(S) OF RESISTANCE 21
2.3.1 Resistance due to Mutations **21**
2.3.2 Resistance due to Efflux **26**
2.3.3 Cross-resistance **28**
2.4 NONCLINICAL PHARMACOKINETICS/PHARMACODYNAMICS 29
2.4.1 Human Pharmacokinetics **29**
2.5 ANIMAL STUDIES 29
2.5.1 In vivo Activity of Bedaquiline in Animal Models 29
2.6 ANTIMICROBIAL CONCENTRATION ASSESSMENT 36
2.6.1 Extra-cellular and Intra-cellular activity of Bedaquiline **36**
2.6.2 Persisters **40**
3.0 IDENTIFICATION AND SUSCEPTIBILITY TEST METHODS AND METHODS FOR THE DETECTION OF RESISTANT ORGANISMS **42**
3.1 SPECIMEN COLLECTION AND SHIPMENT 44
3.1.1 Specimen collection and transport **44**
3.2 IDENTIFICATION 45
3.2.1 Acid-fast bacillus microscopy **45**
3.2.2 Qualitative culture of sputum samples **46**
3.3 ANTI-MYCOBACTERIAL SUSCEPTIBILITY TESTING 48
3.3.1 Drug susceptibility Testing **48**
3.3.2 Susceptibility testing of pyrazinamide **55**
3.3.3 Drug carry-over **56**
3.4 GENOTYPING 56
3.4.1 Sequence of ATP Operon **58**
4.0 ANTIMICROBIAL INTERACTIONS AND FIXED COMBINATION STUDIES **60**
5.0 CLINICAL STUDIES **65**
5.1 STUDY C202 65
5.2 STUDY C208 66
5.2.1 Treatment effect on susceptibility of drugs to background regimen **71**
5.2.2 Other observations from trial C208 ..... **71**
5.3 STUDY C209 72
6.0 PROVISIONAL INTERPRETIVE CRITERIA **76**
7.0 CORRELATION OF PROVISIONAL INTERPRETIVE CRITERIA WITH CLINICAL OUTCOME **77**
7.1 SUSCEPTIBILITY TEST QUALITY CONTROL DATA 78
8.0 ESTABLISHMENT OF BREAKPOINTS FOR USE IN LABELING **80**
9.0 REFERENCES **81**
10.0 LABEL **87**

Reference ID: 3225917

1.0 INTRODUCTION

The subject of this New Drug Application (NDA) is bedaquiline (TMC207, R207190) a new anti-tuberculosis drug for the treatment of multi-drug resistant tuberculosis (MDR-TB). Bedaquiline is oxidatively metabolized to an *N*-monodesmethyl metabolite (M2) that is also active against *Mycobacterium tuberculosis* but to a lesser degree then the parent compound bedaquiline. Bedaquiline is used in conjunction with at least three other anti-tuberculosis drugs to which *M. tuberculosis* isolate is susceptible.

Bedaquiline is a diarylquinoline that binds to ATP (adenosine 5'-triphosphate)-synthase 3 and inhibits the production of energy in *Mycobacterium* cells which results in the death of the bacteria.. The activity of bedaquiline *in vitro*, inhibits both actively replicating and non-replicating susceptible and resistant *M. tuberculosis*.

In January 2005 TMC was granted orphan-drug designation. It was granted fast-track designation on April 2011.

M. tuberculosis is an acid–fast bacterium that is the causative agent of tuberculosis (TB) in humans. It is a highly communicable airborne disease. With the advent of HIV infection with mycobacteria has increased in both poor nations and industrialized countries.[1] The World Health Organization (WHO) estimated that there were approximately 8.7 million new tuberculosis (TB) cases worldwide in 2011 up from 8.3 million new cases in 2000. The World Health Organization also estimated that in 2011 there were approximately 1.4 million deaths from tuberculosis with about 1 million in HIV negative individuals. Additionally, there were approximately 60,000 cases of multi-drug resistant tuberculosis (MDR-TB) worldwide[2]. The prevalence of TB in the USA in 2010 was 11,181. There were less than 100 cases of MDR-TB in the USA in 2011 (CDC)[3].

The recommended tuberculosis treatment schedule is 2 months of isoniazid, rifampin, ethambutol and pyrazinamide followed by 4 to 7 months of isoniazid and rifampin. Effective as this widely accepted treatment regimen has been it has some significant drawbacks (86% successful treatment in 2008). It has been found to be too slow to manage the TB epidemic, expensive for direct observation therapy (DOT) and inconvenient for the patient to use. Consequently, there is a high rate of patient non-compliance. The outcome of non-compliance is increased death rates and the emergence of multi-drug resistant-TB

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[1] Corbett, et al. 2003. The Growing Burden of Tuberculosis: Global Trends and Interactions With the HIV Epidemic. Arch. Int. Med. 163: 1009-1021.

[2] World Health Organization. Global Tuberculosis Report 2012.

[3] Centers for disease Control, Decrease in Reported *Tuberculosis* Cases MMWR *2010*; 59 (No.10); 289-294 October 25, 2010

(MDR-TB). MDR-TB is defined as infection with an isolate of *M. tuberculosis* that is resistant to isoniazid (INH) and rifampin (RIF)/rifampicin (RMP), the two most important drugs used to treat drug-susceptible TB. The MDR-TB isolates may also demonstrate resistance to other first-line anti-TB drugs, such as pyrazinamide (PZA), ethambutol (EMB), and streptomycin (SM). Resistance to important drugs such as INH and RMP leads to longer durations of combination therapy with a variety of other agents which are less effective and which have significant toxicities. MDR-TB-infected subjects are currently treated with at least 5 second-line TB drugs for an extended period of time, lasting up to 2 years. Furthermore, extensively drug-resistant TB (XDR-TB), i.e. MDR-TB with additional resistance to aminoglycosides or capreomycin and fluoroquinolones (the two most important classes of second-line TB drugs), has recently emerged as a public health threat. Classical or standard treatment for MDR-TB is usually divided into two treatment phases, a 4 to 6-month intensive phase in which an injectable aminoglycoside is administered along with three or four other (second-line) drugs, including a fluoroquinolone. This is followed by a continuation phase without an aminoglycoside to achieve a total therapy (intensive and continuation phase) of 18 to 24 months. The continuation period may be shortened if 12 months of treatment is given after documentation of sputum culture conversion from positive to negative. Inclusion of bedaquiline in a treatment regimen is expected to shorten the treatment schedule.

2.0 NON-CLINICAL INFORMATION

In non-clinical studies bedaquiline has been used in 2 forms the free base (R207910 or JNJ-16175328-AAA) or as the fumarate salt (R403323 or JNJ-16175328- AEP). Throughout this document, these forms are referred to as (i) bedaquiline, which was used in the early stages of development of the drug and (ii) bedaquiline-fumarate used in the later phases. The major metabolite M2 (= R405786 or JNJ-28325583-AAA), was dosed and/or measured in several *in vivo* studies and evaluated in a few *in vitro* studies

2.1 ANTIMICROBIAL SPECTRUM OF ACTIVITY

Studies have shown that bedaquiline may be effective in the treatment of infections by *M. tuberculosis, M. leprae*, *M. ulcerans*, *M. avium complex*, *M. kansasii*, *M. fortuitum* and *M. abscessus*[4]. Andries et al. studied the activity of bedaquiline against various strains of *M. tuberculosis* and other species of mycobacteria. Oleic acid albumin dextrose catalase (OADC)-enriched Middlebrook 7H11 agar containing 2-fold dilutions of bedaquiline was used. The agar plates were inoculated with 10^4 to 10^5 colony forming units (CFU)/mL of the test mycobacterium and incubated at 30^0C or 37^0C for 3 to 42 days (depending on the *Mycobacterium* species). The minimum inhibitory concentration (MIC) defined as the lowest concentration of drug that inhibited 99% growth was measured. The MICs for the reference strain, *M. tuberculosis* H37Rv, and 6 susceptible clinical isolates were ≤ 0.120 mcg/mL. The *in vitro* activity of bedaquiline against isolates resistant to isoniazid, rifampin, streptomycin, ethambutol, pyrazinamide or moxifloxacin was similar to that against susceptible isolates

[4]Andries, et al. 2005. A diarylquinoline drug active on the ATP synthase of *M. tuberculosis.* Science. 307: 223-227.

(Table 1). The basis for classifying the isolates as resistant was not specified. Low bedaquiline MICs (≤ 0.5 mcg/mL) were observed against mycobacterium species other than *M. tuberculosis*.

Table 1: Minimum Inhibitory Concentration (MIC_{99}) of Bedaquiline against different *Mycobacterium* species

Mycobacterial species	Number of strains	Range of MICs for multiple strains (μg/ml)	Median MIC (μg/ml)
M. tuberculosis, H37Rv	1	—	0.030
M. tuberculosis, fully susceptible clinical isolates	6	0.030–0.120	0.060
M. tuberculosis resistant to isoniazid	7	0.003–0.060	0.010
M. tuberculosis resistant to rifampin	1	—	0.030
M. tuberculosis resistant to isoniazid and rifampin	2	0.030–0.030	0.030
M. tuberculosis resistant to isoniazid and streptomycin	1	—	0.010
M. tuberculosis resistant to ethambutol	1	—	0.010
M. tuberculosis resistant to pyrazinamide	1	—	0.030
M. tuberculosis resistant to fluoroquinolone	2	0.060–0.120	0.090
M. bovis	1	—	0.003
M. avium/M. intracellulare (MAC)	7	0.007–0.010	0.010
M. kansasii	1	—	0.003
M. marinum	1	—	0.003
M. fortuitum	5	0.007–0.010	0.010
M. abscessus	1	—	0.250
M. smegmatis	7	0.003–0.010	0.007
M. ulcerans	1	—	0.500

Source: NDA 204384

2.1.1 Activity against other genera of bacteria

Andries et al., 2005 in a study performed in 2004 using NCCLS [now the Clinical and Laboratory Standards Institute (CLSI)] methodology, also tested the activity of bedaquiline (R207910) against a limited number of isolates of gram-positive and gram-negative bacteria. The MIC was defined as the concentration of the drug that inhibited 99% of the bacterial growth.

It was found that the MIC of this drug was much higher for bacteria other than *M. tuberculosis*. This indicated that bedaquiline *in vitro* had better activity against *Mycobacterium* spp. Because of the small number of isolates tested for some species the results should be interpreted with caution. Table 2 shows the results of bedaquiline activity against non-mycobacterium isolates of bacteria.

Table 2: MIC of bedaquiline against selected strains of gram-positive and gram-negative bacteria

Bacterial species	Number of strains tested	Median MIC mcg/mL
Corynebacterium jeikeium	1	4
Corynebacterium urealyticum	1	4
Helicobacter pylori	20	4
Nocardia asteroides	1	>16
Nocardia farcinica	1	>16
Escherichia coli	1	>32
Haemophilus influenzae	1	>32
Streptococcus pneumoniae	10	>32
Staphylococcus aureus	1	>32

Modified from Andries 2005

2.1.2 Activity of metabolites

N-momodesmethylmetabolite (M2) is the main circulating metabolite of bedaquiline. M2 was tested in an agar medium to compare its activity with that of the parent compound. The strain H37Rv at approximately 100 CFU/mL was tested on 7H10 agar supplemented with OADC and in 7H9 broth. The concentrations of drug tested in the agar medium were 0.006 mcg/mL to 1.0 mcg/mL for both bedaquiline and M2. For the 7H9 broth bedaquiline was tested at a concentration of 0.0075 mcg/mL to 0.12 mcg/mL and M2 at a concentration of 0.009 mcg/mL to 0.6 mcg/mL. The MIC in agar was measured in duplicate. The MIC of M2, was 0.1 mcg/mL when measured in both 7H10 agar and 7H9 broth media. The MIC for bedaquiline was 0.025 mcg/mL and 0.020 mcg/mL when measured in agar and 0.015 mcg/mL when measured in 7H9 broth. This indicates that M2 is approximately 4 to 6 times less active than the parent compound. Another metabolite N-didesmethyl (M3) has a MIC of 5.6 mcg/mL in both agar and liquid media against H37Rv that is 93 to 280 times higher than the parent compound bedaquiline.

2.2 MECHANISM(S) OF ACTION

The mechanism by which bedaquiline exhibits anti-mycobacterium activity was investigated by Andries *et al.,* 2005. In this study, the genomes of bedaquiline (TM207) resistant *M. tuberculosis* strain BK12 and *M. smegmatis* strains R09 and R10 were sequenced and compared to their respective parent susceptible strains. A point mutation was identified at amino acid position 63 (alanine to proline change) of the F0 subunit of ATP synthase protein encoded by the *atpE* gene in the *M. tuberculosis* resistant strain BK12 (Figure 1A). Similarly, a point mutation was identified at amino acid position 32 (aspartic acid to valine) in the resistant *M. smegmatis* strains R09 and R10 (Figure 1B). These finding suggest that bedaquiline inhibits mycobacterium ATP synthase – the enzyme that is responsible for the production of ATP by oxidative phosphorylation. It inhibits the enzyme by binding to the subunit c of the enzyme[5]. Subunit c of ATP synthase is responsible for the flow of protons (H^+ or Na^{++}) from the intercristae region in mitochondria and the periplasmic space in bacteria to the mitochondrial matrix and the bacterial cytoplasm, respectively[6]. When wild type *M. smegmatis* was transformed with constructs containing the mutant *atpE* gene, the bedaquiline MIC increased by 1000-fold confirming the finding that mycobacterium ATP synthase is the target (Table 3). Analysis of bedaquiline mutants of *M. tuberculosis* and *M. smegmatis* showed that there were no mutations in the DNA gyrase regions *gyrA* and *gyrB*, sequences in which quinolone resistance develops (data not shown). This suggests that the mechanisms of action of bedaquiline and quinolones against *Mycobacterium* spp. are different and that cross-reaction is unlikely to occur.

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[5] Koul et al.2007. Diarylquinolines target subunit c of mycobacterial ATP synthase. Nat Chem Biol. 3 (6): 323-324.

[6] Fillingame, R. and O. Y. Dmitriev. 2002. Structural model of the transmembrane F0 rotary sector of H_-transporting ATP synthase derived by solution NMR and intersubunit cross-linking in situ. Biochim. Biophys. Acta 1565:232–245.

Other Complementation studies. Wild-type (WT) *M. smegmatis* was transformed by electroporation with a plasmid containing ATP F0 from *M. smegmatis* mutant R09 (D32V) (MT) and cultured in Mueller–Hinton broth with kanamycin (50 mcg/mL, for maintaining the plasmid) with or without bedaquiline (R207910 sometimes abbreviated as J), (10 X MIC_{90}). Mueller–Hinton plates containing serial dilutions of bedaquiline (R207910 or J) were inoculated with these cultures. After incubation CFU were counted and MIC_{90} values calculated. *M. smegmatis* WT and R09 were compared with *M. smegmatis* WT transformants that contain the mutant ATP F0 operon in forward (MTf) and reverse (MTr) direction to lacZ promoter on pSUM41, respectively.

Figure 1: Alignment of protein sequences of residues from *atpE* gene of susceptible and mutant strains of *M. tuberculosis* and *M. smegmatis*.

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Mtb-S = drug sensitive strain of MTB (H37Rv). Mtb-R = drug resistant strain of MTB (BK12), Msm-S *M. smegmatis* sensitive , MsmR09/MsmR10 *M. smegmatis* R09/ and R10 resistant strains (Source: Andries et al. 2005)

(b) (4)

(Source IND 69600)

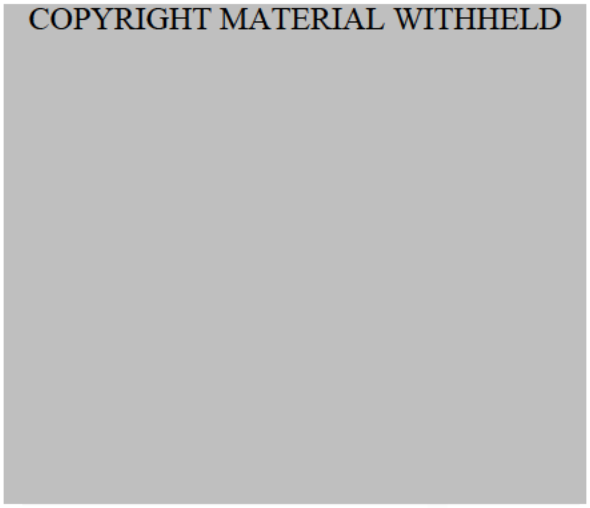
Table 3: Complementation studies of bedaquiline with *Mycobacterium smegmatis*

Strain	Concentration J (pIC_{90})	Concentration J (µg/ml)	Inhibition (%)	MIC_{90}
M. smegmatis (WT)	Control	0.0		
	8.3	0.003	99.9	0.003
	7.3	0.03	100.0	
	6.3	0.30	100.0	
	5.3	3.0	100.0	
M. smegmatis (R09 mutant)	Control	0.0		
	8.3	0.003	0.0	
	7.3	0.03	0.0	
	6.3	0.30	0.0	
	5.3	3.0	99.7	3.0
M. smegmatis (WT MTf)	Control	0.0		
	8.3	0.003	1.3	
	7.3	0.03	5.3	
	6.3	0.30	3.9	
	5.3	3.0	100.0	3.0
M. smegmatis (WT MTr)	Control	0.0		
	8.3	0.003	0.0	
	7.3	0.03	0.0	
	6.3	0.30	0.0	
	5.3	3.0	100.0	3.0

Source: Table NDA 204384

Koul et al., (2007) in an effort to confirm that bedaquiline (R207910) interferes with bacterial metabolism related to the ATP synthase enzyme, measured the cellular ATP in wild-type *M. tuberculosis*. It was found that during exposure of bedaquiline susceptible bacteria there is over-expression of genes that encode ATP synthase leading to a decrease in susceptibility to bedaquiline. The *atpE* genes from a bedaquiline resistant mutant led to a increase >100-fold increase in the MIC_{90} in *M. smegmatis* whereas over-expression of the wild-type subunit c control did not lead to a change in MIC_{90}. It was found that there was a dose dependent decrease in ATP (Figure 2). The positive control ATP synthase inhibitor dicyclohexylcarbodiimide (DCCD) totally blocked the production of ATP while the negative controls isoniazid and rifampin had no effect on the production of ATP.

Figure 2: Dose dependent decrease in cellular ATP of wild-type *M. tuberculosis* with treatment of bedaquiline



Source: Koul et al, 2005

The α and ß subunits of ATP synthase were identified as the specific targets for bedaquiline action by a number of methods. One method was the isolation of two proteins of molecular weights 53 and 59 kDa by immobilization of amine analogs of bedaquiline using Sepharose resin then elution by affinity chromatography, followed by resolution of the two units by gel electrophoresis and analysis by mass spectrometry. The specific binding site, subunit c was later identified because of the strong binding response equilibrium dissociation constants of 500 nM (purified wild-type subunit c) and 1,500 nM (mutant subunit c *atpE*) to the amine analog of the bedaquiline construct. Other binding experiments were undertaken to confirm the binding site.

2.2.1 Latent tuberculosis

The potential for bedaquiline (R207910) to be efficacious in the treatment of latent tuberculosis was demonstrated by exposure of dormant *M. tuberculosis* bacterial cells to bedaquiline in a study by Koul et al., (2008). The study showed that bedaquiline killed dormant bacteria in spite of the fact that transcription in the ATP synthase (target for bedaquiline) operon is down regulated and there is a lower cellular ATP level during this phase of growth. The authors showed that the non-replicating bacteria have residual ATP synthase that can be attacked by minute concentrations of bedaquiline. This results in a greater reduction in the synthesis of ATP leading to critical levels of ATP and subsequent death of the cell. The authors tested *M. tuberculosis* H37Rv, *M. smegmatis*, and *M. bovis* BCG strains obtained from the American Type Tissue Culture Collection (ATCC) in the study. They determined the MIC for several diarylquinolines compounds[7]. The minimal bactericidal concentration MBC_{90} (mcg/mL)

[7] Lenaerts, et al.. 2005. Antimicrob. Agents Chemother. 49, 2294–2301.

Reference ID: 3225917

defined as 90% of aerobic growth killed (using CFU counts) after 5 days of treatment. The results showed that 90% of the dormant bacteria were killed after 5 days of treatment.

The cells were driven to dormancy using oxygen deprivation by three methods:
(1) gradual depletion of oxygen for 18 days in tightly sealed and stirred tubes.
(2) drastic depletion of oxygen as in an anaerobic chamber for 7 days
(3) nitric oxide treatment 150 µM of a NO donor (diethylenetriamine/nitric oxide (DETA/NO) for 2 hours.

The cells were then grown in the presence of either 0.1 mcg/mL or 10 mcg/mL bedaquiline. RNA was extracted from dormant as well as aerobically grown *M. tuberculosis* cells and the total cellular and ATP synthesis activity was measured from both types of cells. Figure 3 shows the effect of 10 mcg/mL bedaquiline in wild type dormant cells and bedaquiline - resistant strain of *M. tuberculosis* BK12. Dicyclohexylcarbodiimide (DCCD) a nonspecific ATP synthase inhibitor showed a drastic killing effect. In Figure 3A, 10 mcg/mL bedaquiline caused a 1.8-$\log_{10}$ reduction in dormant cells, in Figure 3B there was a 2-$\log_{10}$ reduction. In Figure 3C where the bacteria were subjected to drastic oxygen reduction there was a CFU reduction of 1.3-$\log_{10}$. There was no reduction when the cells are treated with 0.1 mcg/mL bedaquiline[8] .

Figure 3: Effect of bedaquiline on wild type and resistant BK12 *M. tuberculosis strains* grown under oxygen depletion or nitric oxide stress

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Source: Koul et al. 2008.

[8] Koul, et al. 2008. Diarylquinolines are bactericidal for dormant mycobacteria as a result of disturbed ATP homeostasis. J. Biol. Chem. 283:25273–25280

Figure 4 shows the killing activity of bedaquiline compared to that of isoniazid in both dormant and aerobically grown *M. tuberculosis*. Dormant and aerobically grown cells were treated with 10 mcg/mL bedaquiline and 10 mcg/mL isoniazid. Killing was monitored at Days 1, 4, 7, and 14 by CFU counts on 7H10 plates. The reduction was calculated against untreated cells. The dormant *M. tuberculosis* cells treated with bedaquiline were below the limit of detection by 14 days (10^2CFU/mL).

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Source Figure from Koul, et al. 2008

2.2.2 In vitro activity of bedaquiline with mycobacterium strains

The *in vitro* activity of bedaquiline against 41 susceptible and 44 multi-drug resistant (MDR, resistant to at least rifampin and isoniazid) *M. tuberculosis* strains was measured by Huitric *et al.,* 2005, (poster presentation at 45th ICAAC). The minimum inhibitory concentration (MIC) defined as the concentration of the drug that showed no growth compared to control was ≤ 0.128 mcg/mL for all 85 isolates (Figure 5). The bedaquiline MIC for susceptible and MDR strains ranged from 0.002 to 0.064 mcg/mL and 0.004 to 0.128 mcg/mL, respectively.

Figure 5: Bedaquiline MICs of 41 susceptible and 44 MDR *M. tuberculosis* strains.

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A susceptibility study by Huitric et al. (2007) tested 85 isolates of *Mycobacterium* species 44 of which were MDR-TB isolates (one was identified as extensively drug resistant (XDR), 41 were drug susceptible (DS) *M. tuberculosis* isolates; 22 *M. avium* and 28 from other non-tuberculous mycobacterium (NTM) isolates against bedaquiline. The MIC was defined as the first concentration at which no bacterial growth was visible. Table 4 shows the MIC of M. *tuberculosis* from three studies - Hutric et al. 2007, Andries et al., 2005 and study C209. The MIC of the 109 susceptible and drug resistant strains of *M. tuberculosis* range from ≤ 0.008 mcg/mL to 0.012 mcg/mL. Table 5 shows the MIC_{50} and MIC_{90} against the *M. tuberculosis* and NTM isolates. The MIC_{50} and MIC_{90} of the susceptible and MDR were 0.03 mcg/mL and 0.06 mcg/mL, respectively. The single XDR-TB isolate had a MIC_{50} and MIC_{90} of 0.03 mcg/mL and 0.06 mcg/mL, respectively. Bedaquiline showed strong inhibitory activity against 8/20 NTM species with MICs ≤ 0.03 mcg/mL. Figure 6 shows the MIC distribution of the isolates.

Table 4: In vitro activity of bedaquiline against 109 strains of *M. tuberculosis*

Bacterium	*M. tuberculosis* Resistance subtypes	No. of strains	Bedaquiline MIC mcg/mL			
			MIC Range	MIC_{50}	MIC_{90}	MIC_{95}
M. tuberculosis	DS-TB	65	≤ 0.008-0.012	0.03	0.06	0.06
	MDR-TB	44	≤ 0.008-0.012	0.03	0.06	0.06
	All	109	≤ 0.008-0.012	0.03	0.06	0.06
NDA submission						

Table 5: MIC_{50} and MIC_{90} ranges for 85 *M. tuberculosis* clinical isolates and 20 non-tuberculous mycobacterium strains

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[a] All but three species are susceptible to Bedaquiline (R207910) with MICs of _0 5 _g/ml
Source Table: Hutric et al., 2007

Figure 6: The MIC Distribution of 132 Bedaquiline-susceptible *Mycobacterium* strains

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Comparison of the protein sequence of the *atpE* gene of *Mycobacterium* strains susceptible and resistant to bedaquiline showed mutations in the gene that could have possibly resulted in drug resistance. In one case an amino acid mutation - arginine in the sensitive strains was replaced by valine in the resistant strain.

2.2.3 Effect of bedaquiline on ATP production in human cells

The safety of bedaquiline for humans was demonstrated by Haagsma et al., (2009) in ovarian cancer cells from cell line OVCAR3 and *M. smegmatis mc*^{2} 155. The authors showed that bedaquiline has a > 20,000-fold lower affinity for human mitochondrial ATP synthase than it has for the mycobacterium ATP synthase. Activity of bedaquiline was detected only in high concentrations (200 μM) of the drug where there was a 35% inhibition of ATP production. Greater than 90% inhibition of ATP in human cells was noted in two non-selective ATP synthase inhibitors *N*,*N* -dicyclohexyl-carbodiimide (DCCD: 5 μM for humans, 100 μM for *M. smegmatis*) and oligomycin (1 μM) that were used as controls that inhibited ATP synthesis. When the effect of bedaquiline was tested on *M. smegmatis* ATP synthesis was inhibited by nanomolar concentrations of bedaquiline, complete inhibition of *M. smegmatis* was achieved by 100 nM of bedaquiline. Figures 7A and 7B show the effect of bedaquiline on human and *Mycobacterium* cells. The ATP synthesis in the mitochondria of human cancer cells that was inhibited in the presence of 0.1 μM of bedaquiline was very little and showed a 35% increase in 200 μM of bedaquiline whereas in the presence of 0.0025 μM of bedaquiline there was > 10% inhibition of ATP synthesis. The IC_{50} for mycobacterium strains was 0.01 μM and the IC_{50} for the human cancer cells was >200 μM.

Figure 7: Effect of bedaquiline on ATP synthesis by mitochondria from a human cell line (A) compared to that for inverted membrane vesicles of *Mycobacterium smegmatis(B)*

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Source: Figure from Haagsma, et al. 2009.

2.3 MECHANISM(S) OF RESISTANCE

2.3.1 Resistance due to Mutations

The study by Andries et al., (2005), indicates that mutation in the *atpE* gene of the ATP synthase is the likely cause of resistance in *Mycobacterium spp.* The mutations occur at two specific points, Ala63 →Pro (A63P) for *M. tuberculosis* and Asp32 → Val (D32C) for *M. smegmatis*. A more recent study by Huitric et al., (2010) assessed the *in vitro* development of resistance of *M. tuberculosis* to bedaquiline. The study utilized six clinical isolates of *M. tuberculosis* and *H37Rv* reference strain. These strains were tested with 10 X, 30 X and 100 X the MIC of bedaquiline (0.03 mcg/mL).

The MICs of the strains were determined after 4 weeks of incubation. At a concentration of 0.3 mcg/ml (10 X the MIC), the mutation rates ranged from 4.7 $x10^{-7}$ to 8.9 x 10^{-9} mutations per cell division. At a concentration of 1.0 mcg/mL (30 X the MIC), the mutations rates ranged from 3.9 x 10^{-8} to 2.4 x 10^{-9} mutations per cell division. No resistant mutants were obtained at 3 mcg/mL (100 X the MIC)[9]. This suggests that the rate of mutation against bedaquiline decreases with the increase in the concentration of the antibiotic. This means for therapeutic purposes if the selected dose range is above 100 X the MIC (3 mg/mL) and non-toxic, the chance of reduction of mutations and consequently resistance will be reduced. The MICs of the mutants ranged from 0.12 to 3.84 mcg/mL representing 4- to 128-fold increases in the MICs. Mutants of *M. tuberculosis* and *M. smegmatis* resistant to bedaquiline were selected *in vitro* to

[9] Huitric, et al. 2007. *In vitro* antimycobacterial spectrum of a diarylquinoline ATP synthase inhibitor. Antimicrob Agents Chemother 2007, 51: 4202-4204.

quantify the proportion of resistant mutants arising by comparison with rifampin and to assess the cross-resistance pattern (Andries et al., 2005). Different inocula (10^8, 10^9, and 10^{10} CFU/mL) of *M. smegmatis mc²155* and *M. tuberculosis H37Rv* strains were used to inoculate brain heart infusion and Middlebrook 7H11 broth containing 10% OADC. The bedaquiline MICs for *M. smegmatis* and *M. tuberculosis* were 0.003 mcg/mL and 0.03 mcg/mL, respectively. The rifampin MIC for *M. smegmatis* and *M. tuberculosis* was 0.5 mcg/mL. Concentrations of bedaquiline and rifampin equivalent to 2 X, 4 X, 8 X and 16 X MIC were used for selection and quantitation of resistant mutants. The proportions of bedaquiline resistant mutants were comparable to those of rifampin-resistant *M. tuberculosis* mutants that emerged (10^{-7} to 10^{-8}). The authors have stated that the susceptibility of bedaquiline-resistant *M. tuberculosis* strains remained unchanged relative to other anti-TB agents[10]. At 4 X MIC (0.12 mcg/mL) the mutation rate for *M. tuberculosis* was 5 x 10^{-7} and at 8 X MIC (0.24 mcg/mL) the mutation rate was 5 x 10^{-8}. The applicant also stated that there were no mutations in gyrA and *gyrB* regions where quinolone resistance usually develops.

It was determined that resistance was due to mutations in this gene but that the rate of mutation decreased as the concentrations of bedaquiline increased. From the sequences of 53/97 of the resistance mutants, the *atpE* gene showed five single point mutations. The mutations came from substitutions of five different amino acids: Asp28 →Val, Ala63 →Pro, Ile66 →Met, Asp28 →Pro, or Glu61→Asp. Table 6 shows the resistance patterns of mycobacterium treated with bedaquiline (R207910). Overall, the results suggest a potential for development of resistance. One explanation for the reduction in mutations as the concentration of the antibiotic increased is that the drug activity is concentration dependent. Thus at lower concentrations the antibiotic tended to be more bacteriostatic allowing the bacilli to live long enough to mutate whereas the higher concentrations of the drug killed the bacteria before there is time to mutate.

[10] Andries, et al. 2005. A diarylquinoline drug active on the ATP synthase of *M. tuberculosis.* Science. 307: 223-227.

Table 6: Resistance mutations in the *atpE* gene and related MIC levels

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Source: Table Hutric et al., 2010

Segala et al. (2012) identified the amino acid residues involved in the binding of bedaquiline to its target. Figure 8A shows the structure of bedaquiline and the point of binding to the ATP synthase molecule, shown as an arrow, at aspartate (Asp) or glutamate (Glu) residue. Figure 8B is a ribbon diagram with relevant amino acids involved in *M. tuberculosis* resistance to bedaquiline and their positions. This study has shown that six distinct substitutions aspartate (Asp)28→glycine (Gly), aspartate (Asp) 28→alanine (Ala), leucine (Leu)59→valine (Val), glutamate (Glu) 61→aspartate (Asp), alanine (Ala)63→proline (Pro), and isoleucine (Ile)66→methionine (Met), have been identified in the subunit c in the ATP synthase as the cause of resistance to bedaquiline.

Figure 8. A: Structure of bedaquiline and the point of binding to the ATP synthase molecule B: Crystallographic structure of H+/Na+ binding site implicated in *M. tuberculosis* bedaquiline resistance

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(Source Segala et al.2012)

Segala et al. used mutant strains of *M. tuberculosis H37Rv* and *M. smegmatis mc*^{2}*155* and clinical isolates from *M. tuberculosis, M. abscessus and M. fortuitum* to identify mutations that resulted in resistance to bedaquiline. Mutants for *M. smegmatis,* MIC 0.007 mcg/mL, were selected from strains that were resistant to individual concentrations of bedaquiline between 0.003 mcg/mL and 1 mcg/mL. For *M. fortuitum,* MIC = 0.01 mcg/mL, the mutants were selected from tests at individual concentrations between 0.015 mcg/mL to 0.5 mcg/mL and for *M. abscessus*, MIC= 0.25 mcg/mL, the mutants were selected from tests performed at concentrations between 0.25 mcg/mL and 8 mcg/mL. For *M. tuberculosis*, MIC = 0.015 to 0.06 mcg/mL, the mutant strains were selected from concentrations between 0.03 mcg/mL and 0.5 mcg/mL. The DNA extracted from cell suspensions of the mutants under various drug concentrations were examined for mutations in the subunit c of the ATP synthase gene, a*tpE.* The frequency in the mutations ranged from 10^{-8} to 5.10 X 10^{-8} regardless of the species. Sixteen percent (4/25) of the *M. tuberculosis H37Rv* mutants demonstrated mutations in amino acid residues Ala63→ Pro. The mutants obtained from the clinical isolates of *M. tuberculosis* showed the following 5 mutations with amino acid substitutions Asp28→Gly, Leu59→Val, Glu61→Asp, Ala63→Pro, and Ile66→Met. Table 7 shows the phenotypic and genotypic properties of the resistant mutants. Figure 9 shows the amino acid sequences of the ATP synthase gene. The substitution of Ala63 → Pro results in the greatest effect in the mycobacterium subunit c. This mutation caused an increase in the MIC value for *M. tuberculosis* by 133-fold and for *M. abscessus* an increase by 64-fold. It is felt that substitution of Ala63 plays a key role in resistance of mycobacterium to bedaquiline [Petrella et al. (2006), Hutric et al. (2007)]. Figure 9 also shows the sequence of the ATP synthase c subunit and the mutations resulting to resistance. The amino acid residues and their positions previously reported in resistance testing of *M. tuberculosis* positions are at 28, 61, 63 and 66. The author stated that 38/82 (46%) of the mutants obtained from *M. tuberculosis* and *M. abscessus* showed no mutations in the *atpE* gene. The author also stated that these mutants were selected

at low concentrations of the drug. This indicates that other mechanisms apart from mutations in the *atpE* gene many also be responsible for resistance to bedaquiline in *M. tuberculosis and M. abscessus*. Additionally, resistance mutations can also be found in other genes or proteins. In isoniazid-resistant *Mycobacterium* mutations are found not only in *inhA,* the target gene, but also in *katG* and other genes. [11]

Table 7: Phenotypic and genotypic characteristics of mutants resistant to bedaquiline obtained from two reference strains (*M. tuberculosis* H37Rv and *M. smegmatis* mc^2155) and five clinical isolates (*M. tuberculosis* P, D, and LQ, *M. fortuitum* B, and *M. abscessus* V)

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Source: Segala et al, 2012

[11] Telenti et al. 1997. Genotypic assessment of isoniazid and rifampin resistance in *M. tuberculosis*: a blind study at reference laboratory level. Clin. Microbiol. 35. 719-723

Figure 9: Multiple sequence alignments of the subunit c of the ATP synthase gene

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M. abscessus (Mabs), *M. tuberculosis* (Mtub), *M. fortuitum* (Mfor), *M. smegmatis* (Msme), *E. coli* (Ecol), *Spirulina platensis* (Spla), and *I. tartaricus* (Itar). Source : Segala et al. 2012

The mechanism of low-level-drug resistance was not shown in the previous study by Segala et al., to be caused by mutations in the c subunit of the ATP synthase gene.

2.3.2 Resistance due to Efflux

Another means of resistance of *M. tuberculosis* to bedaquiline was investigated by Ramon-Garcia et al., 2009. The effect of efflux pumps in the resistance to bedaquiline.

The efflux pump is a nonspecific transporter mechanism of both prokaryotic and eukaryotic cells that are not necessarily similar in structure, by which molecules are extruded from the cell. Included in this are antibiotics used to inhibit the growth of micro-organisms. This action is a significant means of drug resistance in bacteria and a possible mechanism of resistance of *M. tuberculosis*. Efflux pumps are divided into five superfamilies with the ABC superfamily considered the primary efflux pump system. Efflux pumps consist of two membrane spanning domains which are hydrophobic, two cytoplasmic nucleotide binding domains. Figure 10 shows a prokaryotic importer and exporter.

Ramon-Garcia et al. constructed a P55 efflux pump mutant from the *M. bovis* BCG strain. They found that the deletion of the *Rv1410* gene that encodes the P55 efflux pump made strains more susceptible to toxic agents including rifampin. Table 8 shows that the inactivation of *p55* gene caused greater susceptibility to rifampin. In KOP55, the efflux pump mutant, the MIC is lower for all formulations of rifampin than it is in the wild type. In KOP55 PAZ23, the regenerated strain where the expression of *p55 gene* is restored, the MIC is also restored to that of the wild type. The results indicate that P55 efflux pump induces low-level resistance to rifampin and that susceptibility increases when the *p55* gene is inactivated. Additional tests indicate that the energy needed for efflux pumping is obtained from the proton and electrochemical gradient across the bacterial cell membrane.

The authors found that the nucleotide sequences of the *M. tuberculosis Rv1410c* and *Rv1411c* genes are similar to those of *M. bovis BCG1471c* and *BCG1472c*. The results from *M. bovis* BCG can be extended to the efflux mechanism in *M. tuberculosis*.

Figure 10: Organization of prototypical ABC transporter

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Source Figure:Braibant et al., 2000

Table 8: Antimicrobial susceptibilities of *M. bovis* BCG P55 strains

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Source: Table Ramon-Garcia et al. 2009.

DaSilva et al., (2011) reviewed the information on mycobacterium efflux pumps. He found the *LfrA* efflux pumps in *M. smegmatis*, the *TetV* against *tetracycline* and the *Tap* that affect aminoglycosides. *M. smegmatis* was generally used as a model in the reported studies.

Guilfoile and Hitchinson (1991) found that when the gene cluster drrl\-drrB-drrC was over expressed in *M. smegmatis* it exported many clinically relevant antibiotics. Braibant et al., (2000) identified at least 12 putative genes in *M. tuberculosis* that encoded ABC transporters. Braibant et al., (2000) found that in the *M. tuberculosis* genome there were at least 45 nucleotide binding domains (NBDs) in 44 different proteins, 45 membrane-spanning domains (MSDs) in 44 different proteins and 15 substrate binding proteins (SBPs) parts of ABC transporters which they tried to assemble by gene fusion and phylogenetic trees into ABC transporters. Twenty-six ABC transporters were reconstructed by the authors in *M. tuberculosis* among them were also found 6 antibiotic transporters, 4 of which were exporters similar to many of the already identified antibiotic resistance systems. Therefore efflux pumps are a possible means of *M. tuberculosis* resistance to bedaquiline.

2.3.3 Cross-resistance

Andries at al. (2005), evaluated cross-resistance. Bedaquiline showed similar *in vitro* activity against *M. tuberculosis* clinical isolates resistant to the known anti-TB drugs (isoniazid, rifampin, streptomycin, ethambutol, pyrazinamide, or fluoroquinolones) as well as MDR-TB strains thereby suggesting no cross-resistance.
In another study to investigate cross-resistance between bedaquiline and other TB drugs. The researchers measured and compared the MICs of *H37Rv* and the resistant mutant *BK12* to a group of anti-tuberculosis drugs. The drugs were isoniazid, rifampin, ethambutol, streptomycin, PA-824, amikacin, moxifloxacin. The results show that the MICs against *BK12,* the mutant strain, to bedaquiline were similar to those obtained with *H37Rv*, the susceptible strain, for isoniazid, streptomycin and moxifloxacin. The MIC was a two-fold dilution lower in the case of rifampin, and a two-fold dilution higher for PA-824, ethambutol and amikacin i.e. essentially similar, because a dilution difference can represent a difference in technique, indicating no cross-reactivity. Table 9 shows these results.

Table 9: Comparison of the MICs of different antibiotics that tnhibited 99% of the growth of the Bedaquiline-resistant and susceptible mutant

Compounds	MIC (µg/ml)	
	M. tuberculosis H37Rv	*M. tuberculosis* BK12
TMC207	0.03	4.00
Isoniazid	0.12	0.12
Rifampin	0.50	0.12
Ethambutol	2.00	4.00
Streptomycin	1.00	1.00
Amikacin	1.00	2.00
Moxifloxacin	0.25	0.25
PA-824	0.12	0.25

Source Table : NDA 204384

2.4 NONCLINICAL PHARMACOKINETICS/PHARMACODYNAMICS

2.4.1 Human Pharmacokinetics

This summary of pharmacokinetics was extracted from the section of the background document prepared by Dr. Dakshina Chilukuri, Clinical Pharmacology. The clinical pharmacology program consisted of 16 trials that evaluated the PK, drug interactions and PK/PD of bedaquiline. Pharmacokinetic parameters of bedaquiline are summarized in Table 10 (Table 1 of the clinical pharmacology document) for healthy volunteers.

Table 10: Pharmacokinetic parameters of bedaquiline in healthy volunteers

PK Property	PK Parameter	
Dose-proportionality	PK dose-proportional for doses 10 – 700 mg	
Absorption	T_{max} (median)	~5 hr
	$t_{1/2\ term}$	~ 4-5 months
	Food Effect	High fat meal increased C_{max} and AUC by 2-fold.
Distribution	Protein Binding	> 99%
Metabolism	Pathways	Metabolized to M2 and M3 by CYP3A4.
Excretion	Fecal excretion is the major route of elimination.	

Source Table. Chilukuri. FDA

Absorption: The exposure to bedaquiline increases approximately 2-fold when administered under fed conditions. Bedaquiline is recommended to be administered with food to ensure optimal absorption and exposure. The effect of food on exposure of bedaquiline was determined based on a standard meal as opposed to the high fat meal that is usually recommended per the FDA guidance.

Distribution: After reaching C_{max}, bedaquiline concentrations decline tri-exponentially with a long terminal half-life ($t_{1/2,\ term}$) of approximately 4-5 months; however, the effective half-life of bedaquiline is approximately 24-30 hours, based on the approximately 2-fold accumulation after 2 weeks of q.d. dosing. Bedaquiline is highly protein bound (>99%) in human plasma. The apparent volume of distribution of bedaquiline in the central compartment was estimated to be around 164 L. The apparent volume of distribution in the peripheral compartments was estimated to be around 178 L, 3010 L and 7350 L indicating that bedaquiline is extensively distributed in the body.

2.5 ANIMAL STUDIES

2.5.1 In vivo Activity of Bedaquiline in Animal Models

The *in vivo* activity of bedaquiline was measured using two murine (non-established/acute and established) models.

Non-established/acute infection murine model*:* In the non-established/acute model of TB infection, normal female Swiss mice were infected with 2 X 10^7 CFU/mL of *M. tuberculosis* strain *H37Rv* (Andries *et al.,* 2005). The strain *H37Rv* is susceptible to all anti-mycobacterium drugs. The bedaquiline MIC against the *H37Rv* strain was 0.03 mcg/mL. Treatment was initiated the day after intravenous (i.v.) infection with *M. tuberculosis.* The mice were treated orally with different doses of bedaquiline (also referred to as J) for 5 days per week for one month. Mice treated with isoniazid (25 mg/kg) served as positive controls.
The spleen weight, gross lung lesions and bacterial burden in the spleen and lungs were measured. In this model, untreated mice are expected to die within 4 weeks of infection. The authors have stated that the minimal dose of bedaquiline that prevented mortality was 1.5 mg/kg, and the minimal effective dose (MED) preventing gross lung lesions was 6.5 mg/kg. The actual survival data and lung lesion scores were not shown. In mice receiving doses of 12.5 mg/kg, the bacterial load per organ was reduced from 5 to 2 $\log_{10}$ units ($p < 0.0014$) Figure 11. At 12.5 mg/kg and 25 mg/kg, bedaquiline was significantly more active ($p < 0.0014$) than isoniazid.

Figure 11: Minimal effective oral dose of bedaquiline (J) and effect of dosing frequency in the non-established infection murine TB model determined after 4 weeks of treatment.

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1x/W = once per week. Values are means ± SD (Source Figure :Andries et al., 2005)

Rouan et al., studied the effects of bedaquiline and its active derivative *N*-desmethyl bedaquiline (M2)[12] using *M. tuberculosis H37Rv* infected female Swiss mice. The mice were infected by inoculation of 0.2 mL of 10^7 bacteria in the tail and then randomized into one of the two treatment groups or a control group. Suspensions of bedaquiline or the *N*-desmethyl bedaquiline were administered by gavage. The mice were sacrificed at the end of treatment

[12] Rouan et al. 2012. Pharmacokinetics and pharmacodynamics of bedaquiline and its N-desmethyl metabolite in a murine model of tuberculosis. Antimicrob Agents Chemother. 2012 ; 56 (3): 1444-1451.

and bacterial counts were performed on lung tissue. The MIC and MBC (>99.9% killing) and fractional bactericidal concentrations (FBC) were determined for the effect of combination of the parent compound, bedaquiline, and its *N*-desmethyl metabolite. PK studies were done on uninfected females divided into groups that received the same treatment as the infected mice but for 5 days a week and male mice that were administered 30mg/kg of bedaquiline.
The effect of the combination of bedaquiline and its metabolite *N*-desmethyl bedaquiline was additive and dose dependent. The MIC and MBC were 0.06 mcg/mL and 0.4 mcg/mL for bedaquiline and 0.3 mcg/mL and 2.0 mcg/mL for *N*-desmethyl bedaquiline, respectively. Table 11 shows the cell count after 4 weeks of treatment. Sterilization was effected by 50 mg/kg of bedaquiline.

TABLE 11: Bacterial count (mean ±standard deviation) in the lungs of mice after 4 weeks of treatment with bedaquiline or *N*-desmethyl bedaquiline given 5 days per week

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Source Table : Rouan et al. 2012

Table 12 shows the pharmacokinetic parameters obtained in plasma after a single administration of 30 mg/kg to Swiss mice. The maximum concentration of bedaquiline is greater than that of the M2 metabolite.

Table 12: Bedaquiline and *N*-desmethyl Bedaquiline pharmacokinetic parameters obtained after single administration of 30 mg/kg bedaquiline to Swiss mice

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Source Table: Rouan et al. 2012

Figure 12 shows that there is no significant difference in CFU among the various frequencies of dosing. Table 13A shows that the bacterial load in the lung was dose dependent after 4 weeks of treatment. Table 13B shows a similar effect after 6 weeks of treatment with bedaquiline.

Figure 12: Relationship between the mycobacterium counts versus bedaquiline weekly dose schedule

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Source Figure: Rouan et al. 2012

Table 13: Relationship between dose and bacterial cell counts in the lungs of mice.when treated with bedaquiline and/or its M2 metabolite

(A) Bacterial count (mean ± standard deviation) in the lungs of mice after 4 weeks of treatment with bedaquiline or *N*-desmethyl bedaquiline given 5 days per week. Source Tables A/B: Rouan et al. 2012

(B)Bacterial count (mean±standard deviation) in the lungs of mice after 6 weeks of treatment with bedaquiline given at different dosing schedules

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Ibrahim et al., 2007 studied the effect of treatment with bedaquiline (R207910) as a single drug and in combinations of two and three drugs. The study used bedaquiline (R207910), isoniazid (INH), rifampin (RIF), pyrazinamide (PZA) or moxifloxacin (MXF). The combinations were R207910-PZA, R207910-PZA-INH, R207910-PZA-RIF, or R207910-PZA-MXF and the reference regimen was RIF-MXF-PZA. Four hundred and forty 4-week old, female Swiss mice were infected intravenously with 0.5 mL of approximately 2 x 10^6 CFU/mL of *M. tuberculosis H37Rv*.

After two weeks of infection, the mice were randomized into 21 groups – 5 monotherapy with each of the drugs listed above, 8 groups of two-drug combination - RIF-INH, RIF-PZA, INH-PZA, RIF-MXF, R207910-RIF, R207910-INH, R207910Z-PZA or R207910-MXF), and 7 groups of three combinations - RIF-INH-PZA, RIF-MXF-PZA, R207910-INH-RIF, R207910-MXF-RIF, R207910-INHPZA, R207910-RIF-PZA, or R207910-MXF-PZA. The negative control group comprised 40 untreated mice. The test mice were treated by oral gavage for 2 months.

The doses of the drugs used were chosen to result in AUCs similar to those achieved in humans. Therefore the animals were treated with the following doses by body weight R207910 = 25 mg/kg, RIF = 10 mg/kg; PZA =150 mg/kg; MXF = 100 mg/kg; and INH =25 mg/kg. The mice surviving to the end of treatment were euthanized.

Efficacy was assessed by the killing of mice at various time-points during the study cycle to assess the severity of infection. Severity of infection was assessed by the survival rate, weight of the spleen, the gross lesions in the lung and the number of CFU/mL in the lungs. Ten control mice were sacrificed at Day1 after infection, 20 at Day 14. From each treatment group 10 mice were sacrificed 1 and 2 months after treatment initiation.

The results show that all untreated mice died between 5 and 35 days after infection. Tables 14, 15 and 16 show the results of treatment with 1, 2 and 3 drugs after 5 days of treatment. The data show that 20% of the mice treated with bedaquiline (R207910 = J) survived to 2 months whereas none of the mice treated with other monotherapy survived. There was no decrease in bacterial count of the mice treated with INH and PZA. Treatment with two drugs resulted in 100% survival in mice treated with a combination of bedaquiline (J) and PZA and at least 70% of the mice treated with the three-drug combination that included bedaquiline (J) survived. The activity decreased when bedaquiline (J) and PZA were combined with MXF, RIF and INH separately.

The results indicate that bedaquiline was effective in eliminating *M. tuberculosis* from Swiss mice. They also indicated that the bactericidal effect of INH was similar to that of MXF when either was added to the combination of bedaquiline and PZA. INH and RIF are limited in TB treatment because of development of resistance, toxicity, hypersensitivity, and/or drug-drug interactions.

Table 14 : Bacterial counts in the lungs after monotherapy given 5 days/week for murine TB[a]

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Table 15: Bacterial counts and proportion of mice with positive cultures in the lungs after treatment with two-drug combinations given 5 days/week for murine TB[a]

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Source Table 12, 13, 14 Ibrahim et al 2007

Whereas the previous study used first-line drugs, Lounis et al., 2006 in a similarly designed study used combinations of second-line drugs with bedaquiline to treat subjects' multi-drug resistant tuberculosis. Bedaquiline (R207910) was expected to treat subjects who were unable to use rifampin (RIF) and isoniazid (INH) because of toxicity or subjects who were also being treated with antiviral drugs. Usually, when subjects were unable to use INH and RIF the alternate regimen recommended by the World Health Organization was amakacin (AMK), ethionamide (ETH), a fluoroquinolone (such as moxifloxacin), and PZA. The authors state that this combination is less effective than the standard RIF-INH-PZA.
The drugs and doses used in the study were bedaquiline (R207910 = 25 mg/kg), amikacin (AMK = 150 mg/kg), pyrazinamide (PZA = 150 mg/kg), moxifloxacin (MXF = 100 mg/kg), ethionamide (ETH = 50 mg/kg), rifampin (RIF = 10 mg/kg), isoniazid (INH = 25 mg/kg). The doses were chosen to be equivalent to those administered to humans. *M. tuberculosis* strain *H37Rv* at a concentration of 5 x 10^6 CFU/mL in 0.5 mL was used to inject the tail vein of 230 female, 4-week old, Swiss mice. The mice were then randomly assigned to six control and five treatment groups each with 20 to 30 mice. One of the control groups was a negative control (untreated). Treatment commenced 2 weeks after infection and was administered 5 days per week. The treatment groups are listed in Table 17. There are 10 groups 2 of which received single drug treatments, 3 triple treated groups and 5 groups in which the animals were treated with 4 drugs.

Table 17: Treatment groups used in Swiss mice study of second-line Drug combinations

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Source Table : Lounis et al. 2006

Baseline information was obtained from the sacrifice of 10 uninfected and 10 infected mice on Days 1 and 14 after infection. Efficacy was assessed by the survival rate, the weight of the spleen, gross lesions of the lung and the number of CFU/mL after cultures. The cultures comprised serial dilutions on Lowenstein-Jensen slants of sections of the spleen and lung from sacrificed mice. After 2 months the entire undiluted suspension obtained from each individual organ of animals treated with bedaquiline was plated on LJ media. The bactericidal effect was defined as a statistically significant decrease in the mean number of CFU at the end of the treatment cycle compared to the pre-treatment value.
The untreated animals survived for 21 to 28 days. All of the treated animals survived except those that died from the gavage accidents. The increase in the weight of the spleen was significant ($p < 0.05$) from an average of 112 mg (±14 mg) a day after infection to 631 ± 121mg two weeks post infection. This increase in weight indicated active infection. The decrease in the spleen after one month of treatment was also significant ($p < 0.05$). Table 18 shows the colony counts of the bacteria from the spleen. After one month of monotherapy with bedaquiline the colony count was reduced by approximately 4 $\log_{10}$ CFU significantly ($p < 0.05$). All of the combinations in which bedaquiline was included were more effective in killing bacteria than those without for example the combination of RHZ compared to RHZJ at both 1 and 2 months of treatment. The same effect was found in the lung cultures. Overall all combinations containing bedaquiline were significantly ($p = 0.05$) more effective than those without this drug. In this study regimens with INH and RIF that included bedaquiline resulted in lung and spleen negativity within 2 months.

Table 18: CFU counts (log_{10}) in the spleens and lungs of mice after 1 and 2 months of treatment and proportions of mice displaying negative cultures

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Source Table: Lounis et al 2006

2.6 ANTIMICROBIAL CONCENTRATION ASSESSMENT

One of the mechanisms of resistance of mycobacterium in the body is the inhibition of phagolysosomes in the alveolar macrophages by which they may be ingested. The bacteria live and proliferate in the macrophages and release large numbers of bacteria when the macrophages lyse. Additionally, the macrophages, $CD4^+$ T cells and other cells wall off the bacilli and form granulomatous lesions. A drug that can penetrate the cells and inhibit the bacilli is highly important.

2.6.1 Extra-cellular and Intra-cellular activity of Bedaquiline

Dhillon et al., (2010) compared the activity of bedaquiline against bacteria found extra-cellularly and intra-cellularly within the mouse peritoneal macrophages and the J774A.1 macrophage-like cell line. *M. tuberculosis*, strain *H37Rv*, was the infecting bacterium. The final concentrations of drugs tested ranged from 0.006 – 1.0 mcg/mL or 0.25 - 32 mcg/mL dissolved in culture medium. Drug free controls were also used.

The monolayers used to study the intracellular activity of *M. tuberculosis* in the mouse peritoneal macrophages were prepared from the peritoneal cavities of recently killed specific pathogen free BALB/c mice (Harland UK Ltd, Bichecster, UK). The procedures for the study in J774A.1 and the BALB/c peritoneal macrophages were similar except for the days on which the bacterial counts were performed. Figures 13, 14, and 15 show the results of the experiments.

Figure 13: The bactericidal action of bedaquiline on on Extracellular cultures of *M. tuberculosis*

Figure 14: The bactericidal action of bedaquiline cultures of *M. tuberculosis in J774 cells*

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Sporce Figure : Dhillon et al.2010

Figure 15: The bactericidal action of bedaquiline on cultures of *M. tuberculosis* in mouse peritoneal macrophages

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Source Figure : Dhillon et al.2010

The bactericidal activity of bedaquiline on extracellular bacteria was slow and showed no difference between the concentration of 0.006 mcg/mL to 0.12 mcg/mL in which growth was found and the bactericidal concentrations of 0.25 to 32 mcg/mL until 21 days of treatment. With the J774 cells and the primary mouse peritoneal macrophages the bactericidal effect was

evident very early and showed a difference between the concentrations of antibiotic at approximately 5 to 7 days. The rapidity of the bactericidal activity in the intracellular environments is probably influenced by the richness of the medium in which the activity was taking place leading to a shorter lag phase in the growth of the bacteria. The environment in the human body in which the drug is expected to act is also rich. Additionally, there are fewer molecules of ATP within the cell than there are on the outside of the cell resulting in the rapid depletion of intracellular ATP. There appeared to be growth inhibition in a concentration dependent manner. Table 19 shows a comparison of the decrease in the bacterial count (CFU) of *M. tuberculosis* strain *H37Rv* when treated with bedaquiline in the 3 different environments – extracellularly, J774 cells and mouse peritoneal cells.

Table 19: Results of the rate of decrease in CFU of *Mycobacterium spp*. during incubation with bedaquiline in the extracellular environment, JM774 cells and mouse peritoneal cells

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Source Table: Dhillon et al, 2010

In another study, the *in vivo* activity of bedaquiline was examined in the same established infection murine model.[13] Female Swiss mice (n = 440) were infected intravenously with the same strain of *M. tuberculosis*, however, the inoculum concentration was 10-fold lower (2 x 10^6 CFU). Two weeks after infection, mice received monotherapy with either bedaquiline (J), rifampin (R), isoniazid (H), pyrazinamide (Z), or moxifloxacin (M) 5 days per week by oral gavage for 2 months. All surviving mice were killed after 2 months of treatment. Untreated mice (n = 40) were used as controls. The survival rate, spleen weight, gross lung lesions, and number of colony forming units (CFU) in the lungs were measured. All untreated mice died between Days 20 and 50. In monotherapy seven out of twenty mice treated with Z died between Days 8 and 22. One mouse each treated with R or H also died between Days 1 to 8 (Table 20).

[13]Ibrahim et al. 2007. Synergistic activity of R207910 combined with pyrazinamide against murine tuberculosis. Antimicrob Agents Chemother. 51: 1011-1015.

Table 20: Survival of mice treated with various antimycobacterial drugs in different treatment groups.

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® = Rifampin 10 mg/kg; (H) = Isoniazid 25 mg/kg. Source Table : Ibrahim et al. 2007

The mean spleen weight of infected mice increased from 149 ± 21 mg to 524 ± 98 mg during the first 2 weeks of infection. At one month of treatment with bedaquiline (R207910), rifampin, isoniazid, moxifloxacin or pyrazinamide, the mean spleen weights decreased from 524 mg to 350 mg. No further reduction in spleen weights was noted at the end of 2 months of treatment. All mice had massive gross (++) lung lesions at 2 weeks of infection. The authors have stated that there was a reduction of lesions after one month of therapy with the different treatment regimens but the data on lung lesion scores for the different treatment regimens were not included. The mean CFU count in the lungs was 7.2 $\log_{10}$ at 2 weeks of infection. At the end of two months of therapy, the bacterial burden in the lungs of mice treated with bedaquiline alone was significantly lower than rifampin, isoniazid, moxifloxacin or pyrazinamide alone ($p<0.01$). In Table 21B monotherapy with bedaquiline (J) is used as a comparator and in 21C bedaquiline in combination with pyrazinamide is used as a comparator.

Table 21: The effect on *M. tuberculosis* strains from treatment with various combinations of antituberculosis drugs in bacterial counts (log_{10} cfu) in the lung after 1 and 2 months

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Source Tables; Ibrahim et al. 2007

2.6.2 Persisters

Lenaerts et al., (2007) undertook a study to identify the location of *M. tuberculosis* persister subpopulation. The authors found that treatment with conventional therapy for tuberculosis for 6 weeks slightly (1.7 log_{10}) reduced the bacilli in the lung but treatment with bedaquiline for 6 weeks usually resulted in sterilization in of both primary and secondary lesions. The few remaining bacilli in some cases were found to be extracellularly located in primary necrotic lesions with incomplete dystrophic calcification. The study used *M. tuberculosis H37Rv*. The drugs used were INH, RIF, PZA and bedaquiline (R207910). The female Hartley, approximately 600 gm guinea pigs were inoculated intra-nasally with a Madison chamber aerosol generation device that delivered 20 to 30 bacteria directly to the lungs of the guinea

pigs. After 30 days of infection, five guinea pigs were sacrificed to determine the bacterial load prior to treatment. The control group of animals were treated for 5 days a week with 1 mL of 40% sucrose. The standard treatment was INH (10 mg/kg) + RIF (12 mg/kg) + PZA (25 mg/kg). This dose was not meant to be equivalent to human doses. Bedaquiline (R207910) was administered at 5, 10,15 mg/kg in 1 mL of 40% sucrose. Six days after the completion of treatment the animals were euthanized and the right cranial lung lobe was homogenized and cultured on Lowenstein-Jensen plates and incubated at 37^0C. After 4 weeks, the growth was assessed. Table 22 shows the bacterial load at the completion of treatment. Of the five infected guinea pigs treated with bedaquiline at 15 mg/kg, two in each the lung and spleen had viable bacteria. In the lung of the animals treated with 10 mg/kg, 2/5 and 1/5 had viable bacteria were isolated from the lung and spleen, respectively. Although the bedaquiline was significantly more effective in eliminating bacteria compared to the standard dose with 5/5 animals resulting in viable bacteria in both the lung and spleen, viable bacteria were isolated from some animals treated with bedaquiline.

Table 22: Bacterial numbers in whole lungs and spleens of drug resistant *M. tuberculosis* in guinea pigs treated with various anti-mycotuberculosis drugs including bedaquiline (R207910).

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Source Table: Lenaertes et al. 2007

Histology was performed on sections of the left cranial lung lobe and the sections were stained with either hematoxylin and eosin or acid-fast stain. The location of the acid-fast bacteria was investigated by examination of 10 random fields of the lung and the primary granuloma. In the primary lesion of the untreated animal AFB were found primarily extracellularly within the uncalcifiied necrotic tissue whereas in the secondary lesions they were found dispersed extracellularly and intracellularly. However, in the bedaquiline treated animal the AFB were found primarily in the acellular rim of the necrotic region. Figure 16 shows bacteria found extracellularly in the acellular uncalcified necrotic tissues in the lung of the guinea pig model.

Figure 16: Location of acid-fast bacilli in the acellular rim of primary granuloma

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.Source Figure: Lenaerts et al. 2007

3.0 IDENTIFICATION AND SUSCEPTIBILITY TEST METHODS AND METHODS FOR THE DETECTION OF RESISTANT ORGANISMS

The methods used in this application for the identification and susceptibility testing are outlined in this section. The applicant defined multi-drug resistant *M. tuberculosis* as confirmed pulmonary infection by an isolate of *M. tuberculosis* resistant to both RMP and INH by previous screening from a TB treatment facility within the preceding 6 months. Subjects infected with extensively drug resistant tuberculosis (XDR-TB) were allowed to enter the trial. Figure 17 shows the algorithm for testing and Table 23 shows the flowchart of microbiology testing. Identification of *M. tuberculosis* is routinely performed only from Day 1 samples.

Figure 17: Test algorithm for Multi-drug resistant *M. tuberculosis*

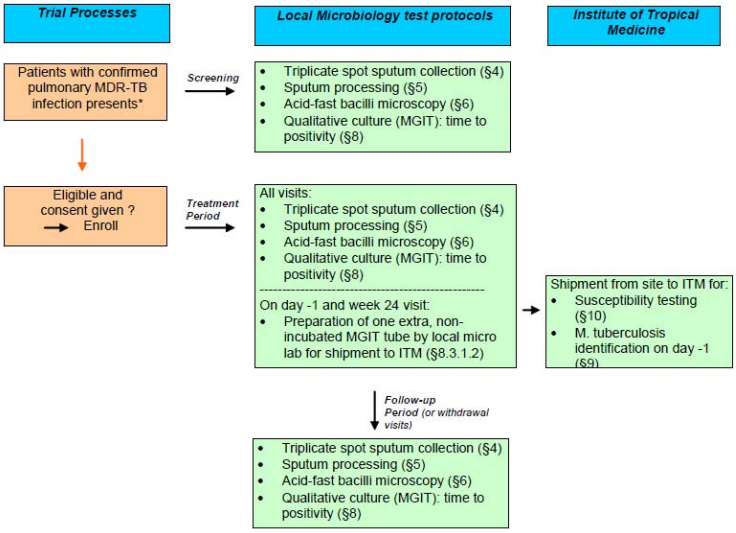

Table 23: Flow-chart for microbiological testing of sputum samples in this study

Test	Lab	Test outcome (and/or wording in CRF)	Screening Visit	Treatment Period TMC207 + BR									Follow-up Period with BR								In case of withdrawal	Follow-up after withdrawal
				Day -1	Day 1	Week 2	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Week 36	Week 48	Week 60	Week 72	Week 84	Week 96	Week 108	Week 120		
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
Previous screening from a TB treatment facility that confirms pulmonary MDR-TB infection.	Local1	Is the patient diagnosed as having resistance to at least both RMP and INH by previous screening from a TB facility? ○ No ○ Yes	Confirmation within 6 months before enrolment																			
1. AFB sputum smear (triplicate) 2. Qualitative sputum culture: time to positivity (triplicate)	Local	1. No AFB seen, Negative – Scanty – 1+ – 2+ – 3+ 2. time to signal or no growth or contaminated or no results	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Preparation of one extra MGIT tube (non-incubated) for refrigerated shipment to ITM	Local	nap		X								X										
M. *tuberculosis* identification	ITM	Identification: M. *tuberculosis* complex or other strain		X																		
Susceptibility testing for - TMC207 - and other TB drugs	ITM	- MIC result - susceptible to/ resistant to		X								X										
Genotyping (only when needed)	UPMC	Relapse or reinfection	will be done once a patient has converted his cultures which become positive again																			

Source Table : NDA 204384

3.1 SPECIMEN COLLECTION AND SHIPMENT

3.1.1 Specimen collection and transport

Three sputum samples were collected at each time point on Day 1 and Week 24 for acid-fast bacteria (AFB) smear, culture and susceptibility testing. The samples were visually examined for acceptability and within 15 minutes of collection, stored in a refrigerator (b) (4).

The specimens were processed in a (b) (4) laboratory and under a biological safety cabinet (BSC) (b) (4) They were decontaminated and liquefied for testing in the MGIT.

Specimens were shipped from the local laboratories to the central laboratory (b) (4) . Table 24 shows the local testing sites and the turn-around-times for specimens sent from the local sites to the central laboratory in (b) (4). On Day1 and Week 24 an additional MGIT tube was inoculated with decontaminated sputum and shipped (b) (4) to the central laboratory (b) (4).

Table 24: List of local testing sites and the turn-around-times from the local sites to the central laboratory (b) (4)

Country	City	Turn around Time (hours)
(b) (4)		

Reference ID: 3225917

3.2 IDENTIFICATION

3.2.1 Acid-fast bacillus microscopy

Two methods were used for microscopy.

(1) The fluorescent stain Auramine O was used to stain the sputum samples. The smear was decolorized with acid alcohol and counterstained with potassium permanganate. A positive mycobacterium result is demonstrated by bright yellow stain against a black background (Figure18). The results are reported semi-quantitatively. Negative smears are counterstained by Zeihl-Neelsen. The sensitivity and specificity of this staining method compared to culture positivity is approximately 75% and 89%, respectively.[14]

(2) Ziehl-Neelsen. Uses Carbol fuchsin which is decolorized with acid alcohol and counterstained with methylene blue. The acid-fast bacilli appear red against a bluish background (Figure 18). The results are reported semi-quantitatively as shown in Table 25. Quality control comprised of *M. tuberculosis* – positive and *E.coli* – negative controls. The sensitivity of this staining method ranges from 86 to 93% and specificity 96 to100%.[14,15]

Table 25: Semi-quantitative reporting for acid-fast bacteria on sputum smears

Auramine O		Ziehl-Neelsen	
Number of AFB seen	Report	Number of AFB seen	Report
None	No AFB seen	None	No AFB seen
1-9 per 10 fields	Scanty	1-9 per 100 fields	Scanty
1-9 per field	1+	1-9 per 10 fields	1+
10-90 per field	2+	1-9 per field	2+
> 90 per field	3+	> 9 per field	3+

[14] Tarhan et al. 2003. Comparison of auramine-rhodamine and Erlich-Ziehl-Neelsen staining methods for the diagnosis of tuberculosis] Mikrobiyol Bul. 37 131-136.

Figure 18: Auramine-Rhodamine and acid-fast stains of mycobacteria

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3.2.2 Qualitative culture of sputum samples

These were performed in the Mycobacteria Growth Indicator Tube (MGIT) 960 for detection of growth of mycobacteria and for susceptibility testing. The BACTEC 960 holds 960 tubes in three drawers A, B, and C each inoculated tube is entered into a specific station that is recorded in the instrument.

The sputum samples are inoculated into 7 mL of liquid media contained in MGIT tubes that are imbedded with a fluorescent sensor (tris 4, 7-diphenyl-1, 10-phenonthroline ruthenium chloride pentahydrate) at the base of the tube. The medium is supplemented with OADC and antibiotics - Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim, Azlocillin (PANTA). The antibiotics inhibit the growth of contaminating bacteria and fungi allowing the slowly growing mycobacteria to grow unimpeded.

The fluorescence that occurs when growth appears in a tube is controlled by the amount of oxygen in the tube. In uninoculated tubes with no growth, the oxygen binds the fluorescent material and quenches the fluorescence. Micro-organisms growing in the tube deplete the oxygen and cause the fluorescent compound to emit light. A photo sensor in the instrument measures the amount of light emitted. The emitted light corresponds to the amount of oxygen utilized by the organism.

The inoculated MGIT tubes are placed in racks and read on a continuous cycle of 60 minutes. A row of light emitting diodes (LED) below the tubes activate the sensors and readings are taken. A positive tube is detected almost immediately when a cutoff value of 75 growth units (GU) is reached. When a tube is flagged the technologist extracts the tube and confirms that it is a true positive by turbidity or the presence of granules and an AFB smear. The growth is cultured on solid medium and tests for identification of the growth commence. At a GU of 75 the *M. tuberculosis* growth in the tube approximates 10^5 to 10^6 CFU/mL of medium. A true negative is reported if a tube remains negative for 42 days. In this study, a positive result was assigned to tubes that gave a signal in < 42 days and the growth was identified as *M. tuberculosis.* Contaminated tubes also became positive in < 42 days but the growth was not identified as *M. tuberculosis*.

Reference ID: 3225917

When a tube became positive, as indicated by fluorescence in the MGIT, the isolates were identified according to the protocol of the Institute of Tropical Medicine (ITM), Antwerp. Initially two MGIT tubes are inoculated with decontaminated sputum sample one is placed in the MGIT instrument at the testing site and the other is sent to ITM where it is incubated at 37^0C. When a true positive signal is obtained from the MGIT the incubated tube at the ITM is used to inoculate two plain Lowenstein Jensen (LJ) medium tubes and one LJ with pyruvate (Stonebrink). All inoculated media are incubated at 37^0C for 4 to 6 weeks and the colonies are tested for identification by a number of tests that include but are not limited to:

- Rate of growth – *M. tuberculosis* takes 3 to 4 weeks to grow on LJ medium
- Pigment production - *M. tuberculosis* does not produce a pigment
- Use of growth inhibitors e.g. (i) thiophene carboxylic acid hydrazide (TCH) *M. tuberculosis* not inhibited (ii) Hydroxylamine - *M. tuberculosis* inhibited
- Biochemical tests such as urease and tween 80 hydrolysis, catalase and nitrate reduction tests.

Table 26 shows the sensitivity of culture systems, liquid and solid, with respect to the isolation of *Mycobacterium* species. The table shows that the liquid media have an 88% and 91% sensitivity compared to solid media 76% .[15] The shortest time to detection of growth occurred in MGIT 960 (Table 27).

Table 26: Sensitivity of culture systems according to *Mycobacterium species*

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Source Table: Cruciani et al. 2004,

[15] Cruciani et al. 2004. Meta-Analysis of BACTEC MGIT 960 and BACTEC 460 TB, with or without Solid Media, for Detection of Mycobacteria. J. Clin. Microbiol. 42: 2321–2325

Reference ID: 3225917

Table 27: Time to detection of *Mycobacterium* species from inoculation of sputum sample.

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One study investigated 2,567 consecutive sputum samples to determine if mycobacteria were present[16]. The study showed that the contamination rate of the egg based Lowenstein-Jensen medium was 17% whereas that of MGIT 960 was 10% and that of the BACTEC 460 was 3.7% The same study showed that the isolation rate for *M. tuberculosis* on the MGIT 960 was 88%, BACTEC 460 92% on Lowenstein-Jensen 74%.

3.3 ANTI-MYCOBACTERIAL SUSCEPTIBILITY TESTING

3.3.1 Drug susceptibility Testing

Agar proportion method.
It is necessary in the discussion of anti-mycobacterium susceptibility testing to make reference to the agar proportion testing method. The agar proportion method is referred to in the Clinical and Laboratory Standards Institute (CLSI) document Susceptibility Testing of Mycobacteria, Nocardiae and other Aerobic Actinomycetes: Approved Standard- 2nd ed 2011 (CLSI M24-A2). The agar proportion method is used as the standard against which new susceptibility tests for anti-mycobacterium drugs are measured. The agar proportion method on these media, 7H10 and 7H11, is not recommended for PZA since PZA is active in an acid environment in which many tubercle bacilli cannot survive.

[16] Tortoli et al. 1999. Use of BACTEC MGIT 960 for Recovery of Mycobacteria from Clinical Specimens: Multicenter Study J Clin Microbiol. 37:3578–3582.

Tortoli et al. (2002) first evaluated the BACTEC MGIT 960 system for susceptibility testing with the BACTEC 460TB method and the agar plate method using Middlebrook 7H11. Four anti-tuberculosis drugs were tested - streptomycin, rifampin, isoniazid and ethambutol. The author tested 133 primarily clinical strains that were confirmed as *M. tuberculosis* by DNA probe, niacin and nitrate reduction tests. The test strains included 120 fresh clinical isolates, eight resistant strains and four reference strains each resistant to one of the major anti-mycobacterium agents (ATCC 35820, streptomycin resistant; ATCC 35822, isoniazid resistant; ATCC 35838, rifampin resistant; ATCC 35837, ethambutol resistant), and the fully susceptible *H37Rv* reference strain (ATCC 27294). Strains that were resistant were tested twice. The agar proportion method was used to resolve discrepancies.

Middlebrook 7H11 plates were used with Sensi discs impregnated with the antibiotics - streptomycin, 2 and 10 mcg/mL of agar; isoniazid, 0.2 and 1 mcg/mL of agar; rifampin, 5 mcg/mL of agar; ethambutol, 5 and 10 mcg/mL of agar. Purity plates were set up from the broths on 7H11. The results showed that there was complete agreement in 106/133 strains. There was a higher prevalence of resistance in MGIT 960 but the majority of discrepancies were due to contamination of the MGIT 960 broth. Table 28 shows the results of sensitivity and specificity of susceptibility testing for BACTEC MGIT 960 and BACTEC 460TB. The sensitivity was defined as the ability to detect resistance and specificity (defined as the ability to detect susceptibility). The comparator was the "gold standard" agar proportion method. Both instruments performed well; the sensitivities and specificity ranged from 96% to 99% and 82 % to 100% respectively for MGIT 960 and 99% to 100% and 82% to 100% for the BACTEC 460TB.

Table 28: Sensitivity and specificity of the BACTEC MGIT 960 and 460TB systems

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Source Table :Tortoli et al. 2002

Agar method

Susceptibility of *M. tuberculosis* to bedaquiline was first determined by measuring the minimal inhibitory concentration (MIC) of bedaquiline using the agar proportion method on solid medium 7H11. Equal volumes of the organism subcultured in 7H9 broth supplemented in OADC were diluted to a concentration of McFarland # 1 standard. The concentration of bacteria tested in the drug-containing medium was 0.1 mL of the undiluted suspension, 10^{-1} and 10^{-2}. The concentration of bacilli added to the drug-free control was 0.1 mL of 10^{0}, 10^{-1},

10^{-2} 10^{-3}. The final concentrations of the test drug in the 7H11 media were 2, 1, 0.5, 0.25, 0.12, 0.06, 0.03, 0.015 and 0.008 mcg/mL.

Susceptibility was defined as the lowest concentration of bedaquiline (R207910) that inhibited the growth of 99% of *M. tuberculosis.* The susceptibility or resistance of the bacteria was determined based on the percentage of growth on the drug-containing medium compared to the drug-free medium. *Mycobacterium* growth in the presence of drug >1% of the control indicated resistance. Susceptibility was described as growth ≤ 1%.

The bedaquiline MIC on 7H11 was equal to, or higher than 0.48 mcg/mL. The growth by the agar proportion method is quantitated as follows:

Confluent (500 or more colonies)	4+
Almost confluent (200 to 500 colonies)	3+
100 to 200 colonies	2+
50 to 100 colonies	1+
Less than 50 colonies	Actual count

The author also performed susceptibility testing of bedaquiline using 7H11 in another way. bedaquiline was diluted in DMSO to obtain 0.0075 mcg/mL, 0.015 mcg/mL, 0.03 mcg/mL, 0.06 mcg/mL, 0.12 mcg/mL, 0.24 mcg/mL, 0.48 mcg/mL. For each isolate there was a set of all bedaquiline dilutions and a set of drug-free control tubes. The inoculum used in all bedaquiline drug-containing dilutions was 0.1 mL of 10^{-0}, and 10^{-1} and for the control tubes 10^{-0},10^{-1},10^{-2} ,10^{-3}. The inoculated 7H11 tubes were incubated at 37^{0}C in 5% O_2 for up to 21 days while for the growth control tubes incubation was allowed up to 42 days. The percentage of bacteria that grew on each drug concentration tube, the resistant bacteria, was calculated. The MIC was defined as the lowest concentration of the drug that inhibits 99% of the growth. The applicant calculated the MIC using the following formula:

$$\textbf{RATIO COLONIES} = \frac{\textbf{Colony count on drug medium at } 10^{-0}}{\textbf{Colony count on control medium at } 10^{-2}} = \textbf{MIC (if} \leq \textbf{1\%)}$$

For each concentration tested:
- If the RATIO COLONIES is ≤ 1%, this concentration is the MIC.
- If the RATIO COLONIES is > 1%, this concentration is not the MIC and the following concentration has to be read. The control strain was *H37Rv*.

<u>REMA method</u>
A third method used in the study for determining MIC was performed using 7H9-S liquid medium - Resazurin Microtiter Assay (REMA) supplemented with OADC. The REMA method is a rapid colorometric anti-mycobacterium susceptibility test particularly for multi-drug resistant strains. The test is conducted in a microtiter plate. A study of the efficiency of

Reference ID: 3225917

the test to detect the activity of first–line drugs was undertaken by Palomino et al., in 2002. The researchers used 80 clinical isolates of *M. tuberculosis* to evaluate their susceptibility to INH and RIF and compared the results to those of the proportion method. Sixty-five of the samples were obtained from subjects who lived in Peru where there is a high prevalence of MDR-TB and 15 samples were obtained from a TB reference laboratory in Bolivia. The concentrations of INH used ranged from 0.03 mcg/mL to 1.0 mcg/mL and for RIF 0.06 mcg/mL to 2.0 mcg/mL. The inoculum comprised fresh Lowenstein-Jensen isolated strains diluted to the turbidity of McFarland # 1 standard concentration of bacteria and were incubated for up to 42 days and then further diluted 1:20. One hundred microliters of both diluted drug and inoculum were added to each well and incubated for 7 days. Positive results were indicated by a change in color of reasurin. Table 29 shows the empirical critical concentration of the drugs used in the REMA assay. A growth control that contained no antibiotic was included along with a sterility control.

Table 29: Empirical critical concentrations used to test the activity of first and second-line anti-TB drugs in the REMA assay

Drugs	Cut-off point by REMA
INH	0.25 μg/ml
RMP	0.5 μg /ml
EMB	4 μg/ml
SM	1 μg/ml
ETH	2.5 μg/ml
CAP	2.5 μg/ml
OFLO	2 μg/ml
KAN	2.5 μg/ml
LNZ	4 μg/ml
AMX+CVA (AGT)	8 μg/ml+4 μg/ml
CFZ	1 μg/ml
TCZ	2 μg/ml

When the MIC is > to the cut-off point the strain is considered resistant.

Fifty–four of the isolates that were resistant to INH by the agar proportion method resulted in REMA MICs of ≥ 0.25 mcg/mL, 45 of these strains had an MIC of ≥ 1.0 mcg/mL. The agar proportion method showed that 26 strains were susceptible to INH the REMA method showed that 25/26 of these had MICs of ≤ 0.06 mcg/mL. For RIF, 49 strains were resistant by the agar proportion method and had an MIC of 0.5 mcg/mL by the REMA method. The agar proportion method showed that there were 31 strains susceptible to RIF all of which had an MIC of 0.25 mcg/mL by the REMA method. Table 30 shows the results of this study. The sensitivity, specificity and positive predictive values of the REMA were all 100%.

Table 30: Minimum Inhibitory Concentration of Isoniazid and Rifampin for 80 *M. tuberculosis* isolates tested by REMA that were resistant by agar proportion method.

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Source Table : Palomino et al. 2002

In a study by Martin et al., (2003) the efficiency of detecting susceptibility of five second-line drugs was evaluated. The drugs were ethionamide (ETH), kanamycin monosulfate (KAN), capreomycin sulfate (CAP), ofloxacin (OFX), and *para*-aminosalicylic acid (PAS) and clinical isolates of *M. tuberculosis*. The correlation between the REMA results and the proportion method showed complete agreement for KAN, PAS, and OFX and a specificity of 100%, sensitivity ranging from 96.8 % to 100% except for CAP where the sensitivity was 84.2%. The results of the proportion method were available after 3 weeks whereas those for the REMA were available after 8 days.
When small populations (0 to 16%) of resistant strains of *H37Rv* were mixed with susceptible strains of the same tubercle bacilli the REMA method was able to detect the small populations of resistant strains (Figure 19).

Figure 19: Sensitivity of REMA to detect 0 to 16% of resistant strains among susceptible H37Rv *M. tuberculosis*

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Source Figure: Andries et al.

In this study, the REMA test used the same concentration of working drug solutions as the proportion method above. The bacterial concentration matched McFarland #1 standard - approximately 10^8 CFU/mL. Each well in the microtiter plate was inoculated with 100 µL each of 7H9 broth, the specific concentration of bedaquiline (R207910) and a 1:10 dilution of the test bacterium. No drug was added to the growth control (positive). No inoculum was added to

the negative control. The tests were incubated at 37^0C for 7 days then 30 µL of 0.01 to 0.02% sterile reasurin were added and the culture reincubated overnight. Growth in a specific concentration of the drug was indicated by a change in color of the liquid culture medium in the well from blue to pink. The MIC of bedaquiline (R207910) was defined as the lowest concentration of the drug in which there was no change in color i.e. the color of the solution in the well remained blue.

The 7H9 liquid assay is performed in a similar manner to the REMA but with the omission of reasurin. As such, there is no color change to indicate growth instead; growth is measured by the visualization of turbidity.

The radiometric broth method employs the BACTEC 460 instrument to compare the growth index from untreated control cultures to cultures grown in the presence of a drug by the amount of $^{14}CO_2$ produced. The disadvantage of the BACTEC 460 is the regulations associated with the use of radioactive material. This instrument uses ^{14}C labeled substrate. Because of the regulations a system that is non-radioactive BACTEC MGIT 960 system was developed.
For susceptibility testing in the MGIT 960 two tubes are inoculated – a growth control tube, without antibiotic, and a test tube with known quantity of antibiotic. If the drug concentration inhibits growth the tube with the antibiotic will remain negative i.e. show no fluorescence because there is no bacterium to utilize the oxygen and cause fluorescence. On the other hand in the drug-free (growth control) tube the bacteria will grow, utilize the oxygen and the tube will fluoresce.

Andries et al., (2005) measured the activity of bedaquiline against isolates of *M. tuberculosis* that were fully susceptible and isolates that were resistant to first-line drugs individually as well as MDR-TB isolates using a BACTEC 460TB radiometric method.
In the same study by Andries *et al.,* 2005 the *in vitro* activity of bedaquiline against *M. tuberculosis* and other *Mycobacterium* species was measured. Oleic acid-albumin-dextrose-catalase (OADC)-enriched Middlebrook 7H11 agar containing 2-fold dilutions of bedaquiline was used. The agar plates were inoculated with 10^4 to 10^5 CFU and incubated at 30^0C or 37^0C for 3 to 42 days depending on the *Mycobacterium* species. The minimum inhibitory concentration (MIC) defined as the lowest concentration of drug that inhibited 99% growth was measured. The MICs for the reference strain, *M. tuberculosis H37Rv*, and 6 susceptible clinical isolates were ≤ 0.120 mcg/ml (Table 31). The *in vitro* activity of bedaquiline against isolates resistant to isoniazid, rifampin, streptomycin, ethambutol, pyrazinamide or moxifloxacin was similar to that against sensitive isolates. The basis for classifying the strains as resistant was not specified. Low bedaquiline MICs (≤ 0.5 mcg/ml) were observed against other *Mycobacterium* species.
The MIC values of other anti-mycobacterium agents against the *Mycobacterium* species were not provided for comparison.

Table 31: MICs of bedaquiline against different *Mycobacterium* species.

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Source Table: Andries et al. 2005

The authors also measured the activity of bedaquiline against 50 *M. tuberculosis* isolates including isolates of MDR-TB (resistant to at least rifampin and isoniazid) using the BACTEC radiometric system. Standard anti-mycobacterium agents were used as controls. All isolates were inhibited at a concentration of 0.1 mcg/ml bedaquiline (Table 32).

Table 32: Susceptibility of drug-resistant *M. tuberculosis* to two concentrations (0.1 and 0.01 mcg/mL) of Bedaquiline, as measured by the BACTEC radiometric system.

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Source Table : Andries et al. 2005

Hain Test

In trial C208 Stage 1 the MTBDR plus test (Hain) was used for screening of subjects for eligibility for inclusion in the trial. MTBDR plus test is a rapid, slide, molecular test that can detect resistance of *M. tuberculosis* to rifampicin and /or isoniazid from smear-positive sputum

samples or from cultures. The results can be obtained in 5 hours. The sensitivity for rifampin and isoniazid resistance are 95.5% and 81.8% respectively with a specificity of 100% [17]
The MTBDRsl is a molecular assay that detects resistance to ethambutol, aminoglycosides/cyclic peptides and fluoroquinolones using mutation in the respective genes. The sensitivity in detecting resistance to fluoroquinolones is 75.6%, ethambutol 64.2% and kanamycin 100%. The specificity for all three of these drugs is 100%.[18]

3.3.2 Susceptibility testing of pyrazinamide

Susceptibility testing of pyrazinamide in the MGIT 960. *In vitro* pyrazinamide is active only at low pH values (< 6.0) at which many *M. tuberculosis* isolates are inhibited. As such, the agar proportion method is not satisfactory for the susceptibility testing of PZA. Testing of PZA using the BACTEC 460TB is the CLSI currently recommended reference method for PZA susceptibility testing. The BACTEC MGIT 960 PZA kit uses a fluorescent indicator rather than radioactivity. PZA is tested at a concentration of 100 mcg/mL as in the MGIT460TB. The concentration of bacteria used to inoculate the MGIT tube is 0.5 mL of 1:5 suspension of 0.5 McFarland standard while the growth control tube is inoculated with 0.5 mL of a 1:10 dilution of a 0.5 McFarland standard. Blood agar plates are used for specimen sterility controls while a growth control tube (without antibiotic) is incubated for each strain along with each MGIT test tube. The positive quality control organism is *M. tuberculosis* ATCC 27294. Table 33 shows a comparison of the results of PZA testing between BACTEC 460TB and BACTEC 960 PZA. There is at least 88% agreement between the two methods. The reproducibility of the results of PZA susceptibility tests in BACTEC MGIT 960 is 94%. The agreement with CDC challenge testing panel was 96.8% (Becton Dickinson).

Table 33: Comparison of the results of pyrazinamide testing between the BACTEC 460TB and the BACTEC 960

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[17] Huang et al. 2009. Performance assessment of the GenoType MTBDRplus test and DNA sequencing in detection of multidrug-resistant *M. tuberculosis* . J Clin Microbiol. 47: 2520-2524.

[18] Vo et al. 2010. Evaluation of the MTBDRsl test for detection of second 1 line drug resistance in *M. tuberculosis*. J. Clin. Microbiol. 53:4835-4839.

3.3.3 Drug carry-over

The inhibition of bacterial growth *in vitro* by means of high concentrations of an inhibitor was investigated because bedaquiline is highly protein bound. It was therefore important to determine the effect of carry-over so as to prevent the over estimation of the *in vivo* effect of the drug. The carry-over effect can be demonstrated when sputum or organs from infected individuals are cultured for *M. tuberculosis*. Lounis et al. (2008) studied the effect of protein-enriched media on the prevention of carry-over of bedaquiline. The author determined the average MIC (concentration that killed 99% of the initial inoculum resulting in the absence of colonies after 4 weeks) of *M. tuberculosis* where bedaquiline was incorporated into the media. The media used in the study were unsupplemented Middlebrook 7H11 medium, Middlebrook 7H11 agar containing 5% bovine serum albumin (BSA) and Lowenstein-Jensen (LJ) medium. Middlebrook 7H11 medium (with 10% oleic acid, albumin, dextrose, and catalase [OADC]) was used as a control. Bedaquiline was added to the medium to provide three-fold dilutions ranging from 0.001 to 9 mcg/ mL for the Middlebrook media and 1 to 50 mcg/ mL for LJ medium. The average MICs were: 0.03 mcg/ mL for unsupplemented 7H11 agar, 1 mcg/ mL for 7H11 agar supplemented with 5% bovine serum albumin, and 14.33 mcg/ mL for Lowenstein-Jensen medium. Bedaquiline was then added to the inoculum of the mycobacteria suspended in either sputum or phosphate buffered saline rather than to the culture medium. The maximal noninhibitory concentration (MNIC), the highest concentration that resulted in the survival of >99% of the original inoculum, and the MICs were determined. The three-fold concentrations of bedaquiline used for *M. tuberculosis* ranged from 0. 11 mcg/mL to 394 mcg/ mL. The repeated MICs were 0.97 mcg/ mL for 7H11 agar, 32.33 mcg/ mL for 7H11 agar with 5% BSA, and 96.33 mcg/mL for Lowenstein-Jensen medium. The control mycobacteria were *M. smegmatis* and *M. tuberculosis*. The sponsor states that both protein enriched media were able to prevent drug carry-over effects.

3.4 GENOTYPING

Genotyping of *M. tuberculosis* isolates. This protocol was used for four major purposes.

- testing for relapse during the study
- testing for reinfection
- testing for discrimination between relapse and reinfection
- clarifying the accuracy in reporting of a drug susceptibility test (DST) from the central laboratory (b) (4) for a patient on whom the MGIT test performed locally was negative. This determined if a the DST was generated on the baseline isolate or a contaminant.

(b) (4)

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(b) (4)

[redacted]

4.0 ANTIMICROBIAL INTERACTIONS AND FIXED COMBINATION STUDIES

Some of the studies that describe the effect of drug combinations can be found in reviews of publications by Ibrahim et al. (2007) and Andries et al. (2005) described on pages 31 and 35 respectively of this review.

Reddy et al., (2010), studied the interactions between bedaquiline and SQ109 with each other and with rifampin. SQ109 [*N*-geranyl-*N*_-(2-adamantyl) ethane-1,2-diamine] is a new diamine

anti-tuberculosis drug candidate.[20,21] The mechanism of action of SQ109 is not well known but it is found to be active at the cell wall of *M. tuberculosis*. Both bedaquiline and SQ109 are bactericidal against MDR-TB.[22,23] SQ109 has a half-life of 60 hours at a dose of 300 mg/day (unpublished data). Bedaquiline has a half life of 24 hours at 400 mg/day.[24]

The study showed that SQ109 improved the rate of killing by bedaquiline of *M. tuberculosis H37Rv* between 4-to 8-fold. There was a synergistic effect for both drugs and enhancement of bedaquiline post-antibiotic treatment by 4 hours. The authors found that there was no antagonistic activity when the two drugs were used together. Figure 20 shows that the addition of SQ109 at a concentration of 0.5 X MIC (0.32 mcg/mL) to the culture of *M. tuberculosis* rifampin-resistant strain 2482 (MIC >100 mcg/mL) to bedaquiline (100 mcg/mL) inhibited the growth >99%. Reddy et al. used three strains of bacteria in the study - *M. tuberculosis H37Rv,* the *M.tuberculosis H37Rv* bacterial luciferase reporter strain (pSMT1) [25] and *M. smegmatis* mc^2155 in a checkerboard assay.[26,27]

The MICs were determined by BACTEC 460 radiometric method and microdilution.[28,29] The author investigated synergistic, antagonistic and additive interactions of bedaquiline and

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[20] Protopopova et al. 2005. Identification of a new antitubercular drug candidate, SQ109, from a combinatorial library of 1,2-ethylenediamines.J. Antimicrob. Chemother. 56**:**968–974.

[21] Lee et al. 2003. Combinatorial lead optimization of [1,2]-diamines based on ethambutol as potential antituberculosis preclinical candidates. J. Comb.Chem. 5:172–187.

[22] Chan et al. 2004. In vitro post antibiotic effects of rifapentine, isoniazid, and moxifloxacin against *M. tuberculosis*. Antimicrob. Agents Chemother. 48:340–343.

[23] Ibrahim et al. 2007. Synergistic activity of R207910 combined with pyrazinamide against murine tuberculosis. Antimicrob Agents Chemother. 51: 1011-1015.

[24] Andries et al. 2005. A diarylquinoline drug active on the ATP synthase of *M. tuberculosis.* Science. 307: 223-227.

[25] Snewin et al. 1999. Assessment of Immunity to Mycobacterial Infection with Luciferase Reporter Constructs. Infect. Immun. 67:4586 – 4593.

[26] Chen et al. 2006. Synergistic interactions of SQ109, a new ethylene diamine with front-line antitubercular drugs in vitro. J. ournal of Antimicrob. Chemother. 58: 332–337,

[27] Heifets, L. B. (ed.). 1991. Drug susceptibility in the chemotherapy of mycobacterial infections, CRC Press, Boca Raton, FL.

[28] Wallace et al. 1986 Susceptibility testing of slowly growing mycobacteria by a microdilution MIC method with 7H9 broth. J. Clin. Microbiol. 24:976–981

[29] Luna-Herrera et al. 1995. In vitro activity of the benzoxazinorifamycin KRM-1648 against drug-susceptible and multidrug-resistant tubercle bacilli. Antimicrob. Agents Chemother. 39:440–444.

SQ109, bedaquiline and RIF, SQ109 and RIF. Table 36 shows the synergistic activity of the two-drug combinations. The MIC by broth microdilution for *H37Rv* was 0.5 mcg/mL for SQ109, 0.25mcg/mL for bedaquiline, and 0.0078 mcg/mL for RIF.
By BACTEC the MICs were 0.25 mcg/mL (SQ109), 0.062 mcg/mL (bedaquiline) and 0.062 mcg/mL (rifampin). The MICs by BACTEC were lower for SQ109 and bedaquiline but higher for rifampin. The fractional inhibitory index ΣFIC showed an increase in bedaquiline inhibitory activity for *H37Rv* of 4-fold in one assay and 8-fold in the other assay. The combination of bedaquiline and RIF showed no change in the inhibitory activity of either drug but RIF did show synergism when combined with SQ109. None of the combinations was antagonistic.

Figure 20: Growth profile of Rifampin resistant *M. tuberculosis* 2483 treated with rifampin and SQ109

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Table 36: Synergy between SQ109, bedaquiline, and rifampin in two-drug combinations against *M. tuberculosis*

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Source Table Reddy et al. 2010

In the three–drug combination the concentrations of RIF that were added to the checkerboard titrations of bedaquiline and SQ109 were: 0.5 X, 0.2 X, 0.1 X and 0.05 X the MIC. RIF showed indifference when added to the combination of bedaquiline and SQ109.
The rate of killing by single and combination drugs was assessed using a relative light unit drugs was assessed using a relative light unit (RLU) assay. The rate of killing was defined as the time taken for a drug to eliminate >90% of the initial inoculum of bacteria or to decrease the initial metabolic activity by >90%. The organism used was the *M. tuberculosis H37Rv* bacterial luciferase reporter strain (strain pSMT1). A decrease of > 90% in metabolic activity was achieved by 4 X and 8 X bedaquiline MIC by Day 1. The bacterial count was 0.003% of the inoculum by the end of Day 2. Killing occurred in Day 1 even in the combination with 2 X MIC of bedaquiline. Combinations with RIF did not increase the killing more than each single drug alone. Table 37 shows the results of the drugs on *M. tuberculosis H37Rv*. The time-kill activity was also checked by a growth index test using the BACTEC 460 radiometric growth assay. Table 38 shows the results of the radiometric growth assay. Table 39 shows the rate of killing by $\log_{10}$ CFU counts of *M. tuberculosis*.

Table 37: Rate of killing of *M. tuberculosis* pSMT1 by SQ109, bedaquiline, or rifampin, alone and in combination, assessed as RLUs

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Source Table : Reddy et al. 2010

Table 38: Rate of killing of *M. tuberculosis* by SQ109, bedaquiline, and rifampin alone and combination, assessed by BACTEC
radiometric growth assay

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Source Table : Reddy et al. 2010

Table 39: Rate of killing of *M. tuberculosis* determined from log_{10} CFU counts

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Source Table : Reddy et al. 2010

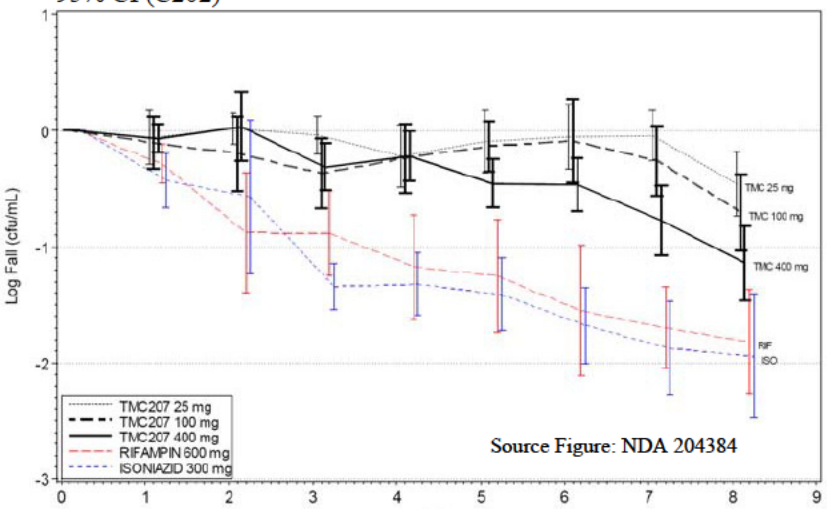
5.0 CLINICAL STUDIES

Phase I safety, tolerability and pharmacokinetic studies were conducted with bedaquiline in 265 healthy individuals in 8 single dose trials with doses as high as 800 mg. In another study 57 subjects were enrolled in 3 multiple dose trials with once daily doses of up to 400 mg for a maximum duration of administration of 15 days. Phase I trials were also conducted with 16 HIV – 1 positive individuals.

5.1 STUDY C202

Phase IIa (C202) is a randomized, open-label clinical trial that assessed the early bactericidal activity of bedaquiline, The study involved 75 treatment naïve individuals with sputum smear positive pulmonary TB. In that dose ranging study 45 subjects were divided into 3 bedaquiline treatment groups and 30 were assigned to two control groups treated separately with rifampin or isoniazid. Individual bedaquiline doses of 25 mg, 100 mg or 400 mg were administered daily for 7 days. The bactericidal activity was measured by the number of CFU in the sputum compared to the baseline count. The mean daily values for the change in CFU over the 7 day period for the 25 mg , 100 mg, 400 mg of bedaquiline were –0.01, -0.04, and –0.11 log_{10} CFU/day while the respective 7 day changes in CFU were –0.04, -0.26, and –0.77 respectively. The values for the control groups were –0.24 log_{10} CFU/day for the rifampin group and –0.27 log_{10} CFU/day for the isoniazid group. Figure 21 shows the daily changes in sputum CFU counts. The dose of 400 mg was most effective.

Figure 21: Changes in $\log_{10}$ sputum CFU counts from baseline over time with 95% CI (C202)



5.2 STUDY C208

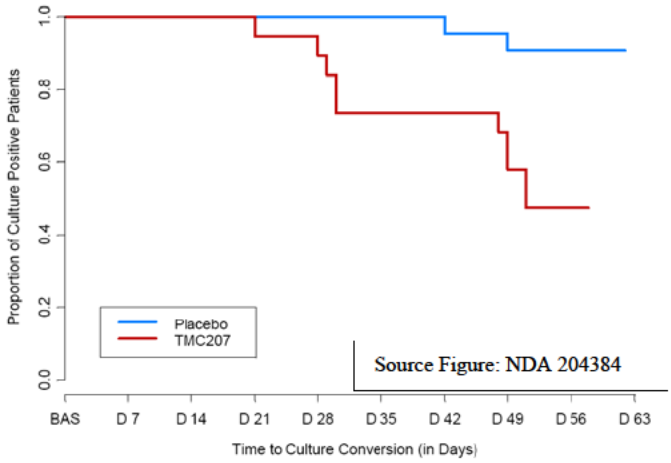
Phase IIB trial (C208) is an ongoing Phase II, randomized, double-blind, placebo-controlled Multi-center trial with bedaquiline. The two stage study was designed to evaluate the anti-bacterial activity, safety and tolerability of bedaquiline in 161 subjects. Stage 1 was exploratory and lasted 8 weeks while Stage 2 was confirmatory and lasted 24 weeks. The study sites were located in Asia, Europe, Latin America and South Africa. The subjects comprised newly diagnosed persons with sputum smear positive pulmonary infection from multi-drug resistant *M.tuberculosis* (MDR-TB). The bedaquiline dosage was 400 mg administered once daily for the first two weeks and then 200 mg three times per week for 6 weeks. The placebo group was treated with a background regimen of ethionamide, kanamycin, pyrazinamide, ofloxacin and terizidone for 8 week. After 8 weeks of treatment with either bedaquiline or placebo both groups received the background regimen for an additional 68 to 88 weeks (a total of 72 to 96 weeks) of treatment. For the confirmatory stage each group of subjects was evaluated at 24 weeks after treatment was initiated.

The primary outcome was the time to sputum culture conversion which was defined as the number of days between the initiation of treatment for MDR-TB and the first of two (≥25 days apart) consecutive negative sputum cultures before the end of the double-blind treatment period. The secondary objective was to determine the number of CFU in the sputum samples. Thirty-four subjects from 2 sites were studied to determine the CFU in the sputum. The overnight sputum samples of the subjects were pooled and cultured. The cultures were tested by MGIT.

When bedaquiline was added to the 5 drug treatment regimen 10/21 subjects in the Stage 1 treatment period became culture negative as compared to 2/23 treated with the background

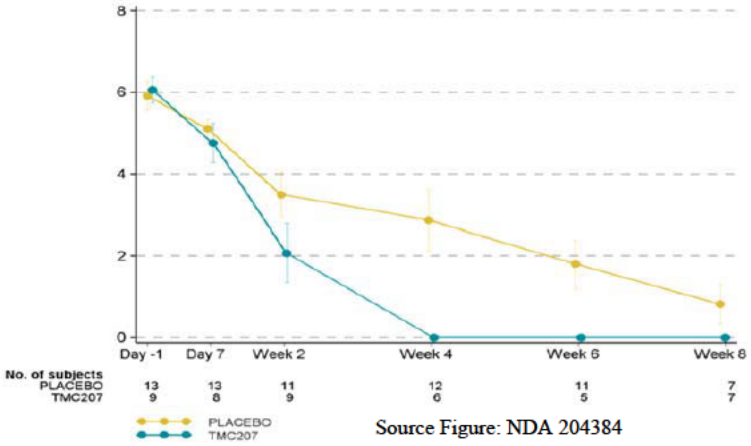
regimen only. The difference was statistically significant (p=0.034). The subjects in the bedaquiline treatment group also converted faster (starting 21 days for bedaquiline versus 42 days for the background regimen) than the background regimen group. Figure 22 shows the time to culture negativity at 8 weeks.

Figure 22: Time to culture negativity by MGIT in subjects treated with background regimen either containing bedaquiline or placebo during the 8 week period. (Study 208)



Only 22/34 subjects selected were eligible for quantitative (CFU) sputum determination; the sputum samples of 10/34 subjects had no bacteria at baseline and 2/34 were positive at baseline only, therefore the applicant determined that the samples of these 12 subjects could not be evaluated and were excluded from that study. Six subjects treated with bedaquiline were negative at 4 weeks and remained negative at 8 weeks. The 12 subjects on placebo had median counts of 3 $\log_{10}$ CFU after 4 weeks of treatment and continued to decrease by 1 $\log_{10}$ by the end of 8 weeks. Four subjects withdrew. Figure 23 shows the mean sputum CFU after 8 weeks. The sputum smears of all bedaquiline treated patients were negative by 4 weeks whereas it took more than eight weeks before most smears from subjects treated with the background regimen only became negative.

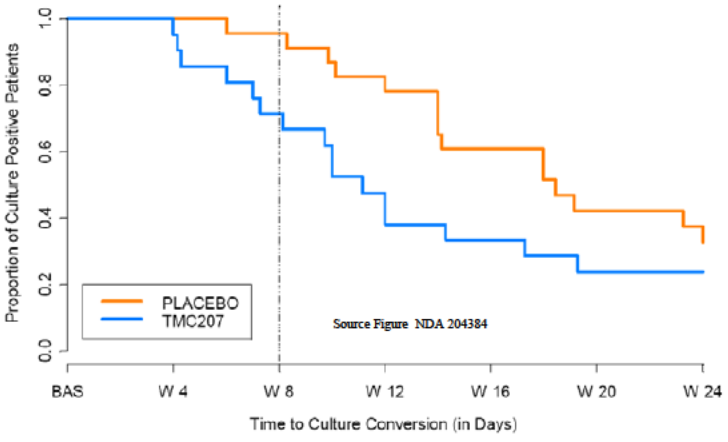
Figure 23: The mean sputum CFU from 22 subjects treated with bedaquiline + background regimen, placebo + background regimen



Source Figure: NDA 204384

Ninety percent of the 6 subjects treated for 8 weeks with bedaquiline plus a background regimen of kanamycin, ethionamide, pyrazinamide, ofloxacin and terizidone and an additional 16 weeks of treatment with the background regimen remained culture negative. This is in comparison to 69.7% of those who were treated with background regimen and placebo from the start of treatment. Figure 24 shows the results of the treatment. The number of subjects in each arm is small and therefore the results should be interpreted with caution. The mean time to positivity was 8 days for both bedaquiline and placebo treated cultures.

Figure 24: Time to culture negativity of subjects treated with background regimen + bedaquiline or placebo for 8 weeks and evaluated at 24 weeks as determined by MGIT



Subjects dropping out during the 24W period are carried forward as not converting

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Susceptibility tests for C208 stages 1 and 2 were performed by two methods the REMA and the 7H11 agar test. Table 40 shows the MIC of baseline isolates tested by 7H11. The baseline was defined as the last sample prior to dosing and the endpoint as the last available sample post-treatment. Paired isolates were collected on 1 bedaquiline treated subject and 9 from the placebo group. The isolate in the bedaquiline group had a 4-fold increase in MIC (0.06 mcg/mL to 0.24 mcg/mL). Of the 9 isolates from the placebo group 2 had decreased MIC, (0.015 to 0.0075 mcg/mL and 0.06 to 0.03 mcg/mL) 3 remained the same at 0.06 mcg/mL (2) and 0.015 mcg/mL(1), 4 isolates had increased MICs (0.06 to 0.12 mcg/mL (2) and 0.015 to 0.024 mcg/mL(2). The MIC_{90} performed on 7H11 agar was ≤ 0.12 mcg/mL.

Table 40: MIC of Baseline isolates of *M. tuberculosis* by the agar method

	TMC207/BR N = 19			Placebo/BR N = 22			All Subjects N = 41		
Baseline TMC207 MIC[a]	**n**	**%**	**$\%_{cum}$**	**n**	**%**	**$\%_{cum}$**	**n**	**%**	**$\%_{cum}$**
0.0075 µg/mL	1	5.3	5.3	1	4.5	4.5	2	4.9	4.9
0.015 µg/mL	2	10.5	15.8	6	27.3	31.8	8	19.5	24.4
0.03 µg/mL	5	26.3	42.1	3	13.6	45.5	8	19.5	43.9
0.06 µg/mL	7	36.8	78.9	7	31.8	77.3	14	34.1	78.0
0.12 µg/mL	2	10.5	89.5	3	13.6	90.9	5	12.2	90.2
0.24 µg/mL	0	0	89.5	2	9.1	100	2	4.9	95.1
0.48 µg/mL	1	5.3	94.7	0	0	100	1	2.4	97.6
> 0.48 µg/mL	1	5.3	100	0	0	100	1	2.4	100

N = number of subjects with data; n = number of subjects with that result; $\%_{cum}$ = cumulative %
Baseline is defined as the last available assessment before first intake of study medication.
[a] The following TMC207 concentrations were tested on solid medium (7H11 agar): 0.0075, 0.015, 0.03, 0.06, 0.12, 0.24, and 0.48 µg/mL. Source Table:: NDA 204384

The baseline MICs by the REMA method are shown in Table 41. The MIC_{90} was similar to that of the agar method (0.125 mcg/mL). Paired samples were obtained on 11 subjects 2 from the bedaquiline group and 9 from the placebo group. Of these, one subject in the test group had a one dilution increase (0.0156 mcg/mL to 0.0313) mcg/mL and one had a one dilution decrease (0.0625 mcg to 0.0313 mcg/mL). A one dilution change in results could also be attributed to technical error. The MICs of 4 of the subjects treated with the placebo had an increase in MIC two of them with MICs > 4-fold increase. There was one subject in whom there was a decrease in MIC and four subjects in whom the MIC remained the same. From study C208 only 3 isolates had > 4-fold increase in MIC over the baseline. Only one treated with bedaquiline showed an increase in MIC > 4-fold. The isolate was resistant to all other drugs at baseline except ofloxacin (OFL) but developed resistance to capreomycin (CAP) and kanamycin (KAN) at 8 weeks. In this study the MICs values obtained by bedaquiline are shown in Table 41. Table 42 shows the MICs obtained by both the agar and REMA methods at baseline. The MIC_{90} for the agar method is higher than that of the REMA method. Table 43

shows a comparison of the results of TB susceptibility tests from study C208B analyzed by Dr. Xianbin Li.

Table 41: MIC of Baseline isolates of *M. tuberculosis* by the REMA method

	TMC207/BR N = 19			Placebo/BR N = 22			All Subjects N = 41		
Baseline TMC207 MIC[a]	**n**	**%**	**$\%_{cum}$**	**n**	**%**	**$\%_{cum}$**	**n**	**%**	**$\%_{cum}$**
0.0078 µg/mL	2	10.5	10.5	2	9.1	9.1	4	9.8	9.8
0.0156 µg/mL	4	21.1	31.6	6	27.3	36.4	10	24.4	34.1
0.0313 µg/mL	6	31.6	63.2	5	22.7	59.1	11	26.8	61.0
0.0625 µg/mL	5	26.3	89.5	5	22.7	81.8	10	24.4	85.4
0.125 µg/mL	0	0	89.5	2	9.1	90.9	2	4.9	90.2
0.25 µg/mL	2	10.5	100	2	9.1	100	4	9.8	100

N = number of subjects with data; n = number of subjects with that result; $\%_{cum}$ = cumulative %
Baseline is defined as the last available assessment before first intake of study medication.
[a] The following TMC207 concentrations were tested in liquid medium (REMA method): 0.0039, 0.0078, 0.0156, 0.0313, 0.0625, 0.125, 0.25, 0.5, and 1 µg/mL. Note that tested concentrations for which n = 0 in all columns are not shown in the table. Source Table:: NDA 204384

Table 42: MIC ranges by method at baseline and follow-up

Parameter	Range of MICs mcg/mL	
	REMA	AGAR
Baseline	0.0078 to 0.25 µg/mL	0.0075 to > 0.48 µg/mL
Follow-up	0.0156 to 0.0625 µg/mL	0.0075 to 0.24 µg/mL
MIC_{50}	0.0313	0.06
MIC_{90}	0.125	0.12

Table 43: Comparison of results 0f C208 by Agar proportion and REMA methods

MIC(MINIMAL INHIBITORY CONCENTRATION)	MSMETHOD(METHOD OF TEST OR EXAMINATION)		
Frequency	AGAR PROPORTION	REMA	Total
0.0039 UG/ML	0	44	44
0.0075 UG/ML	24	0	24
0.0078 UG/ML	0	92	92
0.015 UG/ML	196	0	196
0.0156 UG/ML	0	294	294
0.03 UG/ML	349	0	349
0.0313 UG/ML	0	534	534
0.06 UG/ML	572	0	572
0.0625 UG/ML	0	325	325
0.12 UG/ML	198	0	198
0.125 UG/ML	0	52	52
0.24 UG/ML	24	0	24
0.25 UG/ML	0	34	34
0.48 UG/ML	24	0	24
>0.48 UG/ML	2	0	2
Total	1389	1375	2764
Frequency Missing = 6884			

Source Table : Dr Xianbin Li

5.2.1 Treatment effect on susceptibility of drugs to background regimen

The placebo comprised a background regimen – cycloserine (CS).ethionamide (ETH), kanamycin (KAN), ofloxacin (OFL) pyrazinamide (PZA), and terizidone (TRD). Resistance to drugs in the background regimen was determined by the agar proportion method except for pyrazinamide which was performed by MGIT 960 as outlined in susceptibility methodology. Paired samples were available for one subject in the bedaquiline treatment group and 10 in the placebo group. The one bedaquiline treated subject had been diagnosed as having MDR-TB at baseline and was shown to develop pre-extensively drug-resistant *M. tuberculosis* (pre-XDR-TB) at the endpoint. Three placebo treated subjects who were also diagnosed with MDR-TB at baseline also became pre-XDR-TB at the endpoint. Pre-XDR-TB is defined as MDR-TB (resistant to INH and RIF) with resistance to at least one of the second-line injectable drugs (AMK, KAN, CAP) or to any fluoroquinolone but not to anti-tuberculosis drugs in both groups.

5.2.2 Other observations from trial C208

Nine subjects, (two bedaquiline and 7 placebo treated) failed to convert. All of these subjects had prematurely discontinued treatment. Six of the subjects had no post-baseline samples, two placebo subjects were diagnosed with pre-XDR-TB at baseline. At baseline both bedaquiline non-converted subjects showed resistance to PZA. Cavitation was present in 1/2 bedaquiline treated and all 7 of the placebo treated subjects.

There were six subjects who relapsed. All of the subjects showed resistance to PZA at baseline. Cavitation >2 cm was noted in both bedaquiline treated subjects and 2/4 placebo treated subjects.

5.3 STUDY C209

Bedaquiline-TiDP13-C209 was an open label, unblinded multi-center trial of bedaquiline administered as part of a MDR-TB regimen. The major objective was to evaluate safety, tolerability, and efficacy of bedaquiline as part of a multi-drug regimen in the treatment of subjects with MDR-TB. Specifically evaluating the efficacy of treatment with 2 fixed doses of bedaquiline using the BACTEC 460. The secondary objective was to determine the MIC of a set of *M. tuberculosis* drug-resistant and drug-susceptible strains. The subjects were treated with bedaquiline in addition to the subject's individualized background regimen. The sponsor proposed to treat 225 smear-positive MDR-TB subjects for 24 weeks with bedaquiline comprising 400 mg once daily for two weeks and 200 mg three times per week for the next 22 weeks. The subjects were then followed for 24 weeks while being treated with only the background regimen. Microbiology assessment included smears, cultures in a MGIT and solid media susceptibility tests by REMA and agar from samples collected as spots. Microbiological efficacy was determined as cure, treatment completed, death, treatment default, treatment failure and transfer out and relapse.

The inoculum comprised 0.1 mL of a McFarland Standard #1 suspension of growth inoculated into the BACTEC vial. The control consisted of 0.1 mL of a 1/100 bacterial suspension in phosphate buffered saline (PBS) added to the BACTEC vial. The concentration of bedaquiline in the test medium was 0.1 mcg/mL and 0.01 mcg/mL. The following drugs and concentrations were also used as comparators in the study. Rifampin (RIF): 2 mcg/mL, Isoniazid (NH): 0.2 mcg/mL, Ethambutol (EMB): 5 mcg/mL, Streptomycin (SM): 4 mcg/mL. Table 44 lists the resistant patterns of the bacterial strains tested with the 2 concentrations of bedaquiline. Ten isolates were drug-susceptible, 10 were mono-, dual-, or triple-resistant, and 30 were multi-drug resistant. Table 45 shows the distribution of strains that were susceptible to 0.1 mcg/mL and 0.01 mcg/mL of bedaquiline. All of the susceptible strains (10/10) of *M. tuberculosis* were susceptible to 0.1 mcg/mL of bedaquiline but only 1/10 was susceptible to 0.01 mcg/mL. All of the resistant strains were susceptible to 0.1 mcg/mL of bedaquiline. However, > 50% of the resistant strains were also susceptible to 0.01 mcg/mL of bedaquiline.

The susceptibility of clinical isolates to bedaquiline was also determined using 7H10 agar. The inoculum, the same as above (1.0 µL of a McFarland #1 standard) was inoculated on to a 10% OADC-enriched 7H10 plate and incubated for 21 days. The results show that there was no difference in susceptibility between the isolates since both susceptible and resistant isolates had a MIC of ≤ 0.128 mcg/mL. Additionally, all except one of 85 isolates were inhibited by ≤ 0.064 mcg/mL of bedaquiline. Table 46 shows the MICs against susceptible and drug-resistant strains of *M. tuberculosis* performed by agar and BACTEC 460 methods.

Table 44: Resistance patterns of the 50 *M. tuberculosis* clinical isolates tested with two fixed concentrations of Bedaquiline.

Strain ID	RIF	INH	STR	EMB	Susc	Origin
P 24/94	R	R	R	R	MDR	Estonia
P 8/94	R	R	S	S	MDR	Estonia
R 12/95	R	R	R	R	MDR	Russia
S 148/97	R	R	R	R	MDR	Estonia
S 156/97	R	R	R	R	MDR	Estonia
S 39/95	R	R	R	R	MDR	Nepal
S 42/95	R	R	R	R	MDR	China
S 46/95	R	R	S	S	MDR	Somalia
S 96/77	R	R	R	S	MDR	Sweden

Source Table : NDA204384

Table 45: Susceptibility of Drug-sensitive and Drug-resistant *M. tuberculosis* to Two concentrations of Bedaquiline, as measured by the BACTEC radiometric system

Resistance pattern Median MIC	Number of strains	Number of strains inhibited by 0.1 µg/ml	Number of strains inhibited by 0.01 µg/ml
M. tuberculosis, fully susceptible isolates	10	10	1 (10%)
M. tuberculosis, resistant isolates	40	40	22 (55%)
Multidrug-resistant *M. tuberculosis* (resistant to at least RIF and INH)	30	30	17 (57%)
M. tuberculosis resistant to INH, RIF, SM and EMB	13	13	8 (62%)
M. tuberculosis resistant to INH	38	38	20 (53%)
M. tuberculosis resistant to RIF	30	30	17 (57%)
M. tuberculosis resistant to SM	25	25	15 (60%)
M. tuberculosis resistant to EMB	20	20	12 (60%)

Source Table : NDA204384

Table 46: MICs of bedaquiline against susceptible and resistant strains of *M. tuberculosis* by BACTEC 460 and agar

MIC (µg/ml)	SIRE-S Strains (41 in total)	MDR Strains (Total 44)	All Strains (Total 85)
0.002	1	0	1
0.004	1	1	2
0.008	0	2	2
0.016	8	17	25
0.032	22	13	35
0.064	9	10	19
0.128	0	1	1
0.256	0	0	0

Source Table : NDA204384

C209 included another study designed to determine the MIC of bedaquiline against drug-sensitive and drug resistant *M. tuberculosis* clinical isolates and to determine the susceptibility of the mutant BK12 which is resistant to bedaquiline. Bk12 was developed from *H37Rv* in the presence of bedaquiline. The authors tested the MICs with the concentrations of antibiotics shown in Table 47.

Table 47: Concentration of drugs used to test *M. tuberculosis* strains.

Drug	Concentration (mcg/mL)
Bedaquiline	S = 0.0015 to 1 R = 0.003 to 16
Rifampin	S = 0.05 to 4 R = 2 to 256
Ofloxacin	0.25 to 64
PA-824	0.007 to 1
Isoniazid	0.015 to 16
Ethambutol	0.5 to 32
Streptomycin	0.03 to 8
Amikacin.	0.03 to 8
Moxifloxacin	: 0.03 to 8

R = resistant strains, S = sensitive strains Source Table : NDA204384

The MICs of the following *M. tuberculosis* strains were tested *H37Rv* strain, 6 fully susceptible clinical isolates, 7 INH-resistant clinical isolates, 1 RIF-resistant clinical isolate, 2 INH and RIF-resistant clinical isolates, 1 INH and SM-resistant clinical isolate, 1 EMB-resistant clinical isolate, 1 PZA-resistant clinical isolate, 2 fluoroquinolone-resistant clinical isolates. In addition, 1 clinical isolate of *M. bovis* was tested.
The inoculum of 10^2 to 10^3CFU/mL was plated on 7H11 supplemented with 10% OADC. The MIC of the strains tested are shown in Table 48. The MICs of the susceptible strains ranged from 0.03 to 0.06 mcg/mL. The bedaquiline MICs for resistant INH and RIF strains were also 0. 03 to 0.06 mcg/mL. But the MICs of strains that were resistant to PZA, SM, EMB and FQ ranged from 0.01 to 0.12 mcg/mL.

Agar method. The susceptibility cut-off was < 1% for all antibiotics except PZA. For PZA the cut-off was < 10% difference of CFU between the drug-free medium and the drug-containing medium of Lowenstein-Jensen. The inoculum was diluted to 10, 1000 and 100,000-fold from which 0.2 mL was plated on both the drug-free and drug-containing (INH, RIF, SM, EMB or PZA) media. It should be noted that susceptibility tests of PZA on agar might not be accurate. The concentrations of drug tested were INH: 0.1, 0.2, 1 and 10 mcg/mL, RIF: 40 mcg/mL, SM: 4 mcg/mL, EMB: 2 mcg/mL, PZA: 200 mcg/mL. Table 48 shows the MIC of bedaquiline that inhibited 99 % of the growth. of *M. tuberculosis* strains that were susceptible and resistant to other anti-mycobacterium drugs. The MICs ranged from 0.03 to 0.12 mcg/mL. for both drug susceptible and drug resistant strains.

Table 48: MICs of bedaquiline that inhibited 99% of the growth of different strains of *Mycobacteria*

Mycobacterial species	Number of Strains	Range of MICs for multiple strains (µg/ml)	Median MIC (µg/ml)
M. tuberculosis, H37Rv	1	-	0.03
M. tuberculosis, fully susceptible clinical isolates	6	0.03-0.12	0.06
M. tuberculosis resistant to INH	7	0.03-0.06	0.06
M. tuberculosis resistant to RIF	1	-	0.03
M. tuberculosis resistant to INH and RIF	2	0.03-0.03	0.03
M. tuberculosis resistant to INH and SM	1	-	0.01
M. tuberculosis resistant to EMB	1	-	0.01
M. tuberculosis resistant to PZA	1	-	0.03
M. tuberculosis resistant to FQ	2	0.06-0.12	0.09
M. bovis	1	-	0.003

Source Table : NDA204384

When the clinical history suggested resistance genetic tests were performed to confirm susceptibility or resistance. The genes used for testing resistance to INH were *inhA* and katG., for RIF the gene was *rpoB*, streptomycin *rpsL*, for PZA *pncA* and fluoroquinolone *gyrA.*

5.4 STUDIES C208 Stage 2 AND C209 SELECTED COMBINED RESULTS

The *atp* operon gene from isolates with an increase in titer of at least 4-fold or failed to convert was sequenced. As such, 11 isolates from the bedaquiline arm were sequenced Isolates from subject 208-3004 had a 4-fold increase in titer from baseline to 8 Week but at 24 Week had a successful outcome. There were three samples for 208-4465 with pre-XDR isolates - baseline, 8 Week and 24 Week. The MICs were 0.06 mcg/mL, 0.06 mcg/mL and 0.25 mcg/mL respectively (agar) and 0.015 mcg/mL, 0.015 mcg/mL and 0.25 mcg/mL respectively (REMA). At 24 Week the outcome was failure and the isolate was still classified as Pre-XDR. Isolates 209-0269, 209-0038 also PreXDR at baseline had a 4–fold increase in titer at 24 Week by both methods and failed at 24 Week while retaining the Pre-XDR status. Isolate 209-0263 although initially relapsed had a successful outcome at 24 Week. All of the isolates except one that failed to convert or that originated from subjects who relapsed were Pre-XDR or XDR. Table 49 shows the baseline and assessment time-points and *M. tuberculosis* status of the bedaquiline treated isolates that failed to convert. The table 49 (9) was prepared by Dr. Xianbin Li, statistician, FDA.
There were five subjects in the bedaquiline arm with agar baseline MICs ≥ 0.25 mcg/mL and one in the placebo arm, Of the five subjects three had successful outcomes at Week 24. All except one subject had MDR-TB. The subject with XDR-TB also had a paired sample at Week 24 which resulted in a MIC of 0.5 mcg/mL by both tests. The results are depicted in Table 50.

Table 9: Summary of Subjects' Isolates With at Least 4-Fold Increase in TMC207 MIC and *atp* Operon Sequencing Results (Clinical Trials C208 Stage 1, C208 Stage 2 and C209, mITT Subjects)

Subject CRF ID	Outcome (No Overruling[a] – All Available Data)[b]	MIC (REMA) µg/mL			MIC (Agar) µg/mL			Extent of Resistance at BL	*atp* Operon Variance in Coding Regions Between Strains (BL vs Post-BL)
		BL	Post-BL	F	BL	Post-BL	F		
C208 (Stage 1)									
208-3004	relapse	0.0313	0.0156 (W8)	0.5x	0.06	0.24 (W8)	4x	$MDR_{S&R}$	no coding variation
C208 (Stage 2)									
208-4465	failure to convert	0.0156	0.25 (W24)	16x	0.06	0.24 (W24)	4x	Pre-XDR	no coding variation
C209									
209-0269	failure to convert	0.0625	0.25 (W24)	4x	0.06	0.48 (W24)	8x	Pre-XDR	no coding variation
209-0038	failure to convert	0.0625	0.25 (W24)	4x	0.06	> 0.48 (W24)	> 8x	Pre-XDR	no coding variation
209-0050	failure to convert	0.0313	0.50 (W24)	16x	0.06	0.48 (W24)	8x	Pre-XDR	no coding variation
209-0182	failure to convert	0.0625	0.25 (W24)	4x	0.06	> 0.48 (W24)	> 8x	XDR	no coding variation
209-0265	relapse	0.0313	0.25 (W24)	8x	0.06	0.48 (W24)	8x	XDR	no coding variation
209-0128	failure to convert	0.0625	0.50 (W24)	8x	0.06	> 0.48 (W24)	> 8x	XDR	no coding variation
209-0157	responder	0.0313	0.50 (W24)	16x	0.06	0.48 (W24)	8x	XDR	no coding variation
209-0267	failure to convert	0.0625	1 (W24)	16x	0.06	> 0.48 (W24)	> 8x	XDR	no coding variation

BL = baseline; F = fold increase in MIC compared with baseline (derived from the non-rounded MIC values); W = week
Subjects for whom baseline and post-baseline isolates were genotypically different were excluded from this table.
[a] In the no overruling analysis, the discontinuation information was not taken into account and conversion status prior to discontinuation is used.
[b] All Available Data Selection: analyses for the Phase IIb trials based on this data selection took into account all available data in the interim database (i.e., up to the cut-off date of the analyses for the trials C208 Stage 2 and C209) or in the final database (for trial C208 Stage 1).

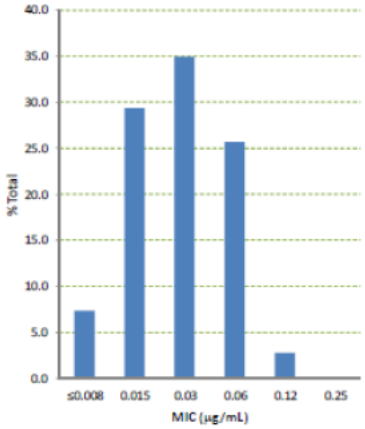
Table 50: Clinical outcome of subjects with Baseline MICs equal to or greater than0.25 cg/mL

Subject Identification No.	TB Status	Susceptibility Test Method		Clinical Outcome
		Agar (mcg/mL)	REMA (mcg/mL)	
208-1204	MDR	0.5	0.15	Success
208-4246	MDR	0.25	0.25	Failure
209-0091	MDR	0.5	0;25	Success
209-0282	MDR	0.5	0.25	Success
209-0311*	XDR	0.5	0.5	Failure

6.0 PROVISIONAL INTERPRETIVE CRITERIA

Information for the provisional interpretive data was drawn from Andries et al., 2007 and the non-clinical study report C209. These studies tested 109 *M. tuberculosis* isolates of which 44 were MDR-TB. Figure 25 shows the distribution of all 109 strains tested by the agar method. From the results of these data the MIC_{90} susceptibility cut-off value is $\leq$ 0.12 mcg/mL

Figure 25: The MIC distribution of 109 strains of *M. tuberculosis* tested by the agar proportion method



Source Figure: NDA 204384

7.0 CORRELATION OF PROVISIONAL INTERPRETIVE CRITERIA WITH CLINICAL OUTCOME

The MIC when tested by the REMA method using a standard inoculum and standard concentration of bedaquiline was ≤ 0.25 mcg/mL and for the agar method on 7H11 the MIC was ≤ 0.5 mcg/mL. Isolates of M. *tuberculosis* with equal or lower MICs to those stated above by method are described as susceptible and have a great possibility of being inhibited *in vivo* by the relevant concentration of the drug at the infection site. In the C208 study the average plasma concentration for a 400 mg dose of bedaquiline after 2 weeks was 1.37 mcg/mL this means that both MICs are lower than the drug concentration in the plasma and at this dose should inhibit the growth of *M. tuberculosis* isolates.

Results of study C208 and C209 show that there was less than 100% correlation between the MICs of the isolates and the clinical outcomes of the subjects. Of the subjects who failed in study C208 none had MICs > 0.5 mcg/mL and of 29 subjects who failed in study C209 two had MICs >0.5 mcg/mL when the susceptibilities were performed on agar. For the REMA method in study C208 the MICs of none of the failures was above 0.25 mcg/mL and only 3/29 in C209 was above 0.25 mcg/mL. One of the subjects who failed had MICs as low as 0.015 mcg/mL (agar) and ≤ 0.008 mcg/mL (REMA). On the other hand none of the subjects with successful outcomes had *M. tuberculosis* MICs > 0.5 mcg/mL (agar) and only one had an MIC > 0.25 mcg/mL(REMA). One patient in the agar group had > 4-fold increase in titer but this did not show in the REMA method. Resistance as defined by MIC values greater than the breakpoints did not appear to play a role in treatment failures. Tables 51, 52, and 53 show the number of isolates for which susceptibility was performed by method, the ranges and the clinical outcomes.

Table 51: Correlation of Provisional of Provisional Interpretive Criteria with Clinical Outcome-Study C208 Stage 1 Evaluation at Week 8 Evaluation from baseline MIC values

Clinical Outcome	7H 11Agar Method		REMA Method	
	No (%)	MIC range (mcg/mL)	No (%)	MIC range (mcg/mL)
Success	16 (84%)	≤ 0.008 – 0.12	16 (84%)	≤0.008 – 0.06
Failure	3 (16)	0.015 - 0.5	3 (16%)	≤0.008 - 0.25

Table 52: Correlation of Provisional of Provisional Interpretive Criteria with Clinical Outcome- Study C208 Stage 2 at Week 24 Evaluation from baseline values.

Clinical Outcome	Agar Method		REMA Method	
	No (%)	MIC range (mcg/mL)	No (%)	MIC range (mcg/mL)
Success	50/52 (75%)	≤0.008-0.5	51/52 (75%)	≤0.008-0.06
Failure	16/16 (24%)	0.015 - 0.25	16/16 (24%)	≤0.008 - 0.25

Table 53: Correlation of Provisional of Provisional Interpretive Criteria with Clinical Outcome- Study C209 at 24 Week Evaluation from baseline values.

Clinical Outcome	Agar Method		REMA Method	
	No (%)	MIC range (mcg/mL)	No (%)	MIC range (mcg/mL)
Success	138/141 (81%)	≤0.008 – 0.5	137/141 (81%)	≤0.008- 0.5
Failure	29 (17%)	0.015 - >1	28 (17%)	≤0.008 – 0.25

7.1 SUSCEPTIBILITY TEST QUALITY CONTROL DATA

M. tuberculosis strain *H37Rv* (ATCC 27294) a pan-susceptible strain was used for quality control (QC). In order to determine quality control ranges *M. tuberculosis H37Rv* was tested against H710 and H711 agar and H 9 broth. There were 18 pieces of quality control data for the REMA tests performed in preclinical studies and 27 for the proportion method. Figures 26 and 27 show the distributions of the data for REMA and proportion methods and Table 54 the compiled susceptibility data. The most common MIC for both the proportion and REMA methods were 0.06 mcg/mL.

Figure 26: MIC distribution of bedaquiline obtained from 18 pre-clinical studies using *M. tuberculosis H37Rv* by REMA methodology

Figure 27:MIC distribution of bedaquiline obtained from 27 pre-clinical studies using *M. tuberculosis H37Rv* by agar proportion methodology

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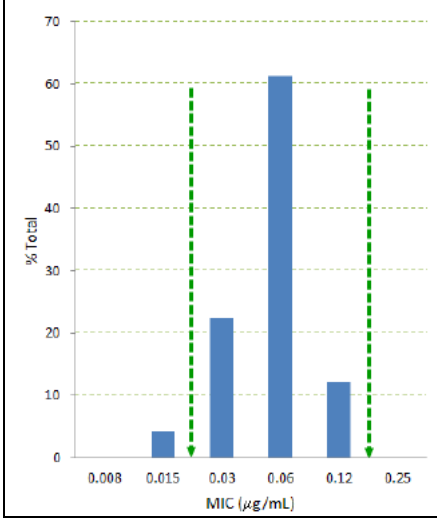
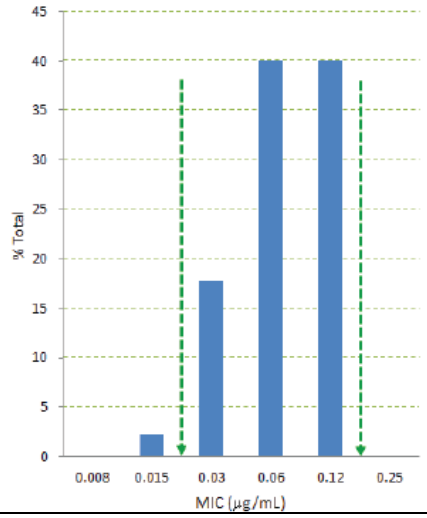
Andries et al., 2005. Study Report

Table 54: Bedaquiline Quality Control Ranges Obtained from Pre-Clinical Studies Using Both REMA and Agar Proportion Methods and *M. Tuberculosis H37Rv*

Organism	Provisional Bedaquiline MIC (µg/mL)	
	Agar	REMA
M. tuberculosis H37Rv	0.03 – 0.12	0.03 – 0.12

Quality control data for the clinical trials were compiled from the results of clinical trials C208 and C209. For the agar method all 18 of the test results were included and the MIC was 0.12 mcg/mL. For the REMA method the concentration to which most strains was susceptible was 0.06 mcg/mL but this included only 29/31 (93.5%) of the strains tested. In order to include 95% of the results a 4-dilution range was used.

To obtain an overall quality control range for the agar method and the REMA methods, the results of the pre-clinical and clinical quality control values for each method were combined. Figure 28 and 29 shows the results of the combined QC data from 45 strains for the agar proportion method and 31 strains for the REMA method, respectively. The data are based on the mode for both methods which was 0.06 mcg/mL.

Figure 28: Combined non-clinical and clinical MIC data of bedaquiline against H37Rv using the REMA method	Figure 29: Combined non-clinical and clinical MIC data of bedaquiline against H37Rv using the agar method
	

8.0 ESTABLISHMENT OF BREAKPOINTS FOR USE IN LABELING

The establishment of breakpoints did not include PK/PD parameters. The MICs were determined using standardized methods in broth such as the microdilution technique, REMA method and Middlebrook 7H11 agar, and standardized inoculum. The data for the establishment of breakpoints were obtained from the results of trials in C208 and C209. When Middlebrook 7H11was used for susceptibility testing no preclinical isolate was found with an MIC for bedaquiline that was > 0.5 mcg/mL therefore the 7H11 agar breakpoint was set at ≤ 0.5 mcg/mL. When the REMA method was used only one isolate resulted in an MIC of > 0.25 mcg/ leading to the REMA breakpoint of ≤ 0.25 mcg/mL. The proposed interpretive criteria for bedaquiline are shown in Table 55.

Table 55: Proposed Interpretive criteria for Bedaquiline

	Bedaquiline MIC (µg/mL)	
	7H11 Agar	**REMA**
Pathogen	Susceptible Only (S)	Susceptible Only (S)
M. tuberculosis	≤ 0.5	≤ 0.25

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/s/

LYNETTE Y BERKELEY
12/04/2012

Product Quality Microbiology Review

25 OCT 2012

NDA: 204-384

Drug Product Name
Proprietary: Sirturo (proposed)
Non-proprietary: Bedaquiline

Review Number: 1

Dates of Submission(s) Covered by this Review

Submit	Received	Review Request	Assigned to Reviewer
29 JUN 2012	29 JUN 2012	02 JUL 2012	05 JUL 2012
12 SEP 2012	12 SEP 2012	N/A	N/A
19 SEP 2012	19 SEP 2012	N/A	N/A
25 OCT 2012	25 OCT 2012	N/A	N/A

Applicant/Sponsor
Name: Janssen Therapeutics, a Division of Janssen Products, LP
Address: 1125 Trenton-Harbourton Road
Titusville, NJ 08560
Representative: Gary Lewis
Telephone: 908-218-6014

Name of Reviewer: Jessica G. Cole, PhD

Conclusion: Recommend approval.

Product Quality Microbiology Data Sheet

A. 1. TYPE OF SUBMISSION: New 505(b)(1) NDA

2. SUBMISSION PROVIDES FOR: New molecular entity NDA with a priority review

3. MANUFACTURING SITE:

Kemwell Biopharma Pvt. Ltd.
34th KM, Tumkur Road,
Teppada Begur,
Nelamangala Taluk,
Bangalore- 562 123 India

4. DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:

- 100 mg uncoated tablet for oral administration

5. METHOD(S) OF STERILIZATION: Non-sterile dosage form

6. PHARMACOLOGICAL CATEGORY: Treatment of multi-drug resistant pulmonary tuberculosis

B. SUPPORTING/RELATED DOCUMENTS: None.

C. REMARKS: This submission is in the eCTD format. The following information was requested in the 16 July 2012 Product Quality Microbiology filing review. The applicant responded on 12 September and 19 September 2012.

Microbiology Comment

1. Provide the verification studies that support the proposed microbiological test methods.

(b) (4)

[redacted]

The following information request was sent to the project manager on 20 September 2012 and a response was received on 25 October 2012.

We refer to your amendment received 12 September 2012, specifically the response to FDA Question #2. The method verification studies requested by the FDA in this question were for the microbial enumeration

Reference ID: 3211868

studies, specifically the method verification studies for USP<61> and <62> assays. Please submit the method verification studies which support your planned release test for microbiological purity.
Provide the maximum duration of the (b) (4) steps in the drug product manufacturing process (b) (4)

Additional Comment: If in the future you propose a reduction in microbial limits testing we recommend that you have a microbial specification for the drug substance at that time.

filename: N202438R1.doc

Reference ID: 3211868

Executive Summary

I. Recommendations

A. Recommendation on Approvability – This application is recommended for approval on the basis of product quality microbiology.

B. Recommendations on Phase 4 Commitments and/or Agreements, if Approvable – Not applicable.

II. Summary of Microbiology Assessments

A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology – This is a (b) (4) . The final tablet is uncoated and meets the USP microbial limits recommendations in USP<1111>.

B. Brief Description of Microbiology Deficiencies – Not applicable.

C. Assessment of Risk Due to Microbiology Deficiencies - Not applicable.

III. Administrative

A. Reviewer's Signature _____
Jessica G. Cole, PhD

B. Endorsement Block _____
Bryan Riley, PhD
Microbiology Team Leader

C. CC Block
N/A

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Reference ID: 3211868

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/s/

JESSICA COLE
11/02/2012

BRYAN S RILEY
11/02/2012
I concur.

MICROBIOLOGY FILING CHECKLIST

NDA Number: 204384 **Applicant: Janssen Therapeutics** **Stamp Date: 06/29/2012**

Drug Name: TMC207 **NDA Type: 505(b)(1) Priority Review**

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comments
1	Is the microbiology information (preclinical/nonclinical and clinical) described in different sections of the NDA organized in a manner to allow substantive review to begin?	X		
2	Is the microbiology information (preclinical/nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin?	X		
3	Is the microbiology information (preclinical/nonclinical and clinical) legible so that substantive review can begin?	X		
4	On its face, has the applicant submitted *in vitro* data in necessary quantity, using necessary clinical and non-clinical strains/isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?	X		
5	Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling?			Not critical to analysis of microbiology
6	Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions?	X		
7	Has the applicant submitted the clinical microbiology datasets in a format which intents to correlate baseline pathogen with clinical and microbiologic outcome?	X		
8	Has the applicant submitted draft/proposed interpretive criteria/breakpoint along with quality control (QC) parameters and interpretive criteria, if applicable, in a manner consistent with contemporary standards, which attempt to correlate criteria with clinical results of NDA/BLA studies, and in a manner to allow substantive review to begin?	X		
9	Has the applicant submitted a clinical microbiology dataset in an appropriate/standardized format which intents to determine resistance development by correlating changes in the phenotype (such as *in vitro* susceptibility) and/or genotype (such as mutations) of the baseline pathogen with clinical and microbiologic outcome?	X		
10	Has the applicant used standardized or nonstandardized methods for measuring microbiologic outcome? If	X		

MICROBIOLOGY FILING CHECKLIST

	Content Parameter	Yes	No	Comments
	nonstandardized methods were used, has the applicant included complete details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?			
11	Has the applicant submitted draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	X		
12	Has the applicant submitted annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package?	X		
13	Have all the study reports, published articles, and other references been included and cross-referenced in the annotated draft labeling or summary section of the submission?	X		
14	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?	X		One published article Relevant to microbiology is written in French. However, it is understandable.

IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE?
__yes._____

If the NDA is not fileable from the microbiology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Lynette Y. Berkeley 07/24/2012
Reviewing Microbiologist Date

Fred Marsik, Ph.D. 24 Jul 12
Microbiology Team Leader Date

File name: 5_Microbiology Filing Checklist for a NDA or Supplement 010908

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/s/

LYNETTE Y BERKELEY
07/24/2012

FREDERIC J MARSIK
07/24/2012

PRODUCT QUALITY MICROBIOLOGY FILING CHECKLIST

NDA Number: 204-384 **Applicant:** Janssen Therapeutics **Letter Date:** 29 June 2012

Drug Name: TMC207 (Bedaquiline) **NDA Type:** 505(b)(1) Priority Review **Stamp Date:** 29 June 2012

The following are necessary to initiate a review of the NDA application:

	Content Parameter	Yes	No	Comments
1	Is the product quality microbiology information described in the NDA and organized in a manner to allow substantive review to begin? Is it legible, indexed, and/or paginated adequately?	X		This is a non-sterile tablet intended for the treatment of multi drug resistant pulmonary tuberculosis.
2	Has the applicant submitted an overall description of the manufacturing processes and microbiological controls used in the manufacture of the drug product?	X		
3	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?		X	
4	Has the applicant submitted microbiological specifications for the drug product and a description of the test methods?	X		The applicant proposes skip lot testing after successful manufacture of the first 10 production batches.
5	Has the applicant submitted the results of analytical method verification studies?		X	
6	Is this NDA fileable? If not, then describe why.	X		

Comment to be sent to the applicant:

1. Provide the verification studies that support the proposed microbiological test methods.
2. (b) (4)

16 July 2012

Jessica G. Cole, PhD Reviewing Microbiologist Date

Bryan Riley, PhD Microbiology Team Leader Date

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/s/

JESSICA COLE
07/16/2012

BRYAN S RILEY
07/16/2012
I concur.