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July 28, 2020

The Controller of Patents
The Patent Office
Boudhik Sampada Bhawan, Plot No. 32,
Sector 14, Dwarka, New Delhi-110078

Re: **REPRESENTATION U/S 25(1) OF THE PATENTS ACT – BY THE DELHI NETWORK OF POSITIVE PEOPLE AGAINST INDIAN PATENT APPLICATION NO. 201817014361 DATED 05/10/2016**
APPLICANT: THE GLOBAL ALLIANCE FOR TB DRUG DEVELOPMENT INC

Dear Sir,

We submit herewith a Representation under Section 25(1) of the Patents Act, 2005 along with Form 7A.

The Controller is requested to take the documents on record and proceed further in the matter and keep the Petitioner advised of each and every step taken in the matter.

We crave the leave of the Controller to submit additional documents or evidence or if necessary to support any of the averments in the representation as may be necessitated in the proceeding.

Lastly, we request the Controller to grant an opportunity of being heard before the above representation is finally decided.

Thanking you,

RAJESHWARI H.
AGENT FOR OPPONENT
RAJESHWARI AND ASSOCIATES

Encl:

- Form 7;
- Opposition; and
- List of documents

C.C.:
S MAJUMDAR & CO.

BEFORE THE CONTROLER OF PATENTS, THE PATENT OFFICE, NEW DELHI

In the matter of Section 25(1) of The Patents Act,1970 as amended by The Patents (Amendment)Act 2005;

And

In the matter of Rule 55 of The Patents Rules 2003 as amended by the Patent (Amendment) Rules, 2006

And

IN THE MATTER of Indian Patent Application No. 201817014361 dated 05/10/2016 in the name of THE GLOBAL ALLIANCE FOR TB DRUG DEVELOPMENT INC**.**

REPRESENTATION BY:

THE DELHI NETWORK OF POSITIVE PEOPLE
A1-5, HOUSE NO. 141, GALI NO. 3,
IGNOU MAIN ROAD, NEB SARAI
NEW DELHI - 110068

.....OPPONENTS/ PETITIONERS

VS.

THE GLOBAL ALLIANCE FOR TB DRUG DEVELOPMENT INC.
40, WALL STREET
NY, 10005 U.S.A.

…..APPLICANT/RESPONDENT

PRE-GRANT OPPOSITION BY THE DELHI NETWORK OF POSITIVE PEOPLE

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8.	Copy of Power of Authority	Will follow

Dated this 28th day of July, 2020

RAJESHWARI H.
AGENT FOR OPPONENT
OF RAJESHWARI AND ASSOCIATES

To,
The Controller of Patents
The Patent Office, Delhi

FORM 7A
THE PATENTS ACT,
1970 (39 OF 1970)
AND
THE PATENTS RULES, 2003
REPRESENTATION FOR OPPOSITION TO GRANT OF PATENT
[See Rule 55]

We, **THE DELHI NETWORK OF POSITIVE PEOPLE,** an Indian citizen of A1-5, House No. 141 Gali No. 3, IGNOU Main Road, Neb Sarai, New Delhi - 110068, India; hereby give representation by way of opposition to the grant of patent in respect of Indian Patent Application 201817014361 filed on 16/04/2018 titled **COMBINATION ANTIBACTERIAL COMPOSITION AND SHORT COURSE ANTIBACTERIAL REGIMEN** and published under Section 11A on **07/09/2018** belonging to THE GLOBAL ALLIANCE FOR TB DRUG DEVELOPMENT INC., of 40, Wall Street, NY 10005 (US), on the following grounds:

1. That the invention claimed in any and all claims of the complete specification was published before the priority date of the claim in India or elsewhere in any other document – Section 25(1)(b);
2. That the invention claimed in any and all claims of the complete specification was publicly known or publicly used in India before the priority date of the claim – Section 25(1)(d);
3. That the invention claimed in any and all claims of the complete specification is obvious and clearly does not involve any inventive step – Section 25(1)(e);
4. That the subject of any and all claims of the complete specification is not an invention within the meaning of this Act, or is not patentable under this Act – Section 25(1)(f);
5. That the complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed- Section 25(1)(g);
6. That applicants deliberately did not disclose to the Controller the information required by Section 8 or has furnished the information which in any material particular was false to their knowledge – Section 25(1)(h).

(**Detailed grounds are set out in the Opposition as attached**)

My address for service in India is:

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MOBILE NO: 9910206718

Dated, this 28th day of July, 2020

RAJESHWARI H.
AGENT FOR OPPONENT
OF RAJESHWARI AND ASSOCIATES

To
The Controller of Patents,
The Patent Office, New Delhi

BEFORE THE CONTROLLER OF PATENTS, THE PATENT OFFICE,DELHI

In the matter of Section 25(1) of The Patents Act,1970 as amended by The Patents (Amendment) Act 2005;

And

In the matter of Rule 55 of The Patents Rules 2003 as amended by thePatent (Amendment) Rules, 2006

And

IN THE MATTER of Indian Patent Application 201817014361 dated 05/10/2016 in the name of THE GLOBAL ALLIANCE FOR TB DRUG DEVELOPMENT INC**.**

REPRESENTATION BY:

THE DELHI NETWORK OF POSITIVE PEOPLE
A1-5, HOUSE NO. 141, GALI NO. 3
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.....OPPONENTS/ PETITIONER

VS.

THE GLOBAL ALLIANCE FOR TB DRUG DEVELOPMENT INC.
40, WALL STREET
NY, 10005 U.S.A.

…..APPLICANT/RESPONDENT

REPRESENTATION BY WAY OF PRE-GRANT OPPOSITION UNDER SECTION 25(1) OF THE PATENTS ACT, 1970

We, **THE DELHI NETWORK OF POSITIVE PEOPLE**, an Indian organization, hereby submit my representation by way of oppostion to the grant of patent in respect of application no. 201817014361 filed on 16/04/2018 entitled “COMBINATION ANTIBACTERIAL

COMPOSITION AND SHORT COURSE ANTIBACTERIAL REGIMEN**"** on the following grounds.

STATEMENT OF CASE OF OPPONENT

1. The Opponent, **THE DELHI NETWORK OF POSITIVE PEOPLE**, is a community based non-profit organisation representing the needs of people living with HIV/AIDS ("PLHAs") and Hepatitis C (HCV), and is registered as a Trust under Registration No. 8525, Additional Book No. 1423/1-23 IV Sub Registrar, New Delhi, with its registered address at Flat no. A1-5, Property 141 Gali No. 3, Harijan Colony, Neb Sarai, New Delhi, 110068.

2. The Opponent is a PLHIV (People Living with HIV) network working extensively in the area of access to medicines. The Opponent's work includes but is not limited to service delivery, treatment literacy and community empowerment. The main focus and emphasis is advocating for access to medicines as they believe every individual should get treatment and no one should suffer and die due to lack of medicines. Of main concern to the Opponent, is the impact of product patent protection on access to effective and affordable medicines for people not just in India but across the developing world.

3. Cognisant of public health concerns, Parliament introduced certain provisions, while passing the Patents (Amendments) Act, 2005 to amend the Patents Act, 1970 (hereinafter referred to as the "Patents Act"), to ensure that patents are granted only for genuine inventions – which can either be product or processes only (refer definition of invention in S.2(1)(j) that states that 'invention means a new product or process…' and when read in conjunction with S.3(i), bring a complete bar to patenting of any method of treatment). The statute thus, does not allow patenting of medical use and also seeks to prevent "ever-greening", i.e. creation or extension of monopolies through patent terms by obtaining patents for minor or routine modifications.

4. The Opponents firmly believe that a proper application of the patentability standards set out in Section 3 as well as those embodied in Section 2(1)(j) and Section 2(1)(j)(a) of the Patents Act, in a manner that fully carries out the objectives of the Amending Act, will result in the rejection of the present application in its entirety. The Opponents, therefore, humbly request

the Hon'ble Patent Controller to scrutinise the present application with appropriate care, as its decision will determine whether millions of people will have access to affordable life-saving treatment.

5. As per Section 25(1), a pre-grant representation can be instituted by any person as long as an Application is still under prosecution. As per the on-line status check on IPASS site, the present Application has not matured into a patent as of the date of filing of this pre-grant representation. Hence, the present pre-grant opposition being filed by PLHIV is validly filed and is not time barred. A copy of the complete specification with claims as originally filed (with 10 claims) and downloaded from IPASS is attached as **Annexure 1**.

FILING DETAILS OF THE PRESENT APPLICATION

6. The Opponent has reviewed the file available at the IPASS system of the Indian Patent Office in respect of 201817014361 and notes that this Indian application filed at the Patent Office, Delhi – is a national phase filing of afore-mentioned PCT and claims priority from a US provisional filing # 62/241,280 dated 14/Oct/2015. According to the information available therein, following are the relevant details:

Application Details	
APPLICATION NUMBER	201817014361
APPLICANT NAME	**THE GLOBAL ALLIANCE FOR TB DRUG DEVELOPMENT INC.**
DATE OF FILING	16/Apr/2018
PUBLICATION DATE (U/S 11A)	07/Sep/2018
TITLE OF INVENTION	COMBINATION ANTIBACTERIAL COMPOSITION AND SHORT COURSE ANTIBACTERIAL REGIMEN
PCT Publication number	WO2017066053

PCT INTERNATIONAL FILING DATE	05/Oct/2016
PCT INTERNATIONAL APPLICATION NUMBER	PCT/US2016/055414
REQUEST FOR EXAMINATION DATE	11/Oct/2018
PRIORITY DATE	14/Oct/2015
FIRST EXAMINATION REPORT DATE	11/Dec/2019
REPLY TO FER DATE	04/June/2020

TUBERCULOSIS: BACKGROUND

7. The bacterium Mycobacterium Tuberculosis (MTB) causes Tuberculosis (TB). This is an infectious disease. Tuberculosis generally affects the lungs, but can also affect other parts of the body. When infections do not show symptoms, it is termed as latent tuberculosis. The Government of India has a Tuberculosis division at the Central Government level and for the year 2019, India had a total 24.04 lac notified cases of tuberculosis[1]. The WHO estimates that India has an estimated incidence of 26.9 lakh cases in 2019. WHO's 'Global tuberculosis report 2019' available at the WHO site[2] gives detailed data and estimates on Tuberculosis patient numbers, prevalence etc. As of 2018, one quarter of the world's population is thought to have latent infection with TB and within this population, India accounts for slightly more than 25% of the total number of infected populace.

8. Since tuberculosis is a disease emanating from a bacterium, the treatment regime consists of administering anti-bacterial medicines, over a prolonged period of time. If patients are found to have developed resistance to existing treatment regime, the case is termed as multi-drug

[1]https://tbcindia.gov.in/showfile.php?lid=3538
[2]WHO report is available at:
<https://apps.who.int/iris/bitstream/handle/10665/329368/9789241565714-eng.pdf?ua=1>

resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB). The resistance can either be primary i.e. resistance developed before the initiation of treatment or secondary resistance developed after the initiation of anti-tuberculosis treatment in patients. Active tuberculosis (i.e. Patients with active symptoms), requires several drugs (involving multiple antibiotics) to be co-administered in a regime for a period of minimum 6 months or more.

CURRENT TREATMENT REGIME

9. The current treatment regimen is a combination of multiple drugs and the current medications used to treat tuberculosis include[3]:
    a. Isoniazid
    b. Rifampicin (Rifampin)
    c. Ethambutol (Myambutol)
    d. Pyrazinamide

    Multiple research/ trials are on-going to assess suitability of other/ newer drugs to treat tuberculosis. These research attempts focus on repurposing older drugs (for instance: Linezolid, Moxifloxacin, Clofazimine) or finding new anti-bacterial drugs (e.g. Bedaquiline, Delaminid or Sutezolid) and below are some of the alternative/ new drugs that are being used / considered for use as add-on therapy to the current drug-resistant combination treatment, including:
    e. Bedaquiline
    f. Linezolid
    g. Pretomanid

DISCUSSION ON DEVELOPMENT OF NEW COMBINATION THERAPIES

10. Before moving forward to the current Specification and claims contained therein, it must be noted that most of the above drugs are old drugs that have been disclosed many years ago for use in the anti-biotic or specifically in the tuberculosis space. The basic patents covering each of the drugs that feature in the current Specification, are as follows.

[3]https://www.mayoclinic.org/diseases-conditions/tuberculosis/diagnosis-treatment/drc-20351256

a. Linezolid – US5688792 granted in the year 1997
b. Pretomanid – US5668127 granted in the year 1997, US6087358 granted in the year 2000;
c. Bedaquiline – US7498343 granted in the year 2009
d. Pyrazinamide seems to be covered in US2675385 from the 1950s.

11. US20110190199 (hereinafter **D3** or '199) is a patent specification carrying a publication dated 04/Aug/2011 that discloses and claims combination therapy for tuberculosis. The patent focuses on combining Sutezolid(compound I) + multiple drugs for the treatment of tuberculosis. These drugs include Bedaquiline and Pretomanid and optionally Pyrazinamide as well.Linezolid is acknowledged therein as an earlier approved oxazolidinone antibiotic while Sutezolidisdisclosed as a newoxazolidinoneantibiotic.While the '199 focuses on use of Sutezolid along with bedaquiline + pretomanid, using of alreadyapprovedoxazolidinoneclass drugs is not precluded(approvedLinezolid in place of newer Sutezolid).

12. Para no. 0034 of the '199 discloses use of Sutezolid with Bedaquiline (TMC-207) and Pyrazinamide. Para. 0080-84 discloses a pharmaceutical composition comprising Sutezolid and anti-TB drugs including Bedaquiline, Pretomanid and Pyrazinamide. '199 further discloses aspects of synergy arising from combining drugs - at para 144-7:

However, a synergistic effect is observed when the compound of formula (I) [sutezolid] *of the invention is administered in combination with at least two agents useful in the treatment of tuberculosis. ..*

Such synergy is advantageous in that it may allow for administration of each of the components in the combination in an amount less than that would be required if administered individually which may reduce the likelihood of adverse events or unpleasant side effects. Alternatively, such synergy may shorten the duration of treatment for TB.

Thus, administration of both the compound of [sutezolid] *of the invention and the at least two agents useful in the treatment of tuberculosis, ... was found to produce an effect, which results in improved treatment of tuberculosis as compared to the effect when the compound of* [sutezolid] *of the invention alone, when the at least two agents useful in the*

treatment of tuberculosis administered individually or when the at least two agents useful in the treatment of tuberculosis are administered in combination with one another.

Para 0167 discloses use of Sutezolid with Pretomanid and Pyrazinamide and at para 0175, it discloses use of Sutezolid with Bedaquiline and Pyrazinamide.

13. Three of the drugs mentioned in the present Specification (and claim 1) were investigated as a multi-agent combination drugs for the treatment of tuberculosis as early as 2012. Refer: *"**14 day bactericidal activity of PA-824 (Pretomanid), bedaquiline, pyrazinamide and moxifloxacin combinations: a randomized trial***" that carries a publication date of 23/July/2012[4].

14. In late 2012[5], the U.S. Food and Drug Administration (US FDA) approved Sirturo (Bedaquiline) as part of combination therapy in adults in following language:

    'SIRTURO should only be used in combination with at least 3 other drugs to which the patient's MDR-TB isolate has been shown to be susceptible in vitro.'

15. As seen from the 2012 approval, the combination treatment methods and need for a shorter drug regime and using newer drugs with existing drugs have been scientifically studied and approved. For example, "***Principles for designing future regimens for multidrug resistant tuberculosis***" carrying a publication[6] date of 25/Oct/2013 specifically states (refer Abstract):

    'Regimens should contain at least one new class of drug; be broadly applicable for use against MDR and extensively drug-resistant Mycobacterium tuberculosis complex strains; contain three to five effective drugs, each from a different drug class; be delivered orally; have a simple dosing schedule; have a good side-effect profile that allows limited monitoring; last a maximum of 6 months; and have minimal interaction with anti-retrovirals.'

16. Coming to use of Linezolid in the Tuberculosis regime, use of Linezolid (LZD) against drug-resistant Tuberculosis treatment was already disclosed and so was the possibility of exploring the use of this drug at lower dosage to minimize the adverse side effects. This comes in from

[4]10.1016/S0140-6736(12)61080-0
[5]https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/204384Orig1s000Lbl.pdf
[6]doi: http://dx.doi.org/10.2471/BLT.13.122028

a 17/July/2012 document[7] titled: "***WHO Group 5 Drugs for the treatment of Drug Resistant Tuberculosis: Unclear Efficacy or Untapped Potential".***

17. Problems linked to prolonged use of Linezolid and alternatives for such use in form of intermittent use, dose reduction were also studied long before the priority date for present Specification. For instance, the Supplementary Appendix[8] to "***Linezolid for Chronic Extensively Drug-Resistant TB***" –a 2012 document (hereinafter **D4**) - discusses re-administration of linezolid after one or two week of drug holiday at table 4 (page 13 of Supplement) as well as the possibility of discontinuing linezolid for a period of time to overcome the problem of adverse side effects, when administered to TB patients.

18. The clinical efficacy of Linezolid when its dosage was reduced from 600mg to 300mg and the approach of its intermittent dosing while continuing to maintaining its efficacy was disclosed in a 2015 document[9] - "***New anti-tuberculosis drugs and regimens:2015 Update***".

19. "**Archive History for NCT02333799**" (hereinafter **D1**) is a document downloaded from Clinicaltrial.gov website. This document carries a publication date of 08/June/2015. It is a dataset entry on a clinical trial bearing the official title, '*A Phase 3 Open-label Trial Assessing the Safety and Efficacy of BedaquilinePlus PA-824 Plus Linezolid in Subjects With Pulmonary Infection of Either Extensively Drug-resistant Tuberculosis (XDR-TB) or Treatment Intolerant / Non-responsive Multi-drug Resistant Tuberculosis (MDR-TB).*' This experimental trial had patients who were given the following treatment/ intervention:

    'Bedaquiline + PA-824 + Linezolid

    bedaquiline 400 mg *once daily for 2 weeks then 200mg 3 times per week plus* ***PA-824 200mg*** *once daily plus* ***linezolid 1200mg*** *once daily.'*

    Thus, **D1** categorically discloses treating of tuberculosis using Pretomanid + Bedaquiline + Linezolid.

[7]DoI: 10.1093/infdis/jis460)
[8]2012 (E) (N Engl J Med 2012;367:1508-18. DOI: 10.1056/NEJMoa1201964)
[9]DoI: 10.1183/23120541.00010-2015

20. "Nix-TB: Testing a New Potential Treatment for XDR-TB" (hereinafter **D2**) is document that carries a publication of 06/May/2015. It is a factsheet about a treatment regime that discusses a new study ('*Nix-TB trial*'). The document, at page 2 categorically mentions the following:

> *'Nix-TB tests a three-drug regimen consisting of **bedaquiline**, which received conditional regulatory approval in several high-TB disease burden countries; the novel antibacterial drug compound **pretomanid**, which is being tested in multiple clinical trials for TB; **and linezolid**, an oxazolidinone that has been used off-label to treat'*

Thus, **D2** also categorically discloses treating of tuberculosis using Bedaquiline + Pretomanid + Linezolid.

21. The US FDA approved Pretomanid Tablets in combination with bedaquiline and linezolid for the treatment of a specific type of highly treatment-resistant tuberculosis (TB) of the lungs in 2019[10]. Several newer drugs like Delaminid, Telacebec etc. are also being investigated for treatment in this disease and most of them have pending patent applications in India.

THE SPECIFICATION & ALLEGED INVENTION

22. The Specification for the present Application runs into approx. 33 odd pages of text. The Specification gives the following statement, generally:

> *'The invention relates generally to combinations of compounds with antibacterial activity and, more specifically, with anti-tuberculosis properties. In particular, it relates to chemically stable combinations of anti-bacterial agents linezolid, bedaquiline and pretomanid, and optionally with pyrazinamide, in a short-course oral dosage regimen for the treatment of tuberculosis.'*

23. The Specification acknowledges that present treatment regime for treating tuberculosis goes up to 2 years. The need or the problem that Specifications seeks to solve is discussed later, in following words:

[10]https://www.fda.gov/news-events/press-announcements/fda-approves-new-drug-treatment-resistant-forms-tuberculosis-affects-lungs

'a need exists in the art for novel regimens that are more effective than the current first-line regimen for drag-susceptible TB, thereby shortening and simplifying treatment for pulmonary TB irrespective of resistance to existing drugs.'

24. The solution or answer the Specification brings to the public is then mentioned as:

'Provided is a pharmaceutical composition, comprising a therapeutically effective amount of each of linezolid, bedaquiline and pretomanid, and optionally pyrazinamide, or a pharmaceutically acceptable salt of each thereof, and a pharmaceutically acceptable carrier.

Also provided is a method for the treatment of tuberculosis, comprising the step of administering to a patient in need thereof a therapeutically effective amount of each of linezolid, bedaquiline and pretomanid, and optionally pyrazinamide, or a pharmaceutically acceptable salt of each thereof, and a pharmaceutically acceptable carrier. In one embodiment of the invention, linezolid is removed from the treatment regimen after one or two months. In another embodiment linezolid can be re-administered after one or two week drug holiday.'

25. A key aspect of the Applicant's invention is centred on the point that when Linezolid is used in combination with Bedaquiline +Protemanid, it adds bactericidal and sterilizing activity and apparently even when Linezolid is administered only for two months, it is still able to retain the sterilizing activity in the treatment. The Specification also notes that Linezolid can be re-administered after one or two week interruption (treatment holiday) in the treatment regime. However, **D4** already disclosed these points.

26. The Specification specifically acknowledges that a regimen of 'bedaquiline + pretomanid + linezolid' was already initiated in the NiX-TB Trial (hereinafter **D2**). It discusses various examples and specifically discusses the concept of synergy in context of using multiple drugs, at page 8.

27. The Opponent submits that a very large part of this Specification is a verbatim reproduction from another 2004 patent document that has no bearing to Tuberculosis or present 3-4 drug regime for treating tuberculosis. A closer analysis (after reading the below copied sections) thus make it clear that the Specification does not cover any patentable invention under Indian law at all and given the above state of art (using multi-agent drug regime in the treatment of tuberculosis, possible drug combinations to be co-administered for achieving clinical efficacy and also various approaches explored in reducing the treatment duration, information on intermittent use of Linezolid), the Opponent submits that present Specification is an attempt to seek unmerited patent protection, over existing knowledge.

28. The Opponent submits that the present Specification is nothing but a discussion of an already known drug administration regime enveloped within a (heavily copied) Specification – that is thereafter converted into the present Specification; and thus this Specification does not consists of any genuine patentable product or process. The below table provides a comparison of the text copied exactly from the other patent document and included in the present Specification.

Present Specification	WO2004064846
Entire page 9 of present Specification *'When tradenames are used applicants intend to independently include the trade name product and the active pharmaceutical ingredient (s) of the trade name product.* *The term "chemical stability" means that the three antibacterial agents in combination are substantially stable to chemical degradation. Preferably, they are sufficiently stable in physical combination to permit commercially useful shelf life of the combination product Typically, "chemically stable" means that a*	**Please refer specification:** *'When tradenames are used herein, applicants intend to independently include the tradename product and the active pharmaceutical ingredient(s) of the tradename product.* *The term "chemical stability" means that the two primary antiviral agents in combination are substantially stable to chemical degradation. Preferably, they are sufficiently stable in physical combination to permit commercially useful shelf life of the*

first component of the mixture does not act to degrade a second component when the two are brought into physical combination to form a pharmaceutical dosage form. More typically, "chemically stable" means that the acidity of a first component does not catalyzes or otherwise accelerate lite acid decomposition of a second or a third component.	*combination product. Typically, "chemically stable" means that a first component of the mixture does not act to degrade a second component when the two are brought into physical combination to form a pharmaceutical dosage form. More typically, "chemically stable" means that the acidity of a first component does not catalyzes or otherwise accelerate the acid decomposition of a second component.*
The terms "synergy'' and "synergistic" mean that the effect achieved with the compounds used together is greater than the sum of the effects that results from using the compounds separately, i.e. greater than what would be predicted based on the two active ingredients administered separately. *A synergistic effect may be attained when the compounds are: (1) co-formulated and administered or delivered simultaneously in a combined fonmuation; (2) delivered by alternation or m parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when ie compounds are administered or delivered sequentially, e.g. in separate tablets, pills or capsules, or by different injections in separate syringes, in general, during alternation therapy, an effective dosage of*	*The terms "synergy" and "synergistic" mean that the effect achieved with the compounds used together is greater than the sum of the effects that results from using the compounds separately, i.e. greater than what would be predicted based on the two active ingredients administered separately.* *A synergistic effect may be attained when the compounds are: (1) co-formulated and administered or delivered simultaneously in a combined formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or delivered sequentially, e.g. in separate tablets, pills or capsules, or by different injections in separate syringes. In general, during alternation therapy, an effective dosage of*

each active ingredient is administered sequentially, i.e, serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together. *A synergistic antibacterial effect denotes an antibacterial effect which is greater than the predicted purely additive effects of the individual compounds of the combination.*	*each active ingredient is administered sequentially, i.e. serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.* *A synergistic anti- viral effect denotes an antiviral effect which is greater than the predicted purely additive effects of the individual compounds of the combination.*
"Bioavailability" is the degree to which, the pharmaceutically active agent becomes available to the target tissue after the agent's introduction into the body. *Enhancement of the bioavailability of a pharmaceutically active agent can provide a more efficient and effective treatment for patient because, for a given dose, more of the pharmaceutically active agent will be available at the targeted tissue sites.*	*"Bioavailability" is the degree to which the pharmaceutically active agent becomes available to the target tissue after the agent's introduction into the body. Enhancement of the bioavailability of a pharmaceutically active agent can provide a more efficient and effective treatment for patients because, for a given dose, more of the pharmaceutically active agent will be available at the targeted tissue sites.*
Page 10 of present Specification *The compounds of the combinations of the invention may be referred to as "active ingredients" or "pharmaceutically active agents."*	**Please refer specification:** *The compounds of the combinations of the invention may be referred to as "active ingredients" or "pharmaceutically active agents."*
The term "prodrug" as used herein refers to any compound that when administered to a biological system generates the drug substance, i.e. active ingredient, as a result of spontaneous chemical reaction(s), enzyme	*The term "prodrug" as used herein refers to any compound that when administered to a biological system generates the drug substance, i.e. active ingredient, as a result of spontaneous chemical reaction(s), enzyme*

catalyzed chemical reaction(s), and/or metabolic chemical reaction(s).	*catalyzed chemical reaction(s), and/or metabolic chemical reaction(s).*
"Prodrug moiety" means a labile functional group which separates from the active inhibitory compound during metabolism, systemically, inside a cell, by hydrolysis, enzymatic cleavage, or by some other process (Bundgaard, Hans, "Design and Application of Prodrugs" in Textbook of Drug Design and Development {1991), P. Krogsgaard-Larsen and H. Bundgaard, Eds. Harwood Academic Publishers, pp. 113-1 1).	*"Prodrug moiety" means a labile functional group which separates from the active inhibitory compound during metabolism, systemically, inside a cell, by hydrolysis, enzymatic cleavage, or by some other process (Bundgaard, Hans, "Design and Application of Prodrugs" in Textbook of Drug Design and Development (1991), P. Krogsgaard- Larsen and H. Bundgaard, Eds. Harwood Academic Publishers, pp. 113-191).*
Prodrug moieties can serve to enhance solubility, absorption and lipophilicity to optimize drag delivery, bioavailability and efficacy. A "prodrug" is thus a covalently modified analog of a therapeutically active compound.	*Prodrug moieties can serve to enhance solubility, absorption and lipophilicity to optimize drug delivery, bioavailability and efficacy. A "prodrug" is thus a covalently modified analog of a therapeutically-active compound.*
Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., McGraw-Hill Dictionary of Chemical Terms (1984) McGraw-Hill Book Company, New York; and Eliel, E, and Wilen, S., Stereochemistry of Organic Compounds (3994) John Wiley & Sons, Inc., New York.	*Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., McGraw-Hill Dictionary of Chemical Terms (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., Stereochemistry of Organic Compounds (1994) John Wiley & Sons, Inc., New York.*
Many organic compounds exist in optically	*Many organic compounds exist in optically*

active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chirai center(s). The prefixes d and 1 or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or 1 meaning that the compound is levorotatory.	*active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes R and S, d and 1 or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or 1 or S meaning that the compound is levorotatory.*
A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these compounds, called stereoisomers, are identical except that they are mirror images of one another. A specific stereoisomer is also referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemale. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.	*A compound prefixed with (+) or d or R is dextrorotatory. For a given chemical structure, these compounds, called stereoisomers, are identical except that they are mirror images of one another. A specific stereoisomer is also referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.*
Page 11 of present Specification *The term "chiral" refers to molecules which have the property of non- superimposability of the mirror image partner, while the term*	**Please refer Specification** *The term "chiral" refers to molecules which have the property of non- superimposability of the mirror image partner, while the term*

"achiral" refers to molecules which are superimposable on their mirror image partner.	*"achiral" refers to molecules which are superimposable on their mirror image partner.*
Any reference to any of the compounds in the compositions of the invention also includes any physiologically acceptable salt thereof. Examples of physiologically acceptable salts and their physiologically functional derivatives include salts derived from an appropriate base, such as an alkali metal (for example, sodium), an alkaline earth (for example, magnesium), ammonium and NX4+ (wherein X is C_1-C_4 alkyl), or an organic acid such as fumaric acid, acetic acid, succinic acid. *Physiologically acceptable salts of an hydrogen atom or an amino group include salts of organic carboxylic acids such as acetic, benzoic, lactic, fumaric, tartaric, maleic, malonic, malic, isethionic, lactobionic and succinic acids; organic sulfonic acids, such as methanesnlfonic, ethanesulfonic, benzenesulfonie and p-toluenesulfonic acids; and inorganic acids, such as hydrocliloric, sulfuric, phosphoric and sulfamic acids. Physiologically acceptable salts of a compound of anhydroxy group include the anion of said compound in combination with a suitable cation such as NaT and X4 * (wherein X is independently*	*Any reference to any of the above compounds also includes a reference to a physiologically acceptable salt thereof. Examples of physiologically acceptable salts of GS-7340, emtricitabine and their physiologically acceptable derivatives include salts derived from an appropriate base, such as an alkali metal (for example, sodium), an alkaline earth (for example, magnesium), ammonium and NX + (wherein X is C_1-C_4alkyl).* *Physiologically acceptable salts of an hydrogen atom or an amino group include salts of organic carboxylic acids such as acetic, benzoic, lactic, fumaric, tartaric, maleic, malonic, malic, isethionic, lactobionic and succinic acids; organic sulfonic acids, such as methanesulfonic, ethanesulfonic, benzenesulfonic and p-toluenesulfonic acids; and inorganic acids, such as hydrochloric, sulfuric, phosphoric and sulfamic acids. Physiologically acceptable salts of a compound of anhydroxy group include the anion of said compound in combination with a suitable cation such as Na+ and NX4+ (wherein X is independently selected from H or a C1-C4 alkyl group).*

selected from H or a. C1-C4 alkyl group). *For therapeutic use, salts of active ingredients of the combinations of the invention will be physiologically acceptable, i.e. they will be salts derived from a physiologically acceptable acid or base. However, salts of acids or bases which are not physiologically acceptable may also find use, for example, in the preparation or purification of a physiologically acceptable compound. All salts, whether or not derived from a physiologically acceptable acid or base, are within the scope of the present invention.* *The combination may be formulated in a unit dosage formulation comprising a fixed amount of each active pharmaceutical ingredient for a periodic, e.g. daily, dose or subdose of the active ingredients.* *Pharmaceutical formulations according to the present invention comprise a combination according to the invention together with one or more pharmaceutically acceptable carriers or excipients and optionally other therapeutic agents.*	*For therapeutic use, salts of active ingredients of the combinations of the invention will be physiologically acceptable, i.e. they will be salts derived from a physiologically acceptable acid or base. However, salts of acids or bases which are not physiologically acceptable may also find use, for example, in the preparation or purification of a physiologically acceptable compound. All salts, whether or not derived from a physiologically acceptable acid or base, are within the scope of the present invention.* *The combination may be formulated in a unit dosage formulation comprising a fixed amount of each active pharmaceutical ingredient for a periodic, e.g. daily, dose or subdose of the active ingredients.* *Pharmaceutical formulations according to the present invention comprise a combination according to the invention together with one or more pharmaceutically acceptable carriers or excipients and optionally other therapeutic agents.*
Page 12 *Pharmaceutical formulations containing the active ingredient may be in a form suitable*	**Please refer Specification:** *Pharmaceutical formulations containing the active ingredient may be in any form suitable*

for the intended method of administration. When used for oral use for example, tablets, troches, lozenges, aqueous or oil suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs may be prepared (Remington's Pharmaceutical Sciences (Mack Publishing Co., Easton, Pa.).	*for the intended method of administration. For example, tablets, troches, lozenges, aqueous or oil suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs may be prepared for oral administration (Remington's Pharmaceutical Sciences (Mack Publishing Co., Easton, PA).*
Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including antioxidants,, sweetening agents, flavoring agents, coloring agents and. preserving agents, in order to provide a palatable preparation. *Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipient or auxiliary agents which are suitable for manufacture of tablets are acceptable.*	*Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including antioxidants, sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide a palatable preparation.* *Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipient which are suitable for manufacture of tablets are acceptable.*
Page 13, 2nd para onwards: *Formulations for oral use may he also presented as hard gelatin capsules where the active ingredient is mixed with an inert, solid diluent, for example pregelatinized starch, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active*	**Please refer Specification:** *Formulations for oral use may be also presented as hard gelatin capsules where the active ingredient is mixed with an inert solid diluent, for example pregelatinized starch, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active*

ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.	*ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.*
Aqueous suspensions of the invention contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include a suspending agent, such as sodium earboxymethylcellulose, methylcellulose, hydroxypropylmethylcelluose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethyleneoxycetanol), a condensation product of ethylene oxide with a partial ester derived from, a fatty acid and a hexitol anhydride (e.g., polyoxyethylenesorbitanmonooleate). *The aqueous suspension may also contain one or more preservatives such as ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents,*	*Aqueous suspensions of the invention contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include a suspending agent, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcelluose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethyleneoxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylenesorbitanmonooleate).* *The aqueous suspension may also contain one or more preservatives such as ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose, sucralose, or saccharin.*

such as sucrose, sucralose or saccharin. *Oil suspensions may be formulated by suspending the active ingredient in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin.*	*Oil suspensions may be formulated by suspending the active ingredient in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin.*
At pg. 14 – top para: *The oral suspensions may contain a thickening agent, such as beeswax, hard paraffin or eetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid, BHT, etc.*	**Please refer Specification:** *The oral suspensions may contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid, BHT, etc.*
Dispersible powders and granules of the invention suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent, and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those disclosed above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.	*Dispersible powders and granules of the invention suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent, and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those disclosed above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.*
The pharmaceutical compositions of the invention may also be in the form of oil -in-	*The pharmaceutical compositions of the invention may also be in the form of oil-in-*

water emulsions or liposome formulations. The oily phase may be a vegetable oil, such as olive oil or arachis oil, a mineral oil, such as liquid paraffin, or a mix lure of these. *Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitanmonooleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylenesorbitanmonooleate.* *The emulsion may also contain sweetening and flavoring agents. Syrups and elixirs may be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.*	*water emulsions or liposome formulations. The oily phase may be a vegetable oil, such as olive oil or arachis oil, a mineral oil, such as liquid paraffin, or a mixture of these.* *Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitanmonooleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylenesorbitanmonooleate.* *The emulsion may also contain sweetening and flavoring agents. Syrups and elixirs may be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.*
The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a time-release formulation intended for oral administration to humans may contain approximately 1 to 1000 mg of active	*The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a time-release formulation intended for oral administration to humans may contain approximately from about 1 to 1000*

material compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95% of the total compositions (weight: weight). *The pharmaceutical composition can be prepared to provide easily measurable amounts for administration.* *As noted above, formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion.*	*mg of active material compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95% of the total compositions (weight: weight).* *The pharmaceutical composition can be prepared to provide easily measurable amounts for administration.* *As noted above, formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion.*
At pg. 15 – para 2: *The combinations of the invention may conveniently be presented as a pharmaceutical formulation in a unitary dosage form. A convenient unitary dosage formulation contains the active ingredients in any amount from 1 mg to 1 g each, for example but not hunted to, 10 mg to 500 mg for each, active ingredient In certain embodiments, 50 mg to 300 mg of each active ingredient can be used.*	**Please refer Specification:** *The combinations of the invention may conveniently be presented as a pharmaceutical formulation in a unitary dosage form. A convenient unitary dosage formulation contains the active ingredients in amounts of from about 1 mg to 1 g each, for example, 100 mg to 300 mg.*

Segregation of active ingredients in pharmaceutical powders and granulations is a widely recognized problem that can result in inconsistent dispersions of the active ingredients in final, dosage forms. Some of the main factors contributing to segregation are particle size, shape and density. Segregation is particularly troublesome when attempting to formulate a single homogenous tablet containing multiple active ingredients having different densities and different particle sizes.	*Segregation of active ingredients in pharmaceutical powders and granulations is a widely recognized problem that can result in inconsistent dispersions of the active ingredients in final dosage forms. Some of the main factors contributing to segregation are particle size, shape and density. Segregation is particularly troublesome when attempting to formulate a single homogenous tablet containing multiple active ingredients having different densities and different particle sizes.*
Glidants are substances that have traditionally been used to improve the flow characteristics of granulations and powders by reducing interparticulate friction, See Lieberman, Lachman, & Schwartz, Pharmaceutical Dosage Forms: Tablets, Volume 1, p, 177- 178 (1989), incorporated herein by reference.	*Glidants are substances that have traditionally been used to improve the flow characteristics of granulations and powders by reducing interparticulate friction. See Lieberman, Lachman, & Schwartz. Pharmaceutical Dosage Forms: Tablets. Volume 1, p. 177-178 (1989), incorporated herein by reference.*
Glidants are typically added to pharmaceutical compositions immediately prior to tablet compression to facilitate the flow of granular material into the die cavities of tablet presses, *Glidants include: colloidal silicon dioxide, asbestos free talc, sodium aluminosilicate, calcium silicate, powdered cellulose, microcrystalline cellulose, corn starch,*	*Glidants are typically added to pharmaceutical compositions immediately prior to tablet compression to facilitate the flow of granular material into the die cavities of tablet presses.* *Glidants include: colloidal silicon dioxide, asbestos free talc, sodium aluminosilicate, calcium silicate, powdered cellulose, microcrystalline cellulose, com starch,*

sodium benzoate, calcium carbonate, magnesium carbonate, metallic stearates, calcium stearate, magnesium stearate, zinc stearate, stearowet C, starch, starch 1500, magnesium lauryl sulfate, and magnesium oxide.	*sodium benzoate, calcium carbonate, magnesium carbonate, metallic stearates, calcium stearate, magnesium stearate, zinc stearate, stearowet C, starch, starch 1500, magnesium lauryl sulfate, and magnesium oxide.*
The novel compositions of the present invention may contain glidants to effect and maintain homogeneity of active ingredients during handling prior to tablet compression.	*The novel compositions of the present invention may contain glidants to effect and maintain homogeneity of active ingredients during handling prior to tablet compression.*
At pg. 16 – para 1: *Compositions of the present invention are administered to a human or other mammal in a safe and therapeutically effective amount as described herein. These safe and therapeutically effective amounts will vary according to the type and size of mammal being treated and the desired results of the treatment.*	**Please refer specification:** *Compositions of the present invention are administered to a human or other mammal in a safe and effective amount as described herein. These safe and effective amounts will vary according to the type and size of mammal being treated and the desired results of the treatment.*
At pg. 16 – para 2: *'Any of the various methods known by persons skilled, in the art for packaging tablets, caplets, or other solid dosage forms suitable for oral administration, that will not degrade the components of the present invention, are suitable for use in packaging. The combinations may be packaged in glass and plastic bottles. Tablets, caplets, or other solid dosage forms suitable for oral administration may be packaged and contained in various packaging materials*	**Please refer specification:** *'Any of the various methods known by persons skilled in the art for packaging tablets, caplets, or other solid dosage forms suitable for oral administration, that will not degrade the components of the present invention, are suitable for use in packaging. The combinations may be packaged in glass and plastic bottles. Tablets, caplets, or other solid dosage forms suitable for oral administration may be packaged and contained in various packaging materials*

optionally including a dessicant e.g. silica gel.	*optionally including a dessicant, e.g. silica gel.*
Packaging may be in the form of unit dose blister packaging. For example, a package may contain one blister tray of tenofovir DF and another blister tray of emtricitabine pills, tablets, caplets, or capsule. A patient would take one dose, e.g. a pill, from one tray and one from the other.	*Packaging may be in the form of unit dose blister packaging. For example, a package may contain one blister tray of GS-7340 and another blister tray of emtricitabine pills, tablets, caplets, or capsule. A patient would take one dose, e.g. a pill, from one tray and one from the other.*
Alternatively, the package may contain a blister tray of the co- formulated combination of tenofovir DF and emtricitabine in a single pill, tablet, caplet or capsule. As in other combinations and packaging thereof, the combinations of the invention include physiological functional derivatives of tenofovir DF and FTC.	*Alternatively, the package may contain a blister tray of the co-formulated combination of GS-7340 and emtricitabine in a single pill, tablet, caplet or capsule. As in other combinations and packaging thereof, the combinations of the invention include physiological functional derivatives of GS- 7340 and emtricitabine.*
The packaging material may also have labeling and information related to the pharmaceutical composition printed thereon. Additionally, an article of manufacture may contain a brochure, report, notice, pamphlet, or leaflet containing product information. This form of pharmaceutical information is referred to in the pharmaceutical industry as a "package insert." A package insert may be attached to or included with a pharmaceutical article of manufacture. The	*The packaging material may also have labeling and information related to the pharmaceutical composition printed thereon. Additionally, an article of manufacture may contain a brochure, report, notice, pamphlet, or leaflet containing product information. This form of pharmaceutical information is referred to in the pharmaceutical industry as a "package insert." A package insert may be attached to or included with a pharmaceutical article of manufacture. The*

package insert and any article of manufacture labeling provides information relating to the pharmaceutical composition. The information and labeling provides various forms of information utilized by health-care professionals and patients, describing the composition, its dosage and various other parameters required by regulatory agencies such as the United States Food and Drug Agency.'	*package insert and any article of manufacture labeling provides information relating to the pharmaceutical composition. The information and labeling provides various forms of information utilized by health-care professionals and patients, describing the composition, its dosage and various other parameters required by regulatory agencies such as the United States Food and Drug Agencies.'*

29. As noted above, pages 10-14 – while copied in wholesale from prior art, discuss general aspects on formulating a pharmaceutical formulation for this combination of drugs in very general terms – by discussing excipients, type of composition etc.

30. Going further, the Specification has no human clinical data / efficacy / efficiency data points pertaining to any of the 06 composition claims. The Specification has the 'Examples' section starting at page 16. All the examples (total 4) were only treatment regimen run on mice and at no point of time was a specific/ single 'composition' covering all the 3 (or 4) drugs was disclosed/ formulated as a 'composition' for human administration, disclosed in the Specification. The Specification notes (at pg. 17-18):

> *'Treatment was administered once daily, 5 days per week, by gavage. ... Each drug was administered once daily, by gavage, 5 days per week. For all combinations, PMD was administered immediately after the dose of BDQ +/- PZA, which were formulated together (9. 12). Oxazolidinones were administered at least 4 hours later.'*

The term gavage refers to supplying a nutritional substance by means of a small plastic feeding tube passed through the nose or mouth into the stomach.

31. There is no specific guidance given at any stage in the entire Specification on what type of composition (subject matter of claim 1) is sought to be prepared or actually prepared or contains what specific excipients (tablet/ capsule/ injection- containing what drug and excipient components) and hence this entire discussion is not the '*sweat of the inventor's*

brow'. The entire discussion on composition aspect in the Specification – viz. pages 10-15 and examples section- does not support any specific or even a single composition that was ever made / can be made- comprising anti-TB drugs. The Opponent states that by no stretch of imagination can the disclosed regimen be equated to an actual 'composition' sought to claimedin claim 1 nor can such regimen disclosure be equated to a product or process (as required under the Patents Act).

As mentioned earlier, each of the drugs individually as well as in a multi-agent drug regimen have been used in treatment of antibacterial diseases (including tuberculosis) and the present claims are only an attempt to mask a 'method of use'/ regime as a composition and secure a patent for the same.

THE CLAIMS OF IN201817014361 & EXAMINATION AT PATENT OFFICE:

32. The PCT Application was originally published with 16 claims. Claim 1 was an independent claim for a pharmaceutical composition, with claims 2, 3, 13, 15, 16 as dependent on claim 1; while claim 4 was an independent claim for a method of use, with claims 5-12, and 14 being dependent on this method of use claim 4.

33. The Applicant filed the Indian Application with 10 claims (from the original 16 PCT claims). The composition claims and independent method of use claims were retained but the dependent claims for the method viz. 5/6/7/10/11/12 were deleted. The First Examination Report was issued on 11/Dec/2019. The Applicant responded to this Report on 03/June/2020 and also carried out modifications to the claims. Original claims 4 to 10 were deleted (i.e. the method claim was deleted). New dependent claims 4 to 6 – for composition- were added. Remaining claims were renumbered – and currently 06 claims – all allegedly pertaining to a pharmaceutical composition are present in the Application.

34. Claim 1 is the sole independent claim and covers a composition that has at least 3 specific drugs (Linezolid, BedaquilineandPretomanid) and such composition optionally may have a 4^{th} drug- pyrazinamide. Claims 2 -6 are dependent on claim 1. The present pre-grant opposition is filed against the 06 claims as currently submitted by Applicant.

GROUNDS OF OPPOSITION:

S.25(1)(b) Lack of Novelty/ publicly known in India.

S.25(1)(b) states that an application can be opposed on the ground that

'*the invention so far as claimed in any claim of the complete specification has been published before the priority date of the claim –*

(i) in any specification filed in pursuance of an application for patent made in India on or after the 1st day of January, 1912; or

(ii) in India or elsewhere, in any document'

This section covers lack of novelty as a ground for refusal of claims.

35. Claim 1 has following components:

    a. a pharmaceutical composition, comprising
    b. a therapeutically effective amount of each of linezolid,
    c. bedaquiline, and
    d. pretomanid, and
    e. optionally pyrazinamide,

    or a pharmaceutically acceptable salt of each thereof,

    and a pharmaceutically acceptable carrier.'

    Claim 1 does not state what this alleged composition is norspecific dosage amount for the 3 drugs. The later claims also do not cover any specific type of composition or excipients for use in making any composition or even a bare mention of what type of composition is claimed (e.g. tablet/ capsule/ injection).

36. The impugned Application claims priority from US provisional filing # 62/241,280 originally dated 14/Oct/2015. If a single prior art reference, published before this date, discloses the 3 drugs being administered in a clinical setting for treating tuberculosis, then the claim(s) stands anticipated by such reference.

37. The Opponent submits that **D1** anticipates every mandatory component of present claim 1. As noted earlier, this experimental trial of **D1**had patients being administered the following treatment/ intervention:

'Bedaquiline + PA-824 + Linezolid

bedaquiline 400 mg *once daily for 2 weeks then 200mg 3 times per week plus* ***PA-824 200mg*** *once daily plus* ***linezolid 1200mg*** *once daily.'*

Thus, **D1** categorically discloses every single component of claim 1 - treating tuberculosis using Pretomanid + Bedaquiline + Linezolid(and their dosages) and thus destroys novelty of claim 1.

38. The Opponent submits that**D2**(independent of **D1**)also anticipates claim1 on every component. **D2** ('*Nix-TB trial*')at page 2 categorically mentions the following:

'Nix-TB tests a three-drug regimen consisting of ***bedaquiline****, which received conditional regulatory approval in several high-TB disease burden countries; the novel antibacterial drug compound* ***pretomanid****, which is being tested in multiple clinical trials for TB;* ***and linezolid****, an oxazolidinone that has been used off-label to treat'*

Thus, **D2** categorically discloses every single component of claim 1 – a treatment regimen using Pretomanid + Bedaquiline + Linezolid for treating tuberculosis and it thus destroys novelty of claim 1.

39. When an independent claim is deemed not patentable, dependent claims arising from such independent claims would also fall if they do not bring in any patentable limitation. The dependent claims (2-6) do not bring in any patentable distinction to the independent claim when compared with **D1** or **D2**. **D1** clearly shows that the drugs are administered via individual tablets and their dosages and hence anticipates claims 4 and 5 that cover the invention in the form of multiple, discrete drug dosages.

40. The Opponent submits that claims 1—6 of present Application are anticipated by **D1** and/or **D2,**and hence are liable to be rejected in entirety on account of S.25(1)(b)(ii) – that the claimed invention was already published in India/ elsewhere in a document.

S.25(1)(d) Publicly known / used in India.

S.25(1)(d) states that an application can be opposed on the ground that

'the invention so far as claimed in any claim of the complete specification was publicly known or publicly used in India before the priority date of that claim'

This section covers public knowledge/ use in India prior to priority date, as a ground for refusal of claims.

41. **D1** / **D2** categorically disclose every single component of claim 1 - treating tuberculosis using Pretomanid + Bedaquiline + Linezolid to a reader. The Opponent submits that both **D1** and / or **D2** were publicly available via the internet, in India before the priority.Accordingly these documents render the claimed invention - publicly known prior to the priority date. The Opponent submits that based on availability of **D1**/ **D2** on the internet and accessibility in India, claims 1-6 are liable to be refused in entirety, under the ambit of S.25(1)(d).

S.25(1)(e) Obviousness / lacking an inventive step.

S.25(1)(e) states that an application can be opposed on the ground that

'*the invention so far as claimed in any claim of the complete specification is obvious and clearly does not involve any inventive step …*'

This section covers obviousness or lack of inventive step as a ground for refusal of claims.

42. The Hon'ble Supreme Court's decision in *Biswanath Prasad RadheyShyam v/s Hindustan Metal Industries*[11] is the leading decision on analysis of inventive step. The Court specifically said:

> *"It is important to bear in mind that in order to be patentable an improvement on something known before or a combination of different matters already known, should be something more than a mere workshop improvement; and must independently satisfy the test of invention or an 'inventive step'.*
>
> *To be patentable the improvement or the combination must produce a new result, or a new article or a better or cheaper article than before. The combination of old known integers may be so combined that by their working inter-relation they produce a new process or improved result.*

[11][(1979) 2SCC 511]

Mere collocation of more than one integers or things, not involving the exercise of any inventive faculty, does not qualify for the grant of a patent."

Very importantly, the Apex Court quoted an old case (Rickmann v. Thierry[12])

'It is not enough 'that the purpose is new or that there is novelty in the application, so that the article produced is in that sense new, but there must be novelty in the mode of application. By that, I understand that in adopting the old contrivance to the new purpose, there must be difficulties to be overcome, requiring what is called invention, or there must be some ingenuity in the mode of making the adoption'.

43. The Intellectual Property Appellate Board has ruled[13] on obviousness / inventive step in the context of claims covering combination of pharmaceutical substances. That case covered a patent for combination of two drugs (Bimatoprost and Timolol) for treatment of ocular hypertension- without any specific dosage or excipient in claim 1. The prior art disclosed a combination of Latanoprost and Timolol for glaucoma.The IPAB held the claims as obvious as the end result of the combination was merely additive and that a person skilled in the art would have tried such a combination. So, taking guidance from the above SC and IPAB Judgements, it is clear that the invention or combination of known integers must produce a new or improved result.

44. **D3** discloses use of Sutezolid in combination with multiple drugs, including Protemanid, Bedaquiline and Pyrazinamide. It specifically states that use of combination results in synergistic effects.BothSutezolid and Linezolid are oxazolinones.

45. There is absolutely no discussion in present Specification on how/ which 'composition' allegedly sought to be claimed in present claims is actually made or how the claimed composition can be multiple discrete units as well. Present Specification does not disclose any specific hurdle that the claimed 'composition' jumps over or what is the actual composition or how is it better (inventive) over any prior art composition.

[12](1896)14 Pat. Ca. 105
[13]Case: *Ajanta Pharma v. Allergan Inc.* [ORA/21/2011/PT/KOL]

46. **D1** discloses every single mandatory component of the present claim 1and gives information about a clinical study that analysed the 3 drug regimen to patients with Tuberculosis and also gives the specific drug dosages for the 3 drugs (Bedaquiline at 400mg, Pretomanid at 200 mg and Linezolid at 200 mg. The Specification gives comparative data from the mice study while **D1** did not have data from the human study, as of the date of publication. However, the animal model data is not a part of the claims.

47. The Applicant, while responding to the Controller on inventive step objections in the First Examination Report, argued that the shorter time duration / administration and discontinuation of Linezolid as distinguishing aspect over prior art. The Opponent has already discussed various prior arts that discuss Linezolid being used in short duration mode. Importantly, use of Linezolid in short mode or for partial point of time is not a component of claim 1 or the later claims. Interestingly, while the Applicant seeks to distinguish by asserting that prior art does not mention what type of 'composition' is administered – whether single or plurality of unit dosages or adding of the 4^{th} drug, the Applicant does not admitthatthe Specification itself does not disclose even a single, actual 'composition', so the claims fall on that count.

48. **D4** is a part of the state of the art and clearly discusses Linezolid and how by reducing the dosage of Linezolid and its intermittent administration, helps in achieving clinical efficacy *vis-a-vis* addressing the adverse side-effects and finally combining more than 3 or 4 anti-TB drugs in a treatment regime to shorten the duration of TB treatment.

49. The Opponent submits that the cited prior art - **D3**+ **D1**<u>or</u>**D3** + **D2**<u>or</u>**D3** + **D1**+**D2**, in combination with the existing knowledge of the person skilled in the art(**D4**) discloses a combination of Linezolid with Bedaquiline and Pretomanid and renders the invention claimed in claims 1-6, as obvious and devoid of inventive step, thus rendering claims 1-6 as liable to rejectionin entirety, under the ambit of S.25(1)(d).

S.25(1)(f) Not an invention/ Not patentable under the Act.

S.25(1)(f) states that an application can be opposed on the ground that

'*the subject of any claim of the complete specification is not an invention within the meaning of this Act, or is not patentable under this Act*'

Chapter II of the Patents Act covers 'Inventions not patentable'. Specifically, S.3 states:

> *'3. What are not inventions.- The following are not inventions within the meaning of this Act,--*
>
> *...*
>
> *(d) the mere discovery of a new form of a known substance which does not result in the enhancement of the known efficacy of that substance or the mere discovery of any new property or new use for a known substance or of the mere use of a known process, machine or apparatus unless such known process results in a new product or employs at least one new reactant.*
> Explanation.—*For the purposes of this clause, ... combinations ... shall be considered to be the same substance, unless they differ significantly in properties with regard to efficacy;*
>
> *(e) a substance obtained by a mere admixture resulting only in the aggregation of the properties of the components thereof or a process for producing such substance;*
>
> *...*
>
> *(i) any process for the medicinal, surgical, curative prophylactic, diagnostic, therapeutic or other treatment of human beings to render them free of disease or to increase their economic value of that of their products.'*

S.3 is a chapter covering inventions thatmay pass the test of S.2(1)(ja) and hence patentable as such, they are still not patentable inventions by virtue of a deeming fiction.

3(d) discussion

50. The Opponent submits that prior art document(s) **D1**, **D2** and **D3** – each individually disclose a combination regiment of the 3 drug for administration to patients. For a claim to pass the S.3(d) threshold, the Specification has to contain data which shows that the claimed combination is significantly differing with regard to efficacy as compared to the prior art (specifically, **D1** or **D2**). Thus, it is incumbent uponthepresent Specification to show data on how the combination of the substances claimed therein is beyond prior art disclosure on the touchstone of 'significantly differing in properties with regard to efficacy'.The present

Specification has no discussion on how the alleged invention of claims 1-6 (the combination regimen) issignificantly different when compared to **D1** or **D2**.

51. The examples in the Specification look at the efficacy when a drug from the oxazolidinone class is combined with the Bedaquline + Pretomanid. Thus, 3oxazolidinone class drugs (Tidozolid, Linezolid and Sutezolid) are combined with Bedaquline + Pretomanid and the efficacy of such regimen, is disclosed. The examples indicate that, in general, oxazolidones exhibit similar sterilizing activity, refer page no. 20, para no.2, line no.7 and 8. The Specification further notes Linezolid has similar activity to Sutezolid when used in combination with Bedaquline + Pretomanid (refer example 2, page no.21).

52. When compared to Bedaquline + Pretomanid alone, addition of oxazolidones significantly increased bactericidal activity at 1 and 2 months (refer page no. 23, Example 3, para no. 2, line no. 12, 13 and 14). The given data does not indicate any superiority of the claimed pharmaceutical composition Linezolid +Bedaquline + Pretomanid over the already known pharmaceutical composition of anti-TB drugs Sutezolid+Bedaquline + Pretomanid.

53. Applicant's argument that discovery of use of Linezolid in combination with Bedaquline + Pretomanid displayed bactericidal and sterilizing activity is baseless as it is not substantiated by the data include in the specification. The present Specification only contains test data on mice (no human data) and there is no discussion on how this regime / alleged inventiondifferswhen compared to **D1** or **D2**.

54. The Opponent submits that the decision in App. 2751/DEL/2006 applies to the present claims. The '2751 claimed a composition of Ivabradine + Amlodipine without any specific composition / excipients. The IPO rejected the claims with the following reasoning:

    Also no improvement in the therapeutic efficacy of present compound as compared to its prior art compound has been provided. In fact both the compounds. Pharmaceutical composition comprising a selective and specific sinus node If current inhibitor and a calcium inhibitor use to treat angina which is also the activity shown by the prior art compound. The applicant submits that clinical study pages 6 to 8 of the description of the present patent application but it is not sufficient to prove that the present invention have

better therapeutic efficacy over prior art. In view of above I state that the subject matter for application no. 2715/DEL/2006 is not patentable under section 3(d).

55. Likewise, IPO's decision in 1996/DEL/2006 is equally applicable to present claims. The '1996 covered a combination of Agomelatine + Valproate in composition form. The IPO rejected this combination with following reasoning:

However the applicant has not provided any argument and experimental proof of any enhancement of the above properties and significant improvement in therapeutic efficacy, i.e. to say no comparative experimental data is available in the specification to prove the improvements are significant and the composition is efficacious than the earlier one. In such circumstances of failure to prove efficacy the composition as claimed is merely a new form of the known substances which is not Patentable U/S 3(d) of the Patent Act.

56. The Opponent submits that the Specification does not contain any data for enhanced efficacy for the combination composition covered in claims 1-6, when compared with the disclosure of **D1** or **D2**. Thus, the claims 1-6 are liable to be rejected S. 25(1)(f) when read in conjunction with S.3(d).

3(e) discussion

57. The Opponent submits that claims 1-6 are deemed not patentable as they are hit by S.3(e). The Specification does not disclose any specific 'composition' or any process to make any such specific 'composition'. What the Applicant has claimed is actually a combination regimen of 3 drugs (and optionally a 4^{th} drug) that was already known in **D1** that categorically gave the specific drugs and their dosage ranges for use in the treatment of tuberculosis. The Applicant has not disclosed details for even a single, specific composition that displays synergistic relation between the drug components and the excipients. All the components of the claimed composition have been disclosed in the same dosage range in **D1** and perform their individual functions and there is no surprising or unexpected effect that is resultant of aggregating such components over **D1**.

58. Paragraph 10.13 of the Guidelines for Examination of Patent Applications in the Field of Pharmaceuticals states:

"It is a well-accepted principle of Patent Law that mere placing side by side of old integers so that each performs its own proper function independently of any of the others is not a patentable combination, but that where the old integers when placed together has some working inter-relation producing a new or improved result, then there is patentable subject matter in the idea of the working interrelationship brought about by the collocation of the integers".

59. The IPO has consistently held a position that composition claims have to be specific with regard to the components of the composition. IPO's following decisions throw some light on patentability of composition claims:

1324/DELNP/2006

'Synergism of the combination/composition has also not been proved. It is an undue burden for a skilled person, in trying to assess the scope of the matter for which protection is sought in the claim concerned, to provide a complete overview of all known (and future) compounds functionally defined or very generally indicated as above from the multitude of possible compounds, even when provided with means to do so in the application. Claim 1 Composition of a compound whose activity results in a direct or indirect activation of the alpha 2 receptor (notably an alpha-2 agonist) combined with an alpha 1 adrenergic receptor antagonist, for treatment of pain . Therefore the composition as claimed in claim 1 fall U/S 3(e) of the Act.

314/MUM/2008

'The composition claimed by the applicant is a mere admixture of above mentioned ingredients without showing any synergism. The combination does not result in any enhanced additive effect….

The question of efficacy and or synergism are matters of scientific facts which are required to be embodied in the specification so that the said characteristics are apparent from the specification. The applicant vaguely claimed that the composition is a

synergistic composition but no support in this regard was provided in the specification. …

In the absence of synergism between the defined components , which applicant failed, the claimed composition of the alleged application is considered mere admixture as defined under clause (e) of Section 3 of the Act.'

1997/DEL/2006

It is observed that the composition comprising agomelatine… and reboxetine used for preparation of pharmaceutical compositions for preparing medicaments as claimed in Claim 1 of the present application is not patentable under Section 3(e) of the Indian Patent Act, 1970 as amended by the Patents (Amendment) Act, 2005, as it is a mere admixture of two or more known substances which does not fulfil the requirement to be patentable under Section 3(e) of the Act.

Further, no comparative data is provided that the composition has any beneficial / superior or surprising effect over the prior art. Therefore Claim 1 of the application is not patentable under the provisions of the Section 3(e) of the Indian Patent and the claimed composition is a admixture of different ingredients without demonstration of any synergistic effect. The applicant also failed to provide any specific amount /ratio of the different ingredients use for the composition.

60. The mice data in the Specification, even if viewed as a support to overcome the admixture test of S.3(e), does not really help the Applicant as the discussion in the Specification is merely a direct outcome of the regime already mentioned in **D1** and is not an evidence of surprise or synergy by itself by present Specification when compared with **D1**.

61. When the claims only cover a mere admixture of components in a 'composition' (which itself is never disclosed) such composition claimsfailand hence claims 1-6 are liable to be rejected S. 25(1)(f) when read in conjunction with S.3(e).

3(i) discussion

62. A claims falls under statutory bar of 3(i) once it is clear that the claim only covers a regimen/ method or use/ method of treatment. The Opponent submits that present claims 1-6 are not patentable due to the statutory bar of S.3(i). These claims, while worded as a 'composition', do not go beyond attempting to patent an already known drug regimen or method of use – using a combination of drugs to treat tuberculosis.

63. PresentSpecification does not even disclose a single composition that can be equated with 'a composition' of claim 1/6 or 'plurality of unit dosages' of 4/5. There is no actual 'composition in the claims and all that is covered is 'a drug regimen' that is already disclosed in **D1** or **D2**. Once this analysis of the components of claim 1 is analysed with the actual disclosure of the Specification, it is apparent that the claims are only a drug regimen, then the claims necessarily fail under the lens of S.3(i).

64. Paragraph 10.20 of the 'Guidelines for Examination of Patent Applications in the Field of Pharmaceuticals' has also noted this practise:
"In the field of pharmaceuticals, it is noticed that method of treatments are often claimed in the guise of composition claims."

65. The Opponent submits that clever drafting of use claims crafted as composition claims is not legally valid and submits the following decisions from the IPO:

417/DEL/2011

Objection:

1. Claim 1 mention about the dosage of the peptide is to be from 5 to 40 µg and this dosage aids in the treatment of cancer. Therefore the subject matter of claim 1 is objected under section 3(i) of the Patents Act, 1970.

This claim was later modified to a 'composition' claim and the IPO rejected the same.

201714032478

Aclaim for ‘combination’ of 2 drugs Cenicriviroc + Tropifexorfor‘use in treating or preventing a fibrotic or cirrhotic disease’ was objected and the claim was finally disallowed.

66. The Opponent submits that claims 1-6 are not ‘composition’ claims but merely a clever attempt of couching a method into a composition format and hence claims 1-6 are liable to be rejected S. 25(1)(f) when read in conjunction with S.3(i).

S.25(1)(g)Insufficiency or lack of clarity in description or manner for performing the invention.

S.25(1)(g) states that an application can be opposed on the ground that

‘*the complete specification does not sufficiently and clearly describe the invention or the method by which it is to performed*’

67. At this juncture, the Opponent submits that the present Specification does not sufficiently and clearly describe the manner in which the claimed invention is to be performed. A very large part of the Specification – especially the discussion on composition- is copied from a previous publication. The Specification does not even disclose a single ‘working’ composition that was actually produced or a composition that would align to the invention sought to be protected in claims 1-6. On the one hand, each of the 06 claims specifically cover ‘a composition’; the Specification does not disclose what this actual ‘composition’ is (i.e. is it a tablet/ capsule or injection?) or any specific manufacturingprocessto make such claimed composition (for instance, tableting via wet granulation or making capsules etc.).

68. Accordingly, the Opponent submits that present Specification does not sufficiently describe or support the claimed invention (viz. any actual composition or any actual method to make a specific composition) and henceclaims1-6 of the present Specification are liable to be rejected in entirety u/s 25(1)(h).

S.25(1)(h) – Non disclosure of information required u/s 8:

S.25(1)(h) states that an application can be opposed on the ground that

‘the Applicant has failed to disclose to the Controller the information required by section 8 or has furnished the information which in any material particular was false to his knowledge.’

69. The Opponent submits that Applicant has deliberately not provided detailed particulars and copies of all the relevant office actions/ responses and examination reports pending against the corresponding foreign filings of this case to the Patent Office.

70. The Division Bench of the Delhi HC has elucidated the law relating to S.8 submissions (though that case dealt with revocation u/s 64) in *SukeshBehl vs. Koninklijke Phillips Electronics*[14]. At Para 40, the Hon'ble Court stated:

> *'40. For the aforesaid reasons, we are of the view that the power to revoke a patent under Section 64(1) is discretionary and consequently it is necessary for the Court to consider the question as to whether the omission on the part of the plaintiff was intentional or whether it was a mere clerical and bonafide error.'*

Hence only a *bona-fide* and clerical error in context of S.8 filings is pardonable.

71. The Applicant's latest Form 3discloses 12 active family members (excluding present Indian application) and gives a common status for all 12 – as simply, 'Pending'. But Opponent submits that critical, negative developments have not been reported by the Applicant.Specifically, the Applicant has hidden thedetailed legal position for two key jurisdictions, as notedbelow:

EP3362068	**US20180280401**
Applicant has not disclosed the following actions: 1. Negative 'Supplementary Search Report' dated 16/May/2019 2. Claims amended in mid-Dec 2019	Applicant has not disclosed the following: 1. Non-final rejection dated 31/Dec/2018, 28/June/2019 and final rejection dated 08/Jan/2020 2. New claims and request for continued examination dated 08/July/2020

72. The clear attempt to hide negative search/ examination report is not merely a clerical or administrative error. This is an attempt to secure an Indian patent while hidingthe negative examination report of the EPO andthe US PTO. The Opponent submits that since the Applicant has wilfully hidden prejudicial US PTO and EPO examination reports from the

[14] FAO(OS) No.16 OF 2014, Order dated 07/Nov/2014

Indian Patent Office, this is a clear and *mala-fide* violation of Applicant's obligations strict u/s 8 and hence the Controller has to refuse the present application,in its entirety.

PRAYERS

73. In the fact and circumstances of the case, the Opponent now prays as follows:

a. that the Controller take the present Opposition on record;

b. that the Indian Patent Application No. 201817014361, be rejected in entirety under Section 25(1) of the Patents (Amendment) Act, 2005;

c. that we be allowed to file further documents as evidence if necessary to support our case;

d. that we be given an opportunity of a personal hearing in this matter before any final orders are passed;

e. that we be provided copies of any further submissions/ claim amendments /response by Applicant;

f. that we be allowed to make further submissions, amend pleadings, add or edit grounds of opposition in case the Applicant makes any amendments in the claims or files any response;

g. any other reliefs that may be just and fair in favour of the Opponent.

Dated this the 28th day of July 2020

RAJESHWARI H.
AGENT FOR THE OPPONENT
RAJESHWARI & ASSOCIATES

TO
THE CONTROLLER,
THE PATENT OFFICE BRANCH
DELHI

FORM 2

ANNEXURE - 1

THE PATENTS ACT, 1970
(39 of 1970)
&
THE PATENTS RULES, 2003

COMPLETE SPECIFICATION
(See section 10, rule 13)

"COMBINATION ANTIBACTERIAL COMPOSITION AND SHORT-COURSE ANTIBACTERIAL REGIMEN"

THE GLOBAL ALLIANCE FOR TB DRUG DEVELOPMENT, INC. of 40 Wall St New York, NY 10005, US;

The following specification particularly describes the invention and the manner in which it is to be performed.

COMBINATION ANTIBACTERIAL COMPOSITION AND SHORT-COURSE ANTIBACTERIAL REGIMEN

Field of the Invention

The invention relates generally to combinations of compounds with antibacterial activity and, more specifically, with anti-tuberculosis properties. In particular, it relates to chemically stable combinations of anti-bacterial agents linezolid, bedaquiline and pretomanid, and optionally with pyrazinamide, in a short-course oral dosage regimen for the treatment of tuberculosis.

All documents cited to or relied upon below are expressly incorporated herein by reference.

Background of the Invention

Mycobacterium tuberculosis is the causative agent of tuberculosis ("TB"), a devastating infectious disease. It is estimated that about 2 million TB patients die each year globally. Failure to properly treat tuberculosis has caused global drug resistance in *mycobacterium tuberculosis* and thus rendering some medications ineffective.

Approximately half a million new cases of multidrug-resistant (MDR) tuberculosis occur annually (1). Current recommendations call for up to 2 years of treatment with second-line drugs that are poorly tolerated, toxic, more difficult to administer and less effective than the 6-month, so-called short course regimen for drug-susceptible TB. Regimens containing at least 6 drugs, including newer fluoroquinolones in high doses, an injectable agent, clofazimine (CFZ), pyrazinamide (PZA) and high-dose isoniazid (INH) have shown potential as effective 9-month regimens in MDR-TB cases with minimal bacillary resistance to second-line drugs (2-4). However, these regimens remain quite cumbersome to administer and are not expected to be as effective in the setting of resistance to fluoroquinolones and/or injectable agents (3).

Agents from 2 novel classes recently received conditional regulatory approval for use in MDR-TB: the diarylquinoline bedaquiline (BDQ) and the nitroimidazole derivative delamanid. Aside from some mutations known to confer cross-resistance between BDQ and CFZ (5, 6), these agents are not known to exhibit cross-resistance with other TB drugs. It was recently reported that the 3-drug regimen of BDQ plus pretomanid (PMD; formerly known as PA-824),

the second nitroimidazole to enter phase 3 clinical trials, and the oxazolidinone sutezolid (SZD; formerly known as PNU-100480) have greater sterilizing activity than the first-line regimen of rifampin (RIF), INH and PZA in a murine model of TB (7-10). The 3-drug combo was more active than any of its 2-drug components, indicating that SZD contributes important activity. However, SZD has only completed a single phase 2a trial of its early bactericidal activity (8) and its further development cannot be assured.

Until recently, linezolid (LZD) was the only marketed oxazolidinone antibiotic. It has proven efficacy in salvage therapy for recalcitrant cases of MDR-TB patients, but its usage has been curtailed by dose- and duration-dependent toxicity. Novel regimens based on 3 or more oral agents, one of which is LZD, with little or no pre-existing resistance would provide simpler, more universally active regimens. Thus, a need exists in the art for novel regimens that are more effective than the current first-line regimen for drug-susceptible TB, thereby shortening and simplifying treatment for pulmonary TB irrespective of resistance to existing drugs.

Summary of the Invention

Provided is a pharmaceutical composition, comprising a therapeutically effective amount of each of linezolid, bedaquiline and pretomanid, and optionally pyrazinamide, or a pharmaceutically acceptable salt of each thereof, and a pharmaceutically acceptable carrier.

Also provided is a method for the treatment of tuberculosis, comprising the step of administering to a patient in need thereof a therapeutically effective amount of each of linezolid, bedaquiline and pretomanid, and optionally pyrazinamide, or a pharmaceutically acceptable salt of each thereof, and a pharmaceutically acceptable carrier. In one embodiment of the invention, linezolid is removed from the treatment regimen after one to two months. In another embodiment, linezolid can be re-administered after a one to two week drug holiday.

Detailed Description of the Invention

It is to be understood that the descriptions of the present invention have been simplified to illustrate elements that are relevant for a clear understanding of the present invention, while eliminating, for the purpose of clarity, many other elements found in typical pharmaceutical compositions. Those of ordinary skill in the art will recognize that other elements and/or steps are desirable and/or required in implementing the present invention. However,

because such elements and steps are well known in the art, and because they do not facilitate a better understanding of the present invention, a discussion of such elements and steps is not provided herein. The disclosure herein is directed to all such variations and modifications to such elements and methods known to those skilled in the art. Furthermore, the embodiments identified and illustrated herein are for exemplary purposes only, and are not meant to be exclusive or limited in their description of the present invention.

Despite the conventional understanding that it is necessary to continuously administer antibacterial agents over a period of many months to obtain the desired antibacterial effect, the inventors surprising discovered that LZD in a multi-agent dosage form can be administered for one to two months and still retain sterilizing activity. More specifically, the inventors discovered that LZD adds bactericidal and sterilizing activity in formulation comprising BDQ and PMD. Its additive sterilizing activity was indistinguishable from that of SZD as shown in Example 1. In Example 3, in which the bacterial burden and the dose of PMD were higher, LZD did not appear as effective as SZD, although the difference was not statistically significant. Thus, the impact of substituting LZD for SZD in terms of duration needed to cure mice is small. This finding constitutes the basis for moving the evaluation of the BDQ+PMD+LZD regimen into patients with extensively drug-resistant TB (XDR-TB) or treatment-intolerant or non-responsive MDR-TB in the recently initiated NiX-TB trial.

Given the superior sterilizing activity of BDQ+PMD plus an oxazolidinone compared to the first-line regimen, it is also reasonable to consider whether regimens based on this combination could also shorten the treatment of drug-susceptible TB. It was previously shown that the addition of PZA to the BDQ+PMD+SZD combination produced even greater sterilizing activity, curing nearly all mice after just 6 weeks of treatment (7). However, the contribution of SZD to the 4-drug combination was not confirmed. In Example 2, it was confirmed that addition of LZD to BDQ+PZA+PMD also significantly increases the bactericidal and sterilizing activity of the regimen, curing all mice after 6 weeks of treatment, while at least 5 months of treatment with the first-line regimen is typically required to produce this result under the same condititions.

The 100 mg/kg dose of LZD used in the Examples produced a mean plasma $AUC_{0\text{-}24h}$ comparable to that produced by a dose of 600 mg twice daily in humans (19, 20), the same dose at which treatment is initiated in the NiX-TB trial. This dose has produced unacceptably high rates of myelotoxicity and neuropathy when administered for long durations in salvage regimens

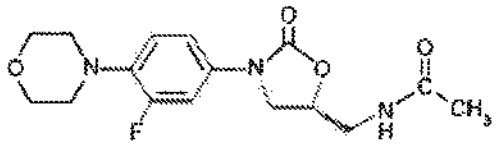
to treat MDR- and XDR-TB (26). Because these toxicities are dose- and duration-dependent, the inventors determined whether the contribution of LZD to the sterilizing activity of these regimens would be adversely affected by reducing the dose and duration of LZD. The potent sterilizing activity of the 4-drug regimen containing PZA in Example 2 was such that all mice were cured after 1.5 months of treatment. Nevertheless, limiting the duration of LZD 100 mg/kg to the first month did not adversely affect LZD's contribution to the sterilizing activity. In the absence of PZA, reducing the LZD dose to 50 mg/kg, which produces a mean $AUC_{0\text{-}24h}$ equivalent to a 600 mg daily dose in humans, reduced the bactericidal activity over the first month of treatment. However, after 2 months of treatment, only small numbers of CFU were recovered irrespective of whether LZD was administered as 100 mg/kg for 1 or 2 months or as 50 mg/kg for 2 months. Moreover, limiting LZD 100 mg/kg to the first month of treatment did not significantly increase the rate of relapse after 2 or 3 months of treatment. These results suggest that use of LZD 600 mg once daily for the first 1-2 months of treatment and/or front-loading therapy with higher doses for even shorter periods may enable LZD to contribute important bactericidal and sterilizing activity before the onset of potentially irreversible neuropathy develops (27, 28).

The inventors further discovered that linezolid can be re-administered at a lower dose after a one to two week interruption (e.g., drug or treatment holiday) based on neuropathy findings and subsequent improvement or, similarly, hematology findings. Clinical doses for pretomanid can be 200 mg qd or 100 mg qd. For bedaquiline, clinical doses can be 400 mg qd for two weeks followed by 200 mg tiw or 200 mg qd. And clinical doses for linezolid are 600 mg bid or 1200 mg qd to start.

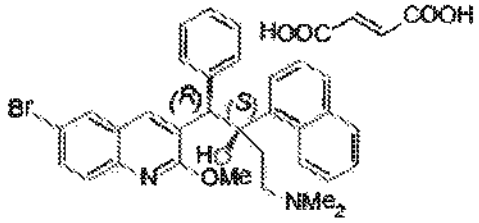
Certain Embodiments of the Invention

The present invention generally relates to a oral dosage combination of linezolid, bedaquiline and pretomanid, optionally with pyrazinamide.

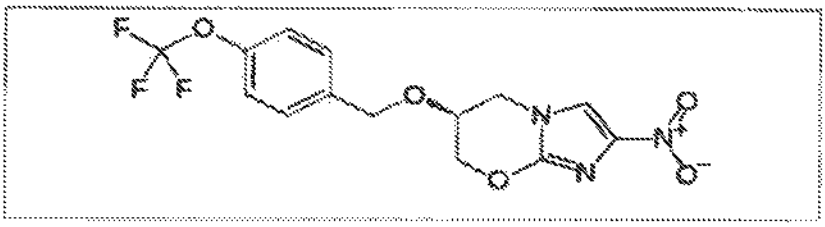
Linezolid is a synthetic antibacterial agent of the oxazolidinone class. The chemical name for linezolid is (S)-N-[[3-[3-Fluoro-4-(4morpholinyl)phenyl]-2-oxo-5-oxazolidinyl] methyl]-acetamide. The empirical formula is $C_{16}H_{20}FN_3O_4$. Its molecular weight is 337.35, and its chemical structure is represented below:



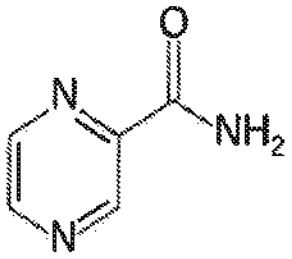
Bedaquiline is a diarylquinoline antimycobacterial drug marketed in fumarate form as SIRTURO. The chemical name of bedaquiline fumarate is (1R, 2S)-1-(6-bromo-2methoxy-3-quinolinyl)-4-(dimethylamino)-2-(1-naphthalenyl)-1-phenyl-2-butanol compound with fumaric acid (1:1). It has a molecular formula of $C_{32}H_{31}BrN_2O_2 \cdot C_4H_4O_4$ and a molecular weight of 671.58 (555.50 + 116.07). The molecular structure of bedaquiline fumarate is the following:



Pretomanid is a novel nitroimidazole anti-bacterial agent currently in development by Global TB Alliance. As a potential TB therapy, it has many attractive characteristics - most notably its novel mechanism of action, its activity *in vitro* against all tested drug-resistant clinical isolates, and its activity as both a potent bactericidal and a sterilizing agent. In addition, the compound shows no evidence of mutagenicity in a standard battery of genotoxicity studies, no significant cytochrome P450 interactions, and no significant activity against a broad range of Gram-positive and Gram-negative bacteria. The IUPAC designation for pretomanid is (6*S*)-2-nitro-6-{[4-(trifluoromethoxy)benzyl]oxy}-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine. Pretomanid has the following structure:



Pyrazinamide (pyrazine-2-carboxamide):



is the pyrazine analogue of nicotinamide and used as an anti-tuberculosis agent. Pyrazinamide is most commonly used for treatment of active tuberculosis (TB) during the initial phase of therapy (generally the first two months of treatment), in combination with other agents. Pyrazinamide demonstrates clinically significant antibacterial activity against *Mycobacterium tuberculosis* and *M. africanum.*

Thus, in one embodiment of the present invention, provided is a pharmaceutical composition, comprising a therapeutically effective amount of each of linezolid, bedaquiline and pretomanid, and optionally pyrazinamide, or a pharmaceutically acceptable salt of each thereof, and a pharmaceutically acceptable carrier.

In another embodiment of the invention, provided is a pharmaceutical composition wherein linezolid is at a dosage of 100 mg/kg.

In another embodiment of the invention, provided is a pharmaceutical composition wherein linezolid is at a dosage of 50 mg/kg.

In a further embodiment of the invention, provided is a method for the treatment of tuberculosis, comprising the step of administering to a patient in need thereof a therapeutically effective amount of each of linezolid, bedaquiline and pretomanid, and optionally pyrazinamide, or a pharmaceutically acceptable salt of each thereof, and a pharmaceutically acceptable carrier.

In another embodiment of the invention, provided is a method for the treatment of tuberculosis, wherein linezolid is administered for up to three months.

In another embodiment of the invention, provided is a method for the treatment of tuberculosis, wherein linezolid is administered for up to two months.

In another embodiment of the invention, provided is a method for the treatment of tuberculosis, wherein linezolid is administered for up to one month.

In another embodiment of the invention, provided is a method for the treatment of tuberculosis, wherein linezolid is administered for up to one to two months at 100 mg/kg per day.

In another embodiment of the invention, provided is a method for the treatment of tuberculosis, wherein linezolid is administered for up to two months at 50 mg/kg per day.

In another embodiment of the invention, provided is a method for the treatment of tuberculosis, wherein linezolid is administered for up to one month at 100 mg/kg per day.

In another embodiment of the invention, provided is a method for the treatment of tuberculosis, wherein linezolid is administered at a dosage of 600 mg once daily for the first one to two months.

In another embodiment,, wherein linezolid is re-administered after a one to two week drug holiday.

In a further embodiment, bedaquiline is at a dosage of 200 to 400 mg qd. In an alternative embodiment, bedaquiline is administered at 400 mg qd for two weeks followed by 200 mg tiw.

In a still further embodiment, linezolid is at a dosage of 600 mg bid or 1200 mg qd.

In another embodiment, pretomanid is at a dosage of 100 to 200 mg qd.

Definitions and Certain Components of the Invention

When trade names are used herein, applicants intend to independently include the trade name product and the active pharmaceutical ingredient(s) of the trade name product.

The term "chemical stability" means that the three antibacterial agents in combination are substantially stable to chemical degradation. Preferably, they are sufficiently stable in physical combination to permit commercially useful shelf life of the combination product. Typically, "chemically stable" means that a first component of the mixture does not act to degrade a second component when the two are brought into physical combination to form a pharmaceutical dosage form. More typically, "chemically stable" means that the acidity of a first component does not catalyzes or otherwise accelerate the acid decomposition of a second or a third component.

The terms "synergy" and "synergistic" mean that the effect achieved with the compounds used together is greater than the sum of the effects that results from using the compounds separately, i.e. greater than what would be predicted based on the two active ingredients administered separately. A synergistic effect may be attained when the compounds are: (1) co-formulated and administered or delivered simultaneously in a combined formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or delivered sequentially, e.g. in separate tablets, pills or capsules, or by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e. serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together. A synergistic antibacterial effect denotes an antibacterial effect which is greater than the predicted purely additive effects of the individual compounds of the combination.

"Bioavailability" is the degree to which the pharmaceutically active agent becomes available to the target tissue after the agent's introduction into the body. Enhancement of the bioavailability of a pharmaceutically active agent can provide a more efficient and effective treatment for patients because, for a given dose, more of the pharmaceutically active agent will be available at the targeted tissue sites.

The compounds of the combinations of the invention may be referred to as "active ingredients" or "pharmaceutically active agents."

The term "prodrug" as used herein refers to any compound that when administered to a biological system generates the drug substance, i.e. active ingredient, as a result of spontaneous chemical reaction(s), enzyme catalyzed chemical reaction(s), and/or metabolic chemical reaction(s).

"Prodrug moiety" means a labile functional group which separates from the active inhibitory compound during metabolism, systemically, inside a cell, by hydrolysis, enzymatic cleavage, or by some other process (Bundgaard, Hans, "Design and Application of Prodrugs" in *Textbook of Drug Design and Development* (1991), P. Krogsgaard-Larsen and H. Bundgaard, Eds. Harwood Academic Publishers, pp. 113-191). Prodrug moieties can serve to enhance solubility, absorption and lipophilicity to optimize drug delivery, bioavailability and efficacy. A "prodrug" is thus a covalently modified analog of a therapeutically-active compound.

Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., *McGraw-Hill Dictionary of Chemical Terms* (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., *Stereochemistry of Organic Compounds* (1994) John Wiley & Sons, Inc., New York. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (−) are employed to designate the sign of rotation of plane-polarized light by the compound, with (−) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these compounds, called stereoisomers, are identical except that they are mirror images of one another. A specific stereoisomer is also referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

Any reference to any of the compounds in the compositions of the invention also includes any physiologically acceptable salt thereof. Examples of physiologically acceptable salts and their physiologically functional derivatives include salts derived from an appropriate base, such as an alkali metal (for example, sodium), an alkaline earth (for example, magnesium), ammonium and NX_4^+ (wherein X is C_1-C_4 alkyl), or an organic acid such as fumaric acid, acetic acid, succinic acid. Physiologically acceptable salts of an hydrogen atom or an amino group include salts of organic carboxylic acids such as acetic, benzoic, lactic, fumaric, tartaric, maleic, malonic, malic, isethionic, lactobionic and succinic acids; organic sulfonic acids, such as methanesulfonic, ethanesulfonic, benzenesulfonic and p-toluenesulfonic acids; and inorganic acids, such as hydrochloric, sulfuric, phosphoric and sulfamic acids. Physiologically acceptable salts of a compound of an hydroxy group include the anion of said compound in combination with a suitable cation such as Na^+ and NX_4^+ (wherein X is independently selected from H or a C_1-C_4 alkyl group).

For therapeutic use, salts of active ingredients of the combinations of the invention will be physiologically acceptable, i.e. they will be salts derived from a physiologically acceptable acid or base. However, salts of acids or bases which are not physiologically acceptable may also find use, for example, in the preparation or purification of a physiologically acceptable compound. All salts, whether or not derived from a physiologically acceptable acid or base, are within the scope of the present invention.

The combination may be formulated in a unit dosage formulation comprising a fixed amount of each active pharmaceutical ingredient for a periodic, e.g. daily, dose or subdose of the active ingredients.

Pharmaceutical formulations according to the present invention comprise a combination according to the invention together with one or more pharmaceutically acceptable carriers or excipients and optionally other therapeutic agents. Pharmaceutical formulations

containing the active ingredient may be in any form suitable for the intended method of administration. When used for oral use for example, tablets, troches, lozenges, aqueous or oil suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs may be prepared (Remington's Pharmaceutical Sciences (Mack Publishing Co., Easton, Pa.). Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including antioxidants, sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide a palatable preparation. Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipient or auxiliary agents which are suitable for manufacture of tablets are acceptable. Suitable excipients or auxiliary agents include but are not limited to, for example, inert diluents, solubilizers, suspending agents, adjuvants, wetting agents, sweeteners, perfuming or flavoring substances, isotonic substances, colloidal dispersants and surfactants, including, but not limited to, charged phospholipids such as dimyristoylphosphatidylglycerin, alginiinic acid, alginates, acacia resin, gum arabic, 1,3-butylene glycol, benzalkonium chloride, colloidal silicon dioxide, cetosteryl alcohol, cetomacrogol emulsifying wax, casein, calcium stearate, cetylpyridine chloride, cetyl alcohol, cholesterol, calcium carbonate, CRODESTAS F-110, which is a mixture of sucrose stearate and sucrose distearate (Croda Inc.), clays, kaolin and bentonite, derivates of cellulose and salts thereof, such as hydroxypropyl methylcellulose (HPMC), sodium carboxymethyl cellulose, carboxymethyl cellulose and salts thereof, methyl cellulose, hydroxyethyl cellulose, hydroxpropyl cellulose, hydroxypropyl methylcellulose phtalate, non-crystalline cellulose, dicalcium phosphate, dodecyltrimethylammonium bromide, dextrane, dialkylester of sodium sulfosuccinate (e.g. AEROSEL OT, American Cyanamid), gelatine, glycerol, glycerol monostearate, glucose, p-isononylphenoxypoly (glycidol), also known as Olin 10-G or 10-GR surfactant (Olin Chemicals, Stamford, Conn.); glucamides such as octanoyl-N-methylglucamide, decanoyl-N-methylglucamide and heptanoyl-N-methylglucamide, lactose, lecithin (phosphatides), maltosides such as n-dodecyl-beta-D-maltoside, mannitol, magnesium sterarate, magnesium aluminium silicates, oils such as cotton oil, seed oil, olive oil, castor oil and sesame oil; paraffin, potato starch, polyethylene glycol (e.g. CARBOWAX 3350, CARBOWAX 1450 and CARBOPOL 9340 (Union Carbide), polyoxyethylene alkyl ester (e.g. macrogolethers such as CETOMACROGOL 1000), polyoxyethylene sorbitol fatty acid esters (e.g. TWEENS, ICI

Specialty Chemicals), polyoxyethylene castor oil derivatives, polyoxyethylene stearates, polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), phosphates, 4-(1,1,3,3-tetramethylbutyl)phenol polymer with ethylene oxide and formaldehyde (also known as TYLOXAPOL, SUPERIONE and TRITON), poloxamers and polaxamines (e.g. PLURONICS F68LF, F87, F108 and TETRONIC 908, available from BASF Corporation, Mount Olive, N.J.), pyranosides such as n-hexyl-beta-D-glucopyranoside, n-decyl-beta-D-glucopyranoside, n-octyl-beta-D-glucopyranoside, quaternary ammonium compounds, silica, sodium citrate, starches, sorbitol esters, sodium carbonate, solid polyethylene glycols, sodium dodecyl sulfate, sodium lauryl sulfate (e.g. DUPONAL P, DuPont), stearic acid, sucrose, tapioka starch, talc, thioglucosides such as n-heptyl-.beta.-D-thioglucoside, tragacanth, triethanolamine, TRITON X-200 (Rohm and Haas); and the like.

Formulations for oral use may be also presented as hard gelatin capsules where the active ingredient is mixed with an inert solid diluent, for example pregelatinized starch, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions of the invention contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include a suspending agent, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropyl methylcelluose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethyleneoxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan monooleate). The aqueous suspension may also contain one or more preservatives such as ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose, sucralose or saccharin.

Oil suspensions may be formulated by suspending the active ingredient in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid

paraffin. The oral suspensions may contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid, BHT, etc.

Dispersible powders and granules of the invention suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent, and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those disclosed above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

According to another embodiment of the invention, provided is a pharmaceutical composition in the form of a dispersible tablet. Dispersible tablets are intended to be dispersed in water before administration, providing a homogeneous dispersion. Dispersible tablets disintegrate within, for example, 3 minutes using water at 15-25°C.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions or liposome formulations. The oily phase may be a vegetable oil, such as olive oil or arachis oil, a mineral oil, such as liquid paraffin, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan monooleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavoring agents. Syrups and elixirs may be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.

The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a time-release formulation intended for oral administration to humans may contain approximately 1 to 1000 mg of active material compounded with an

appropriate and convenient amount of carrier material which may vary from about 5 to about 95% of the total compositions (weight:weight). The pharmaceutical composition can be prepared to provide easily measurable amounts for administration. As noted above, formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion.

The combinations of the invention may conveniently be presented as a pharmaceutical formulation in a unitary dosage form. A convenient unitary dosage formulation contains the active ingredients in any amount from 1 mg to 1 g each, for example but not limited to, 10 mg to 500 mg for each active ingredient. In certain embodiments, 50 mg to 300 mg of each active ingredient can be used.

Segregation of active ingredients in pharmaceutical powders and granulations is a widely recognized problem that can result in inconsistent dispersions of the active ingredients in final dosage forms. Some of the main factors contributing to segregation are particle size, shape and density. Segregation is particularly troublesome when attempting to formulate a single homogenous tablet containing multiple active ingredients having different densities and different particle sizes. Glidants are substances that have traditionally been used to improve the flow characteristics of granulations and powders by reducing interparticulate friction. See Lieberman, *Lachman, & Schwartz, Pharmaceutical Dosage Forms: Tablets, Volume* 1, p. 177-178 (1989), incorporated herein by reference. Glidants are typically added to pharmaceutical compositions immediately prior to tablet compression to facilitate the flow of granular material into the die cavities of tablet presses. Glidants include: colloidal silicon dioxide, asbestos free talc, sodium aluminosilicate, calcium silicate, powdered cellulose, microcrystalline cellulose, corn starch, sodium benzoate, calcium carbonate, magnesium carbonate, metallic stearates, calcium stearate, magnesium stearate, zinc stearate, stearowet C, starch, starch 1500, magnesium lauryl sulfate, and magnesium oxide. The novel compositions of the present invention may contain glidants to effect and maintain homogeneity of active ingredients during handling prior to tablet compression.

Compositions of the present invention are administered to a human or other mammal in a safe and therapeutically effective amount as described herein. These safe and therapeutically effective amounts will vary according to the type and size of mammal being treated and the desired results of the treatment. A "therapeutically effective amount" is an amount effective for treating tuberculosis. The term "treating", with regard to a subject, refers to improving at least one symptom of the subject's disorder. Treating can be curing, improving, or at least partially ameliorating the disorder.

Any of the various methods known by persons skilled in the art for packaging tablets, caplets, or other solid dosage forms suitable for oral administration, that will not degrade the components of the present invention, are suitable for use in packaging. The combinations may be packaged in glass and plastic bottles. Tablets, caplets, or other solid dosage forms suitable for oral administration may be packaged and contained in various packaging materials optionally including a dessicant e.g. silica gel. Packaging may be in the form of unit dose blister packaging. For example, a package may contain one blister tray of tenofovir DF and another blister tray of emtricitabine pills, tablets, caplets, or capsule. A patient would take one dose, e.g. a pill, from one tray and one from the other. Alternatively, the package may contain a blister tray of the co-formulated combination of tenofovir DF and emtricitabine in a single pill, tablet, caplet or capsule. As in other combinations and packaging thereof, the combinations of the invention include physiological functional derivatives of tenofovir DF and FTC.

The packaging material may also have labeling and information related to the pharmaceutical composition printed thereon. Additionally, an article of manufacture may contain a brochure, report, notice, pamphlet, or leaflet containing product information. This form of pharmaceutical information is referred to in the pharmaceutical industry as a "package insert." A package insert may be attached to or included with a pharmaceutical article of manufacture. The package insert and any article of manufacture labeling provides information relating to the pharmaceutical composition. The information and labeling provides various forms of information utilized by health-care professionals and patients, describing the composition, its dosage and various other parameters required by regulatory agencies such as the United States Food and Drug Agency.

Examples

The following examples further describe and demonstrate particular embodiments within the scope of the present invention. Techniques and formulations generally are found in *Remington's Pharmaceutical Sciences* (Mack Publishing Co., Easton, Pa.). The disclosure is further illustrated by the following examples, which are not to be construed as limiting this disclosure in scope or spirit to the specific procedures herein described. It is to be understood that the examples are provided to illustrate certain embodiments and that no limitation to the scope of the disclosure is intended thereby. It is to be further understood that resort may be had to various other embodiments, modifications, and equivalents thereof which may suggest themselves to those skilled in the art without departing from the spirit of the present disclosure and/or scope of the appended claims.

Materials and Methods

Mycobacterial strain. *M. tuberculosis* H37Rv was mouse-passaged, frozen in aliquots and sub-cultured in Middlebrook 7H9 broth with 10% oleic acid-albumin-dextrose-catalase (OADC) (Fisher, Pittsburgh, PA) and 0.05% Tween 80 prior to infection.

Antimicrobials. INH, RIF, PZA, BDQ, PMD and LZD were obtained and formulated for oral administration as previously described (9, 10, 12, 13). SZD (prepared in a PEG-200/0.5% methylcellulose suspension) and TZD (prepared as phosphate prodrug dissolved in water) were synthesized by WuXi (Hubei, China). AZD (prepared as disodium phosphate prodrug dissolved in 0.3% dextrose and 0.9% saline) was provided by AstraZeneca. RWJ (prepared in 0.5%
methylcellulose) was provided by Johnson and Johnson.

Pharmacokinetics of oxazolidinones. Uninfected female BALB/c mice weighing approximately 20 g received single doses of SZD (50 mg/kg), LZD (100 mg/kg), TZD (10 or 20 mg/kg) or AZD (50 or 200 mg/kg). Three mice per dose per time point were sampled by cardiac puncture at 0.5, 1, 2, 4, 8 and 24 hours post-dose (plus an additional 16 hour time point for AZD). Plasma drug concentrations were quantified by a validated LC/MS method performed at AstraZeneca (for AZD only) (14) or Rutgers New Jersey Medical School (for other oxazolidinones). Standards for the different oxazolidinones were provided by the sponsors. At

Rutgers, analytes of interest were extracted by combining 20 µl of mouse plasma with 20 µl of acetonitrole:water (1:1), and 180 µl of methanol:acetonitrile (1:1) containing 10 ng/ml of verapamil (Sigma-Aldrich) as internal standard. The mixture was vortexed and centrifuged, and 100 µL of the supernatant was recovered and combined with 100µL of water for analysis. LC/MS-MS analysis was performed with an Agilent 1260 LC-system coupled to an AB Sciex 4000 Q-trap Mass Spectrometer (positive mode, electrospray ionization), and an Agilent column SB-C8, 2.1 x 30mm, 3.5 µm, with the column temperature fixed at 24°C. Mobile phase A was 0.1% formic acid in 100% H_2O and mobile phase B was 0.1% formic acid in 100% acetonitrile. Injection volumes were routinely 2 µl. The Mass Selective Detector was set to MRM (multiple reaction monitoring) mode using positive polarity ionization, monitoring for the ions of interest SZD (m/z 354.12/312.04), SZD-M1(370.13/238.12), LZD(338.00/235.00), and TZD(371.12/343.00) and the internal standard (m/z 455.4/165.2). The lower limit of quantification for all oxazolidinones was 5 ng/ml. PK parameters were determined by non-compartmental analysis using WinNonlin 6.4 (Certara, Princeton, NJ).

Aerosol infection with *M. tuberculosis*. All animal procedures were approved by the Johns Hopkins University Animal Care and Use Committee. High-dose aerosol infection was performed as previously described (15). Briefly, 5-to-6-week-old female BALB/c mice (Charles River, Wilmington, MA) were infected with *Mycobacterium tuberculosis* H37Rv, using the Inhalation Exposure System (Glas-Col, Terre Haute, IN) and a fresh log phase broth culture (optical density at 600 nm of 0.8-1.0) with the goal of implanting 3.5-4.0 $\log_{10}$ CFU in the lungs of each mouse. Two or three mice from each aerosol infection run (Examples 1, 2, 4) or five mice from the only run (Examples 3a and 3b) were humanely killed 1 day after infection and on the day of treatment initiation (D0) to determine the number of bacteria implanted in the lungs and at the start of treatment, respectively.

Chemotherapy. Mice were block-randomized by aerosol run to experimental arms prior to treatment. Treatment was initiated 14-17 days after infection. Treatment was administered once daily, 5 days per week, by gavage. Except for dose-ranging monotherapy examples, drug doses (in mg/kg) were: INH (10), RIF (10), PZA (150), BDQ (25), PMD (50 or 100), SZD (50), LZD (50 or 100), TZD (10), AZD (125) and RWJ (100) (12, 14, 16-18). Each

drug was administered once daily, by gavage, 5 days per week. For all combinations, PMD was administered immediately after the dose of BDQ +/- PZA, which were formulated together (9, 12). Oxazolidinones were administered at least 4 hours later.

In Example 1, control mice received RIF+INH+PZA for 2 months followed by RIF+INH alone for a total of up to 4 months. To compare the contribution of SZD and LZD, test mice received a 3-drug combination of BDQ and PMD (50 mg/kg) plus either SZD (50 mg/kg) or LZD (100 mg/kg), or each 1- or 2-drug component comprising these 3-drug regimens. One- and two-drug regimens were limited to 1 and 2 months of treatment, respectively, except that the BDQ+PMD combination was given for up to 4 months along with the 3-drug combinations. In Example 2, the contribution of LZD to regimens containing BDQ+PMD plus PZA was assessed. Control mice received BDQ+PZA plus PMD at either 50 or 100 mg/kg. Test mice received BDQ+PZA+PMD (100 mg/kg) plus LZD (100 mg/kg) for up to 2 months. One cohort received LZD for the entire 2 months. Another received LZD for the first month only.

Assessment of treatment efficacy. Efficacy was assessed on the basis of lung CFU counts at selected time points during treatment (a measure of bactericidal activity) and the proportion of mice with culture-positive relapse after treatment completion (a measure of sterilizing activity). Quantitative cultures of lung homogenates were performed in parallel on 7H11 agar enriched with OADC (basic agar) and on basic agar supplemented with 0.4% activated charcoal to reduce drug carryover effects (12). Plates were incubated for up to 42 days at 37°C before final CFU counts were determined. Lung CFU counts were assessed in four or five mice per treatment group at each time point in Examples 1 and 2, respectively. The proportion of mice with culture-positive relapse was determined by holding cohorts of 15-20 mice for 3 additional months after completion of treatment, then sacrificing them to determine the proportion with positive lung cultures, as defined by ≥1 CFU of *M. tuberculosis* detected after plating the entire lung homogenate onto five 7H11 plates with and without 0.4% activated charcoal.

Statistical analysis. CFU counts (x) were log-transformed as (x+1) before analysis and group means were compared by one-way analysis of variance with Dunnett's post-test to

control for multiple comparisons. Group relapse proportions were compared using Fisher's exact test, adjusting for multiple comparisons. GraphPad Prism version 5 (GraphPad, San Diego, CA) was used for all analyses. Use of 15 mice per group for relapse assessment provides greater than 80% power to detect 40 percentage point differences in the relapse rate, after setting α at 0.01 to adjust for up to 5 simultaneous two-sided comparisons. Smaller differences may not be meaningful in terms of shortening the duration of treatment.

Example 1

Contribution of LZD and SZD to novel combinations with BDQ+PMD

Example 1 was performed to evaluate whether the marketed oxazolidinone LZD is able to replace SZD in this combination without loss of efficacy and to clarify the contribution of each drug component to the activity of the combination.

Lung CFU counts during treatment. The mean CFU count (± S.D.) at the start of treatment was 6.17 ± 0.27. The lung CFU counts after 1, 2, and 3 months of treatment are presented in Table 1:

Table 1
Lung CFU counts assessed during treatment and proportion of mice relapsing after treatment completion

	Mean (±SD) $\log_{10}$ CFU count at[a]:					Proportion (%) relapsing after treatment for:		
Drug Regimen	**D-13**	**D0**	**M1**	**M2**	**M3**	**2 mos**	**3 mos**	**4 mos**
Untreated	2.69±0.13	6.17±0.27	6.47±0.06					
2RIF+INH+PZA/ RIF+INH			3.47±0.37	1.59±0.25	0.50±0.51		13/15 (87)	1/20 (5)
BDQ			3.24±0.25					
PMD			4.57±0.22					
LZD			4.97±0.26					
SZD			3.85±0.37					
BDQ+PMD			4.21±0.40	1.62±0.19	0.52±0.36	15/15 (100)	10/15 (60)	2/20 (10)
BDQ+LZD			2.82±0.15	1.91±0.66				
BDQ+SZD			2.88±0.07	0.65±0.50				

PMD+LZD			3.23±0.41	1.48±0.12				
PMD+SZD			1.65±0.33	0.23±0.40				
BDQ+PMD+LZD			3.28±0.65	0.34±0.41	0.00±0.00	12/15 (80)	0/14 (0)	0/20 (0)
BDQ+PMD+SZD			0.94±0.14	0.00±0.00		14/20 (70)	1/14 (7)	

[a] Time points are shown in days (e.g., D-13, day-13; D0, day 0) or months (e.g., M1, 1 month) of treatment.

As expected, RIF+INH+PZA reduced the mean lung CFU count by 2.70 $\log_{10}$ and by 4.58 $\log_{10}$ after 1 and 2 months of treatment, respectively, and left fewer than 10 CFU/mouse after 3 months of treatment. Due to the previously described antagonism of PMD on BDQ activity (7, 12), BDQ+PMD had lower activity than BDQ alone over the first month. However, its activity was virtually identical to that of the first-line regimen over 2-3 months. Addition of SZD significantly increased the initial bactericidal activity of BDQ+PMD ($p< 0.001$), rendering all mice culture-negative between 1 and 2 months of treatment. The activity of BDQ+PMD+SZD was significantly greater than that of RIF+INH+PZA after 1 and 2 months of treatment ($p< 0.001$). Addition of LZD also significantly increased the activity of BDQ+PMD ($p< 0.01$) and produced activity superior to that of RIF +INH+PZA after 2 and 3 months of treatment ($p< 0.001$). All 2-drug combinations had inferior activity compared to the 3-drug combinations of BDQ+PMD plus SZD or LZD at 2 months ($p< 0.01$), confirming that each component drug contributes to the efficacy of the 3-drug combinations. However, the 3-drug LZD-containing regimen was only superior to BDQ+LZD after 2 months of treatment. LZD-containing regimens produced higher CFU counts compared to their SZD-containing comparator regimen.

Relapse after treatment completion. The relapse results are displayed in Table 1. Treatment with the first-line regimen for 3 and 4 months resulted in relapse of 13 (87%) of 15 and 1 (5%) of 20 mice, respectively. The higher cure rate compared to previous examples is due to the lower than usual bacterial burden at the initiation of treatment. Consistent with the CFU count results, treatment with BDQ+PMD produced relapse results similar to the first-line regimen after 3 and 4 months but did not prevent relapse after just 2 months of treatment. However, unlike the more rapid decline in CFU counts with the 3-drug regimen containing SZD compared to LZD, the two BDQ+PMD+oxazolidinone regimens had similar sterilizing activity.

While only the difference between the SZD-containing regimen and BDQ+PMD alone was statistically significant after 2 months of treatment, both oxazolidinone-containing regimens resulted in significantly fewer relapses after 3 months of treatment compared to both BDQ+PMD and the first-line regimen.

Example 2

Contribution of LZD to BDQ+PZA+PMD

It was previously reported the potent sterilizing activity of BDQ+PZA+SZD in mice and the additive sterilizing activity of PMD (7, 9, 10, 12). After observing that LZD had comparable sterilizing activity to SZD in combination with BDQ+PMD in Example 1, LZD could also replace SZD in the BDQ+PZA+PMD+SZD combination. The mean CFU count at the start of treatment was 7.92 ± 0.26. The lung CFU count and relapse results are displayed in Table 2:

Table 2
Lung CFU counts assessed during treatment and proportion of mice relapsing after treatment completion

	Mean (±SD) log_{10} CFU count at[a]:				Proportion (%) relapsing after treatment for:	
Regimen	**D-13**	**D0**	**M1**	**M2**	**1.5 mos**	**2 mos**
Untreated	4.42 ±0.15	7.92±0.26				
RIF+INH+PZA				2.06±0.37		
BDQ+PZA+PMD_{50}			2.91±0.33	0.95±0.38		
BDQ+PZA+PMD			2.93±0.31	0.06±0.13	9/15 (60)	1/15 (7)
1 BDQ+PZA+PMD+LZD/ 1 BDQ+PZA+PMD			0.11±0.24		0/15 (0)	0/15 (0)
BDQ+PZA+PMD+LZD					0/15 (0)	1/15 (7)

[a] Time points are shown in days (e.g., D-13, day-13; D0, day 0) or months (e.g., M1, 1 month) of treatment.

As previously observed, BDQ+PZA+PMD had significantly greater bactericidal activity compared to RIF+INH+PZA ($p< 0.001$). Greater activity was observed when PMD was dosed at 100 rather than 50 mg/kg, but the difference was only noted after 2 months of treatment ($p< 0.01$). The higher PMD dose helped render all mice but one culture negative at this time point. The addition of LZD had a dramatic effect on efficacy, significantly reducing the CFU count at 1 month ($p< 0.001$) and rendering all mice but one culture-negative one month earlier. The addition of LZD to BDQ+PZA+PMD also significantly reduced the proportion of mice relapsing after 1.5 months of treatment from 60% to 0% ($p< 0.001$), irrespective of whether LZD was discontinued after the first month of treatment.

Example 3
Contribution of oxazolidinones to novel combinations with BDQ+PMD

Example 3 was performed to confirm the additive sterilizing activity of SZD and LZD when added to BDQ+PMD in Example 1 and to evaluate whether reducing the LZD dose by 50% or limiting the duration of LZD to the first 1-2 months would significantly affect its contribution.

Lung CFU counts during treatment. The mean CFU count at the start of treatment was 7.74 ± 0.20. The lung CFU counts observed after 1, 2 and 3 months of treatment are presented in Table 3. RIF+INH+PZA reduced the mean lung CFU count by almost 6 $\log_{10}$ over 2 months of treatment. BDQ+PMD alone had modestly lower activity over this period. As previously observed, addition of SZD 50 mg/kg conferred superior activity compared to LZD 100 mg/kg, although both oxazolidinones significantly increased the initial bactericidal activity of BDQ+PMD. However, results with BDQ+PMD+LZD 100 mg/kg were similar whether LZD was discontinued after 1 month or continued for 2 months. Similarly, use of LZD 50 mg/kg in place of 100 mg/kg resulted in higher CFU counts at 1 month, but not 2 months, suggesting that the total LZD dose achieved with 100 mg/kg for 1 month or 50 mg/kg for 2 months sufficiently maximized the contribution of LZD to the regimen. RWJ at 100 mg/kg performed very similarly to LZD at 50 mg/kg, whereas AZD and TZD were somewhat less effective. Compared to BDQ+PMD alone, all oxazolidinones significantly increased bactericidal activity at 1 and 2

months, except TZD at the 1-month time point ($p < 0.05$ for AZD and for TZD at M2; $p < 0.001$ for other oxazolidinones).

Relapse after treatment completion. The relapse results are displayed in Table 3:

Table 3

Lung CFU counts assessed during treatment and proportion of mice relapsing after treatment completion

	Mean (±SD) log_{10} CFU count at[a]:				Proportion (%) relapsing after treatment for:	
Regimen	**D-13**	**D0**	**M1**	**M2**	**2 mos**	**3 mos**
Untreated	3.96±0.08	7.74±0.20				
2RIF+INH+PZA/ 1RIF+INH				1.94±0.27		8/14 (57)
BDQ+PMD			4.48±0.20	2.33±0.30		3/14 (21)
BDQ+PMD+TZD			4.20±0.13	1.67±0.41		
BDQ+PMD+AZD			4.07±0.36	1.43±0.36		
BDQ+PMD+RWJ			3.63±0.18	0.54±0.41		
BDQ+PMD+LZD_{50}			3.48±0.36	0.39±0.26		
1BDQ+PMD+LZD_{100}/ BDQ+PMD			2.69±0.37	0.93±0.49	9/15 (60)	0/15 (0)
2BDQ+PMD+LZD_{100}/ BDQ+PMD				0.66±0.39	6/15 (40)	0/15 (0)
2BDQ+PMD+LZD_{100}/ BDQ+PMD+LZD_{50}						0/12 (0)
BDQ+PMD+LZD_{100}						0/15 (0)
BDQ+PMD+SZD			1.88±0.22	0.00±0.00	1/14 (7)	0/14 (0)

[a] Time points are shown in days (e.g., D-13, day-13; D0, day 0) or months (e.g., M1, 1 month) of treatment.

Treatment with the first-line regimen for 3 months resulted in relapse of 8 (57%) of 14 mice. Treatment with BDQ+PMD produced numerically superior results, with just 3 (21%) of 14 mice relapsing at 3 months, although this difference was not statistically significant. Addition of SZD to BDQ+PMD resulted in only one relapse after just 2 months of treatment, a result that was at least as effective as 3 months of BDQ+PMD and more effective than 3 months of the first-line regimen. Addition of SZD at 50 mg/kg also resulted in fewer relapses than did the addition of LZD 100 mg/kg for 2 months, although the difference did not reach statistical significance. Use of LZD at 100 mg/kg for the first month only was less effective than SZD for 2 months, but not significantly different from LZD 100 mg/kg for 2 months. Similarly, among regimens administered for 3 months, no relapse was observed irrespective of whether LZD 100 mg/kg was continued throughout or discontinued after 1 or 2 months, or replaced with LZD 50 mg/kg after 2 months. In a *post hoc* analysis comparing BDQ+PMD to all BDQ+PMD+LZD regimens, the addition of LZD 100 mg/kg for at least one month resulted in fewer relapses after 3 months of treatment.

Summary of Examples 1-3:

Oxazolidinone pharmacokinetics in BALB/c mice are presented in Table 4:

Table 4

Pharmacokinetic parameters after single dose oral administration of sutezolid, linezolid, tedizolid or AZD5847 in BALB/c mice

Compound (dose, mg/kg)		t1/2 hr	Tmax hr	Cmax ng/mL	AUC0-24hr hr*ng/ml
Sutezolid	**Mean**	**2.8**	**0.5**	**1292**	**3359**
50	SD	0.5	0.0	707	499

Sutezolid M1	**Mean**		**0.5**	**15233**	**31945**
	SD		0.0	7875	5853
Linezolid	**Mean**	**3.0**	**0.5**	**81933**	**243547**
100	SD	2.5	0.0	32240	31577
Tedizolid	**Mean**	**3.0**	**0.7**	**6417**	**40414**
10	SD	0.4	0.3	624	2942
Tedizolid	**Mean**	**3.9**	**0.5**	**11433**	**77698**
20	SD	1.4	0.0	1665	6107
AZD5847	**Mean**	**2.4**	**0.7**	**20433**	**74761**
50	SD	0.2	0.3	2079	6138
AZD5847	**Mean**	**8.6**	**0.7**	**31367**	**220246**
200	SD	2.9	0.3	1270	34953

As previously described (18), oral administration of SZD at 50 mg/kg resulted in rapid and extensive metabolism to the active sulfoxide M1 metabolite (also known as PNU-101603) for which the mean $AUC_{0\text{-}24h}$ and C_{max} were approximately 10 times higher than that of the SZD parent. Overall, the SZD and SZD M1 $AUC_{0\text{-}24h}$ values were similar to or, in the case of SZD, modestly lower than the geometric means observed in TB patients receiving 1,200 mg per day (8). LZD showed the highest exposure among the tested compounds with $AUC_{0\text{-}24h}$ of 244 hr*ug/mL that is comparable to the average among TB patients receiving 1,200 mg daily (19, 20). TZD exposure was dose proportional from 10 mg/kg to 20 mg/kg. For the 10 mg/kg dose evaluated in the efficacy study, the total drug TZD $AUC_{0\text{-}24h}$ of 40 hr*ug/mL was similar to that determined in other infection models (21-23). Interpolating between the mean AZD $AUC_{0\text{-}24h}$ values produced by the 50 and 200 mg/kg doses, the predicted $AUC_{0\text{-}24h}$ of 160-190 hr*ug/mL produced by the 125 mg/kg dose used in the combination efficacy study (Example 3 below) is similar to the steady state $AUC_{0\text{-}24h}$ in healthy human volunteers receiving 800 mg twice daily (24), the highest dose administered in a recent dose-ranging trial of early bactericidal activity. The oxazolidinones were cleared relatively rapidly, with $t_{1/2}$ values between 2.8 to 3.9 hrs. $AUC_{0\text{-}24h}$ values from all the compounds were approximately equal to AUC_{inf} values ($\geq$ 98%), suggesting that no accumulation would be expected after multiple doses in BALB/c mice.

The invention is further described in the following numbered paragraphs:

1. A pharmaceutical composition, comprising a therapeutically effective amount of each of linezolid, bedaquiline and pretomanid, and optionally pyrazinamide, or a pharmaceutically acceptable salt of each thereof, and a pharmaceutically acceptable carrier. [ADD CLINICAL DOSAGES].

2. The pharmaceutical composition according to paragraph 1, wherein linezolid is at a dosage of 100 mg/kg.

3. The pharmaceutical composition according to paragraph 1, wherein linezolid is at a dosage of 50 mg/kg.

4. A method for the treatment of tuberculosis, comprising the step of administering to a patient in need thereof a therapeutically effective amount of each of linezolid, bedaquiline and pretomanid, and optionally pyrazinamide, or a pharmaceutically acceptable salt of each thereof, and a pharmaceutically acceptable carrier.

5. The method according to paragraph 4, wherein linezolid is administered for up to three months.

6. The method according to paragraph 4, wherein linezolid is administered for up to two months.

7. The method according to paragraph 4, wherein linezolid is administered for up to one month.

8. The method according to paragraph 4, wherein linezolid is administered for up to one to two months at 100 mg/kg per day.

9. The method according to paragraph 4, wherein linezolid is administered for up to two months at 50 mg/kg per day.

10. The method according to paragraph 4, wherein linezolid is administered for up to one month at 100 mg/kg per day.

11. The method according to paragraph 4, wherein linezolid is administered at a dosage of 600 mg once daily for the first one to two months.

12. The method according to paragraph 4, wherein linezolid is re-administered after a one to two week drug holiday.

13. The pharmaceutical composition according to paragraph 1, wherein bedaquiline is at a dosage of 200 to 400 mg qd.

14. The method according to paragraph 4, wherein bedaquiline is administered at 400 mg qd for two weeks followed by 200 mg tiw.

15. The pharmaceutical composition according to paragraph 1, wherein linezolid is at a dosage of 600 mg bid or 1200 mg qd.

16. The pharmaceutical composition according to paragraph 1, wherein pretomanid is at a dosage of 100 to 200 mg qd.

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* * *

It is to be understood that the invention is not limited to the particular embodiments of the invention described above, as variations of the particular embodiments may be made and still fall within the scope of the appended claims.

We claim

1. A pharmaceutical composition, comprising a therapeutically effective amount of each of linezolid, bedaquiline and pretomanid, and optionally pyrazinamide, or a pharmaceutically acceptable salt of each thereof, and a pharmaceutically acceptable carrier.

2. The pharmaceutical composition according to claim 1, wherein linezolid is at a dosage of 100 mg/kg.

3. The pharmaceutical composition according to claim 1, wherein linezolid is at a dosage of 50 mg/kg.

4. A method for the treatment of tuberculosis, comprising the step of administering to a patient in need thereof a therapeutically effective amount of each of linezolid, bedaquiline and pretomanid, and optionally pyrazinamide, or a pharmaceutically acceptable salt of each thereof, and a pharmaceutically acceptable carrier.

5. The method according to claim 4, wherein linezolid is administered for up to one to two months at 100 mg/kg per day.

6. The method according to claim 4, wherein linezolid is administered for up to two months at 50 mg/kg per day.

7. The pharmaceutical composition according to claim 1, wherein bedaquiline is at a dosage of 200 to 400 mg qd.

8. The method according to claim 4, wherein bedaquiline is administered at 400 mg qd for two weeks followed by 200 mg tiw.

9. The pharmaceutical composition according to claim 1, wherein linezolid is at a dosage of 600 mg bid or 1200 mg qd.

10. The pharmaceutical composition according to claim 1, wherein pretomanid is at a dosage of 100 to 200 mg qd.

Dated this 13th day of April 2018

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ABSTRACT

“COMBINATION ANTIBACTERIAL COMPOSITION AND SHORT-COURSE ANTIBACTERIAL REGIMEN”

The present invention relates to therapeutic combinations of anti-bacterial agents linezolid, bedaquiline and pretomanid, and optionally with pyrazinamide, in a short-course oral dosage regimen for the treatment of tuberculosis.

ANNEXURE - 2

Trial record **1 of 1** for: nct02333799

Previous Study | Return to List | Next Study

A Phase 3 Study Assessing the Safety and Efficacy of Bedaquiline Plus PA-824 Plus Linezolid in Subjects With Drug Resistant Pulmonary Tuberculosis

ClinicalTrials.gov Identifier: NCT02333799

Recruitment Status : Active, not recruiting
First Posted : January 7, 2015
Last Update Posted : April 24, 2020

Sponsor:
Global Alliance for TB Drug Development

Information provided by (Responsible Party):
Global Alliance for TB Drug Development

Study Details | Tabular View | No Results Posted | Disclaimer | How to Read a Study Record

Tracking Information
First Submitted Date ICMJE
January 6, 2015

First Posted Date [ICMJE]

January 7, 2015

Last Update Posted Date

April 24, 2020

Study Start Date [ICMJE]

March 2015

Actual Primary Completion Date

January 14, 2019 (Final data collection date for primary outcome measure)

Current Primary Outcome Measures [ICMJE]
(submitted: May 15, 2018)

Incidence of bacteriologic failure or relapse or clinical failure through follow up until 6 months after the end of treatment. [Time Frame: Treatment Period: Day 1, Week 1, 2, 4, 6, 8, 12, 16, 20, 26, 30, 34, 39 Follow Up: Month 1, 2, 3, 6, 9, 12, 15, 18, 21, 24]

Bacteriologic failure: During the treatment period, failure to attain culture conversion to negative. Bacteriologic relapse: During the follow-up period, failure to maintain culture conversion to negative status in culture, with culture conversion to positive status with a Mycobacterium tuberculosis (M.tb.) strain that is genetically identical to the infecting strain at baseline. Clinical failure: A change from protocol-specified TB treatment due to treatment failure, retreatment for TB during follow up, or TB-related death. Note: Culture conversion requires at least 2 consecutive culture negative/positive samples at least 7 days apart. Subjects who are documented at a visit as unable to produce sputum and who are clinically considered to be responding well to treatment will be considered to be culture negative at that visit.

Original Primary Outcome Measures [ICMJE]
(submitted: January 6, 2015)

Incidence of bacteriologic failure or relapse or clinical failure through follow up until 24 months after the end of treatment. [Time Frame: Treatment Period: Day 1, Week 1, 2, 4, 6, 8, 12, 16, 20, 26, 30, 34, 39 Follow Up: Month 1, 2, 3, 6, 9, 12, 15, 18, 21, 24]

Bacteriologic failure: During the treatment period, failure to attain culture conversion to negative. Bacteriologic relapse: During the follow-up period, failure to maintain culture conversion to negative status in culture, with culture conversion to positive status with a of Mycobacterium tuberculosis (M.tb.) strain that is genetically identical to the infecting strain at baseline. Clinical failure: A change from protocol-specified TB treatment due to treatment failure, retreatment for TB during follow up, or TB-related death. Note: Culture conversion requires at least 2 consecutive culture negative/positive samples at least 21 days apart. Subjects who are documented at a visit as unable to produce sputum and who are clinically considered to be responding well to treatment will be considered to be culture negative at that visit.

Change History

Complete list of historical versions of study NCT02333799 on ClinicalTrials.gov Archive Site

Current Secondary Outcome Measures ICMJE
(submitted: January 6, 2015)

- Time to sputum culture conversion to negative status through the treatment period. [Time Frame: Day 1, Week 1, 2, 4, 6, 8, 12, 16, 20, 26, Month 1, 2, 3, 6, 9, 12, 15, 18, 21, 24]
- Proportion of subjects with sputum culture conversion to negative status at 4, 6, 8, 12, 16 and 26 or 39 weeks. [Time Frame: Week 4, 6, 8, 12, 16, 26, 39]
- Incidence of Treatment Emergent Adverse Events (TEAEs) presented by incidence, and seriousness, leading to TB related or non-TB related death. [Time Frame: Day 1, Week 1, 2, 4, 6, 8, 12, 16, 20, 26, 30, 34, 39, Follow-up Month 3, 6, 9, 12, 15, 18, 21, 24]
- All Subjects- Pre-dose sampling at weeks 2, 8 and 16 to measure Ctrough levels of bedaquiline, bedaquiline metabolite M2, Linezolid and PA-824. [Time Frame: Weeks 2, 8 and 16]
- Time to sputum culture positivity [Time Frame: Treatment Period: Day 1, Week 1, 2, 4, 6, 8, 12, 16, 20, 26, 30, 34, 39 Follow Up: Month 1, 2, 3, 6, 9, 12, 15, 18, 21, 24]

  If liquid culture in the MGIT platform is used, the rate of change in time to sputum culture positivity (TTP) over time in the Mycobacterial Growth Indicator Tube (MGIT) system in sputum, represented by the model-fitted log(TTP) results as calculated by the regression of the observed log(TTP) results over time.

Original Secondary Outcome Measures ICMJE

Same as current

Current Other Pre-specified Outcome Measures

Not Provided

Original Other Pre-specified Outcome Measures

Not Provided

Descriptive Information

Brief Title ICMJE

A Phase 3 Study Assessing the Safety and Efficacy of Bedaquiline Plus PA-824 Plus Linezolid in Subjects With Drug Resistant Pulmonary Tuberculosis

Official Title ICMJE

A Phase 3 Open-label Trial Assessing the Safety and Efficacy of Bedaquiline Plus PA-824 Plus Linezolid in Subjects With Pulmonary Infection of Either Extensively Drug-resistant Tuberculosis (XDR-TB) or Treatment Intolerant / Non-responsive

Multi-drug Resistant Tuberculosis (MDR-TB).

Brief Summary

The purpose of this study is to evaluate the efficacy, safety, tolerability and pharmacokinetics of bedaquiline plus PA-824 plus linezolid after 6 months of treatment (option for 9 months for subjects who remain culture positive at month 4) in Subjects with either pulmonary extensively drug resistant tuberculosis (XDR-TB), treatment intolerant or non-responsive multi-drug resistant tuberculosis (MDR-TB).

Detailed Description

Not Provided

Study Type [ICMJE]

Interventional

Study Phase [ICMJE]

Phase 3

Study Design [ICMJE]

Allocation: N/A
Intervention Model: Single Group Assignment
Masking: None (Open Label)
Primary Purpose: Treatment

Condition [ICMJE]

Pulmonary Tuberculosis

Intervention [ICMJE]

- Drug: Bedaquiline

  100mg tablets

  Other Names:
  - B
  - TMC-207
- Drug: PA-824

  200mg tablets

  Other Names:
  - Pa
  - pretomanid
- Drug: Linezolid

  Scored 600mg tablets

Other Names:

- L
- Lin

Study Arms [ICMJE]

Experimental: Bedaquiline + PA-824 + Linezolid

bedaquiline 400 mg once daily for 2 weeks then 200mg 3 times per week plus PA-824 200mg once daily plus linezolid 1200mg once daily .

Interventions:

- Drug: Bedaquiline
- Drug: PA-824
- Drug: Linezolid

Publications *

Conradie F, Diacon AH, Ngubane N, Howell P, Everitt D, Crook AM, Mendel CM, Egizi E, Moreira J, Timm J, McHugh TD, Wills GH, Bateson A, Hunt R, Van Niekerk C, Li M, Olugbosi M, Spigelman M; Nix-TB Trial Team. Treatment of Highly Drug-Resistant Pulmonary Tuberculosis. N Engl J Med. 2020 Mar 5;382(10):893-902. doi: 10.1056/NEJMoa1901814.

*** Includes publications given by the data provider as well as publications identified by ClinicalTrials.gov Identifier (NCT Number) in Medline.**

Recruitment Information

Recruitment Status [ICMJE]

Active, not recruiting

Actual Enrollment [ICMJE]
(submitted: May 15, 2018)

109

Original Estimated Enrollment [ICMJE]
(submitted: January 6, 2015)

200

Estimated Study Completion Date [ICMJE]

July 13, 2020

Actual Primary Completion Date

January 14, 2019 (Final data collection date for primary outcome measure)

Eligibility Criteria [ICMJE]

Inclusion Criteria

1. Provide written, informed consent prior to all trial-related procedures (if under 18, include consent of legal guardian).
2. Body weight of ≥35 kg (in light clothing and no shoes).
3. Willingness and ability to attend scheduled follow-up visits and undergo study assessments
4. Provide consent to HIV testing (if an HIV test was performed within 1 month prior to trial start, it should not be repeated as long as documentation can be provided [ELISA and/or Western Blot]. If HIV status is a confirmed known positive, repeated HIV test is not needed provided documentation is available.
5. Male or female, aged 14 years or above.
6. Subjects with one of the following pulmonary TB conditions:

   a. XDR-TB with

   i. documented culture positive (for M.tb.) results within 3 months prior to screening or M.tb. confirmed in sputum based on molecular test within 3 months prior to or at screening;

   ii. documented resistance to isoniazid, rifamycins, a fluoroquinolone and an injectable historically at any time or at screening;

   b. MDR-TB documented by culture positive results (for M.tb.) within 3 months prior to or at screening with documented non-response to treatment with the best available regimen for 6 months or more prior to enrolment who in the opinion of the Investigator have been adherent to treatment and will be adherent to study regimen;

   c. MDR-TB documented by culture positive (for M.tb.) results within 3 months prior to or at screening who are unable to continue second line drug regimen due to a documented intolerance to:

   i. PAS, ethionamide, aminoglycosides or fluoroquinolones;

   ii. Current treatment not listed above that renders subject eligible for the study in the Investigator's opinion.
7. Chest X-Ray picture (taken within a year prior to screening) consistent with pulmonary TB in the opinion of the Investigator.
8. Be of non-childbearing potential or using effective methods of birth control, as defined below:

Non-childbearing potential:

1. Subject - not heterosexually active or practices sexual abstinence; or
2. Female Subject/sexual partner - bilateral oophorectomy, bilateral tubal ligation and/or hysterectomy or has been postmenopausal with a history of no menses for at least 12 consecutive months; or
3. Male Subject/sexual partner - vasectomised or has had a bilateral orchidectomy minimally three months prior to Screening.

Effective birth control methods:

A double contraceptive method should be used as follows:

1. Double barrier method which can include any 2 of the following: a male condom, diaphragm, cervical cap, or female condom (male and female condoms should not be used together); or
2. Barrier method (one of the above) combined with hormone-based contraceptives or an intra-uterine device for the female Subject/partner;
3. and are willing to continue practicing birth control methods throughout treatment and for 6 months (both male and female Subjects) after the last dose of study medication or discontinuation from study medication in case of premature discontinuation.

Note: Hormone based contraception alone may not be reliable when taking investigational medicinal products; therefore, hormone based contraceptives alone cannot be used by female Subjects or female partners of male Subjects to prevent pregnancy.

Exclusion Criteria Medical History

1. Any condition in the Investigator's opinion (i.e., an unstable disease such as uncontrolled diabetes or cardiomyopathy, extra-pulmonary TB requiring extended treatment), where participation in the trial would compromise the well-being of Subject or prevent, limit or confound protocol specified assessments.
2. Abuse of alcohol or illegal drugs, that in the opinion of the Investigator would compromise the Subjects' safety or ability to follow through with all protocol-specified visits and evaluations.
3. In the judgment of the Investigator, the patient is not expected to survive for more than 12 weeks.
4. Karnofsky score < 50 within 30 days prior to entry.
5. Body Mass index (BMI) < 17 kg/m^2
6. History of allergy or known hypersensitivity to any of the trial Investigational Medicinal Products or related substances.
7. HIV infected Subjects having a CD4+ count ≤ 50 cells/µL; For HIV infected Subjects having a CD4+ count >50 cells/µL;

   a. Currently treated with or will need to initiate antiretroviral therapy (ART) which is not compatible with the allowed ARTs and is not considered an appropriate candidate for switching to a regimen of ARVs which is allowed. Examples of allowed treatment include but are not limited to the following. If there are any questions, discuss with the Sponsor Medical Monitor for confirmation of appropriate ARV regimen.

   i. Nevirapine based regimen consisting of nevirapine in combination with any NRTIs;

   ii. Lopinavir/ritonavir (Aluvia™) based regimen consisting of lopinavir/ritonavir (Aluvia™) in combination with any NRTIs;

   iii. The combination of tenofovir/lamivudine/abacavir should be considered in patients with normal renal function to address myelosuppression cross toxicity of idovudine and linezolid;

   iv. An alternate regimen that may be considered if the above are not appropriate is a triple nucleosidase reverse transcriptase inhibitors (NRTI) based regimen consisting of zidovudine, lamivudine and abacavir

may be used with caution. Regimens including zidovudine should be used with special caution as zivovudine and linezolid may both cause peripheral nerve toxicity;

v. Raltegravir in combination with nucleoside reverse transcriptase inhibitors (NRTIs). b. Cannot ensure a 2 week interval between commencing IMP and the start of ART, if not already on ARTs.

8. Having participated in other clinical studies with dosing of investigational agents within 8 weeks prior to trial start or currently enrolled in an investigational study that includes treatment with medicinal agents. Subjects who are participating in observational studies or who are in a follow up period of a trial that included drug therapy may be considered for inclusion.
9. Significant cardiac arrhythmia requiring medication.
10. Subjects with the following at Screening:
    a. QTcF interval on ECG >500 msec. Subjects with QTcF > 450 must be discussed with the sponsor medical monitor before enrolment.
    b. History of additional risk factors for Torsade de Pointes, (e.g., heart failure, hypokalemia, family history of Long QT Syndrome);
    c. Clinically significant ventricular arrhythmias;
    d. Subjects with other cardiac abnormalities that may place them at risk of arrhythmias must be discussed with the sponsor medical monitor before enrolment. Such abnormalities include: Evidence of ventricular pre-excitation (e.g., Wolff Parkinson White syndrome); Electrocardiographic evidence of complete or clinically significant incomplete left bundle branch block or right bundle branch block; Evidence of second or third degree heart block; Intraventricular conduction delay with QRS duration more than 120 msec.
11. Females who have a positive pregnancy test at Screening or already known to be pregnant, breastfeeding, or planning to conceive a child during the study or within 6 months of cessation of treatment. Males planning to conceive a child during the study or within 6 months of cessation of treatment.
12. A peripheral neuropathy of Grade 3 or 4, according to DMID (Appendix 2). Or, subjects with a Grade 1 or 2 neuropathy which is likely to progress/worsen over the course of the study, in the opinion of the Investigator.
13. Concomitant use of Monoamine Oxidase Inhibitors (MAOIs) or prior use within 2 weeks of treatment assignment.
14. Concomitant use of serotonergic antidepressants or prior use within 3 days of treatment assignment if Investigator foresees potential risks for serotonin syndrome when combined with linezolid.
15. Concomitant use of any drug known to prolong QTc interval (including, but not limited to, amiodarone, bepridil, chloroquine, chlorpromazine, cisapride, cyclobenzaprine, clarithromycin, disopyramide, dofetilide, domperidone, droperidol, erythromycin, fluoroquinolones, halofantrine, haloperidol, ibutilide, levomethadyl, mesoridazine, methadone, pentamidine, pimozide, procainamide, quinidine, sotalol, sparfloxacin, thioridazine).
16. Concomitant use of any drug known to induce myelosuppression.

17. Use of any drugs or substances within 30 days prior to dosing known to be strong inhibitors or inducers of cytochrome P450 enzymes(including but not limited to quinidine, tyramine, ketoconazole, fluconazole, testosterone, quinine, gestodene, metyrapone, phenelzine, doxorubicin, troleandomycin, cyclobenzaprine, erythromycin, cocaine, furafylline, cimetidine, dextromethorphan). Exceptions may be made for subjects that have received 3 days or less of one of these drugs or substances, if there has been a wash-out period before administration of IMP equivalent to at least 5 half-lives of that drug or substance.
18. Subjects may have previously been treated for DS/MDR-TB (with specific excepetions for Bedaquiline and/or linezolid as noted below) provided that treatment is/was discontinued at least 3 days prior to treatment assignment.
19. Subjects should not receive more than 2 weeks of bedaquiline or linezolid prior to enrolment/first dose of IMP.

    Based on Laboratory Abnormalities

20. Subjects with the following toxicities at Screening (labs may be repeated) as defined by the enhanced Division of Microbiology and Infectious Disease (DMID) adult toxicity table (November 2007):

    a. serum potassium less than the lower limit of normal for the laboratory;

    b. Hemoglobin level grade 2 or greater (< 8.0 g/dL);

    c. Platelets grade 2 or greater(<75,000/mm3);

    d. Absolute neutrophil count (ANC) < 1000/ mm3;

    e. Aspartate aminotransferase (AST)

    Grade 3 or greater (> 3.0 x ULN) to be excluded;

    Greater than ULN must be discussed with and approved by the sponsor Medical Monitor

    f. Alanine aminotransferase

    Grade 3 or greater (> 3.0 x ULN) to be excluded

    greater than ULN must be discussed with and approved by the sponsor medical monitor ;

    g. Total bilirubin:

    Grade 3 or greater (≥2.0 x ULN), or if ≥1.5 up to 2.0 x ULN when accompanied by an increase in other liver function test (ALT, AST, Alk Phos or GGT);

    1-1.5 x ULN must be discussed with and approved by the sponsor Medical Monitor

    h. Direct bilirubin:

    Greater than ULN to be excluded

    i. Serum creatinine level greater than 2 times upper limit of normal

    j. Albumin <32 g/L

Sex/Gender [ICMJE]

Sexes Eligible for Study:

All

Ages ICMJE

14 Years and older (Child, Adult, Older Adult)

Accepts Healthy Volunteers ICMJE

No

Contacts ICMJE

Contact information is only displayed when the study is recruiting subjects

Listed Location Countries ICMJE

South Africa

Removed Location Countries

Administrative Information

NCT Number ICMJE

NCT02333799

Other Study ID Numbers ICMJE

NiX-TB-(B-L-Pa)

Has Data Monitoring Committee

Yes

U.S. FDA-regulated Product

Not Provided

IPD Sharing Statement ICMJE

Not Provided

Responsible Party

Global Alliance for TB Drug Development

Study Sponsor ICMJE

Global Alliance for TB Drug Development

Collaborators ICMJE

Not Provided

Investigators ICMJE

Principal Investigator:

Dan Everitt, MD

Global Alliance for TB Drug Development

Principal Investigator:

Francesca Conradie, MD

CHRU Themba Lethu Clinic - Helen Joseph Hospital

PRS Account

Global Alliance for TB Drug Development

Verification Date

April 2020

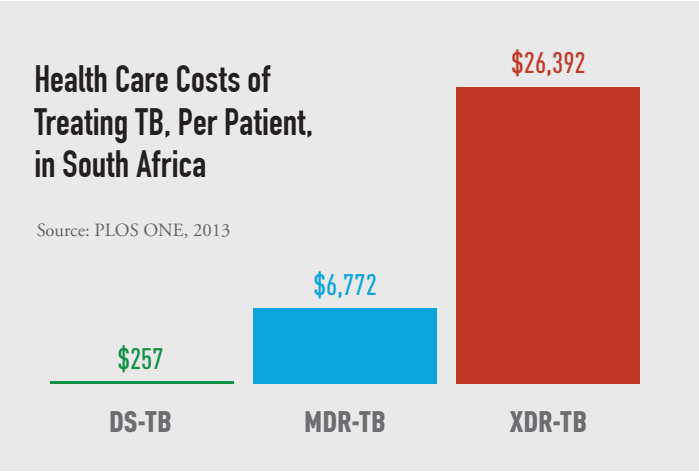
ICMJE **Data element required by the International Committee of Medical Journal Editors and the World Health Organization ICTRP**

ANNEXURE - 3

Nix-TB:
Testing a New Potential Treatment for XDR-TB

Tuberculosis has evolved faster than our medicines

Extensively drug-resistant tuberculosis, or XDR-TB, is a strain of tuberculosis, airborne and infectious, that is resistant to four commonly used anti-TB drugs. Essentially, there is no cure and XDR-TB is often considerd a death sentence. XDR-TB has been confirmed in more than 100 countries around the world. There are an estimated 40,000 people infected with XDR-TB today—nine percent of all multidrug resistant-TB (MDR-TB) cases—and the problem is growing worse. Without new treatments, XDR-TB is emerging as an extremely deadly and costly global health threat that the world is inadequately equipped to tackle.

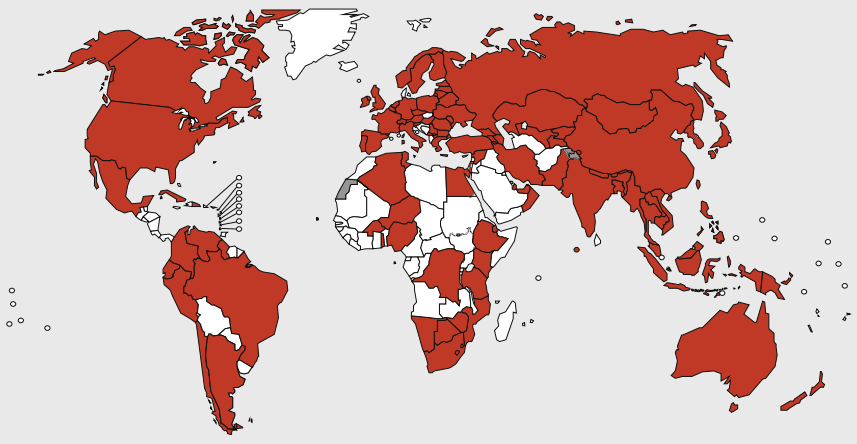


Current care and treatment for XDR-TB

There is no regulatory-approved regimen for curing XDR-TB. Instead, healthcare providers try to individualize treatment, often using antibiotics not normally used for TB, as well as highly toxic medicines not intended to be used for the length of time that TB treatment requires.

Treatment of XDR-TB routinely lasts two years or longer, and consists of thousands of pills plus injections and horrible side effects. It is also extraordinarily costly. In South Africa, for example, the per patient health care cost of XDR-TB is $26,392, four times greater than MDR-TB ($6,772), and 103 times greater than drug-sensitive TB ($257). Drug-resistant TB comprises only 2.2 percent of South African cases, but it consumes 32 percent of the country's total TB budget.

Despite the length, cost, and intensity of the treatment, outcomes are extremely poor. In one study published in the *Lancet* in 2014, after two years of treatment, only a fraction (16 percent) of people with XDR-TB were cured and nearly half (46 percent) died.



XDR-TB patients are often isolated or quarantined because of the public health risk of contagion, a measure that is costly for countries and also takes a massive toll on patients and their families. However, this public health measure has failed to contain XDR-TB since patients who fail on treatment—the vast majority—are often discharged back into their communities, where they risk spreading the disease even further.

Worse, most XDR-TB is not treated at all because the cost and complexity of such programs are out of reach for many health systems in TB-endemic countries.

Nix-TB trial: Hope in research

TB Alliance and partners have launched the world's first clinical trial to study an XDR-TB drug regimen with minimal pre-existing resistance. If successful, the injection-free regimen being tested in Nix-TB could transform XDR-TB treatment, with patients being cured by taking a relatively short, simple, and effective regimen. Importantly, the regimen being tested could reduce the complexity and cost of the treatment to a fraction of what it is today, facilitating the global implementation of XDR-TB treatment in resource-poor nations.

Nix-TB tests a three-drug regimen consisting of bedaquiline, which received conditional regulatory approval in several high-TB disease burden countries; the novel antibacterial drug compound pretomanid, which is being tested in multiple clinical trials for TB; and linezolid, an oxazolidinone that has been used off-label to treat TB. The trial brings hope to those with XDR-TB who have no other treatment options. It includes patients as young as 14 and those who are co-infected with HIV with a CD4 count of 50 or higher.

Nix-TB is an open-label trial that enables patients to be assessed at regular intervals with the aim of being cured in six to nine months. After completing treatment, participants are monitored for two years to ensure they do not relapse. The trial has an adaptive design; if improved treatments become available during the course of the study, they can be incorporated into the trial.

Nix-TB is a partnership between TB Alliance, the sponsor of the trial; Janssen Pharmaceuticals, the discoverer of bedaquiline; and the sites in South Africa where the study is being conducted (Sizwe Hospital, TASK at Brooklyn Chest Hospital, and THINK at Doris Goodwin Hospital.) The study may expand to include other partners and sites.

Pursuing a universal regimen

Nix-TB study is a crucial first step toward establishing a truly "universal" treatment, a regimen to which there is no pre-existing resistance and could therefore treat any type of TB. If the regimen tested in Nix-TB is successful and safe, the study will expand to include people with MDR-TB and then, potentially, people with drug-sensitive TB. Having a regimen that would be usable in such a broad range of TB patients could significantly improve TB control efforts globally.

TB Alliance operates with the support of the Bill & Melinda Gates Foundation, AIDS Clinical Trial Group, UK aid, Irish Aid, UNITAID, the United States Agency for International Development (USAID), Australian Department of Foreign Affairs and Trade (DFAT), European Commission, Global Health Innovative Technology (GHIT) Fund, National Institute Of Allergy And Infectious Diseases (NIAID), and the United States Food and Drug Administration (FDA).
For more information on TB drug development and TB Alliance, please visit www.tballiance.org.

US 20110190199A1

ANNEXURE - 4

(19) **United States**

(12) **Patent Application Publication**

Brickner et al.

(10) **Pub. No.: US 2011/0190199 A1**

(43) **Pub. Date: Aug. 4, 2011**

(54) **COMBINATION THERAPY FOR TUBERCULOSIS**

(75) Inventors: **Steven J. Brickner**, Ledyard, CT (US); **Eric Nuermberger**, Baltimore, MD (US); **Charles K. Stover**, Gaithersburg, MD (US)

(73) Assignees: **Pfizer Inc.**; **The Johns Hopkins University**

(21) Appl. No.: **13/061,233**

(22) PCT Filed: **Aug. 31, 2009**

(86) PCT No.: **PCT/IB09/53796**

§ 371 (c)(1), (2), (4) Date: **Apr. 18, 2011**

Related U.S. Application Data

(60) Provisional application No. 61/093,879, filed on Sep. 3, 2008.

Publication Classification

(51) **Int. Cl.**

A61K 31/541	(2006.01)
A61K 31/7036	(2006.01)
A61K 31/606	(2006.01)
A61K 38/12	(2006.01)
A61P 31/06	(2006.01)

(52) **U.S. Cl.** **514/2.9**; 514/227.8; 514/35; 514/161

(57) **ABSTRACT**

The present invention relates to methods of treating tuberculosis, including multi-drug resistant varieties and latent tuberculosis. More particularly, the present invention relates to a method of treating tuberculosis in a mammal comprising administering to said mammal in need thereof an effective amount of a compound of formula (I), (S)—N-[[3-[3-fluoro-4-(4-thiomorpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide, or a pharmaceutically acceptable salt thereof in combination with at least two agents useful in the treatment of tuberculosis. The present invention also relates to a pharmaceutical composition comprising a therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or solvate thereof, (ii) a therapeutically effective amount of at least one agent useful in the treatment of tuberculosis and (iii) one or more pharmaceutically acceptable carriers or vehicles.

COMBINATION THERAPY FOR TUBERCULOSIS

FIELD OF THE INVENTION

[0001] The present invention relates to methods of treating tuberculosis, including multi-drug resistant varieties and latent tuberculosis. More particularly, the present invention relates to a method of treating tuberculosis in a mammal comprising administering to said mammal in need thereof an effective amount of a compound of formula (I), (S)—N-[[3-[3-fluoro-4-(4-thiomorpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide, or a pharmaceutically acceptable salt thereof in combination with at least two agents useful in the treatment of tuberculosis. The present invention also relates to a pharmaceutical composition comprising: i) a therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof, (ii) a therapeutically effective amount of at least one agent useful in the treatment of tuberculosis and (iii) one or more pharmaceutically acceptable carriers or vehicles.

BACKGROUND OF THE INVENTION

[0002] Tuberculosis (TB) kills approximately 1.6 million people worldwide each year, making it the second leading killer of adults behind HIV. Nearly 500,000 new cases of multidrug-resistant (MDR) TB occur each year, and the recent emergence of extensively drug-resistant (XDR-TB) TB portends new epidemics of untreatable TB. (See Dorman, S. E. et al., Nat. Med 13:295-298 2007, Zignol, M. et al., J Infect. Dis 194:479-485, 2006). New drugs with potent antituberculosis activity, especially against non-multiplying persisters, are needed to shorten the duration of treatment for TB and thereby facilitate the global implementation of directly-observed therapy. (See O'Brien, R. J. et al., Am. J. Respir. Crit Care Med. 163:1055-1058, 2001).

[0003] Currently, the treatment of drug-sensitive tuberculosis consists of administering a combination of at least the following drugs, isoniazid, rifampin, and pyrazinamide. For effective treatment, the above-mentioned drugs are given to a patient in an initial phase of treatment for 8 weeks, during which the drugs are used in combination to kill the rapidly multiplying population of *Mycobacterium tuberculosis* as well as to prevent the emergence of drug resistance. The initial phase of treatment is followed by a continuation or a sterilization phase for 18 weeks during which two or more sterilizing drugs (e.g. isoniazid and rifampin) are given to kill the intermittently dividing population (non-multiplying persisters) of *Mycobacterium tuberculosis*.

[0004] While the above-mentioned combination of drugs together provide treatment against sensitive *Mycobacterium tuberculosis* infection in 4 to 6 months time, such a combination therapy is not always successful, especially in patients harboring drug resistant strains. Also, the long duration of treatment consisting of six months may lead to unpleasant side effects. Further, compliance with the relatively long course of treatment is generally poor. Such non-compliance may lead to treatment failure resulting in development of drug resistance.

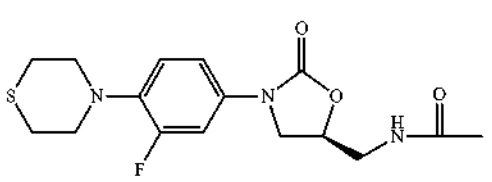
[0005] The oxazolidinones comprise a class of protein synthesis inhibitors that block translation by preventing formation of the initiation complex (for mechanism, see K. Leach et al., Molecular Cell, 26:4, 460-462, 2007). Linezolid (LZD, Zyvox®), the only marketed oxazolidinone, has activity against Gram-positive bacteria and is currently approved for use in complicated skin and skin structure infections and hospital-acquired pneumonia (Zyvox® package insert). However, it is also active against many mycobacterial species, including *Mycobacterium tuberculosis*, for which its MIC ranges from 0.125-1 μg/mL, with an MIC_{50} of 0.5 μg/mL and an MIC_{90} of 1 μg/mL. (See Alcala, L., et al., Antimicrob Agents Chemother 47:416-417, 2003; Cynamon, M. H., et al., Antimicrob Agents Chemother 43:1189-1191, 1999; Fattorini, L., et al., Antimicrob Agents Chemother 47:360-362, 2003). As a result, LZD has been used outside of labeled indications to treat recalcitrant cases of MDR- and XDR-TB. Although several case series suggest that LZD may contribute to successful sputum culture conversion in such cases, its individual activity in TB patients and its precise contribution to combination regimens remain unclear. These studies also demonstrate that the duration of LZD administration may be limited by hematologic and neurologic toxicity that can occur with long-term administration. (See Fortun, J., et al., 56:180-185, 2005; Park, I. N., et al., Antimicrob Chemother 58:701-704, 2006; Zignol, M., et al., J Infect. Dis 194:479-485, 2006). Therefore, new oxazolidinones with more potent in vivo activity against *Mycobacterium tuberculosis* and lower risk of toxicity with prolonged administration are desirable.

[0006] Oxazolidinones with more potent activity against *Mycobacterium tuberculosis* have previously been described. (See Barbachyn, M. R. et al., J Med Chem. 39:680-685, 1996; Sood, R., et al., Antimicrob Agents Chemother 49:4351-4353, 2005). The antituberculosis activity of the compound of formula (I), (S)—N-[[3-[3-fluoro-4-(4-thiomorpholinyl) phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide, was first described in 1996. (See Barbachyn, M. R., et al., J Med Chem. 39:680-685, 1996). Subsequent experiments in a murine model found the compound of formula (I) to be more active than LZD when both drugs were administered at 100 mg/kg, but the clinical relevance of this LZD dose was not established and the activities of the compound of formula (I), and LZD were not clearly different when compared at lower doses. (See Cynamon, M. H., et al., Antimicrob Agents Chemother 43:1189-1191, 1999). Moreover, although the compound of formula (I) appeared to have modest activity when combined with rifampin (RIF) in an acute (early) infection model, the compound of formula (I) had no additional activity when combined with isoniazid (INH). (Cynamon, M. H., et al., Antimicrob Agents Chemother 43:1189-1191, 1999.) Most importantly, the activity of the compound of formula (I), whether alone or in combination with RIF or INH, was only evaluated over the initial 4 weeks of treatment which is insufficient to assess the activity of a compound or combination of agents against non-multiplying persisters which, in turn, ultimately determines the duration of treatment necessary for cure (i.e., prevention of relapse after completion of treatment).

[0007] Hence, there is an urgent need to develop newer regimens that can be used to prevent, treat and/or reduce tuberculosis and/or eliminate the threat of multi-drug resistant tuberculosis or shorten the duration of treatment.

SUMMARY OF THE INVENTION

[0008] The present invention relates to a method of treating tuberculosis in a mammal comprising administering to said mammal in need thereof an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof:



(I)

in combination with at least two agents useful in the treatment of tuberculosis.

[0009] In one embodiment, the at least two agents are selected from the group consisting of isoniazid, rifampin, rifapentine, rifabutin, pyrazinamide, ethambutol, streptomycin, kanamycin, amikacin, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, ciprofloxacin, capreomycin, ethionamide, cycloserine, para-aminosalicylic acid, thiacetazone, clarithromycin, amoxicillin-clavulanic acid, imipenem, meropenem, viomycin, terizidone, clofazimine, TMC207, PA-824, OPC-7683, LL-3858 and SQ-109.

[0010] In another embodiment, one of said at least two agents is selected from the group consisting of isoniazid, rifampin, rifapentine, rifabutin, pyrazinamide, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, ciprofloxacin, clofazimine and ethambutol.

[0011] In yet another embodiment, one of said at least two agents is pyrazinamide.

[0012] In yet another embodiment, one of said at least two agents is rifampin.

[0013] In yet another embodiment, one of said at least two agents is rifapentine.

[0014] In yet another embodiment, one of said at least two agents is PA-824.

[0015] In yet another embodiment, one of said at least two agents is OPC-67683

[0016] In yet another embodiment, one of said at least two agents is TMC-207.

[0017] In yet another embodiment, one of said at least two agents is selected from the group consisting of moxifloxacin, gatifloxacin, levofloxacin, and ofloxacin.

[0018] In yet another embodiment one of said at least two agents is moxifloxacin.

[0019] In a specific embodiment, said at least two agents are pyrazinamide and rifampin.

[0020] In yet another specific embodiment, said at least two agents are pyrazinamide, rifampin and isoniazid.

[0021] In yet another specific embodiment, said at least two agents are pyrazinamide and rifapentine.

[0022] In yet another specific embodiment, said at least two agents are pyrazinamide, rifapentine, and isoniazid.

[0023] In a specific embodiment, said at least two agents are pyrazinamide and moxifloxacin.

[0024] In yet another specific embodiment, said at least two agents are pyrazinamide, moxifloxacin, and rifampin.

[0025] In yet another specific embodiment, said at least two agents are pyrazinamide, moxifloxacin, and rifapentine.

[0026] In yet another specific embodiment, said at least two agents are PA-824 and pyrazinamide.

[0027] In yet another specific embodiment, said at least two agents are PA-824, pyrazinamide and an agent selected from the group consisting of streptomycin, kanamycin, amikacin and capreomycin.

[0028] In yet another specific embodiment, said at least two agents are PA-824 and moxifloxacin.

[0029] In yet another specific embodiment, said at least two agents are PA-824, moxifloxacin and an agent selected from the group consisting of streptomycin, kanamycin, amikacin and capreomycin.

[0030] In yet another specific embodiment, said at least two agents are OPC-67683 and pyrazinamide.

[0031] In yet another specific embodiment, said at least two agents are OPC-67683, pyrazinamide and an agent selected from the group consisting of streptomycin, kanamycin, amikacin and capreomycin.

[0032] In yet another specific embodiment, said at least two agents are OPC-67683 and moxifloxacin.

[0033] In yet another specific embodiment, said at least two agents are OPC-67683, moxifloxacin and an agent selected from the group consisting of streptomycin, kanamycin, amikacin and capreomycin.

[0034] In yet another specific embodiment, said at least two agents are TMC-207 and pyrazinamide.

[0035] In yet another specific embodiment, said at least two agents are TMC-207, pyrazinamide and moxifloxacin or isoniazid.

[0036] In yet another embodiment, said tuberculosis comprises active tuberculosis or latent tuberculosis.

[0037] In yet another embodiment, said active tuberculosis comprises drug sensitive, mono-drug resistant, multi-drug-resistant tuberculosis (MDR) or extensively drug-resistant tuberculosis (XDR).

[0038] In yet another embodiment, the method of the present invention completely eradicates drug-sensitive tuberculosis, mono-drug resistant, multi-drug-resistant tuberculosis, and extensively drug-resistant tuberculosis (XDR) on completion of the treatment.

[0039] In yet another embodiment, said tuberculosis is caused by a *Mycobacterium* infection selected from the group consisting of *Mycobacterium tuberculosis, Mycobacterium bovis* or other related mycobacterial species.

[0040] In yet another embodiment, the method of the present invention prevents relapse of the *Mycobacterium* infection after completion of the treatment.

[0041] In yet another embodiment, the compound of formula (I) and pharmaceutically acceptable salts thereof is administered orally.

[0042] In yet another embodiment, said at least two agents are each administered orally.

[0043] In yet another embodiment, said at least two agents are administered together in a composition.

[0044] In yet another embodiment, said at least two agents are administered separately.

[0045] In yet another embodiment, said at least two agents are administered together and another agent useful for the treatment of tuberculosis is administered separately.

[0046] In yet another embodiment, the compound of formula (I) and pharmaceutically acceptable salts thereof is administered once per day (QD) or twice per day (BID).

[0047] In yet another embodiment, the compound of formula (I) and pharmaceutically acceptable salts thereof is administered once per week, twice per week, thrice per week or every other day.

[0048] In one embodiment, the compound of formula (I) or a pharmaceutically acceptable salt thereof is administered between about 10 mg to about 2000 mg.

[0049] In yet another embodiment, the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered between about 250 mg to about 1000 mg.

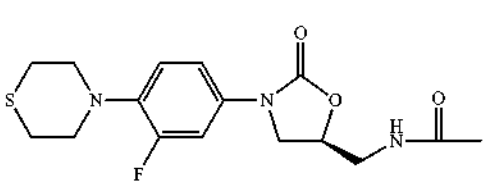
[0050] In yet another embodiment, the compound of formula (I), or a pharmaceutically acceptable salt thereof, is administered between about 600 mg to about 1000 mg.

[0051] In yet another embodiment, the compound of formula (I) or a pharmaceutically acceptable salt thereof is administered between about 25 mg to about 1000 mg.

[0052] In yet another embodiment, the compound of formula (I) or a pharmaceutically acceptable salt thereof is administered between about 50 mg to about 500 mg.

[0053] In yet another embodiment, the compound of formula (I) or a pharmaceutically acceptable salt thereof is administered between about 100 mg to about 500 mg.

[0054] The present invention also relates to a method of treating tuberculosis in a mammal after said mammal has undergone an initial phase of treatment comprising administering to said mammal in need thereof an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof:



(I)

in combination with at least one agent useful in the treatment of tuberculosis.

[0055] In one embodiment, said at least one agent is selected from the group consisting of rifampin, rifapentine, rifabutin, pyrazinamide, ethambutol, streptomycin, kanamycin, amikacin, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, ciprofloxacin, capreomycin, ethionamide, cycloserine, para-aminosalicylic acid, thiacetazone, clarithromycin, amoxicillin-clavulanic acid, imipenem, meropenem, clofazimine, viomycin, terizidone, TMC207, PA-824, OPC-7683, LL-3858 and SQ-109.

[0056] In yet another embodiment, said at least one agent is selected from the group consisting of rifampin, rifapentine, rifabutin, pyrazinamide, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, and ethambutol.

[0057] In a specific embodiment, said at least one agent is pyrazinamide.

[0058] In a specific embodiment, said at least one agent is rifampin.

[0059] In a specific embodiment, said at least one agent is rifapentine.

[0060] In a specific embodiment, said at least one agent is PA-824.

[0061] In a specific embodiment, said at least one agent is OPC-7683.

[0062] In a specific embodiment, said at least one agent is TMC-207.

[0063] In a specific embodiment, said at least one agent is selected from the group consisting of moxifloxacin, gatifloxacin, levofloxacin, and ofloxacin.

[0064] In a specific embodiment, said at least one agent is moxifloxacin.

[0065] In yet another embodiment, said tuberculosis comprises active tuberculosis or latent tuberculosis.

[0066] In yet another embodiment, said active tuberculosis comprises drug-sensitive tuberculosis, mono-drug resistant, multi-drug-resistant tuberculosis (MDR) or extensively drug-resistant tuberculosis (XDR).

[0067] In yet another embodiment, the method of the present invention completely eradicates drug-sensitive tuberculosis, mono-drug resistant, multi-drug-resistant tuberculosis, and extensively drug-resistant tuberculosis (XDR) on completion of the treatment.

[0068] In yet another embodiment, said tuberculosis is caused by a *Mycobacterium* infection selected from the group consisting of *Mycobacterium tuberculosis, Mycobacterium bovis* or other related mycobacterial species.

[0069] In yet another embodiment, the method of the present invention prevents relapse of the *Mycobacterium* infection after completion of the treatment.

[0070] In yet another embodiment, the compound of formula (I) and pharmaceutically acceptable salts thereof is administered orally.

[0071] In yet another embodiment, said at least one agent is administered orally.

[0072] In yet another embodiment, the compound of formula (I) and pharmaceutically acceptable salts thereof and said at least one agent are administered together in a composition.

[0073] In yet another embodiment, the compound of formula (I) and pharmaceutically acceptable salts thereof and said at least one agent are administered separately.

[0074] In yet another embodiment, the compound of formula (I) and pharmaceutically acceptable salts thereof is administered once per day (QD) or twice per day (BID).

[0075] In yet another embodiment, the compound of formula (I) and pharmaceutically acceptable salts thereof is administered once per week, twice per week, thrice per week or every other day.

[0076] In one embodiment, the compound of formula (I) or a pharmaceutically acceptable salt thereof is administered between about 10 mg to about 2000 mg.

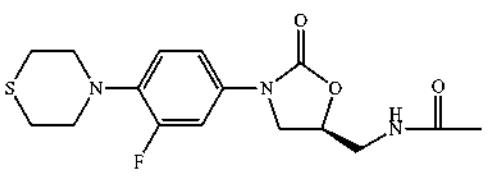
[0077] In yet another embodiment, the compound of formula (I) or a pharmaceutically acceptable salt thereof is administered between about 25 mg to about 1000 mg.

[0078] In yet another embodiment, the compound of formula (I) or a pharmaceutically acceptable salt thereof is administered between about 50 mg to about 500 mg.

[0079] In yet another embodiment, the compound of formula (I) or a pharmaceutically acceptable salt thereof is administered between about 100 mg to about 500 mg.

[0080] The present invention also relates to a pharmaceutical composition comprising:

[0081] i) a therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof:



(I)

[0082] (ii) a therapeutically effective amount of at least one agent useful in the treatment of tuberculosis and

[0083] (iii) one or more pharmaceutically acceptable carriers or vehicles.

[0084] In yet another embodiment, said at least one agent is selected from the group consisting of isoniazid, rifampin, rifapentine, rifabutin, pyrazinamide, ethambutol, streptomycin, kanamycin, amikacin, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, ciprofloxacin, capreomycin, ethionamide, cycloserine, para-aminosalicylic acid, thiacetazone, clarithromycin, clofazimine, amoxicillin-clavulanic acid, imipenem, meropenem, viomycin, terizidone, clofazimine, TMC207, PA-824, OPC-7683, LL-3858 and SQ-109.

[0085] In yet another embodiment, said at least one agent is selected from the group consisting of isoniazid, rifampin, rifapentine, rifabutin, pyrazinamide, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, and ethambutol.

[0086] In a specific embodiment, said at least one agent is pyrazinamide.

[0087] In a specific embodiment, said at least one agent is rifampin.

[0088] In a specific embodiment, said at least one agent is rifapentine.

[0089] In a specific embodiment, said at least one agent is isoniazid.

[0090] In a specific embodiment, said at least one agent is PA-824.

[0091] In a specific embodiment, said at least one agent is OPC-7683.

[0092] In a specific embodiment, said at least one agent is TMC-207.

[0093] In a specific embodiment, said at least one agent is selected from the group consisting of moxifloxacin, gatifloxacin, levofloxacin, and ofloxacin.

[0094] In a specific embodiment, said at least one agent is moxifloxacin.

[0095] In yet another embodiment, the pharmaceutical composition comprises about 10 mg to about 2000 mg of compound of formula (I) or a pharmaceutically acceptable salt thereof.

[0096] In yet another embodiment, the pharmaceutical composition comprises about 250 mg to about 1000 mg of compound of formula (I) or a pharmaceutically acceptable salt thereof.

[0097] In yet another embodiment, the pharmaceutical composition comprises about 600 mg to about 1000 mg of compound of formula (I) or a pharmaceutically acceptable salt thereof.

[0098] In yet another embodiment, the pharmaceutical composition comprises about 25 mg to about 1000 mg of compound of formula (I) or a pharmaceutically acceptable salt thereof.

[0099] In yet another embodiment, the pharmaceutical composition comprises about 50 mg to about 500 mg of compound of formula (I) or a pharmaceutically acceptable salt thereof.

[0100] Additionally, any formulation, including the combinations below, may contain from 250 mg to 1000 mg of the compound of formula (I) or a pharmaceutically acceptable salt thereof or from 600 mg to 1000 mg of compound of formula (I) or a pharmaceutically acceptable salt thereof.

[0101] In yet another embodiment, the pharmaceutical composition comprises about 100 mg to about 500 mg of compound of formula (I) or a pharmaceutically acceptable salt thereof.

[0102] In yet another embodiment, the pharmaceutical composition comprises about 100 mg to about 500 mg of compound of formula (I) or a pharmaceutically acceptable salt thereof and about 600 mg rifampin.

[0103] In yet another embodiment, the pharmaceutical composition comprises about 100 mg to about 500 mg of compound of formula (I) or a pharmaceutically acceptable salt thereof and about 300 mg of isoniazid.

[0104] In yet another embodiment, the pharmaceutical composition comprises about 100 mg to about 500 mg of compound of formula (I) or a pharmaceutically acceptable salt thereof and about 300 mg of isoniazid and about 600 mg of rifampin.

[0105] In yet another embodiment, the pharmaceutical composition comprises about 100 mg to about 500 mg of compound of formula (I) or a pharmaceutically acceptable salt thereof, about 300 mg of isoniazid, about 600 mg of rifampin and about 20-25 mg/kg to about 50-70 mg/kg of pyrazinamide.

[0106] In yet another embodiment, the pharmaceutical composition comprises about 100 mg to about 500 mg of a compound of formula (I) or a pharmaceutically acceptable salt thereof and about 10-15 mg/kg to about 20-30 mg/kg of isoniazid.

[0107] In yet another embodiment, the pharmaceutical composition comprises about 100 mg to about 500 mg of a compound of formula (I) or a pharmaceutically acceptable salt thereof and about 10-15 mg/kg to about 20-30 mg/kg of isoniazid and about 10 mg/kg to about 20 mg/kg of rifampin.

[0108] In yet another embodiment, the pharmaceutical composition comprises about 100 mg to about 500 mg of a compound of formula (I) or a pharmaceutically acceptable salt thereof, about 10-15 mg/kg to about 20-30 mg/kg of isoniazid, about 10 mg/kg to about 20 mg/kg of rifampin and about 15-30 mg/kg to about 50 mg/kg pyrazinamide.

[0109] In yet another embodiment, the pharmaceutical composition comprises about 50 mg to about 250 mg of a compound of formula (I) or a pharmaceutically acceptable salt thereof, about 75 mg isoniazid, about 150 mg of rifampin and about 400 mg of pyrazinamide.

[0110] In yet another embodiment, the pharmaceutical composition comprises about 25 mg to about 250 mg of a compound of formula (I) or a pharmaceutically acceptable salt thereof, about 300 mg of rifampin.

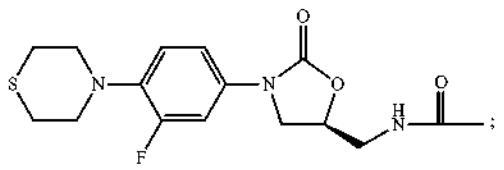
[0111] The present invention also relates to an article of manufacture comprising:

[0112] a packaged composition comprising:

[0113] (a)

[0114] i) a therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof:

(I)



[0115] (ii) a therapeutically effective amount of at least one agent useful in the treatment of tuberculosis and

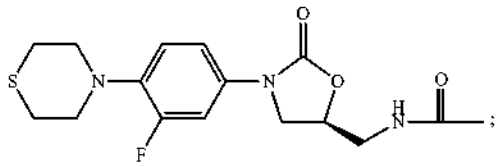
[0116] (iii) one or more pharmaceutically acceptable carriers or vehicles

[0117] (b) an insert providing instructions for administration of the packaged composition of (a) to treat tuberculosis; and

[0118] (c) a container for (a) and (b).

[0119] The present invention also relates to a pharmaceutical package for treating tuberculosis in a mammal which comprises a compound of formula (I) or a pharmaceutically acceptable salt thereof:

(I)



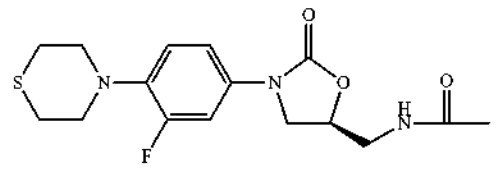
and an insert providing instructions for administering said composition in combination with at least one agent useful in the treatment of tuberculosis.

[0120] These and other aspects, advantages, and features of the invention will become apparent for the following detailed description of the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0121] The present invention relates to a method of treating tuberculosis in a mammal comprising administering to said mammal an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof:

(I)



in combination with at least two agents useful in the treatment of tuberculosis.

[0122] The compound of formula (I) of the invention is disclosed in U.S. Pat. No. 5,880,118, (incorporated in its entirety herein by reference) in Example 1, (S)—N-[[3-[3-fluoro-4-(4-thiomorpholinyl)phenyl]-2-oxo-5-oxazolidinyl] methyl]acetamide. As described in more detail below, the compound of formula (I) of the invention may be administered as the free base or in the form of a salt thereof.

[0123] The phrase "pharmaceutically acceptable salt(s)", as used herein, unless otherwise indicated, includes salts of acidic or basic groups which may be present in the compounds of formula (I).

[0124] For example, the compounds of formula (I) that are basic in nature are capable of forming a wide variety of salts with various inorganic and organic acids. The acids that may be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds of those that form non-toxic acid addition salts, i.e., salts containing pharmacologically acceptable anions, such as the hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, citrate, acid citrate, tartrate, pantothenate, bitartrate, ascorbate, succinate, maleate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and pamoate [i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)] salts.

[0125] Examples of salts include, but are not limited to, acetate, acrylate, benzenesulfonate, benzoate (such as chlorobenzoate, methylbenzoate, dinitrobenzoate, hydroxybenzoate, and methoxybenzoate), bicarbonate, bisulfate, bisulfite, bitartrate, borate, bromide, butyne-1,4-dioate, calcium edetate, camsylate, chloride, caproate, caprylate, citrate, decanoate, dihydrogenphosphate, edetate, edislyate, estolate, esylate, ethylsuccinate, formate, fumarate, gluceptate, gluconate, glutamate, glycollate, glycollylarsanilate, heptanoate, hexyne-1,6-dioate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, y-hydroxybutyrate, iodide, isobutyrate, isothionate, lactate, lactobionate, laurate, malate, maleate, malonate, mandelate, mesylate, metaphosphate, methane-sulfonate, methylsulfate, monohydrogenphosphate, mucate, napsylate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, nitrate, oleate, oxalate, pamoate (embonate), palmitate, pantothenate, phenylacetates, phenylbutyrate, phenylpropionate, phthalate, phospate/diphosphate, polygalacturonate, propanesulfonate, propionate, propiolate, pyrophosphate, pyrosulfate, salicylate, stearate, subacetate, suberate, succinate, sulfate, sulfonate, sulfite, tannate, tartrate, teoclate, tosylate, triethiodode, and valerate salts.

[0126] The invention also relates to base addition salts of the compounds of formula (I). The chemical bases that may be used as reagents to prepare pharmaceutically acceptable base salts of the compounds of formula (I) that are acidic in nature are those that form non-toxic base salts with such compounds. Such non-toxic base salts include, but are not limited to, those derived from such pharmacologically acceptable cations such as alkali metal cations (e.g., potassium and sodium) and alkaline earth metal cations (e.g., calcium and magnesium), ammonium or water-soluble amine addition salts such as N-methylglucamine-(meglumine), and the lower alkanolammonium and other base salts of pharmaceutically acceptable organic amines.

[0127] Hemisalts of acids and bases may also be formed, for example, hemisulphate and hemicalcium salts.

[0128] For a review on suitable salts, see Handbook of Pharmaceutical Salts: Properties, Selection, and Use by Stahl and Wermuth (Wiley-VCH, 2002). Methods for making pharmaceutically acceptable salts of compounds of formula (I) of the invention are known to one of skill in the art.

[0129] As used herein the terms ""formula (I)" and "formula (I) or pharmaceutically acceptable salts thereof"" are defined to include all forms of the compound of formula (I),

including isomers, crystalline and non-crystalline forms, isomorphs, polymorphs, metabolites, solvates, hydrates and prodrugs thereof.

[0130] The term "solvate" is used herein to describe a non-covalent or easily reversible combination between solvent and solute, or dispersion means and disperse phase. It will be understood that the solvate can be in the form of a solid, slurry (e.g., a suspension or dispersion), or solution. Non-limiting examples of solvents include ethanol, methanol, propanol, acetonitrile, dimethyl ether, diethyl ether, tetrahydrofuan, methylene chloride, and water. The term "hydrate" is employed when said solvent is water.

[0131] A currently accepted classification system for organic hydrates is one that defines isolated site, or channel hydrates—see Polymorphism in Pharmaceutical Solids by K. R. Morris (Ed. H. G. Brittain, Marcel Dekker, 1995). Isolated site hydrates are ones in which the water molecules are isolated from direct contact with each other by intervening organic molecules. In channel hydrates, the water molecules lie in lattice channels where they are next to other water molecules.

[0132] When the solvent or water is tightly bound, the complex will have a well-defined stoichiometry independent of humidity. When, however, the solvent or water is weakly bound, as in channel solvates and hygroscopic compounds, the water/solvent content will be dependent on humidity and drying conditions. In such cases, non-stoichiometry will be the norm.

[0133] The invention also relates to prodrugs of the compounds of formula (I). Thus certain derivatives of compounds of formula (I) which may have little or no pharmacological activity themselves can, when administered into or onto the body, be converted into compounds of formula (I) having the desired activity, for example, by hydrolytic cleavage. Such derivatives are referred to as "prodrugs". Further information on the use of prodrugs may be found in Pro-drugs as Novel Delivery Systems, Vol. 14, ACS Symposium Series (T. Higuchi and W. Stella) and Bioreversible Carriers in Drug Design, Pergamon Press, 1987 (Ed. E. B. Roche, American Pharmaceutical Association).

[0134] Prodrugs in accordance with the invention can, for example, be produced by replacing appropriate functionalities present in the compounds of formula (I) with certain moieties known to those skilled in the art as 'pro-moieties' as described, for example, in Design of Prodrugs by H. Bundgaard (Elsevier, 1985).

[0135] Some non-limiting examples of prodrugs in accordance with the invention include:

[0136] (i) where the compound of formula (I) contains a carboxylic acid functionality which is functionalized into a suitably metabolically labile group (esters, carbamates, etc.) compound of formula (I);

[0137] (ii) where the compound of formula (I) contains an alcohol functionality which is functionalized into a suitably metabolically labile group (ethers, esters, carbamates, acetals, ketals, etc.) compound of formula (I); and

[0138] (iii) where the compound of formula (I) contains a primary or secondary amino functionality, or an amide which are functionalized into a suitably metabolically labile group, e.g., a hydrolysable group (amides, carbamates, ureas, phosphonates, sulfonates, etc.) compound of formula (I).

[0139] Further examples of replacement groups in accordance with the foregoing examples and examples of other prodrug types may be found in the aforementioned references.

[0140] The compounds of the formula (I) of the invention may exhibit the phenomena of tautomerism and structural isomerism. For example, the compounds of formula (I) of the invention may exist in several tautomeric forms, including the enol and imine form, and the keto and enamine form and geometric isomers and mixtures thereof. All such tautomeric forms are included within the scope of compounds of formula (I) of the invention. Tautomers exist as mixtures of a tautomeric set in solution. In solid form, usually one tautomer predominates. Even though one tautomer may be described, the present invention includes all tautomers of the compounds of formula (I) of the invention.

[0141] The present invention also includes isotopically-labeled compounds, which are identical to those recited in formula (I) above, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that may be incorporated into compounds of formula (I) include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, fluorine and chlorine, such as, but not limited to, ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{35}S and ^{18}F. Certain isotopically-labeled compounds of formula (I) of the invention, for example those into which radioactive isotopes such as ^{3}H and ^{14}C are incorporated, are useful in drug and/or substrate tissue distribution assays. Tritiated, i.e., ^{3}H, and carbon-14, i.e., ^{14}C, isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium, i.e., ^{2}H, can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically-labeled compounds of formula (I) of the invention may generally be prepared by carrying out the procedures disclosed in the Schemes and/or in the Examples and Preparations below, by substituting an isotopically-labeled reagent for a non-isotopically-labeled reagent.

[0142] The compounds of formula (I) of the invention may exhibit polymorphism. Polymorphs of the compounds of formula (I) of the invention may be prepared by crystallization of a compound of formula (I) of the invention under various conditions. For example, there may be employed various solvents (including water) or different solvent mixtures for recrystallization; crystallization at different temperatures; various modes of cooling ranging from very fast to very slow cooling during crystallization. Polymorphs may also be obtained by heating or melting a compound of formula (I) of the invention followed by gradual or fast cooling. The presence of polymorphs may be determined by solid probe NMR spectroscopy, IR spectroscopy, differential scanning calorimetry, powder X-ray diffraction or other such techniques.

[0143] The minimum amount of the compound of formula (I) of the invention to be administered is an effective amount. The term "effective amount" means the amount of a compound of formula (I) of the invention which prevents the onset of, alleviates the symptoms of, stops the progression of, and/or eliminates a TB infection in a mammal, e.g., a human.

[0144] A therapeutically effective amount of the compound of formula (I) of the invention was found to possess the desired antitubercular properties described below. However,

a synergistic effect is observed when the compound of formula (I) of the invention is administered in combination with at least two agents useful in the treatment of tuberculosis.

[0145] By synergistic effect it is meant that the therapeutic effect of administering a compound of formula (I) of the invention and the at least two agents useful in the treatment of tuberculosis, is greater than the therapeutic effect obtained on administration of the effective amount of either compound of the formula (I) of the invention alone, or the therapeutic effective amount of the at least two agents useful in the treatment of tuberculosis administered individually or in combination.

[0146] Such synergy is advantageous in that it may allow for administration of each of the components in the combination in an amount less than that would be required if administered individually which may reduce the likelihood of adverse events or unpleasant side effects. Alternatively, such synergy may shorten the duration of treatment for TB.

[0147] Thus, administration of both the compound of formula (I) of the invention and the at least two agents useful in the treatment of tuberculosis, for example, at least two of the compounds selected from the group consisting of isoniazid, rifampin, rifapentine, rifabutin, pyrazinamide, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, ciprofloxacin, and ethambutol was found to produce an effect, which results in improved treatment of tuberculosis as compared to the effect when the compound of formula (I) of the invention alone, when the at least two agents useful in the treatment of tuberculosis administered individually or when the at least two agents useful in the treatment of tuberculosis are administered in combination with one another.

[0148] In one embodiment of the invention, administration of both the compound of formula (I) of the invention or a pharmaceutically acceptable salt thereof and at least two of the compounds selected from the group consisting of isoniazid, rifampin, rifapentine, rifabutin, pyrazinamide, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, ciprofloxacin, and ethambutol was found to produce an effect, which results in complete eradication of tuberculosis compared with incomplete eradication when the compound of formula (I) of the invention or the at least two agents useful in the treatment of tuberculosis are administered individually or in combination with one another.

[0149] The term "complete eradication" means no culturable mycobacterium could be observed in the target organ, i.e. lungs of the infected mammals, after the treatment regimen with the combination of the present invention. It is noted that at the end of treatment of infected mammals with the existing drug regimen, i.e. a combination of the at least two agents useful in the treatment of tuberculosis, isoniazid, pyrazinamide and rifampin, a significantly culturable amount of tubercule bacilli is recovered from the target organ i.e. lungs. This is evident from the data in Table 3 below. It is further noted that even after treatment for 4 months with the standard of care, 2 months of isoniazid, pyrazinamide and rifampin followed by 2 months of isoniazid and rifampin, 90% of mice relapse after completion of treatment, meaning that viable bacilli remain at the completion of treatment, even if they cannot be cultured at the time of treatment completion, which is evident from the data in Table 4.

[0150] In one embodiment of the present invention, the at least two agents useful in the treatment of tuberculosis used in conjunction with a compound of formula (I) and pharmaceutical compositions of the invention described herein are as follows: isoniazid, rifampin, rifapentine, rifabutin, pyrazinamide, ethambutol, streptomycin, kanamycin, amikacin, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, ciprofloxacin, capreomycin, ethionamide, cycloserine, para-aminosalicylic acid, thiacetazone, clarithromycin, amoxicillin-clavulanic acid, imipenem, meropenem, clofazimine, viomycin, terizidone, TMC207, PA-824, OPC-7683, LL-3858 and SQ-109.

[0151] In another embodiment, one of said at least two agents is selected from the group consisting of isoniazid, rifampin, rifapentine, rifabutin, pyrazinamide, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, ciprofloxacin, and ethambutol.

[0152] In yet another embodiment, one of said at least two agents is pyrazinamide.

[0153] In yet another embodiment, one of said at least two agents is rifampin.

[0154] In yet another embodiment, one of said at least two agents is rifapentine.

[0155] In yet another embodiment, one of said at least two agents is PA-824.

[0156] In yet another embodiment, one of said at least two agents is OPC-67683

[0157] In yet another embodiment, one of said at least two agents is TMC-207.

[0158] In yet another embodiment, one of said at least two agents is selected from the group consisting of moxifloxacin, gatifloxacin, levofloxacin, and ofloxacin.

[0159] In yet another embodiment one of said at least two agents is moxifloxacin.

[0160] In a specific embodiment, said at least two agents are pyrazinamide and rifampin.

[0161] In yet another specific embodiment, said at least two agents are pyrazinamide, rifampin and isoniazid.

[0162] In yet another specific embodiment, said at least two agents are pyrazinamide and rifapentine.

[0163] In yet another specific embodiment, said at least two agents are pyrazinamide, rifapentine, and isoniazid.

[0164] In a specific embodiment, said at least two agents are pyrazinamide and moxifloxacin.

[0165] In yet another specific embodiment, said at least two agents is pyrazinamide, moxifloxacin, and rifampin.

[0166] In yet another specific embodiment, said at least two agents are pyrazinamide, moxifloxacin, and rifapentine

[0167] In yet another specific embodiment, said at least two agents are PA-824 and pyrazinamide.

[0168] In yet another specific embodiment, said at least two agents are PA-824, pyrazinamide and an agent selected from the group consisting of streptomycin, kanamycin, amikacin and capreomycin.

[0169] In yet another specific embodiment, said at least two agents are PA-824 and moxifloxacin.

[0170] In yet another specific embodiment, said at least two agents are PA-824, moxifloxacin and an agent selected from the group consisting of streptomycin, kanamycin, amikacin and capreomycin.

[0171] In yet another specific embodiment, said at least two agents are OPC-67683 and pyrazinamide.

[0172] In yet another specific embodiment, said at least two agents are OPC-67683, pyrazinamide and an agent selected from the group consisting of streptomycin, kanamycin, amikacin and capreomycin.

[0173] In yet another specific embodiment, said at least two agents are OPC-67683 and moxifloxacin.

[0174] In yet another specific embodiment, said at least two agents are OPC-67683, moxifloxacin and an agent selected from the group consisting of streptomycin, kanamycin, amikacin and capreomycin

[0175] In yet another specific embodiment, said at least two agents are TMC-207 and pyrazinamide.

[0176] In yet another specific embodiment, said at least two agents are TMC-207, pyrazinamide and moxifloxacin or isoniazid.

[0177] It was further found that a synergistic effect is observed when the compound of formula (I) of the invention is administered in combination with at least one agent useful in the treatment of tuberculosis, for example rifampin, during the continuation (sterilization) phase of treatment (after the initial phase of treatment) when non-multiplying persisting bacteria are prevalent. Such synergy is advantageous in that it may shorten the duration of treatment for TB. More significantly, the data below show that on administration of a compound of formula (I) in combination with at least two agents useful for the treatment of TB may shorten the duration of therapy for drug-susceptible TB, mono-drug-resistant TB and multi-drug resistant TB.

[0178] In one embodiment of the present invention, the methods of the present invention provide treating a mammal after the mammal has undergone an initial phase of treatment comprising at least one agent useful in the treatment of tuberculosis used in conjunction with a compound of formula (I).

[0179] In another embodiment, the at least one agent useful in the treatment of tuberculosis selected from the group consisting of rifampin, rifapentine, rifabutin, pyrazinamide, ethambutol, streptomycin, kanamycin, amikacin, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, ciprofloxacin, capreomycin, ethionamide, cycloserine, para-aminosalicylic acid, thiacetazone, clarithromycin, amoxicillin-clavulanic acid, imipenem, meropenem, clofazimine, viomycin, terizidone, TMC207, PA-824, OPC-7683, LL-3858 and SQ-109.

[0180] In yet another embodiment, said at least one agent is selected from the group consisting of rifampin, rifapentine, rifabutin, pyrazinamide, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, and ethambutol.

[0181] In a specific embodiment, said at least one agent is pyrazinamide.

[0182] In a specific embodiment, said at least one agent is rifampin.

[0183] In a specific embodiment, said at least one agent is rifapentine.

[0184] In a specific embodiment, said at least one agent is PA-824.

[0185] In a specific embodiment, said at least one agent is OPC-7683.

[0186] In a specific embodiment, said at least one agent is TMC-207.

[0187] In a specific embodiment, said at least one agent is selected from the group consisting of moxifloxacin, gatifloxacin, levofloxacin, and ofloxacin.

[0188] In a specific embodiment, said at least one agent is moxifloxacin.

[0189] The methods and compositions of the invention are particularly effective against tuberculosis including active tuberculosis and latent tuberculosis. In one example, the active tuberculosis comprises drug-sensitive tuberculosis, mono-drug-resistant tuberculosis, multi-drug-resistant tuberculosis and extensively drug-resistant tuberculosis. In another example, the present invention provides a method to completely eradicate drug-sensitive tuberculosis, mono-drug resistant, multi-drug-resistant tuberculosis, and extensively drug-resistant tuberculosis (XDR) on completion of the treatment.

[0190] In addition, the methods and composition of the invention may be used in conjunction with diagnostic tests to identify the tuberculosis in the mammal which are known to those so skilled in the art. For example, the methods and compositions of the invention may be used in conjunction with the so-called line-probe assay (Hain Life-science GmbH) which may be used to identify genes linked with resistance to rifampin and isoniazid to indicate multi-drug-resistant tuberculosis and/or extensively drug-resistant tuberculosis. Other assays may also be used in conjunction with the methods and composition of the invention, which are known to those so skilled in the art.

[0191] In another embodiment, the methods and compositions of the invention are particularly effective against tuberculosis caused by a *Mycobacterium* infection selected from the group consisting of *Mycobacterium tuberculosis*, *Mycobacterium bovis* or other related mycobacterial species, which would be known by one skilled in the art. In one example, the present invention provides methods and compositions to prevent relapse of the *Mycobacterium* infection after completion of the treatment.

[0192] Such combination may be for simultaneous, separate or sequential use. In one embodiment, the at least two agents (or the at least one agent) useful for the treatment of tuberculosis are administered prior to administration of the compound of formula (I) of the invention. In another embodiment, the at least two agents (or the at least one agent) useful for the treatment of tuberculosis are administered after administration of the compound of formula (I) of the invention. In another embodiment, the at least two agents (or the at least one agent) useful for the treatment of tuberculosis are administered at about the same time as administration of the compound of formula (I) of the invention.

[0193] Separate administration of each compound, at different times and by different routes, in some cases would be advantageous. Thus, the components in the combination i.e. the compound of formula (I) of the invention and the at least two agents (or the at least one agent) in the treatment of tuberculosis need not be necessarily administered at essentially the same time or in any order. The administration can be so timed that the peak pharmacokinetic effect of one compound coincides with the peak pharmacokinetic effect of the other.

[0194] All the active ingredients can be formulated into separate or individual dosage forms which can be co-administered one after the other. Another option is that if the route of administration is the same (e.g. oral) two or more of the active compounds can be formulated into a single form for co-administration, both methods of co-administration, however, being part of the same therapeutic treatment or regimen.

[0195] Preferred agents useful for the treatment of tuberculosis may be as follows: isoniazid, rifampin, rifapentine, rifabutin, pyrazinamide, ethambutol, streptomycin, kanamycin, amikacin, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, ciprofloxacin, capreomycin, ethionamide, cycloserine, para-aminosalicylic acid, thiacetazone, clarithromycin, amoxicillin-clavulanic acid, imipenem, meropenem, clofazimine, viomycin, terizidone, TMC207, PA-824, OPC-7683, LL-3858 and SQ-109. The agents useful for the treatment of tuberculosis can be used in the present invention in a variety of forms, including acid form, salt form, racemates, enantiomers, solvates, and tautomers. The agents useful for the treatment of tuberculosis may be administered by any route useful to administer said agents, which are known to those of skill in the art.

[0196] The invention also relates to compositions of the invention which comprise (i) a therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof, (ii) at least one agent useful in the treatment of tuberculosis and (iii) a pharmaceutically acceptable carriers or vehicles (hereinafter "the compositions of the invention").

[0197] Compositions of the invention that are suitable for administration to a patient in need thereof (e.g., a human) are also referred to herein as "pharmaceutical compositions of the invention."

[0198] The pharmaceutical compositions of the invention may be in any form suitable for administration to a patient. For example, the pharmaceutical compositions of the invention may be in a form suitable for oral administration such as a tablet, capsule, pill, powder, sustained release formulations, solution, and suspension; for parenteral injection as a sterile solution, suspension or emulsion; for topical administration as an ointment or cream; or for rectal administration as a suppository. The pharmaceutical compositions of the invention may be in unit dosage forms suitable for single administration of precise dosages.

[0199] Exemplary parenteral administration forms include solutions or suspensions of active compounds in sterile aqueous solutions, for example, aqueous propylene glycol or dextrose solutions. Such dosage forms can be suitably buffered, if desired.

[0200] In one embodiment, the pharmaceutical compositions of the invention may be in the form of an oral dosage form. Non-limiting examples of oral dosage forms include such as, e.g., chewable tablets, capsules, pills, lozenges, troches, sachets, powders, syrups, elixirs, solutions and suspensions, and the like, in accordance with standard pharmaceutical practice. In another embodiment, the pharmaceutical compositions of the invention can also be delivered directly to a patient's gastrointestinal tract through a nasogastric tube.

[0201] The compound of formula (I) of the invention will be present in the pharmaceutical composition of the invention in an amount sufficient to provide the desired dosage amount in the range described herein. The proportional ratio of compound of formula (I) of the invention to excipients will naturally depend on the chemical nature, solubility and stability of the active ingredients, as well as the dosage form contemplated. Typically, pharmaceutical compositions of the present invention can contain about 20% to about 99% of the compound of formula (I) of the invention by weight.

[0202] In one embodiment, the compound of formula (I) and pharmaceutically acceptable salts thereof is administered between about 10 mg to about 2000 mgln yet another embodiment, the compound of formula (I) and pharmaceutically acceptable salts thereof is administered between about 25 mg to about 1000 mg.

[0203] In yet another embodiment, the compound of formula (I) and pharmaceutically acceptable salts thereof is administered between about 50 mg to about 500 mg.

[0204] In yet another embodiment, the compound of formula (I) and pharmaceutically acceptable salts thereof is administered between about 100 mg to about 500 mg.

[0205] Techniques for formulation and administration of the compound of formula (I) of the instant invention and the compositions of the invention the can be found in Remington: the Science and Practice of Pharmacy, 19th ed., Mack Pub. Co., Easton, Pa. (1995).

[0206] The term "excipient" means an inert material that is combined with the compound of formula (I) to produce a pharmaceutical composition or oral drug dosage form.

[0207] The term "pharmaceutically acceptable excipient" means that the excipient must be compatible with other ingredients of the composition, and not deleterious to the recipient thereof. The pharmaceutically acceptable excipients are chosen on the basis of the intended dosage form.

[0208] The tablets, pills, capsules, and the like may contain excipients selected from binders such as polyvinylpyrrolidone, hydroxypropylmethyl cellulose (HPMC), hydroxypropylcellulose (HPC), sucrose, gelatin, acacia, gum tragacanth, or corn starch; fillers such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate, glycine and starch; disintegrants such as corn starch, potato starch, alginic acid, sodium starch glycolate, croscarmellose sodium and certain complex silicates; lubricants such as magnesium stearate, sodium lauryl sulfate and talc; and sweeteners such as sucrose lactose or saccharin. When a dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier such as a fatty oil. Various other materials may be present as coatings or to modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both.

[0209] In the case of pediatric oral suspensions and sachets, these excipients may comprise suspending aids such as xantham gum or hydroxypropylmethylcellulose, glidants such as colloidal silica, diluents and bulking agents such as silicon dioxide, flavors such as bubble gum, orange, banana, raspberry and golden syrup or mixtures thereof, sweeteners such as aspartame or sugar, and stabilizers such as succinic acid. Powder or granular formulations, such as pediatric suspension formulations and sachets, may be manufactured using techniques which are generally conventional in the field of manufacture of pharmaceutical formulations and in the manufacture of dry formulations for reconstitution into such suspensions. For example a suitable technique is that of mixing dry powdered or granulated ingredients.

[0210] Typically, an effective daily dose (i.e., total dosage over about 24 hours) of the compound of formula (I) of the invention for adults is about 10 mg to about 2000 mg; about 25 mg to about 1000 mg; about 50 mg to about 500 mg; and 100 mg to about 500 mg with or without food. In some cases, it may be necessary to use dosages outside these limits.

[0211] A daily dosage of the compound of formula (I) of the invention is usually administered from 1 to 4 times daily in equal doses.

[0212] In one embodiment, a single dose of compound of formula (I) of the invention is administered per day (i.e., in about 24 hour intervals) (i.e., QD); in another embodiment, two doses of compound of formula (I) of the invention are administered per day (i.e., BID); in another embodiment, three doses of compound of formula (I) of the invention are administered per day (i.e., TID); and in another embodiment, four doses of compound of formula (I) of the invention are administered per day (i.e., (QID); in another embodiment a single dose of compound of formula (I) of the invention is administered every other day (i.e., in about 48 hour intervals), in another embodiment a single dose of compound of formula (I) of the invention is administered twice per week; in another embodiment a single dose of compound of formula (I) of the invention is administered thrice per week.

[0213] In one embodiment, the effective dose of the compound of formula (I) of the invention is administered BID in about 12 hour intervals.

[0214] In another embodiment, the effective dose of the compound of formula (I) of the invention is administered TID in about 8 hour intervals.

[0215] In another embodiment, the effective dose of the compound of formula (I) of the invention for is administered QID in about 6 hour intervals.

[0216] In one embodiment, an effective dose of the compound of formula (I) of the invention is about 25 mg to about 1000 mg which is administered BID in about 12 hour intervals.

[0217] Oral administration is preferred.

[0218] The pharmaceutical composition of the invention in a fixed dose combination comprising a compound of formula

(I) of the invention and at least one agent useful for the treatment of tuberculosis and pharmaceutically acceptable carriers can be prepared by conventional methods in the art. For e.g., a tablet form of the combination can be prepared by any one of skill in the art.

[0219] Some examples of the present invention are combinations and the pharmaceutical compositions which encompass the following non-limiting mixtures:

[0220] a) a compound of formula (I) or a pharmaceutically acceptable salt thereof and pyrazinamide;

[0221] b) a compound of formula (I) or a pharmaceutically acceptable salt thereof and rifampin;

[0222] c) a compound of formula (I) or a pharmaceutically acceptable salt thereof and rifapentine;

[0223] d) a compound of formula (I) or a pharmaceutically acceptable salt thereof, and PA-824;

[0224] e) a compound of formula (I) or a pharmaceutically acceptable salt thereof, and TMC-207;

[0225] f) a compound of formula (I) or a pharmaceutically acceptable salt thereof, and an agent selected from the group consisting of moxifloxacin, gatifloxacin, levofloxacin, ofloxacin moxifloxacin;

[0226] g) a compound of formula (I) or a pharmaceutically acceptable salt thereof, isoniazid and rifampin;

[0227] h) a compound of formula (I) or a pharmaceutically acceptable salt thereof, isoniazid, rifampin and pyrazinamide; and

[0228] i) a compound of formula (I) or a pharmaceutically acceptable salt thereof, rifampin and pyrazinamide.

[0229] Other examples of the present invention are combinations and the pharmaceutical compositions that encompass the following non-limiting mixtures,

[0230] j) about 100 mg to about 500 mg of a compound of formula (I) or a pharmaceutically acceptable salt thereof and about 600 mg of rifampin;

[0231] k) about 100 mg to about 500 mg of a compound of formula (I) or a pharmaceutically acceptable salt thereof and about 300 mg of isoniazid;

[0232] l) about 100 mg to about 500 mg of a compound of formula (I) or a pharmaceutically acceptable salt thereof and about 300 mg of isoniazid and about 600 mg of rifampin;

[0233] m) about 100 mg to about 500 mg of a compound of formula (I) or a pharmaceutically acceptable salt thereof, about 300 mg of isoniazid, about 600 mg of rifampin and about 20-25 mg/kg to about 50-70 mg/kg of pyrazinamide;

[0234] n) about 100 mg to about 500 mg of a compound of formula (I) or a pharmaceutically acceptable salt thereof and about 10 mg/kg to about 20 mg/kg of rifampin;

[0235] o) about 100 mg to about 500 mg of a compound of formula (I) or a pharmaceutically acceptable salt thereof and about 10-15 mg/kg to about 20-30 mg/kg of isoniazid;

[0236] p) about 100 mg to about 500 mg of a compound of formula (I) or a pharmaceutically acceptable salt thereof and about 10-15 mg/kg to about 20-30 mg/kg of isoniazid and about 10 mg/kg to about 20 mg/kg of rifampin;

[0237] q) about 100 mg to about 500 mg of a compound of formula (I) or a pharmaceutically acceptable salt thereof, about 10-15 mg/kg to about 20-30 mg/kg of isoniazid, about 10 mg/kg to about 20 mg/kg of rifampin and about 15-30 mg/kg to about 50 mg/kg pyrazinamide;

[0238] r) about 50 mg to about 250 mg of a compound of formula (I) or a pharmaceutically acceptable salt thereof, about 75 mg isoniazid, about 150 mg of rifampin and about 400 mg of pyrazinamide; and

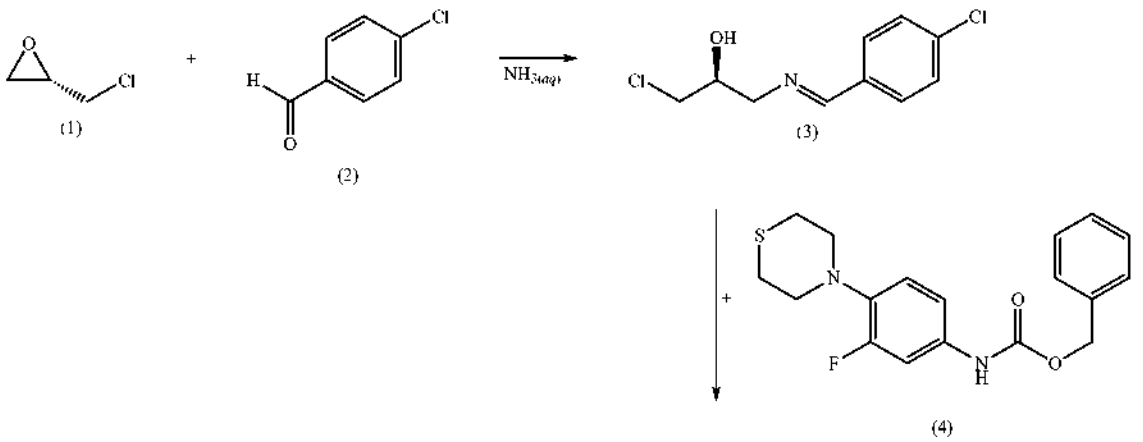
[0239] s) about 25 mg to about 250 mg of a compound of formula (I) or a pharmaceutically acceptable salt thereof, and about 300 mg of rifampin.

[0240] The compounds of formula (I) of the present invention are readily prepared according to synthetic methods familiar to those skilled in the art. The compound of formula (I) of the invention can be prepared in a manner similar to that described for the preparation of Example 1 described in the Examples section in U.S. Pat. No. 5,880,118. In addition, the compound of formula (I) can be prepared by the processes set forth in international Publication WO97/37980 and WO99/24393, both of which are herein incorporated by reference.

[0241] Another example of the preparation of (S)—N-[[3-[3-fluoro-4-(4-thiomorpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide is as follows:

[0242] Scheme 1 illustrates a general synthetic sequence for preparing compounds of the present invention.

SCHEME I



[0243] Scheme 1 illustrates a method of synthesizing compounds of formula (I) of the invention in a multistep synthesis via a compound of formula 6. Referring to Scheme I, the synthesis begins with the formation of intermediate (3) by reacting (S)-epichlorohydrin (1) with a mixture of the appropriately substituted benzaldehyde derivative (2) (preferably 0.5 to 2 eq, most preferably 1 eq) and aqueous ammonia (preferably 0.5 to 3 eq, most preferably 1.5 eq). The reaction is best performed in both protic and aprotic non-nucleophilic and inert solvents such as alcohols (including C_1-C_6 branched and linear alcohols and polyols), ethers (including MTBE, THF, and other C_1-C_6 linear, branched and cyclic ethers) as well as chlorinated solvents such as methylene chloride. MTBE is a preferred solvent. Temperatures in a range from about 15 to about 60° C. are preferred, most preferably between 30 to 50° C. After extractive isolation and concentration, the imine moiety (3) is obtained. It is then crystallized from a second liquid phase, in the presence of non-polar hydrocarbon solvents such as, but not limited to, alkanes, mixtures of alkanes (hexane, heptane, octane, iso-octane and commercially available alkane mixtures), optionally in the presence of aprotic polar solvents, preferably ethereal solvents such as MTBE or aromatic solvents such as toluene or chlorinated solvents such as methylene chloride or mixtures thereof. Preferred solvents are a mixture of MTBE and heptane or a mixture of toluene and heptane. The crystallization process can be conducted at a temperature in a range from ambient temperature (about 18-25° C.) to about 55° C., preferably in a range of 30 to 50° C., more preferably in a range of 38 to 45° C. This crystallization provides surprisingly high yield and affords significantly improved enantiomeric purity after isolation by filtration. (S)-epichlorohydrin (1) and benzaldehyde derivative (2) are commercially available or can be made by methods well known to those skilled in the art.

[0244] The substituted imine moiety (3) is coupled with carbamate (4) (which is known to those skilled in the art, for example see *J. Med. Chem.*, 1996, 39, (3), 680-685 and also Example 2 below, (preferably 1 to 3 eq, most preferably 1.5 to 2 eq)) to provide the corresponding (S)-oxazolidinone imine (5). The reaction is carried out preferably at a temperature in a range from ambient temperature to about 65° C. in the presence of a base with pKa greater than 12, preferably a tertiary alkoxide base, most preferably lithium tert-butoxide and an aprotic non-nucleophilic solvent (preferably DMF, DMAc, acetonitrile, C_1-C_6 linear, branched and cyclic ethers and/or chlorinated solvents and/or mixtures of these solvents, most preferably MTBE or methylene chloride). Most preferably, the temperature is from about 30-60° C. and the reaction time is 2 to 24 hours. Preferably, the (S)-oxazolidinone imine (5), after an aqueous extractive workup, is isolated by filtration from a 1:1 mixture of an ether (including MTBE, THF, and other C_1-C_6linear, branched and cyclic ethers) and water, most preferably MTBE. Alternatively, (5) is isolated after an aqueous extractive workup, by filtration or crystallization from an alcohol (including C_1-C_6linear, branched alcohols and polyols); most preferably isopropanol. Hydrolysis of compound (5) with an aqueous acidic solution provides compound (6) and subsequent acylation provides crude compound (7). Compound (5) is best hydrolyzed with a mixture of water and a strong acid such as hydrochloric acid and the substituted benzaldehyde byproduct is removed by extraction with a water immiscible organic solvent (preferably toluene,

MTBE, methylene chloride or ethyl acetate), most preferably ethyl acetate. The resulting aqueous solution of amine hydrochloride (6) is preferably acylated with acetic anhydride, preferably in the presence of water and a water-immiscible organic solvent (most preferably methylene chloride). The conversion of amine hydrochloride (6) to compound (7) is well known in the literature. (See Brickner, S. J. et. al. *J. Med. Chem.* 1996 39 (3) 673-679, U.S. Pat. No. 5,837,870, U.S. Pat. No. 5,688,792).

[0245] The examples provided below further illustrate and exemplify the compounds of formula (I) of the invention, compositions of the invention and methods of using the compound of the invention. It is to be understood that the scope of the present invention is not limited in any way by the scope of the following examples and preparations.

Example 1

Preparation of (S)-1-chloro-3-[(4-chloro-E-benzylidene)-amino]-propan-2-ol

Method A

[0246] A 5 L three neck round bottom flask equipped with a mechanical stirrer, thermocouple, reflux condenser and heating mantle is charged with 4-chlorobenzaldehyde (351.0 g, 2.5 mol, 1.0 eq.). MTBE (1.5 L) is then charged into the round bottom to give a homogeneous solution. Aqueous ammonia (28 wt %, 252.98 mL, 3.75 mol, 1.5 eq.) is added in a single portion resulting in a white precipitate that turned into a thin slurry within 15 minutes of stirring. (S)-(+)-epichlorohydrin (>99% ee, 196.0 mL, 2.5 mol, 1.0 eq.) is then slowly charged into the vessel. After 40 minutes, the contents are then slowly heated to 43° C. The reaction is stirred at 40° C. for 18 hours at which time 8.4% area of epichorohydrin remained by GC. Upon cooling, the reaction mixture is transferred to a separatory funnel and the layers are separated. The lower aqueous layer is discarded. The organic layer is transferred to a 3 L round bottom flask, concentrated in vacuo to about half the volume (800-900 mL) at which time iso-octane is slowly added from a feed tube (~750 mL) until cloudiness is observed. The biphasic mixture is seeded with ~4 mgs of the title compound. The reaction is cooled with an ice bath for 45 minutes while stirring. The precipitate is collected and rinsed with cold iso-octane (500 mL). The solid is dried for 18 hours at 50° C. under vacuum to give 345.19 g (59% yield) of the title compound as a while solid. GC assay: 100%, 99.7% ee by Chiral SFC). GC (conditions: column-30 meter HP-1, 0.25 mm ID and 0.25 micron film and 15 psi head pressure, 1.0 μl injection size; T_{ini}=70° C., ramp of 20° C./min) T_R (epichlorohydrin)=2.4 min, T_R (4-chlorobenzaldehyde)=4.8 min and T_R (title compound)=9.7 min; HPLC conditions: Chiralpak AD-H 250 mm×4.6 mm column, eluting with 70% CO_2/30% MeOH at 3.0 mL/min, detecting at 255 nm. T_R [title compound]=3.9 min; T_R (enantiomer of title compound)=2.8 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.69 (bs, 2H), 3.80 (m, 2H), 4.15 (s, 1H), 7.41 (d, J=8 Hz, 2H), 7.69 (d, J=8 Hz, 2H), 8.33 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 47.05, 63.09, 70.82, 128.93, 129.39, 134.08, 137.07, 162.30; IR (KBr Pellet) 1630 cm^{-1}.

Method B

[0247] A 5 L three neck round bottom flask equipped with a mechanical stirrer, thermocouple, reflux condenser and heating mantle is charged with 4-chlorobenzaldehyde (375 g, 2.67 mol, 1.0 eq.). Methanol (0.75 L) is added to give a homogeneous solution after warming from 10 to 23° C. Aqueous ammonia (28.4 wt %, 264 mL, 3.95 mol, 1.5 eq.) is added in a single portion resulting in a biphasic solution forming after stirring for 15 minutes at 23 to 26° C. (S)-(+)-epichlorohydrin (99.3% ee, 207 mL, 2.64 mol, 1.0 eq.) is then added in one portion. The reaction mixture is stirred at 23-24° C. for 18 hours, then warmed to 40 to 45° C. and stirred for 2.5 hours at which time 0.26% area of (S)-epichorohydrin remains by GC (GC conditions, 0.050 mL reaction mixture in 1 mL acetonitrile, inject 1 microliter; 15 M DB-1 column, 0.25 mm ID and 0.25 micron film and 15 psi head pressure, 1.0 μl injection size; T_{ini}=38° C., ramp of 10° C./min) T_R (epichlorohydrin)=1.1 min, T_R (4-chlorobenzaldehyde)=6.9 min and T_R (title compound)=16.0 min). The mixture is concentrated in vacuo to a total volume of 1250 mL. Toluene (250 mL) is added and the mixture concentrated in vacuo to a total volume of 1250 mL. Toluene (250 mL) is added and the mixture concentrated in vacuo to a total volume of 1145 mL. Toluene (355 mL) is added and the mixture concentrated in vacuo to a total volume of 900 mL. Toluene (600 mL) is added and the mixture concentrated in vacuo to a total volume of 1120 mL. While maintaining 45 to 50° C., heptane (1500 mL) is added. The resulting biphasic solution is cooled to 45° C. and seeded. The mixture is then further cooled to 38° C. over ½ hour while seeding after every 1 degree of cooling. The mixture is then further allowed to slowly cool to 23° C. over 16 hours. The white crystals are then collected by vacuum filtration and washed with room temperature heptane (180 mL). The product is dried in a nitrogen stream to give the title compound (431.57 g, 70.4%). HPLC 95 area % [Kromasil 150 mm×4.6 mm column, 254 nm, flow rate 1.5 mL/min; A=1000 mL water+0.52 mL trifluoroacetic acid+1.20 mL triethylamine; B=acetonitrile; Isocratic 47: 53 A: B for 5 min then gradient to 100% B over 5 min T_R [title compound]=2.1 min; T_R (4-chlorobenzaldehyde)=2.3 min]; 99.72% ee by Chiral SFC. Chiral HPLC conditions: Chiralpak AD-H 250 mm×4.6 mm column, eluting with 70% CO_2/30% MeOH at 3.0 mL/min, detecting at 255 nm. T_R [title compound]=3.9 min; T_R (enantiomer of title compound)=2.8 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.69 (bs, 2H), 3.80 (m, 2H), 4.15 (s, 1H), 7.41 (d, J=8 Hz, 2H), 7.69 (d, J=8 Hz, 2H), 8.33 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 47.05, 63.09, 70.82, 128.93, 129.39, 134.08, 137.07, 162.30.

Method C

[0248] A 5 L three neck round bottom flask equipped with a mechanical stirrer, thermocouple, reflux condenser and heating mantle is charged with 4-chlorobenzaldehyde (375 g, 2.67 mol, 1.0 eq.). MTBE (1.50 L) is then added to give a homogeneous solution after warming from 9 to 24° C. Aqueous ammonia (28.4 wt %, 265 mL, 3.97 mol, 1.5 eq.) is added in a single portion resulting in a biphasic solution forming after stirring for 15 minutes at 23 to 26° C. (S)-(+)-epichlorohydrin (99.3% ee, 209 mL, 2.67 mol, 1.0 eq.) is then added in one portion. The reaction mixture is stirred at 23-24° C. for 3 days. The phases are separated and the upper phase concentrated under atmospheric pressure from 2000 to 1000 mL total volume (boiling point 58 to 67° C.). While maintaining 45 to 50° C., heptane (1700 mL) is added. The resulting biphasic solution is cooled to 45° C. and seeded. The mixture is then further cooled to 38° C. over ½ hour while seeding after every 1 degree of cooling. The mixture is then further allowed to slowly cool to 23° C. over 1 hour. The snow white

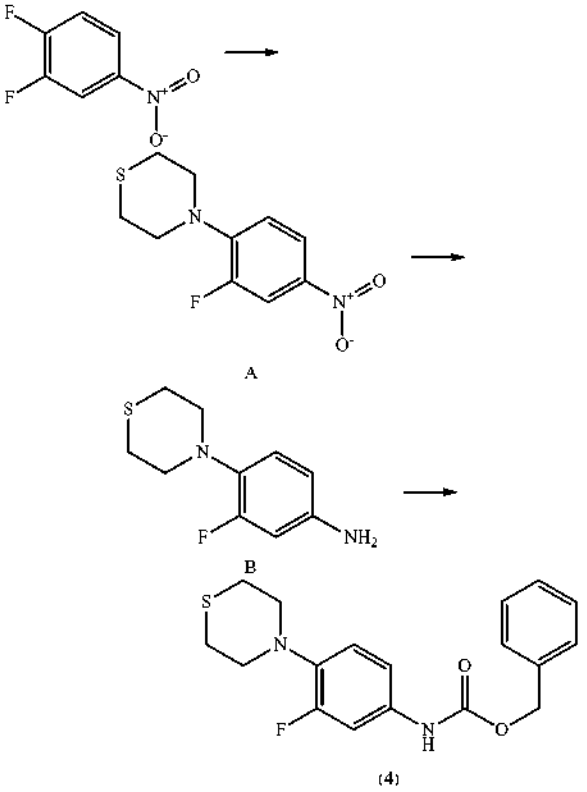
heavy crystals are then collected by vacuum filtration and washed with room temperature heptane (180 mL). The product is dried in a nitrogen stream to give the title compound (462.43 g, 74.7%). HPLC 94 area % [Kromasil 150 mm×4.6 mm column, 254 nm, flow rate 1.5 mL/min; A=1000 mL water+0.52 mL trifluoroacetic acid+1.20 mL triethylamine; B=acetonitrile; Isocratic 47: 53 A: B for 5 min then gradient to 100% B over 5 min. T_R [title compound]=2.1 min; T_R (4-chlorobenzaldehyde)=2.3 min]; 99.92% ee by Chiral SFC. Chiral HPLC conditions: Chiralpak AD-H 250 mm×4.6 mm column, eluting with 70% CO_2/30% MeOH at 3.0 mL/min, detecting at 255 nm. T_R [title compound]=3.9 min; T_R (enantiomer of title compound)=2.8 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.69 (bs, 2H), 3.80 (m, 2H), 4.15 (s, 1H), 7.41 (d, J=8 Hz, 2H), 7.69 (d, J=8 Hz, 2H), 8.33 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 47.05, 63.09, 70.82, 128.93, 129.39, 134.08, 137.07, 162.30.

Example 2

Preparation of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid benzyl ester

[0249] The title compound can be prepared according to the method described in *J. Med. Chem.*, 1996, 39, (3), 680-685 and depicted in SCHEME II.

SCHEME II



[0250] Additional methods for the conversion of intermediate A to 3-fluoro-4-thiomorpholin-4-ylaniline (B) are provided.

Method A

[0251] 4-(2-Fluoro-4-nitrophenyl)thiomorpholine (A, 250 g, 1.03 mole) was charged into a mixture of dioxane (1400 mL), EtOH (1000 mL) and water (600 mL) in a 5000 mL three neck round bottom flask equipped with a mechanical stirrer. Into the stirred mixture was charged ammonium chloride (166 g, 3.1 moles) followed by iron powder (247 g, 4.25 moles), each in single portions. The reaction was warmed to reflux with vigorous stirring. The reaction was heated at reflux for a total of 16 hours and was then allowed to cool to room temperature. The dark mixture was diluted with EtOAc (800 mL), filtered through a pad of celite, and concentrated in vacuo to a pasty residue. The residue was partitioned between brine (1000 mL) and dichloromethane (750 mL). One filtration through celite removed particulates that were interfering with phase separation. The aqueous layer was then extracted with additional dichloromethane (750 mL). The combined organic layers were dried over anhydrous potassium carbonate and concentrated in vacuo to give 225 g of a dark solid. This crude material was dissolved in dichloromethane (1000 mL), treated with 200 g silica gel (230-400 mesh) and the mixture was concentrated to dryness. The plug was filtered over 500 g silica gel (230-400 mesh, packed as a slurry with 20% EtOAc/hexane) eluting with 20-30% EtOAc/hexane while collecting 1000 mL fractions. Fractions 3-11 were combined and concentrated to give 3-fluoro-4-thiomorpholin-4-ylaniline (B, 232 g, 106% yield) as an off-white solid. ^{1}H NMR indicated the desired material along with trace residual solvents to account for the greater than theoretical recovery. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.8 (m, 4H), 3.2 (m, 4H), 3.6 (s, 2H), 6.4 (m, 2H), 6.8 (m, 1H).

Method B

[0252] A 2000 mL Parr shaker flask was charged with 5% sulfided palladium on carbon (Johnson Matthey type A103038-5, 18 g) and 4-(2-fluoro-4-nitrophenyl)thiomorpholine (A, 60 g, 0.25 mole). The mixture was suspended in MeOH (1050 mL) and the reaction was hydrogenated at 50 PSI for 7 h. The catalyst was removed by filtration through celite and the filter cake was washed well with fresh MeOH. The clear gray filtrate was concentrated in vacuo to give 3-fluoro-4-thiomorpholin-4-ylaniline (B, 51.3 g, 98% yield) as a gray solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.8 (m, 4H), 3.2 (m, 4H), 3.6 (s, 2H), 6.4 (m, 2H), 6.8 (m, 1H).

Example 3

Preparation of (5S)-5-{[(4-chlorobenzylidene) amino]methyl}-3-(3-fluoro-4-thiomorpholin-4-ylphenyl)-1,3-oxazolidin-2-one

[0253] The title compound in Example 2 (194 g, 0.56 mole), and the title compound of Example 1 (195 g, 0.84 mole), and lithium tert-butoxide (116 g, 1.4 mole) were charged into a 3000 mL three neck round bottom flask under nitrogen. The reactants were slurried with methyl tert-butyl ether (1200 mL) and the mixture was warmed to 56° C. and stirred for 2 h as a yellow solid gradually formed. The reaction was cooled to room temperature, and diluted with 1200 mL water. The mixture was then stirred vigorously over 60 min as the solid changed from dark yellow to a more pale yellow solid. The mixture was cooled to 10° C., filtered, and the filter cake was washed with ice cold methyl tert-butyl ether (450 mL). The resulting light yellow solid was dried in air for 30 min, then placed in a vacuum oven and dried at 40° C. overnight to afford the title compound (243 g, 99% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.8 (m, 4H), 3.2 (m, 4H), 3.9 (m, 2H), 4.1 (m, 2H), 5.0 (m, 1H), 6.9 (m, 1H), 7.2 (m, 1H), 7.4 (m, 3H), 7.6 (m, 2H), 8.4 (s, 1H).

Example 4

Preparation of N-{[(5S)-3-(3-fluoro-4-thiomorpholin-4-ylphenyl)-2-oxo-1,3-oxazolidin-5-yl]methyl}acetamide

[0254] The title compound in Example 3 (243 g, 0.56 mole) was combined with EtOAc (1300 mL) and water (1300 mL) in a 5000 mL three neck round bottom flask equipped with a mechanical stirrer. The mixture was treated drop-wise with 12N HCl (140 mL, 1.68 moles) and the mixture was stirred vigorously for 1 hour at room temperature. The layers were separated and the aqueous layer was washed with EtOAc (1×500 mL). The resulting aqueous solution containing (S)-5-(aminomethyl)-3-(3-fluoro-4-thiomorpholinophenyl)oxazolidin-2-one hydrochloride was combined with a mixture of dichloromethane (1800 mL) and MeOH (120 mL), and the vigorously stirred mixture was charged with acetic anhydride (132 mL, 1.4 mole) in one portion and subsequently treated drop-wise with 10 N NaOH (200 mL, 2.0 mole) over 15 min. An extremely thick reaction mixture resulted from addition of the base, which gradually thinned as the pH rose and the acylation rapidly progressed. The reaction was stirred vigorously for 1 hour after the mixture resolved to two phases. At that time, 10 M NaOH (160 mL, 1.6 mole) was added drop-wise to the mixture until the pH was stable at 7. The layers were separated, the aqueous layer was extracted with dichloromethane (250 mL), and the combined organic layers were dried over anhydrous potassium carbonate. The volatiles were removed in vacuo to give an off-white solid which was titrated with methyl tert-butyl ether (250 mL), collected, and dried in vacuo to give title compound (5) (186.1 g, 94% yield) as a fine white solid with greater than 98% HPLC purity (retention time=3.93 minutes, HPLC conditions reported below).

[0255] The crude solid was dissolved in warm 6% methanol in dichloromethane (1250 mL) in a 5000 mL three neck round bottom flask equipped with a mechanical stirrer. The solution was warmed to reflux, diluted by the portion-wise (500 mL) addition of 2500 mL isopropanol (IPA), and, in order to maintain reflux, the temperature was ramped to 50-70° C. On completion of this addition of IPA, the reflux condenser was replaced with a short-path distillation head and distillation was continued into a cooled flask. During distillation, a 500 mL portion of fresh IPA was added after 500 mL of distillate was collected to maintain between 2000 and 2500 mL IPA present at all times. After this addition (internal flask temperature dropped to 60° C.) the mixture became slightly cloudy and remained so for the balance of the distillation, becoming increasingly cloudy as the distillate temperature exceeded 70° C.; particulate matter appeared as the distillate temperature exceeded 75° C. The temperature controller was ramped to 85° C. and held there until the conclusion of the distillation. When the distillate was clearly isopropanol alone (82-83° C.) the volume was reduced to 2500 mL hot IPA, the heating mantle was removed, stirring was discontinued, and the paddle was removed from the flask. The mixture was allowed to continue to crystallize as the flask cooled. The white crystalline solid was then collected by filtration, washed with methyl tert-butyl ether (250 mL), and dried in vacuo at 40° C. to afford 180 g (91% yield) of the title compound in greater than 99% HPLC purity (retention time=3.93 minutes, HPLC conditions reported below). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.8 (s, 3H), 2.7 (m, 4H), 3.2 (m, 4H), 3.4 (m, 2H), 3.7 (m, 1H), 4.7 (m, 1H), 7.1 (m, 1H), 7.15 (m, 1H), 7.2 (m, 1H), 8.2 (m, 1H). Mass Spec. $C_{16}H_{20}FN_3O_3S$: m/z 354.1 (M+1).

[0256] HPLC conditions for analyses mentioned in the text: HP Series 1100; Column: Symmetry C8 5 uM 4.6×50 mm; Flow rate 1.2 mL/min; Solvent A: water with 0.1% formic acid, Solvent B: acetonitrile with 0.1% formic acid; Injection volume=10 uL of 1 mg/mL (acetonitrile); Gradient: Solvent B 0-100% over 7 minutes then 100% B for 1 minute; wavelength=254 nm.

Biological Examples

[0257] The following abbreviations are used in the following Examples and have the definitions indicated unless otherwise noted: CFU is colony forming unit; INH is isoniazid; RIF is rifampin; PZA is pyrazinamide; MXF is moxifloxacin, and LZD is linezolid.

[0258] The bacterial strain *Mycobacterium tuberculosis* H37Rv was passaged in mice, frozen in 1 mL aliquots and stored at −80° C. before use. For each infection, an aliquot was thawed and sub-cultured in Middlebrook 7H9 broth supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC) (Difco, Detroit, Mich.) and 0.05% Tween 80 (Sigma, St. Louis Mo.). Female BALB/c mice (Charles River, Wilmington, Mass.), aged four to six weeks were infected via aerosol using the Inhalation Exposure System (Glas-col Inc, Terre Haute, Ind.) and a log phase broth culture with an optical density of approximately 1.0 at 600 nm. Mice were randomized to treatment groups (5 mice per group per time point) after aerosol infection. Untreated mice were routinely sacrificed (i) on the day after infection to determine the number of CFU implanted in the lungs and (ii) on the day of treatment initiation to determine the pre-treatment CFU count.

[0259] The compound of formula (I) of the invention and LZD (obtained from Pfizer Inc. Ann Arbor, Mich. and Groton, Conn.) used in the tests described below was suspended in a solution composed of 5% polyethylene glycol-200 (PEG-200) and 95% methylcellulose (0.5%) in distilled water. MXF was obtained from Bayer (Rolling Meadows, Ill.), PZA was obtained from Fisher, and INH and RIF were obtained from Sigma. Stock solutions were prepared weekly using distilled water. All antibiotic solutions were stored at 4° C.

[0260] Except where otherwise indicated, antibiotics were administered once daily, five days per week in 0.2 ml by gavage. Both oxazolidinone suspensions were sonicated briefly prior to use and shaken between doses. RIF was given 1 hour prior to administration of other drugs to avoid an adverse pharmacokinetic interaction.

Example 5

In Vitro Activity of the Compound of Formula (I)

[0261] The MIC of the compound of formula (I) and LZD was determined by the agar dilution method on Middlebrook 7H11 agar supplemented with 10% OADC (Becton-Dickinson, Sparks, Md.). Plates containing serial two-fold concentrations of the compound of formula (I) and LZD ranging from 0.125 to 4 μg/mL were inoculated with approximately 5×10^5 CFU of *Mycobacterium tuberculosis* H37Rv. CFU were counted after 28 days incubation at 37° C. with 5% ambient CO_2. The MIC was defined as the lowest concentration to inhibit at least 99% of bacterial growth.

[0262] The MIC of the compound of formula (I) and LZD against *Mycobacterium tuberculosis* H37Rv was 0.25 μg/mL.

Dose-Ranging Activity of the Compound of Formula (I) and LZD in an Established Infection Model

[0263] Mice were infected by the aerosol route with 4.44±0.04 $\log_{10}$ CFU. Beginning 13 days after aerosol infection, when the mean lung CFU count was 7.49±0.11 $\log_{10}$ and the mean spleen weight was 105±11 mg, control mice received one of the following treatments: INH at 25 mg/kg, LZD 25 mg/kg to 260 mg/kg (single dose and 130 mg/kg twice daily), and compound of formula (I) 25 mg/kg to 100 mg/kg doses. At the end of the treatment period, 28 days later, only 2 of 5 untreated mice remained alive. The mean spleen weight among those living mice had increased to 237±16 mg.

[0264] In contrast, monotherapy with INH at 25 mg/kg prevented splenomegaly (mean spleen weight=100±1 mg) and death. A dose-dependent decrease in spleen weight relative to untreated controls was observed with increasing doses of LZD between 25 mg/kg (200±47 mg) and 130 mg/kg (102±5 mg). However, spleens from mice treated with LZD 260 mg/kg (whether as a single dose or 130 mg/kg twice daily) were larger than expected (190±52 mg) and similar in size to spleens from mice treated with LZD 25 mg/kg/day. Because the number and size of lung lesions decreased with increasing daily doses of LZD including 260 mg/kg, the splenomegaly observed in mice receiving 260 mg/kg/day was not felt to be due to reduced anti-tuberculosis activity. Treatment with the compound of formula (I) prevented splenomegaly at all doses administered, including the 25 mg/kg dose (mean spleen weight=113±17 mg).

[0265] Surviving untreated mice experienced an increase in CFU counts to nearly 8 $\log_{10}$. Treatment with INH reduced the mean lung CFU count to 5.62 $\log_{10}$, for a log kill of 1.87 compared to the baseline value. All regimens including the compound of formula (I) resulted in a significant reduction in mean CFU count from baseline ($p<0.01$), starting with a 0.78 $\log_{10}$ reduction with 25 mg/kg. At 50 mg/kg, the compound of formula (I) exhibited bactericidal activity (defined by the 2-log-reduction criterion) by reducing the mean CFU count to 5.28 $\log_{10}$, for a log kill of 2.21, greater than that observed with INH. Although LZD also displayed dose-dependent activity, its activity was more limited than that of the compound of formula (I). Only at LZD doses≧100 mg/kg was a significant reduction from the baseline CFU count demonstrated ($p<0.01$). Even at the highest dose tested, 260 mg/kg, LZD did not meet the 2-log-reduction criterion defining bactericidal activity, producing a log kill of only 1.46. The compound of formula (I) was significantly more active than LZD at each dose tested.

Treatment with the compound of formula (I) at 25 mg/kg resulted in a CFU count lower than that observed after treatment with LZD at 25 or 50 mg/kg ($p<0.01$) and not significantly different from that observed after treatment with LZD at 100 or 130 mg/kg. At 50 and 100 mg/kg, the compound of formula (I) was more active than any dose of LZD ($p<0.001$). Dividing the total daily dose of the compound of formula (I) and LZD into two daily doses had no clear effect (i.e., positive or negative) on activity (Table 1).

TABLE 1

Drug	Daily dosing regimen	Mean $\log_{10}$ CFU count (± SD) on: Day 0	Day 28
None	None	7.49 ± 0.11	7.92 ± 0.03
INH	25 mg/kg once		5.62 ± 0.20
Compound of Formula (I)	25 mg/kg once		6.71 ± 0.28
	25 mg/kg twice		5.51 ± 0.14
	50 mg/kg once		5.28 ± 0.53
	50 mg/kg twice		4.89 ± 0.27
	100 mg/kg once		5.07 ± 0.14
LZD	25 mg/kg once		7.55 ± 0.24
	50 mg/kg once		7.33 ± 0.11
	100 mg/kg once		6.75 ± 0.14
	130 mg/kg once		6.57 ± 0.13
	130 mg/kg twice		6.02 ± 0.17
	260 mg/kg once		6.03 ± 0.12

[0266] Hence, the compound of formula (I) demonstrated that it is significantly more active than the human-equivalent dose (i.e. 100 mg/kg) of LZD, the only clinically available oxazolidinone, which is being used off-label for the treatment of MDR- and XDR-TB.

Pharmacokinetics of the Compound of Formula (I)

[0267] To determine the single-dose and steady-state pharmacokinetic profiles of the compound of formula (I) and LZD in the murine model, a sub-study was nested in the above dose-ranging study using mice treated with the compound of formula (I) at 100 mg/kg once-daily or LZD at 130 mg/kg once- or twice-daily. Three mice per group were sacrificed at 0.5, 1, 2, 4, 8 and 24 hours after the first dose of treatment (D1) and at the aforementioned time points with the addition of a 9 hour time point (i.e., 1 hour after the second daily dose) on Day 24 (D24) of treatment. Mice were anesthetized with chloroform and exsanguinated by cardiac puncture. Whole blood was collected on ice and then centrifuged to obtain serum. Acetonitrile was added to the samples before storage in gasketed screwcap tubes at −20° C. D24 samples were collected and processed in the same manner and stored at −20° C. before being sent together with D1 samples to Pfizer (Groton, Conn.) for determination of drug concentrations. The supernatant was injected (10 μL injection volume) onto the LC (Shimadzu SCL-10A, Kyoto, Japan)-MS/MS (Sciex API 3000, Applied Biosystems Group, Foster City, Calif.) using a Hypersil C18 column (5 μm, 50×2.1 mm, Thermo Electron Corp, Waltham, Mass.) and a step gradient consisting of a mobile phase of A: water with 0.05% 5 mM ammonium formate and B: acetonitrile/water/5 mM ammonium formate (80:20:0.05%). The ionization was electrospray positive ion mode. Bioanalytical data were captured using Analyst (Version 1.4.1, Applied Biosystems Group, Foster City, Calif.). Pharmacokinetic calculations were based on mean serum concentrations and performed using the non-compartmental approach (linear trapezoidal rule for AUC calculation with the aid of Watson 7.2 Bioanalytical LIMS [Thermo Electron Corp, Waltham, Mass.]). In the AUC calculations for D24, the plasma level of analytes at time zero was set to the 24-hour concentration. Concentrations of 0 were used for kinetic calculations for all results below the lower limit of quantitation (5 ng/mL).

[0268] Selected pharmacokinetic parameter values for the compound of formula (I) and LZD are presented in Table 2. The 130 mg/kg daily dose of LZD produced a steady state AUC of 379 μg-h/ml, approximately 50% higher than the anticipated steady state AUC observed in humans administered 600-625 mg by mouth twice daily, 215-294 μg-h/ml. (See Gee, T. et a; Antimicrob Agents Chemother 45:1843-1846, 2001; Stalker D J et al, J Antimicrob Chemother

51:1239-46, 2003.) Based on linear kinetics in this dose range, the 100 mg/kg dose of LZD in the mouse represents the upper end of the steady-state AUC range in humans, albeit with a C_{max} that is approximately 3 times higher than that obtained in humans. As expected there was significant first-pass metabolism of the compound of formula (I) to the major sulfoxide metabolite and the minor sulfone metabolite (Barbachyn et al. J Med Chem. 39:680-685, 1996). Because each of these metabolites has an MIC_{90} of 0.5 μg/mL against *M. tuberculosis*, close to the MIC_{90} of 0.25 μg/mL for the compound of formula (I), the concentration of each metabolite was added to that of the parent for the pharmacokinetic analyses. The steady-state AUC for the compound of formula (I) and its metabolites observed with the 100 mg/kg daily dose was approximately 3-fold lower than that observed with LZD at 130 mg/kg. The fact that the 25 mg/kg dose of the compound of formula (I) was as active as this dose of LZD suggests that the compound of formula (I) is 12 times more potent in vivo than LZD despite the similar MICs for both compounds.

TABLE 2

Regimen	C_{max} (μg/mL)	AUC_{0-24} (μg·h/mL)
Compound of formula (I) 100 mg/kg		
Day 1	6.07	7.33
Day 24	4.32	8.74
Sulfoxide metabolite		
Day 1	20.0	37.8
Day 24	16.9	98.8
Sulfone metabolite		
Day 1	0.79	2.58
Day 24	0.66	9.73
Compound of formula (I) + metabolites		
Day 1	24.1	47.8
Day 24	21.7	117
LZD 130 mg/kg		
Day 1	64.9	164
Day 24	58.4	379

Example 6

In Vivo Activity of the Combination

[0269] Mice were aerosol infected with 3.89±0.19 $\log_{10}$ CFU. Beginning 14 days after aerosol infection, when the mean lung CFU count was 7.37±0.05 $\log_{10}$, control mice received one of the following treatments: INH (25 mg/kg) alone, RIF (10 mg/kg) alone, RIF+PZA (150 mg/kg), RIF+INH+PZA, MXF (100 mg/kg)+PZA or RIF+MXF+PZA. Test mice received the compound of formula (I) (100 mg/kg) alone or added to each of the control regimens. Additional mice went untreated to serve as negative controls. Mice were sacrificed after 4 and 8 weeks of treatment for assessment of spleen weights and lung CFU counts. Untreated control mice died during the second month of the experiment.

[0270] The synergistic effect on lung CFU counts of adding the compound of formula (I) to a variety of 1-, 2- and 3-drug regimens is evident in Table 3.

TABLE 3

	Lung $\log_{10}$ CFU counts (±SD)				
	Before treatment	After treatment with the indicated regimen		After treatment with the indicated regimen plus the compound of formula (I) 100 mg/kg/day	
Regimen	Day 0	Day 28	Day 56	Day 28	Day 56
No treatment	7.37 ± 0.12	7.76 ± 0.09	n.d.	4.72 ± 0.17	2.70 ± 0.29
INH		5.79 ± 0.14	4.53 ± 0.24	4.33 ± 0.29	2.87 ± 0.23
RIF		5.74 ± 0.17	4.65 ± 0.24	3.73 ± 0.19	1.29 ± 0.22
RIF-PZA		3.97 ± 0.11	1.05 ± 0.44	3.04 ± 0.22	0.50 ± 0.33
RIF-INH-PZA		4.88 ± 0.09	2.47 ± 0.18	3.54 ± 0.24	0.47 ± 0.20
RIF-MXF-PZA		3.72 ± 0.20	0.61 ± 0.38	3.41 ± 0.18	0.12 ± 0.27
MXF-PZA		5.10 ± 0.13	3.17 ± 0.28	3.33 ± 0.33	0.93 ± 0.34

[0271] Monotherapy with the compound of formula (I) of the invention resulted in a 2.65 $\log_{10}$ reduction in CFU counts from baseline to 4.72 $\log_{10}$ over the first 28 days, whereas INH and RIF monotherapy reduced the lung CFU counts by 1.58 and 1.63 $\log_{10}$ to 5.79 and 5.74 $\log_{10}$, respectively. Remarkably, the compound of formula (I) of the invention continued to exert bactericidal activity during the second month of treatment, reducing the CFU count to 2.70 $\log_{10}$ CFU greater than the activity of INH or RIF alone and similar to the CFU count in mice treated with the standard first-line combination regimen of RIF-INH-PZA which reduced the CFU count to 2.47 $\log_{10}$. The combination of INH and the compound of formula (I) of the invention was no more active than the compound of formula (I) alone, while combining RIF with the compound of formula (I) had a multiplicative effect, resulting in a mean lung CFU count nearly 30 times lower than that observed with the compound of formula (I) alone. The combination of RIF-PZA had activity which reduced the mean lung CFU count to 1.05.

[0272] In this example, the addition of INH to RIF-PZA had a significant antagonistic effect, resulting in mean lung CFU counts of 1.05±0.44 and 2.47±0.18 after 2 months of treatment with RIF-PZA and RIF-INH-PZA, respectively.

This effect could falsely suggest that substituting a new drug for INH in the RIF-INH-PZA regimen has a significant beneficial effect, even if the new drug is completely inactive itself, simply because the antagonistic effect of INH has been removed. In this example, however, the compound of formula (I) of the invention had similarly strong effects whether it was added to RIF-INH-PZA or substituted for INH (i.e., as RIF-PZA-compound of formula (I). After 2 months of treatment, the mean lung CFU counts were 0.47±0.20 and 0.50±0.33 in mice treated with RIF-INH-PZA-compound of formula (I) and RIF-PZA-compound of formula (I), respectively, resulting in a mean lung CFU count approximately 3.5 times lower than the mean lung CFU count in mice receiving RIF-PZA alone ($p<0.05$), providing further evidence that the benefit of substituting the compound of formula (I) of the invention for INH does not come from the removal of the antagonistic influence of INH. The compound of formula (I) of the invention also improved the activity of the RIF-MXF-PZA regimen. Treatment with RIF-MXF-PZA for 2 months resulted in a mean lung CFU count of 0.61±0.38, with 1 of 5 mice culture-negative, while treatment with RIF-MXF-PZA- and the compound of formula (I) resulted in a mean CFU count of 0.12±0.27, with 4 out of 5 mice culture-negative (complete eradication) ($p=0.05$ for difference in mean CFU counts). The difference was of marginal statistical significance, likely due to the low CFU counts, but implies that the compound of formula (I) of the invention may further improve the regimen's sterilizing activity to prevent relapse. Finally, treatment with MXF-PZA-compound of formula (I) for 2 months resulted in a mean lung CFU count of 0.93±0.34, resulting in a mean lung CFU count nearly 175 times lower than that observed without the compound of formula (I) demonstrating that the compound of formula (I) of the invention is able to replace RIF in the treatment-shortening RIF-MXF-PZA regimen without diminishing the regimen's activity.

Example 7

In Vivo Activity of the Combination after Prolonged Administration and Follow-Up

[0273] Mice were aerosol infected with 4.45±0.05 $\log_{10}$ CFU. Beginning 14 days after aerosol infection, when the mean lung CFU count was 7.92±0.15 $\log_{10}$, control mice received RIF-INH-PZA for 8 weeks followed by RIF-INH for 8 weeks. One cohort of test mice received the same regimen to which the compound of formula (I) (160 mg/kg) was added for the entire 16 weeks duration, added for the first 8 weeks only, or added for the entire 16 weeks duration with the removal of INH after the first 8 weeks. Another cohort of test mice received the control regimen to which LZD was added for the entire 16 weeks duration or added for the first 8 weeks only. Additional mice went untreated to serve as negative controls. Mice were sacrificed after 8, 12 and 16 weeks of treatment for assessment of lung CFU counts. Additional mice were held without treatment for 12 weeks after completing 12 or 16 weeks of treatment. Untreated control mice died during the second month of the experiment.

The synergistic effect on lung CFU counts by adding the compound of formula (I) (Abbreviated as "U") to the control regimen during the first 8 weeks is evident in Table 4. The bactericidal effect of the control regimen was increased by 2 orders of magnitude. This effect is quantitatively and qualitatively different from the antagonistic effect of adding LZD.

TABLE 4

	Lung $\log_{10}$ CFU counts (±SD) after treatment for:		Proportion (%) of mice with culture-positive relapse after treatment for:	
Regimen	0 days	8 wks	12 wks	16 wks
Untreated	7.92 ± 0.15			
2 mo. RIF-INH-PZA + 2 mo. RIF-INH		3.17 ± 0.27		18 of 20 (90%)
2 mo. RIF-INH-PZA-U + 2 mo. RIF-INH-U		0.71 ± 0.26	9 of 20 (45%)	1 of 20 (5%)
2 mo. RIF-INH-PZA-U + 2 mo. RIF-U			7 of 20 (35%)	1 of 20 (5%)
2 mo. RIF-INH-PZA-U + 2 mo. RIF-INH			17 of 20 (85%)	7 of 20 (35%)
2 mo. RIF-INH-PZA-LZD + 2 mo. RIF-INH-LZD		4.28 ± 0.24		20 of 20 (100%)
2 mo. RIF-INH-PZA-LZD + 2 mo. RIF-INH				20 of 20 (100%)

[0274] Treatment with regimen including the compound of formula (I) (abbreviated as "U" in Table 4) was associated with a lower likelihood of relapse (the gold standard measure of complete eradication) after completion of 12 and 16 weeks of treatment compared to mice receiving other regimens. After 12 weeks of treatment with the control regimen all mice remained culture-positive and would be expected to exhibit a 100% relapse rate. This is further supported by a 90% relapse rate after 16 weeks of treatment.

[0275] In contrast, groups receiving a regimen including the compound of formula (I) for the entire 16 weeks had an average relapse rate of 40% after 12 weeks and 5% after 16 weeks. When the compound formula (I) was added to the control regimen for only the first 8 weeks, 85% and 35% of mice relapsed after total treatment durations of 12 and 16 weeks, respectively. On the other hand, addition of LZD was antagonistic and prevented complete eradication in any mouse. Overall, these data demonstrate that the compound of formula (I) has synergistic effect when combined with the standard first-line regimen and is capable of shortening the duration of treatment by 1-to-2 months without sacrificing efficacy.

[0276] Hence, the compound of formula (I) has synergistic effect when combined with at least two agents useful for the treatment of tuberculosis. Most importantly, combining the compound of formula (I) with at least two agents for the treatment of tuberculosis dramatically increases bactericidal activity, suggesting that it may shorten the duration of chemotherapy for drug-susceptible TB as well as MDR-TB.

Example 8

Example 8A

Four Week Dose Ranging Study in Combination with Standard First Line Agents, Rifampin, Isoniazid, and Pyrazinamide

[0277] Example 8 describes the results of a study in which mice were infected with *Mycobacterium tuberculosis* similar to the manner described in Example 6 above. Other than the controls, all of the mice were treated with the following three drugs:

[0278] 1) rifampin, 10 mg/kg(R),

[0279] 2) isoniazid, 25 mg/kg(H), and

[0280] 3) pyrazinamide, 150 mg/kg(Z).

[0281] The mice were divided into eight groups (8) and six of the groups received varying doses of the compound of Formula I("U"). Doses ranged from 12.5 mg/kg to 160 mg/kg and are listed in Table 5 below.

[0282] The study was carried out in the following manner. Six-week-old female BALB/c mice were infected by the aerosol route with *Mycobacterium tuberculosis* H37Rv. On that day (D-17), 5 mice were sacrificed to determine the number of CFU implanted in the lungs. Seventeen days later (D0), 5 additional mice were sacrificed to determine the baseline CFU count in the lungs. The remaining mice were randomized to treatment groups, as indicated in Table 5, and started on the specified drug regimen. Treatment was administered once daily, 5 days per week, except for group 8, which received thrice weekly treatment (3/7) throughout. At the conclusion of the 4 week test period, all of the animals were sacrificed for lung CFU counts, except for 80 animals from the Group 4, below. These animals were utilized in an 8 week protocol which is described below in Example 8B.

TABLE 5

Regimen	# Mice	CFU Count Day (−) 17	CFU Count Day 0	CFU Count 4 weeks
Controls				
1) Untreated	15	3.50 ± 0.23	7.71 ± 0.06	dead
2) RHZ	5			4.71 ± 0.17
Test Regimen				
3) $RHZU_{12.5}$	5			4.51 ± 0.16
4) $RHZU_{25}$	85			4.19 ± 0.26
5) $RHZU_{50}$	5			4.32 ± 0.26
6) $RHZU_{100}$	5			4.26 ± 0.25
7) $RHZU_{160}$	5			4.01 ± 0.21
6) $RHZU_{50}$(3/7)	5			4.88 ± 0.33

[0283] As depicted above, the compound formula I demonstrated a dose dependent effect on CFU numbers. The addition of this compound to the standard regimen of RHZ produced further reduction in the number of TB colonies contained within the lung.

Example 8B

Eight Week Dose Ranging Study

[0284] Eighty of the mice from Group 4 (in Example 8A) that had been receiving 25 mg/kg of the compound of formula I (U) along with rifampin(R), isoniazid(H), and pyrazinamide (Z) were evaluated in the second phase of the study. They were assigned to one of the groups described below in Table 6 and the study was continued for an additional 4 weeks (i.e. 8 week results).

TABLE 6

Drug Regimen	# of Mice
1) No treatment	5
2) RH	5
3) R	5
4) H	5
U given with or without R:	
5) U_{160} +/− R	5
6) U_{100} +/− R	5
7) U_{50} +/− R	5
8) U_{25} +/− R	5
9) $U_{12.5}$ +/− R	5
10) U_{50}(3/7) +/− R	5

[0285] As described above, 5 animals served as the control and no further drugs were given. Five were given the combination of rifampin(R) and isoniazid (H), five given isoniazid (H) or rifampin(R) alone. Of the twelve (12) remaining groups, all were given with the compound of formula (I) in doses ranging from 12.5 mg/kg to 160 mg/kg. Six of these groups were also dosed with rifampin (R). The rifampin and isoniazid was dosed in the same manner and in the same amount as in Example 8A.

[0286] At the conclusion of the eight (8) week experiment, all of the animals were sacrificed and CFU lung counts were obtained. The following results were obtained:

TABLE 7

Treatment	Lung CFU
1) Untreated	5.49 ± 0.23
2) Isoniazid(H)	4.46 ± 0.37
3) Rifampin(R)	4.18 ± 0.55
4) R and H	3.84 ± 0.46

[0287] At the initiation of this second phase, the eighty animals had a mean CFU of 4.19+0.26 (based upon the other mice from Group 4 that were sacrificed). Table 7 shows the results obtained with control group, the rifampin(R) only, isoniazid(H) only and the combination of both isoniazid(H) and rifampin(R).

Table 8 shows the results obtained with the mice receiving the compound of formula (I)(U) alone or in combination with rifampin(R).

TABLE 8

Dose of U	CFU-U only	CFU-U and R
$U_{12.5}$	4.99 ± 0.19	3.42 ± 0.40
U_{25}	4.26 ± 0.24	2.83 ± 0.24
U_{50}	3.56 ± 0.15	2.49 ± 0.31
U_{100}	2.88 ± 0.34	1.54 ± 0.45
U_{160}	2.42 ± 0.31	1.26 ± 0.53
U_{50}(3/7)	4.21 ± 0.26	

[0288] The compound of Formula I (U) demonstrated dose-dependent bactericidal activity against persisting tubercle bacilli that remained viable after the initial 4 weeks of treatment with $RHZU_{25}$. The following effects were observed with U monotherapy: a growth-inhibitory effect at 12.5 mg/kg, a bacteriostatic effect at 25 mg/kg, a >0.5 log reduction at 50 mg/kg, a >1 log reduction at 100 mg/kg, and a nearly 2 log reduction at 160 mg/kg. The combination of rifampin(R) and the compound of formula (I) U was synergistic and proved to have strong bactericidal activity against persisters. When the compound of formula (I) U was admin-

istered at doses 25 mg/kg, the combination of it and rifampin R was more effective than the standard regimen of rifampin (R) and isoniazid (H). Results with U alone support the conclusion that U-containing regimens may be capable of significantly shortening the duration of treatment for both multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis.

Example 8C

Additional Dose Ranging Study

[0289] The protocol described above in Example 8A and 8B was carried out on an alternative occasion. The only substantive changes were the number of animals per group and the initial incubation period. Table 9 provides the results obtained

TABLE 9

Regimen	# Mice	CFU Count Day (-) 12	CFU Count Day 0	CFU Count 4 weeks
Controls				
1) Untreated	12	3.26 ± 0.14	6.28 ± 0.08	7.23 ± 0.15
2) RHZ	4			4.58 ± 0.15
Test Regimen				
1) $RHZU_{160}$	4			4.16 ± 0.28
2) $RHZU_{100}$	64			3.93 ± 0.21
3) $RHZU_{50}$	4			4.30 ± 0.27
4) $RHZU_{25}$	4			4.39 ± 0.23
5) $RHZU_{12.5}$	4			4.30 ± 0.25
6) $RHZU_{6.25}$	4			4.39

[0290] Testing continued for an additional 4 weeks with 60 of the mice from Test Group 2 immediately above. The mice either received U alone, at varying doses, or the combination of U and R. The following results were obtained.

TABLE 10

Regimen	# Mice	CFU Count 8 weeks
Controls		
1) Untreated	4	4.91 ± 0.17
2) R	4	4.16 ± 0.65
3) RH	4	3.00 ± 0.16
Test Regimen		
1) U_{160}	4	2.90 ± 0.32
2) RU_{160}	64	2.16 ± 0.20
3) U_{100}	4	3.86 ± 0.53
4) RU_{100}	4	2.35 ± 0.21
5) U_{50}	4	4.59 ± 0.28
6) RU_{50}	4	3.17 ± 0.76
7) U_{25}	4	5.27 ± 0.20
8) RU_{25}	4	4.27 ± 0.33
9) $U_{12.5}$	4	5.30 ± 0.08
10) $RU_{12.5}$	4	3.62 ± 0.40
11) $U_{6.25}$	4	4.93 ± 0.32
12) $RU_{6.25}$	4	4.38 ± 0.16

[0291] As depicted above, the compound formula I demonstrated a dose dependent effect on CFU numbers. The addition of this compound to the standard regimen of rifampin(R) and isoniazid (H) and rifampin (R) alone produced further reduction in the number of TB colonies contained within the lung at both 4 and 8 weeks.

Example 9

[0292] In Example 9 the impact of the compound of formula I on existing first and second line anti-tuberculosis drugs was evaluated. Two doses of the compound were evaluated, 25 mg/kg and 100 mg/kg. The following standard anti-tuberculosis agents were evaluated, at the specified doses:

[0293] 1) isoniazid, 10 mg/kg (H)

[0294] 2) rifampin, 10 mg/kg (R)

[0295] 3) pyrazinamide, 150 mg/kg, (Z)

[0296] 4) ethambutol 100 mg/kg (Eb)

[0297] 5) moxifloxacin 100 mg (M)

[0298] 6) ethionamide 50 mg/kg (Et)

[0299] 7) amikacin 150 mg/kg subcutaneously, (A)

[0300] 8) cycloserine 250 mg/kg twice daily (Cs)

[0301] 9) para-aminosalicyclic acid 750 mg/kg (Ps)

[0302] 10) capreomycin 125 mg/kg subcutaneously (Cap)

[0303] 11) clofazimine 20 mg/kg (C)

The study was carried out in the following manner:

Methods

[0304] Mice: female BALB/c, 6 weeks old

[0305] Infection: aerosol infection with ~10^4 CFU *M. tuberculosis* H37Rv followed by randomization to treatment group

[0306] Treatment: initiated on day 14 after infection (D0) when the CFU counts in the mouse lungs was ~10^8 CFU.

[0307] All drugs were given once daily (except cycloserine—twice daily) by the oral route (except amikacin and capreomycin—subcutaneous injection), 5 days per week.

[0308] Mouse sacrifices for CFU counts in lungs: According to the experimental scheme, 4 mice were sacrificed the day after infection (D-13) and 2 weeks later (D0) to assess the number of implanted CFU and the baseline CFU counts on treatment initiation, respectively. The activity of each regimen was be assessed by lung CFU counts after 4 weeks of treatment.

Results

[0309] Mice were infected with ~4.5 $\log_{10}$ CFU. Fourteen days later the lung CFU count at the initiation of treatment on day zero was approximately 8.3 $\log_{10}$. Untreated mice died within the first 3 weeks of infection (Table 11). The survival of mice treated with pyrazinamide (Z), cycloserine (Cs), and capreomycin (Cap) was not significantly different from untreated controls. Treatment with para-aminosalicylic acid (Ps) delayed but did not prevent mortality. Treatment with ethambutol (Eb) and clofazimine (C) prevented some, but not all, mortality. Treatment with isoniazid (H), rifampin (R), moxifloxacin (M), amikacin (A) and ethionamide (Et) prevented death, as did treatment with the compound of formula I (U) at either dose, whether alone or in combination with other drugs.

TABLE 11

Treatment group	Proportion (%) surviving to Day 42 post-infection	Time to 50% mortality (days)
Any U-containing regimen	4/4 (100%)	N/A
H, R, M, A or Et	4/4 (100%)	N/A
C	3/4 (75%)	N/A
Eb	2/4 (50%)	30
Ps	0/4 (0%)	31

TABLE 11-continued

Treatment group	Proportion (%) surviving to Day 42 post-infection	Time to 50% mortality (days)
Cap	0/4 (0%)	22
Cs	0/4 (0%)	22
Z	0/4 (0%)	21
No treatment	0/4 (0%)	21

[0310] In addition to measuring survival, lung CFU counts were also determined as described above and are reported below in Table 12

TABLE 12

	Mean lung log_{10} CFU count (±) S.D.			
	at treatment	after 4 weeks of treatment with:		
Regimen	initiation (D0)	Regimen alone	Regimen + U 25 mg/kg	Regimen + U 100 mg/kg
1) Untreated	8.36 ± 0.10	N/A	7.17 ± 0.23	5.40 ± 0.10
2) H		7.01 ± 0.14	6.22 ± 0.06	5.79 ± 0.33
3) R		6.23 ± 0.11	5.91 ± 0.21	5.02 ± 0.10
4) Z		N/A	4.73 ± 0.38	≦3.59***
5) Eb		7.41 ± 0.57*	6.86 ± 0.16	5.50 ± 0.13
6) M		6.49 ± 0.12	7.08 ± 0.10	5.33 ± 0.30
7) Et		7.19 ± 0.04	7.18 ± 0.18	5.46 ± 0.15
8) A		7.24 ± 0.14	6.78 ± 0.16	5.18 ± 0.17
9) Cs		N/A	7.19 ± 0.21	5.66 ± 0.11
10) Ps		N/A	6.87 ± 0.21**	5.39 ± 0.32
11) Cap		N/A	6.81 ± 0.13	5.35 ± 0.17
12) C		5.92 ± 0.24**	6.25 ± 0.20	4.91 ± 0.14

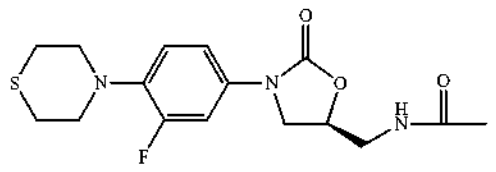
N/A, not available due to death in 4 of 4 mice before W4;
*2 of 4 mice survived;
**3 of 4 mice survived;
***the CFU counts was below the lower limit of detection of 3.40 for 2 of 4 mice

[0311] A review of the data shows that the addition of the compound of Formula I (U), significantly reduced bacterial load in a dose dependent manner when added to standard anti-tubercular drugs. These results suggest that the compound of formula I could be used to improve the efficacy of treatment regimens for drug resistant tuberculosis (either multidrug-resistant or extensively drug-resistant).

[0312] All patents, applications, publications, test methods, literature, and other materials cited herein are hereby incorporated herein by reference in their entireties.

1. Use of a compound of the formula or a pharmaceutically acceptable salt thereof:

(I)



in combination with at least two anti-tuberculin agents in the manufacture of a medicament for the treatment of tuberculosis.

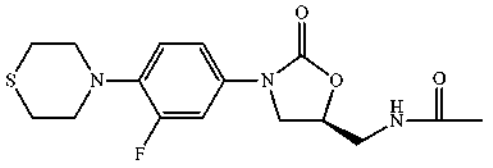
2. The use of claim **1**, wherein said at least two agents is selected from the group consisting of isoniazid, rifampin, rifapentine, rifabutin, pyrazinamide, ethambutol, streptomycin, kanamycin, amikacin, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, ciprofloxacin capreomycin, ethionamide, cycloserine, para-aminosalicylic acid, thiacetazone, clarithromycin, amoxicillin-clavulanic acid, imipenem, meropenem, viomycin, terizidone, TMC207, PA-824, OPC-7683, LL-3858 and SQ-109.

3. The use of claim **1**, wherein one of said at least two agents is selected from the group consisting of isoniazid, rifampin, rifapentine, rifabutin, pyrazinamide, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, ciprofloxacin, and ethambutol.

4. The use of claim **1**, wherein one of said at least two agents are selected from the group consisting of pyrazinamide, rifampin, rifapentine and isoniazid.

5. Use of a of a compound of formula (I) or a pharmaceutically acceptable salt thereof:

(I)



in combination with at least one anti-tuberculin agent in the manufacture of a medicament for treating tuberculosis after a subject has undergone an initial phase of treatment, for tuberculosis.

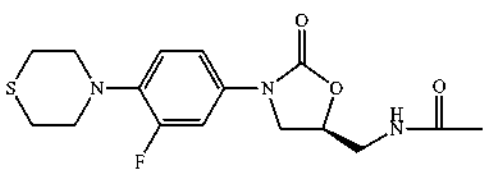
6. The medicament of claim **5**, wherein said at least one agent is selected from the group consisting of rifampin, rifapentine, rifabutin, pyrazinamide, ethambutol, streptomycin, kanamycin, amikacin, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, ciprofloxacin, capreomycin, ethionamide, cycloserine, para-aminosalicylic acid, thiacetazone, clarithromycin, amoxicillin-clavulanic acid, imipenem, meropenem, viomycin, terizidone, TMC207, PA-824, OPC-7683, LL-3858 and SQ-109.

7. The method of claim **5** or **6** wherein said active tuberculosis is selected from the group consisting of drug-sensitive tuberculosis, mono-drug resistant tuberculosis, multi-drug-resistant tuberculosis (MDR) and extensively drug-resistant tuberculosis (XDR).

8. A pharmaceutical composition comprising:

i) a therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof:

(I)



(ii) a therapeutically effective amount of at least one agent useful in the treatment of tuberculosis and,

(iii) one or more pharmaceutically acceptable carriers or vehicles.

9. The pharmaceutical composition of claim **8**, wherein said at least one agent is selected from the group consisting of isoniazid, rifampin, rifapentine, rifabutin, pyrazinamide, ethambutol, streptomycin, kanamycin, amikacin, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, ciprofloxacin, capreomycin, ethionamide, cycloserine, para-aminosalicylic acid, thiacetazone, clarithromycin, amoxicillin-clavulanic acid, imipenem, meropenem, viomycin, terizidone, TMC207, PA-824, OPC-7683, LL-3858 and SQ-109.

10. The pharmaceutical composition of claim **8** or **9**, wherein said at least one agent is selected from the group consisting of isoniazid, rifampin, rifapentine, rifabutin, pyrazinamide, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, and ethambutol.

* * * * *

ORIGINAL ARTICLE

ANNEXURE - 5

Linezolid for Treatment of Chronic Extensively Drug-Resistant Tuberculosis

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ABSTRACT

BACKGROUND

Linezolid has antimycobacterial activity in vitro and is increasingly used for patients with highly drug-resistant tuberculosis.

METHODS

We enrolled 41 patients who had sputum-culture–positive extensively drug-resistant (XDR) tuberculosis and who had not had a response to any available chemotherapeutic option during the previous 6 months. Patients were randomly assigned to linezolid therapy that started immediately or after 2 months, at a dose of 600 mg per day, without a change in their background regimen. The primary end point was the time to sputum-culture conversion on solid medium, with data censored 4 months after study entry. After confirmed sputum-smear conversion or 4 months (whichever came first), patients underwent a second randomization to continued linezolid therapy at a dose of 600 mg per day or 300 mg per day for at least an additional 18 months, with careful toxicity monitoring.

RESULTS

By 4 months, 15 of the 19 patients (79%) in the immediate-start group and 7 of the 20 (35%) in the delayed-start group had culture conversion ($P=0.001$). Most patients (34 of 39 [87%]) had a negative sputum culture within 6 months after linezolid had been added to their drug regimen. Of the 38 patients with exposure to linezolid, 31 (82%) had clinically significant adverse events that were possibly or probably related to linezolid, including 3 patients who discontinued therapy. Patients who received 300 mg per day after the second randomization had fewer adverse events than those who continued taking 600 mg per day. Thirteen patients completed therapy and have not had a relapse. Four cases of acquired resistance to linezolid have been observed.

CONCLUSIONS

Linezolid is effective at achieving culture conversion among patients with treatment-refractory XDR pulmonary tuberculosis, but patients must be monitored carefully for adverse events. (Funded by the National Institute of Allergy and Infectious Diseases and the Ministry of Health and Welfare, South Korea; ClinicalTrials.gov number, NCT00727844.)

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Linezolid (Zyvox, Pfizer) was approved in 2000 for drug-resistant, gram-positive bacterial infections.[1] A member of the oxazolidinone antibiotic class, linezolid inhibits protein synthesis by binding the 23S ribosomal RNA (rRNA) portion of the bacterial 50S ribosomal subunit.[2] In adults, linezolid is administered at a dose of 600 mg twice daily, with phase 3 and postmarketing trials showing an acceptable side-effect and adverse-event profile during the FDA-approved 28 days of therapy.[3] Data on longer-term use are limited, but serious neuropathies (e.g., peripheral and optic neuropathies), myelosuppression, and hyperlactatemia have been observed[4,5] and are considered to be related to the inhibition of mitochondrial protein synthesis.[6,7]

Linezolid exhibits in vitro bacteriostatic activity against *Mycobacterium tuberculosis*, including multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, with a minimum inhibitory concentration of less than 1 μg per milliliter.[8-11] It has only modest activity in murine models of tuberculosis.[12,13] A study of the early and extended bactericidal activity of linezolid showed only minimal early activity (during the initial 2 days) and no late activity (during the subsequent 5 days), with the authors concluding that linezolid had little tissue-sterilizing ability and therefore had a limited role in the treatment of MDR tuberculosis.[14] Despite these characteristics, a number of case reports and retrospective studies suggest that linezolid may be effective in treating MDR and XDR tuberculosis.[15-21] These studies all have important limitations, including a retrospective design, small numbers, the use of linezolid with multiple other active agents, no controls, and limited follow-up. This apparent discrepancy between preclinical data and clinical observations prompted us to undertake a prospective, randomized trial of linezolid in patients with chronic XDR tuberculosis who did not have a response to all other available chemotherapeutic options.

METHODS

STUDY PATIENTS

From December 2008 through May 2011, we enrolled adults, 20 years of age or older, with chronic XDR pulmonary tuberculosis (positive sputum smear and culture) and with confirmed genotypic or phenotypic resistance to isoniazid, rifampin, kanamycin, ofloxacin, and moxifloxacin or a documented nonresponse to treatment, despite test results showing drug susceptibility. Patients were eligible if they had been treated with an unchanged, failing regimen for 6 months or more before enrollment. Exclusion criteria were previous treatment with linezolid, anticipated surgical treatment, a positive test result for the human immunodeficiency virus (HIV), specific baseline laboratory abnormalities, moderate-to-severe peripheral or optic neuropathy, and need for treatment with contraindicated drugs. Additional details regarding the inclusion and exclusion criteria, regimen changes, dose adjustments, study timeline, and adverse events are provided in the Supplementary Appendix and the study protocol, both of which are available with the full text of this article at NEJM.org.

STUDY DESIGN AND OUTCOME MEASURES

This phase 2a, randomized, two-group study was conducted at the National Masan Hospital in Changwon and the National Medical Center in Seoul, South Korea. Patients were randomly assigned to receive linezolid, at a dose of 600 mg per day, in addition to their existing regimen, either immediately or after a 2-month delay. Permuted-block randomization was performed, with stratification according to status with regard to diabetes mellitus (types 1 and 2 included). A 2-month delay was used to minimize the possibility that study effects other than linezolid could account for observed improvement. All patients continued their existing regimen and were hospitalized from the time of enrollment until sputum-culture conversion. The microbiology staff were unaware of treatment assignments throughout the study.

The primary end point was sputum-culture conversion, with data censored at 4 months. Conversion was defined as negative sputum samples on solid (Löwenstein–Jensen) medium for 3 consecutive weeks; culture on liquid medium was performed with the use of the MB/BacT automated mycobacterial culture system. Patients continued taking linezolid at a dose of 600 mg per day until they had negative sputum smears (Ziehl–Neelsen staining) for 2 consecutive weeks or until they had received 4 months of linezolid treatment, whichever came first. Regimen changes, which were not allowed during the 6 months before enrollment, were allowed after sputum-smear conversion and at least 2 months of treatment with linezolid. After conversion to negative

sputum smears (or receipt of 4 months of therapy), patients underwent a second randomization, stratified according to diabetes mellitus status, either to continue receiving linezolid at a dose of 600 mg per day or to receive a lower dose, 300 mg per day, for an additional 18 months or until therapy was stopped owing to side effects or laboratory abnormalities.

If adverse events occurred that were considered to be related to linezolid, a reduction in the dose or a rechallenge at a dose of 300 mg per day was allowed after a limited drug holiday (described in Table 2 in the Supplementary Appendix). Linezolid was administered by means of directly observed therapy during hospitalization. The study staff monitored outpatient adherence by means of videophone or telephone every weekday and also performed pill counts monthly. Patients were treated for at least 18 months after sputum-culture conversion and were followed for an additional 12 months after completing the treatment. Blood was collected for pharmacokinetic analysis[22] of linezolid at both doses.

ADVERSE-EVENT MONITORING

Patients underwent baseline and serial safety evaluations (including complete blood counts, blood chemical measurements, and liver-function tests) weekly until 16 weeks, every 2 weeks from 17 through 24 weeks, and then monthly thereafter. A neurologist evaluated all patients at entry with the use of nerve-conduction studies and was available for repeat consultation if any peripheral neuropathy developed. The Subjective Peripheral Neuropathy Screen, a screening tool used in the treatment of HIV infection (Supplementary Material 2 in the Supplementary Appendix),[23] and clinical neurologic examinations were performed by the study staff at baseline and monthly thereafter. To monitor patients for linezolid-induced optic neuropathy, the study staff performed testing for visual acuity (using the Han test, which is similar to the Snellen chart), contrast sensitivity, and color vision (using Ishihara plates). Patients with any symptoms or abnormal findings were referred to an ophthalmologist.

STUDY OVERSIGHT

Written informed consent was obtained from all participants. The study was approved by the local institutional review boards and by the U.S. National Institute of Allergy and Infectious Diseases and was conducted in accordance with Good Clinical Practice guidelines. A data and safety monitoring board reviewed adverse events and provided management guidance to investigators. All serious adverse events were reported to the data and safety monitoring board, all institutional review boards, and the U.S. and Korean Food and Drug Administrations, per protocol. The study was monitored by an independent clinical research organization. Pfizer provided linezolid at no cost and reviewed the protocol without comment. The authors are fully responsible for the study design, data collection, analysis, completeness of data reporting, fidelity of this report to the study protocol, and interpretation of the data.

STATISTICAL ANALYSIS

From prior case studies, we estimated a culture-conversion rate of more than 90% with linezolid during the first 4 months of therapy, and on the basis of historical data, we assumed that less than 10% of patients would have spontaneous culture conversion without having received linezolid. Thus, we calculated that a sample of 16 patients per group would provide 92% power, assuming a two-sided type 1 error rate of 0.05 and a 10% discontinuation rate. We planned to recruit 20 patients per group to allow for additional loss to follow-up and death. For the primary analysis of time to culture conversion on solid medium, we used a generalized Wilcoxon test[24] and a modified intention-to-treat analysis, which excluded 2 patients who withdrew (owing to baseline neuropathy) before receiving any linezolid. Data from other patients who withdrew were included in the modified intention-to-treat analysis as treatment failures. Ethical concerns regarding the delayed-start group prompted the data and safety monitoring board to request two unplanned interim analyses: one when 16 patients had an end point that could be evaluated and one when 32 had such an end point. For the interim analysis of the primary end point, the Haybittle–Peto rule was used, which specifies a strict significance level of 0.001 as the criterion for stopping the study early because of efficacy and allows for the usual 0.05 level of significance at the final analysis.[25] The boundary for early stopping was not met, and follow-up of the 39 patients in the modified intention-to-treat cohort continued until the planned stopping point.

RESULTS

STUDY PATIENTS

A total of 41 patients underwent randomization, with 21 assigned to the immediate-start group and 20 to the delayed-start group (Fig. 1). Owing to preexisting neuropathy, 2 patients in the immediate-start group were withdrawn from the study before receiving any dose of linezolid and were excluded from the modified intention-to-treat analysis. The remaining 39 patients were predominantly men (72%), with a mean age of 41.2 years (range, 20 to 64), and 36% of the patients had diabetes mellitus (Table 1). On the basis of radiologic testing, 77% of the patients were classified as having "far advanced" tuberculosis, which was defined according to the guidelines of the Korea Centers for Disease Control and Prevention[26] as the presence of disseminated lesions of slight-to-moderate density exceeding the total volume of one lung, or dense and confluent lesions exceeding one third the volume of one lung, or the presence of cavities greater than 4 cm in diameter. Patients had a median of 5 previous treatment episodes for pulmonary tuberculosis (interquartile range, 3 to 8), and their isolates were resistant to a mean of 11 drugs (range, 6 to 15). The baseline characteristics reported in Table 1 were well balanced between the two groups.

PRIMARY OUTCOME

The primary outcome was the time to sputum-culture conversion, with data censored at 4 months. By 4 months, 15 of the 19 patients (79%) in the immediate-start group and 7 of the 20 (35%) in the delayed-start group had conversion to negative sputum cultures on solid medium (P=0.001) (Fig. 2A). Although culture conversion on solid medium is historically the standard used to gauge the effectiveness of a treatment, we also monitored culture results on liquid medium. With data censored at 4 months, 12 of the 19 patients (63%) in the immediate-start group and 11 of the 20 (55%) in the delayed-start group had culture conversion on liquid medium (P=0.07) (Fig. 2B). Liquid culture medium is thought to have higher sensitivity and more reproducible results than solid-culture medium.[27] In our study, however, the culture results on liquid medium had only borderline significance at the per-protocol censored time point (4 months), owing to the higher-than-expected rate of conversion to negative cultures on liquid medium in the delayed-start group.

Combining the two groups, we observed that 34 of the 38 patients who received linezolid (89%) had culture conversion on solid medium by 6 months (Fig. 2C), at a median of 75 days after the start of treatment with linezolid. One patient withdrew before receiving the study drug, owing to a diagnosis of metastatic colon cancer; data from this person were included in the modified intention-to-treat analysis as a treatment failure. As of May 1, 2012, of the 38 patients who received linezolid, 17 were still receiving the treatment per protocol, and 13 had completed treatment, including 6 with no relapse during the treatment period, 4 with no relapse at the 6-month follow-up, and 3 with no relapse at the 12-month follow-up (end of study). Eight patients withdrew early: 4 patients owing to treatment failure, 1 for personal reasons, and 3 owing to adverse events (Fig. 4 in the Supplementary Appendix).

SAFETY

Of the 38 patients who received linezolid, 33 (87%) had clinically significant adverse events; 31 patients (82%) had events that were possibly or probably related to linezolid (Table 2 in the Supplementary Appendix). Most adverse events resolved relatively quickly, and only 3 patients permanently discontinued linezolid owing to drug toxicity (2 patients because of optic neuropathy, and 1 because of anemia). We observed seven episodes of myelosuppression, including anemia and neutropenia, which occurred primarily within the first 5 months (Fig. 3A, and Table 3 in the Supplementary Appendix). In addition, we observed 7 cases of optic neuropathy, 21 cases of peripheral neuropathy, and 1 case of rhabdomyolysis,[28] with these events occurring during the first year of treatment.

Of the 38 patients who received linezolid, 33 (87%) underwent the scheduled second randomization (1 patient had an adverse event requiring withdrawal from the study and 4 had an adverse event requiring dose reduction before undergoing this randomization) (Fig. 1). In the second randomization, 17 patients were assigned to continue receiving the 600-mg daily dose, and 16 to receive 300 mg per day. Of the 17 patients who continued taking 600 mg per day, 15 (88%) had an adverse event related to the study drug, with

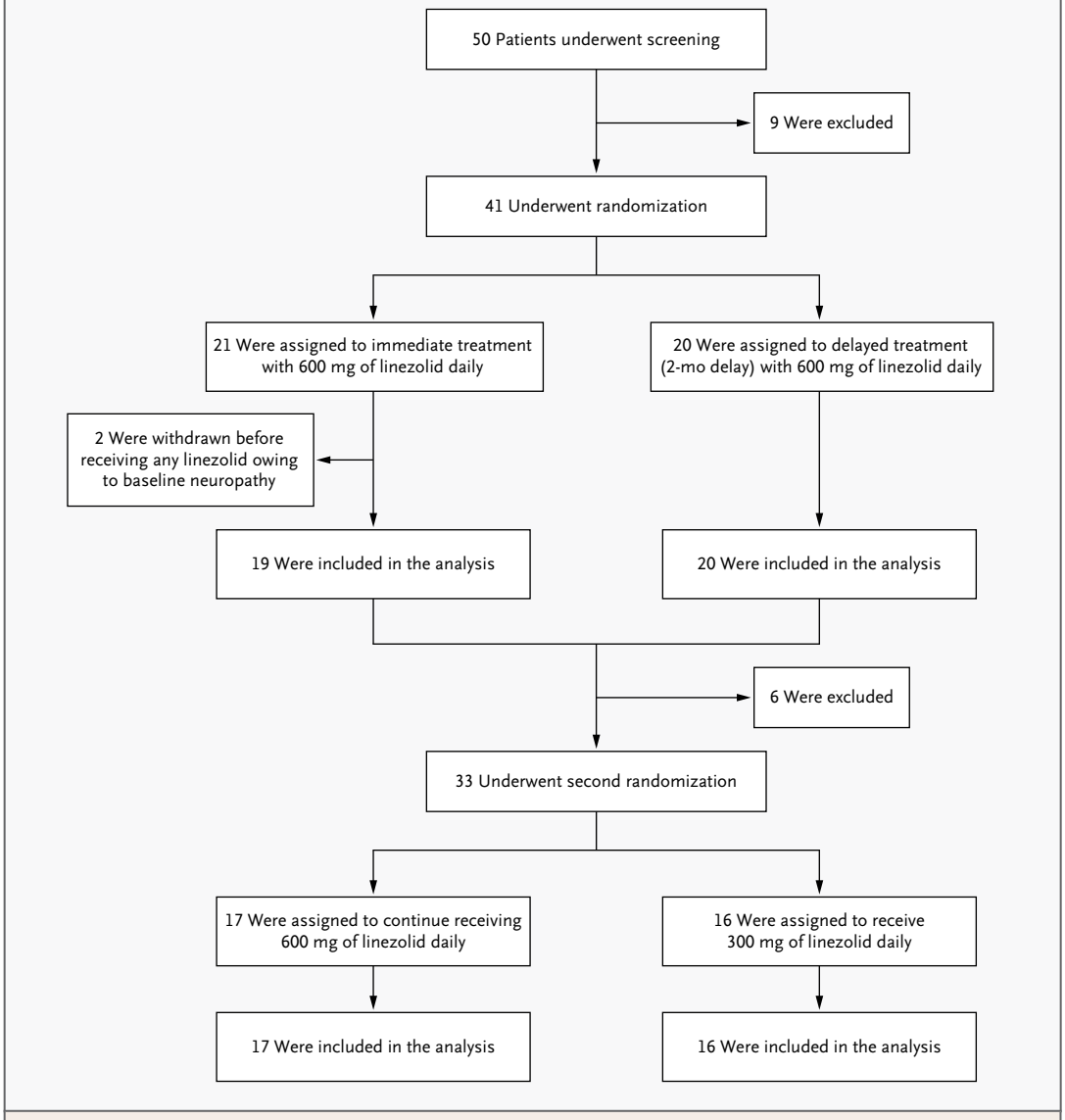

Figure 1. Enrollment, Randomization, and Follow-Up of the Study Patients.

Between December 2008 and May 2011, a total of 50 patients were screened for eligibility and 41 underwent randomization. Two patients were subsequently withdrawn owing to preexisting peripheral neuropathy that was discovered during the baseline examination; the remaining 39 patients were included in the modified intention-to-treat analysis. Two other patients who withdrew before culture conversion were considered to have treatment failure: 1 patient, who had an adverse event requiring a drug holiday, was withdrawn 79 days after starting treatment with linezolid because the drug holiday exceeded the protocol-specified window (28 days before sputum-culture conversion and 42 days after sputum-culture conversion); the other patient was withdrawn 32 days after study entry because of a diagnosis of advanced colon cancer (this patient was in the delayed-start group and had not received any linezolid). Thirty-three patients underwent the second randomization; 17 patients were randomly assigned to continue receiving linezolid at a dose of 600 mg per day, and 16 to receive the reduced dose of 300 mg per day. The 6 patients who did not undergo the second randomization included 4 who had dose reductions due to adverse events before culture conversion and the 2 withdrawn patients mentioned above who were included in the modified intention-to-treat analysis as having treatment failure.

Table 1. Baseline Characteristics of the Study Participants According to Treatment Group.*

Characteristic	Immediate-Start Group (N=19)	Delayed-Start Group (N=20)	Total (N=39)
Age — yr			
Mean	42.1	40.4	41.2
Range	20–64	23–63	20–64
Male sex — no. (%)	12 (63)	16 (80)	28 (72)
Body-mass index†			
Mean	19.6	20.5	20.0
Range	14.9–25.7	14.4–28.1	14.4–28.1
Diabetes mellitus — no. (%)	7 (37)	7 (35)	14 (36)
BCG vaccination scar — no. (%)	14 (74)	17 (85)	31 (79)
Radiographic findings — no. (%)			
Far advanced tuberculosis‡	15 (79)	15 (75)	30 (77)
Cavitary tuberculosis	9 (47)	8 (40)	17 (44)
Bilateral lesions	18 (95)	20 (100)	38 (97)
No. of previous treatment episodes for tuberculosis			
Median	5.0	5.0	5.0
Interquartile range	3.0–8.5	4.0–7.0	3.0–7.3
No. of resistant drugs§			
Mean	11.6	10.4	11.0
Range	8–15	6–14	6–15

* There were no significant between-group differences. BCG denotes bacille Calmette–Guérin.
† The body-mass index is the weight in kilograms divided by the square of the height in meters.
‡ Far advanced tuberculosis was defined according to the guidelines of the Korea Centers for Disease Control and Prevention[26] as the presence of disseminated lesions of slight-to-moderate density exceeding the total volume of one lung, or dense and confluent lesions exceeding one third the volume of one lung, or the presence of cavities greater than 4 cm in diameter.
§ Drug-susceptibility testing for 15 drugs was performed: isoniazid, para-aminosalicylic acid, streptomycin, ethambutol, rifampin, protionamide, cycloserine, kanamycin, amikacin, ofloxacin, levofloxacin, pyrazinamide, rifabutin, moxifloxacin, and capreomycin.

11 subsequently taking the reduced dose of 300 mg per day; of the 16 patients who received 300 mg per day, 11 (69%) had an adverse event related to the study drug. A Cox proportional-hazards analysis showed that, after the second randomization, the group receiving the 600-mg dose was 2.7 times (95% confidence interval, 1.1 to 6.5) as likely to have an adverse event as the group receiving the 300-mg dose (P=0.03) (Fig. 3B, and Fig. 3 in the Supplementary Appendix).

DRUG RESISTANCE

Of the four patients who did not have a response to treatment, three (two in the 300-mg group, and one in the 600-mg group) did not have confirmed culture conversion; the sputum smears and cultures for these three patients improved initially, but they did not have a consistent negative-culture status and were classified as having treatment failure rather than treatment relapse. The fourth patient, who was in the 600-mg group, had a treatment relapse (cultures became negative but turned positive again after 1 year of treatment).

The minimum inhibitory concentration of the corresponding isolates for these four patients increased by a factor of 8 to a factor of 32, as compared with baseline (Fig. 4A). DNA sequencing of these isolates revealed mutations, either in 23S

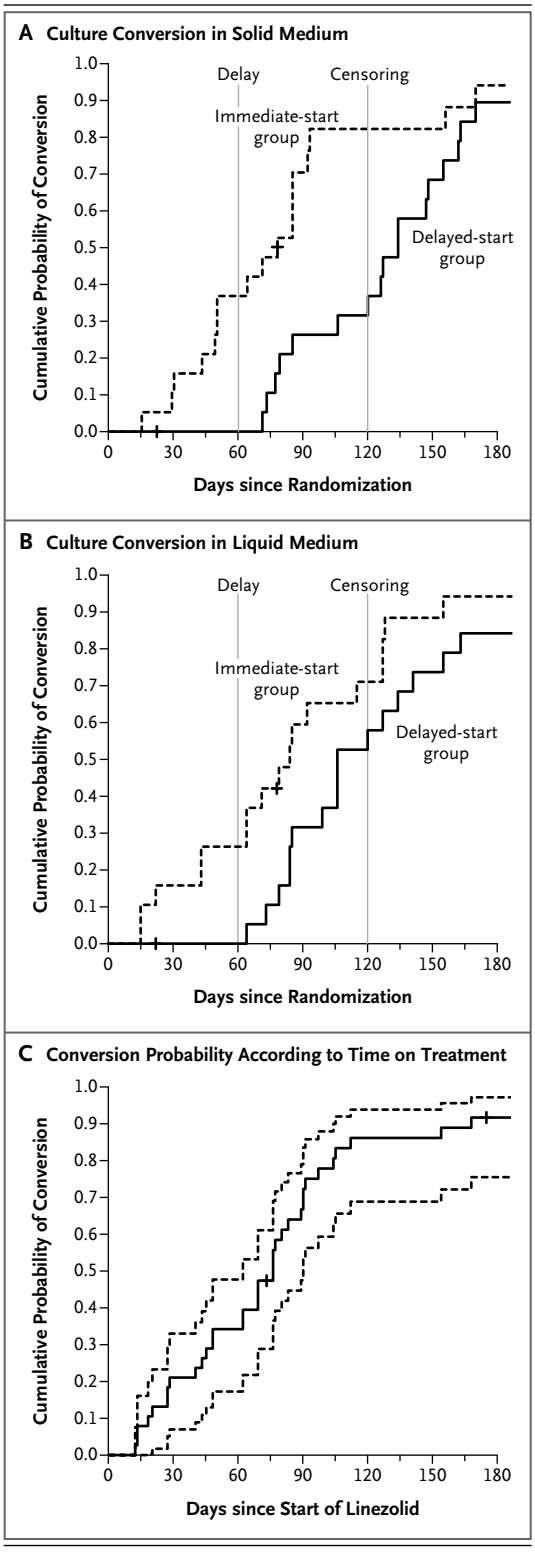

Figure 2. Kaplan–Meier Curves for Culture Conversion According to Time since Randomization.

Panel A shows the results for solid culture medium, and Panel B the results for liquid culture medium. In both panels, the gray vertical lines indicate the start of treatment (at 2 months) in the delayed-treatment group and the time of data censoring (at 4 months). Panel C shows the time to culture conversion on solid medium (solid line) along with the 95% confidence interval (dashed lines) for the 38 participants who received linezolid, according to the duration of linezolid therapy. Tick marks indicate the censored observations at the time of the last follow-up visit with culture results.

rRNA or in the ribosomal protein L3, in all four patients (Fig. 4A). The observed mutations have previously been reported in association with linezolid resistance from *M. smegmatis*[29] and *M. tuberculosis*.[30,31]

PHARMACOKINETICS

The maximal and minimal plasma concentrations that we observed for linezolid (Fig. 4B) are generally in agreement with published pharmacokinetic data in patients with other infectious diseases.[22,32,33] Considering that the plasma protein binding of linezolid is approximately 30%, plasma levels of free linezolid were above the measured minimum inhibitory concentration for each isolate during the entire dosing interval in almost all patients taking 600 mg per day. Among those taking 300 mg per day, the trough level was lower than the mean minimum inhibitory concentration in nine patients, including the two in whom linezolid resistance developed during treatment with that dose. The two doses provided proportional exposures, with a mean (±SD) area under the curve of 180.4±89 μg per milliliter per hour for the 600-mg dose and 91.1±43 μg per milliliter per hour for the 300-mg dose. Using Cox regression, we found no association between the time to culture conversion (measured from the date of the start of treatment with linezolid) and either the peak level (P=0.93) or the trough level (P=0.92), measured after at least 2 weeks of linezolid treatment.

DISCUSSION

In this analysis involving 39 patients with XDR pulmonary tuberculosis who had not had a response to any standard treatment regimen for 6 months or more, we found that the immediate addition of linezolid at a dose of 600 mg per day to the ongoing background treatment regimen had a significant beneficial effect on the time to sputum-culture

conversion on solid medium, as compared with the delayed addition of linezolid at the same dose. During the first 6 months of treatment, 34 of the 39 patients (87%) had confirmed culture conversion, at a median of 76 days.

In studies conducted immediately after the discovery of streptomycin, which is also a protein-synthesis inhibitor, the rate of culture conversion at 3 months was only 19%,[34] whereas in the present study, 60% of patients who received linezolid had negative sputum cultures at 3 months. Monotherapy with isoniazid was also studied throughout the 1950s, and in those studies, less than 32% of patients had sputum-culture conversion during the first 3 months of treatment.[35] First-line quadruple drug therapy (isoniazid, rifampin, pyrazinamide, and ethambutol) has been associated with a mean time to culture conversion on solid medium of 30 to 40 days.[36,37] In patients with MDR tuberculosis who were treated with second-line agents, the conversion rate was substantially lower, and the time to conversion was longer. For example, in a recent study that used a five-drug regimen (kanamycin, ofloxacin, ethionamide, pyrazinamide, and cycloserine), less than 10% of patients had a negative culture after 2 months.[38] In larger studies involving patients with MDR tuberculosis, the estimated median time to culture conversion was 63 days.[39] Linezolid alone would therefore appear to be similar to the five-drug chemotherapy regimen currently used as second-line treatment, and the incorporation of linezolid into second-line regimens may substantially improve culture-conversion rates.

A major concern in undertaking this trial was the emergence of acquired resistance to linezolid because we were adding a single active drug to a failing regimen. In early studies of streptomycin as monotherapy, 35 of 41 patients (85%) had resistant organisms at a mean of 53 days after the initiation of therapy.[34] Likewise, by 3 months, resistant bacilli developed in 83% of the patients who received isoniazid monotherapy.[35] In our study, 4 of the 38 patients who received linezolid for 6 months or more (11%) had apparent acquired resistance. This low frequency of acquisition of resistance may be related to the low rate of observed mutation to linezolid resistance in vitro (estimated at 10^{-9}).[30] The dose of 600 mg per day also maintained linezolid levels above the published mutant-prevention concentration of 0.6 μg per milliliter[40] and may have played a role in reducing the incidence

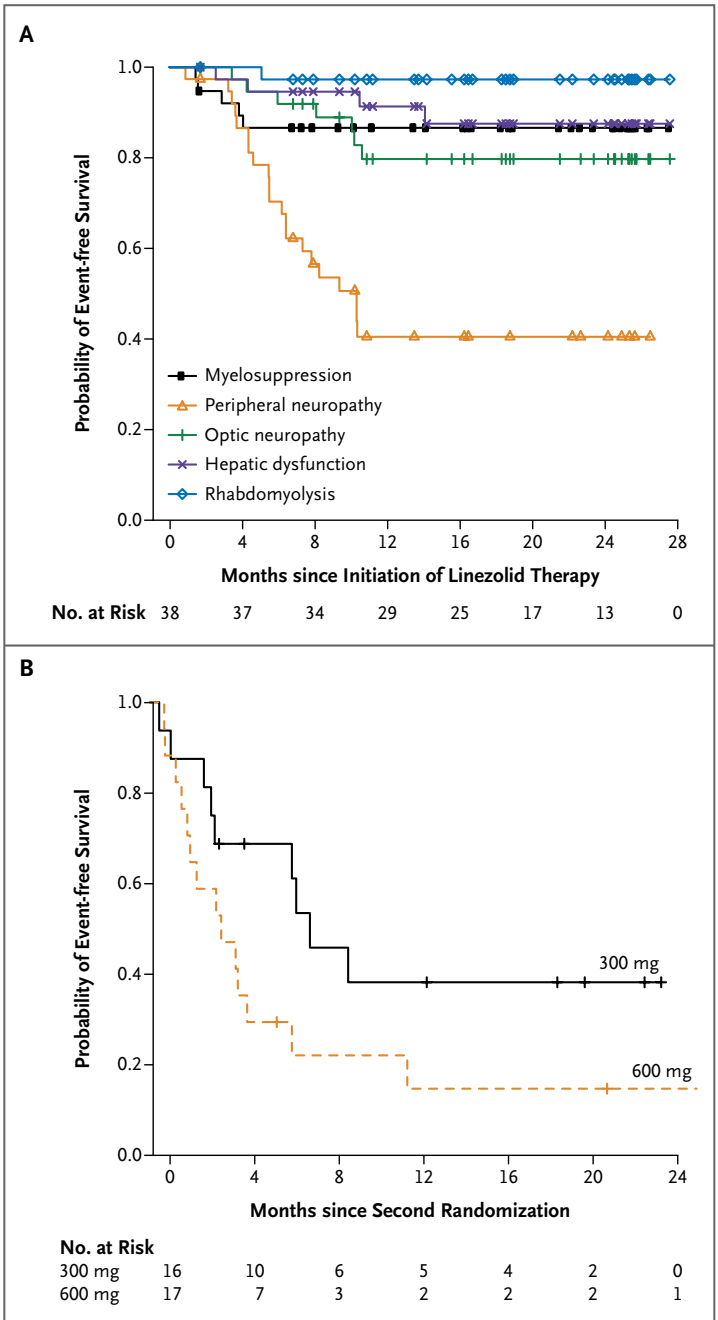

Figure 3. Probability of Event-free Survival over Time.

The Kaplan–Meier curves in Panel A show the time to the onset of clinically significant adverse events that resulted in a drug holiday or dose adjustment during the study. Symbols indicate data-censoring points for patients remaining in the study (see Table 3 in the Supplementary Appendix for detailed risk estimates corrected for person-years of exposure). The curves in Panel B show the time to the first adverse event in patients after the second randomization to either a continuation of the 600-mg daily dose or a reduced dose of 300 mg per day. Tick marks indicate data-censoring points for individual patients who continued to receive the study drug.

A Acquisition of Resistance to Linezolid

Patient No.	Week	MIC (μg/ml)	23S rRNA	*rplC*
1	0	0.5	WT	WT
	25	0.5	WT	WT
	26	16.0	G2447T	WT
2	0	0.25	WT	WT
	20	1.0	WT	WT
	24	2.0	WT	T460C
3	0	0.5	WT	WT
	8	0.5	WT	WT
	12	0.5	WT	WT, T460C
	13	2.0	WT	WT
	15	4.0	WT	T460C
4	0	0.5	WT	WT
	12	0.5	WT	WT
	21	4.0	WT, G2576T	WT
	53	4.0	G2576T	WT

B Pharmacokinetics and MICs of Isolates

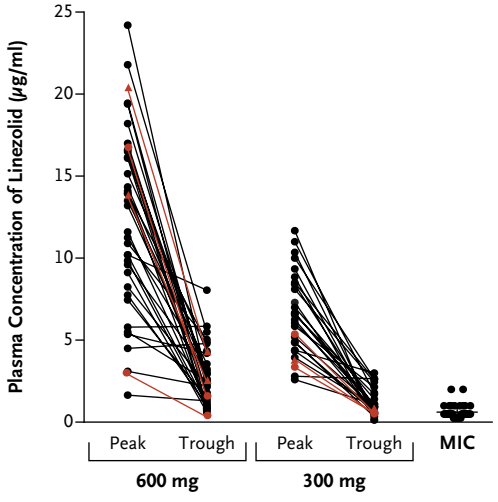

Figure 4. Resistance to and Pharmacokinetics of Linezolid.
Acquired resistance to linezolid was observed in the isolates from four patients; mutations in the linezolid-binding site of the 23S ribosomal RNA (rRNA) developed in two patients, and mutations in the mycobacterial ribosomal protein L3 gene (*rplC*) in the other two (Panel A). Numbers for 23S rRNA indicate the location of mutations according to *Escherichia coli* numbering. The pharmacokinetics of exposure to linezolid were measured for all patients while they were receiving 600 mg per day and for all patients who subsequently received 300 mg per day (Panel B). Plasma concentrations for the four patients in whom resistant organisms developed are shown in red, and the dose the patient was taking at the time of resistance detection is indicated by triangles (600 mg) or circles (300 mg). The minimum inhibitory concentration (MIC) for linezolid in the initial isolates from the patients and the mean MIC (horizontal line) are shown at the right.

of acquired resistance. The relatively small number of clinical isolates with reported linezolid resistance is consistent with this observation.[41]

The significant beneficial effect of linezolid was tempered, as expected, by the high rates of drug-related adverse events, although almost all events resolved quickly with a reduction in the dose or the temporary cessation of linezolid, and only three patients discontinued the study owing to adverse events. As we also expected, adverse events were significantly reduced in patients who subsequently received the reduced dose of 300 mg per day. The pharmacokinetic profile of the 300-mg dose, as compared with the minimum inhibitory concentration of the isolates, showed that this dose was sufficient to maintain serum levels above the minimum inhibitory concentration in most patients, although it is worrisome that three of the patients in whom resistance developed had relatively low exposures while receiving the 300-mg dose (Fig. 4B). Whether the lower dose, which is associated with fewer adverse events, has sufficient potency will need to be further evaluated, along with the possible role of therapeutic drug monitoring. Linezolid shows good pulmonary penetration[42,43] and has been shown to have favorable distribution in infected soft tissue.[44,45] These pharmacokinetic properties may also play a role in maintaining linezolid concentrations that are high enough to prevent the emergence of resistant organisms.

This study is limited by the small number of patients evaluated, particularly the small number of patients who did not have a response to treatment or in whom resistance developed. Although the small numbers of treatment failure and cases of acquired resistance are encouraging, they also preclude more in-depth analyses of the associated risk factors. Balancing the long-term risk–benefit ratio of linezolid requires identifying a dose with sufficient potency but less toxicity.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Lee M, Lee J, Carroll MW, et al. Linezolid for treatment of chronic extensively drug-resistant tuberculosis. N Engl J Med 2012;367:1508-18. DOI: 10.1056/NEJMoa1201964

Linezolid for the Treatment of

Chronic Extensively Drug-Resistant Tuberculosis

Supplementary Appendix

Table of Contents

Supplemental Acknowledgement:

We thank Jae-Joon Yim M.D. (Seoul National University), Barbara Seaworth, M.D. (University of Texas Health Science Center), and Debra Benator, M.D. (Veterans Affairs Medical Center, Washington DC) for their time and expertise for serving as the Safety Monitoring Committee.

Supplemental Material 1: Inclusion and Exclusion Criteria

(1) Inclusion criteria

1) Males and females age 20 and above
2) Documented pulmonary tuberculosis at screening
3) Radiographic evidence of tuberculous disease of the lung(s)
4) History of chronic, AFB positive sputum smears and culture positive TB
5) Mycobacterium species identification as *Mycobacterium tuberculosis*
6) Confirmed resistance to INH, RIF, kanamycin, ofloxacin, and moxifloxacin by genotypic or phenotypic testing OR subjects with documented failure to respond to treatment despite DST susceptibility
7) Failure to respond (after at least 6 months) to an anti-TB drug regimen including any known active agents
8) Willingness to be an inpatient at NMTH until 2 consecutive AFB-negative sputum smears
9) When an outpatient, willing to come back for weekly tests and scheduled follow-up visits
10) Willingness to have samples stored
11) Ability and willingness to give written or oral informed consent

(2) Exclusion criteria

1) Subjects below 20 years of age
2) Subjects who have previously been on LZD
3) Women of childbearing potential, who are pregnant, breast feeding, or unwilling to avoid pregnancy (*i.e.*, the use of appropriate contraception including oral and subcutaneous implantable hormonal contraceptives, condoms, diaphragm, intrauterine

device (IUD), or abstinence from sexual intercourse) [Note: Prospective female participants of childbearing potential must have negative pregnancy test (urine) within 48 hours prior to study entry.]

4) Men who are unwilling to use contraceptives or practice abstinence

5) People with any of the following in their current medical assessments:

   a. Absolute neutrophil count < 1000 cells/mL

   b. White blood cell count (WBC) < 3.0 X $10^3/\mu L$

   c. Hemoglobin < 7.0 g/dL

   d. Platelet count < 75,000 cells/mm^3

   e. Serum creatinine > 2.0 mg/dL

   f. Aspartate aminotransferase (AST or SGOT) >100 IU/L

   g. Alanine aminotransferase (ALT or SGPT) >100 IU/L

   h. Total bilirubin > 2.0 mg/dL

   i. Moderate or severe peripheral or optical neuropathy (or a history of)

   j. HIV-1 or HIV-2 infection

   k. Systemic lupus erythematosus, rheumatoid arthritis, or other connective tissue disease

6) Patients who, in the investigator's judgment, are too ill to participate in the study

7) History of allergy or serious adverse reaction to the LZD formulation used in this study

8) Patients with anticipated surgical intervention

9) The use of any of the following drugs within 30 days prior to study or anticipated use of these drugs within the next 60 days: (Please note, bronchodilators and cough syrup (or similar cough medicines) are allowed before and during the study if blood pressure is monitored regularly, per Contraindications, p.12, of the Zyvox Package Insert.)

   a. Selective serotonin reuptake inhibitors (SSRIs)

   b. Monoamine oxidase inhibitors (MAOIs)

Supplemental Table 1: Initial regimen, final regimen, susceptible drugs at entry*, Treatment status and follow up

Subject ID	Entry regimen	Current/Final regimen	Susceptible drugs at entry	Treatment outcome†/status, study status and follow up
F101	H,R	R,Mfx,Clr	Cs, S, Mfx	Cure, completed study without relapse
F102	Z,Cs,Mfx	Cs,Mfx,Clr,Amx/Clv	None	Cure, withdrew after end of therapy (EOT) plus 6 months without relapse
F103	H,Pto,Cs,Lfx	Na‡	Cs	Withdrawn due to SAE (anemia) before culture conversion to negative
F104	H,Lfx	Mfx,Clr,Amx/Clv	None	Cure, completed study without relapse
F105	H,E,Cs,Mfx,Clr	E,Mfx	Z,PAS, S	Cure, lost to follow-up after EOT
F106	Pto,Cs,PAS,Clr,Amx/Clv,Km	Cs,Mfx,Clr,Amx/Clv	Cs,Km,Cm,Am	Cure, lost to follow-up after EOT
F108	H,R,Lfx	Na	Pto,Cs,S,Km,Cm,Am,Rb	Withdrawn prior to receiving study drug after being diagnosed with metastatic colon cancer
F109	Pto,Mfx,PAS,Clr,Rb	Pto,Cs,Mfx,PAS,Clr,Km	Cs,Km,Cm,Am,Rb	Cure, awaiting EOT plus 6 month without relapse
F110	H,R	Mfx,Clr,Amx/Clv	Pto,Mfx,PAS,S,Cm,Rb	Cure, awaiting EOT plus 12 month without relapse
F111	Pto,Cs,Lfx,PAS	Pto,Mfx,Clr,Amx/Clv	None	Treatment failure, never converted sputum culture to negative
F112	R,Z,Mfx,PAS	Z,Mfx,Amx/Clv,Am	Pto,PAS,Km,Cm,Am	Withdrawn due to AE (optic neuropathy) after becoming culture negative

F113	H	H,Clr,Amx/Clv	E,Cs	Treatment Failure. Initially converted sputum culture to negative but reverted to positive after primary endpoint
F114	Pto,Cs,Lfx,Clr	Am	Km,Cm,Am	On therapy, culture negative
F115	Pto,Cs,Mfx,Clr,Amx/Clv,Rb	Cs,Mfx,Clr,Amx/Clv,S	E,S,Rb	On therapy, culture negative
F116	Pto,Cs,Lfx	Na	Z,PAS,Km,Cm,Am	Treatment failure, never converted sputum culture to negative
F117	H,R,Z,Pto,Cs,Mfx	Mfx,Clr,Amx/Clv,Am	Km,Am	On therapy, culture negative
F118	Z,Pto,Cs,Mfx,PAS,Km	Z,Mfx,Clr,Amx/Clv,Km	PAS	On therapy, culture negative
F119	Z,Pto,Cs,Mfx,Rb	Clr,Amx/Clv,S	Z,Lfx,Mfx,PAS,S	On therapy, culture negative
F120	E,Cs,Lfx	Mfx,Clr,Amx/Clv,Am	Z,Mfx,PAS,Km,Cm,Am	On therapy, culture negative
F121	None	Mfx,Clr,Amx/Clv,Am	Mfx,PAS,Km,Cm,Am	Withdrawn before EOT for personal reasons
F122	Cs,Lfx,PAS,Clr,S	Lfx,PAS,Clr,S	Z,Ofx,Lfx,Mfx,S,Km,Cm,Am,Rb	On therapy, culture negative
F123	E,Z,Cs,Mfx	Cs,Mfx,Clr,Amx/Clv,S	S	On therapy, culture negative
F124	Pto,Cs,Lfx	Mfx,Clr,Amx/Clv,Am	Pto,Km,Cm,Am	On therapy, culture negative
F125	Z,Cs,Mfx,Clr,Rb	Na	Mfx,PAS,S,Km,Cm,Am	On therapy, culture negative
F126	Z,Pto,Cs,Lfx,Rb	Mfx,Clr,Amx/Clv,Km	Rb	On therapy, culture negative
F201	H,R	R,Clr,Amx/Clv,S	S	Cure, completed study without relapse
F202	Mfx,Clr	Mfx,Clr,S	Cs,Mfx,PAS,S	Cure, lost to follow-up after EOT plus 6 month without relapse

F203	Z,Pto,Cs,Clr,Amx/Clv	Pto,Cs,Mfx,Clr,Amx/Clv	E,Mfx,S,Km,Am	Cure, awaiting EOT plus 12 month without relapse
F204	Pto,Cs,Mfx	Pto,Cs,Mfx,Amx/Clv,S	PAS,S	Cure, awaiting EOT plus 6 month without relapse
F205	Pto,Cs,Lfx,PAS,Clr,S	H,Mfx,Clr,Amx/Clv,Am	Km,Cm,Am	Cure, awaiting EOT plus 6 month without relapse
F206	Pto,Cs,Mfx,PAS	Cs,Mfx,PAS	Z,Cs,PAS,Km,Cm,Am	Cure, awaiting EOT plus 6 month without relapse
F208	H,E,Z,Mfx,Clr	E,Mfx,Clr,Amx/Clv,Km	E,Lfx,Mfx,PAS,S,Km,Cm,Am,Rb	On therapy, culture negative
F209	E,Pto,Cs,Lfx,PAS	Amx/Clv,Am	E,Km,Cm,Am,Rb	On therapy, culture negative
F210	Z,Pto,Cs,PAS,Rb	Z,Clr,Amx/Clv,Am	Km,Cm,Am	Withdrawn due to AE (optic neuropathy); initially became culture negative on LZD but reverted after discontinuation of LZD due to AE
F211	Z,Mfx,PAS,Clr,Amx/Clv	No change	Cs,Lfx,PAS,Cm,Am,Rb	On therapy, culture negative
F212	None	Z,Mfx,PAS,Clr,Am	H,Cs,PAS,Cm,Am	On therapy, culture negative
F213	Pto,Cs	Z,Mfx,Clr,Amx/Clv,Km	E,S,Km,Cm,Am	On therapy, culture negative
F214	E,Pto,Mfx,Rb	Na	PAS,Km,Cm,Am,Rb	Treatment failure, never converted sputum culture to negative
F215	H,Pto,Lfx	Pto,Mfx,Clr,Amx/Clv,S	E,PAS,S,Rb	On therapy, culture negative

* Abbreviation of drugs as following: isoniazid (H), rifampicin (R), ethambutol (E), pyrazinamide (Z), prothionamide (Pto), cycloserine (Cs), ofloxacin (Ofx), levofloxacin (Lfx), moxifloxacin (Mfx), p- aminosalicylic acid (PAS), clarithromycin (Clr), amoxicillin/clavulanate (Amx/Clv), streptomycin (S), kanamycin (Km), amikacin (Am) capreomycin (Cm), rifabutin (Rb), linezolid(Lzd)

† Status as of May 1, 2012. Cure and treatment failures were defined using WHO criteria.

Supplemental Figure 1: Study timeline (Immediate arm)

Evaluation	Scrn	Entr	1w	2w	3w	4w	5w	6w	7w	8w	9w	10w	11w	12w	13w	14w	15w	16w	5m	6m	7m	Ev 2 mo	EOT	EOT+ 6m	EOT+ 12m	Early wdrl
Clinical examination	O	O				O								O				O	O	O	O	O	O	O	O	O
Medical history	O																									
HIV ELISA	O																									
AFB smear		O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
TB culture		O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
DST	O																									
Radiologic imaging	CT or PET/CT scan was performed according to arm and participation of substudy for PET/CT																									
Chemistries	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Hematology	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
PK (blood/sputum)		O		O	O	O	O									O	O	O	O							O
MTB lipid (urine/blood/sputum)		O	O		O		O		O		O		O		O		O		O				O	O	O	
T-SPOT-TB		O	O		O		O		O		O		O				O			O			O	O	O	
Whole blood killing assay		O			O				O																	O
Mitochondrial Assay		O			O		O		O		O		O		O		O		O	O	O	O				O
TB RNA		O	O	O		O																				
Neurologic examination	O					O				O				O				O	O	O	O	O	O	O	O	O
Optic test		O				O				O				O				O	O	O	O	O	O	O	O	O
Pregnancy test	O									O										O					O	O

Supplemental Figure 2: Study timeline (Delayed arm)

Evaluation	Scrn	Ent	Weeks																							Months			Ev 2 mo	EOT	EOT+6m	EOT+12m	Early wdr
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	7	8	9					
Clinical examinatio	O	O				O				O				O				O				O				O	O	O	O	O	O	O	O	O
Medical history	O																																	
HIV ELISA	O																																	
AFB smear		O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
TB culture		O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
DST	O																																	
Radiologic imaging	CT or PET/CT scan was performed according to randomized arm and participation in substudy for PET/CT																																	
Chemistries	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Hematology	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
PK (blood /sputum)										O		O	O	O	O									O	O	O	O							O
MTB lipid (urine/bloo d/ sputum)		O								O	O		O		O		O		O		O		O		O		O	O		O	O	O	O	
T-SPOT-TB		O								O	O		O		O		O		O		O				O			O		O	O	O	O	
Whole blood		O											O				O																	O
Mitochondr ial Assay		O			O								O		O		O		O		O		O		O		O	O	O	O				O
TB RNA		O									O	O		O																				
Neurologic examinatio	O					O				O				O				O				O				O	O	O	O	O	O	O	O	O
Optic test		O				O				O				O				O				O				O	O	O	O	O	O	O	O	O
Pregnancy test	O									O								O										O				O	O

Supplemental Table 2: All clinically significant adverse events* regardless of relationship to linezolid and number reported as serious adverse events (SAEs)

Event	Total	Reported as SAE
Peripheral neuropathy	21	6
Myelosupression	7	7
Optic Neuropathy	7	7
Hepatic dysfunction	4	3
Hyperglycemia	3	0
Pneumonia	3	3
Uric acid elevation	2	0
Rhabdomyolysis	2	2
Other †	7	5
Total	**56**	**33**

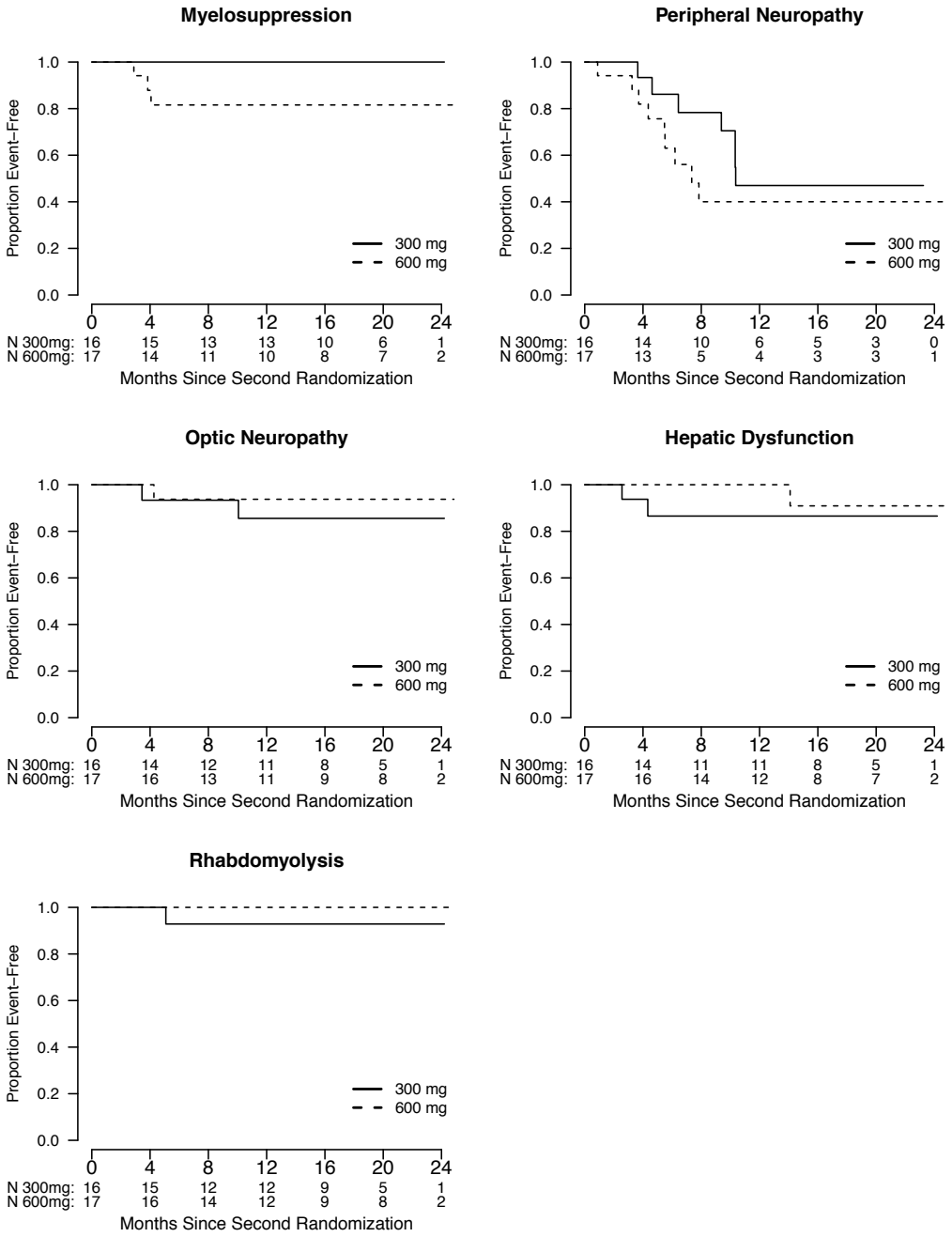
*Clinically significant adverse events were defined as follows: Peripheral neuropathy, all subjects with an SPNS score≥2, or any SPNS score of one that resulted in a dose adjustment; Optic neuropathy > grade 2; other AEs ≥ grade 3; and other < grade 3 events reported as SAEs at the investigators discretion (dizziness n=1 and reaction to psychotropic medications n=1)

† dizziness (n=1), reaction to psychotropic medications (n=1), colon cancer with metastasis (n=1), esophageal hemorrhage due to Mallory-Weiss Tear (n=1), hemoptysis (n=1), cataract (n=1) and diarrhea (n=1)

All adverse events were graded using the DAIDS Toxicity Tables, located at http://rsc.tech-res.com/safetyandpharmacovigilance/gradingtables.aspx.

Recurrent events in a single subject were only counted once.

Supplemental Figure 3: Kaplan-Meier survival curves of type of adverse event and time since 2nd randomization, stratified by treatment arm



Supplemental Table 3: Frequency of clinically significant adverse events possibly or probably related to linezolid‖

Event	0-4 Mo						4-8 Mo						8-12 Mo					
	N*	PY†	Rate	L0‡	L300§	L600||	N	PY	Rate	L0	L300	L600	N	PY	Rate	L0	L300	L600
Myelosuppression	5	12.0	0.42	0.0	1.3	10.7	1	10.1	0.10	0.0	6.8	3.3	0	8.4	0.00	0.4	6.7	0.9
Peripheral Neuropathy	5	12.1	0.41	0.1	1.6	10.4	10	8.2	1.22	0.0	5.8	2.4	5	4.7	1.07	0.1	3.7	0.7
Optic Neuropathy	1	12.5	0.08	0.1	1.7	10.7	2	10.7	0.19	0.0	7.4	3.3	3	8.0	0.38	0.0	6.5	1.5
Hepatic Enzyme Elevation	1	12.4	0.08	0.1	1.5	10.8	1	10.9	009	0.0	7.5	3.4	1	8.6	0.12	0.4	7.0	0.9
Rhabdomyolysis	0	12.5	0.00	0.1	1.7	10.8	1	11.3	0.09	0.0	7.9	3.4	0	9.0	0.00	0.4	7.3	0.9

*N, number of events observed during the specified time interval from linezolid start; †PY, Person-Years of study interval; ‡L0, Person-Years of subjects receiving no linezolid during the study interval; §L300, person-years of subjects receiving 300 mg linezolid during the study period; ||L600, person-years of subjects receiving 600 mg linezolid during the study period

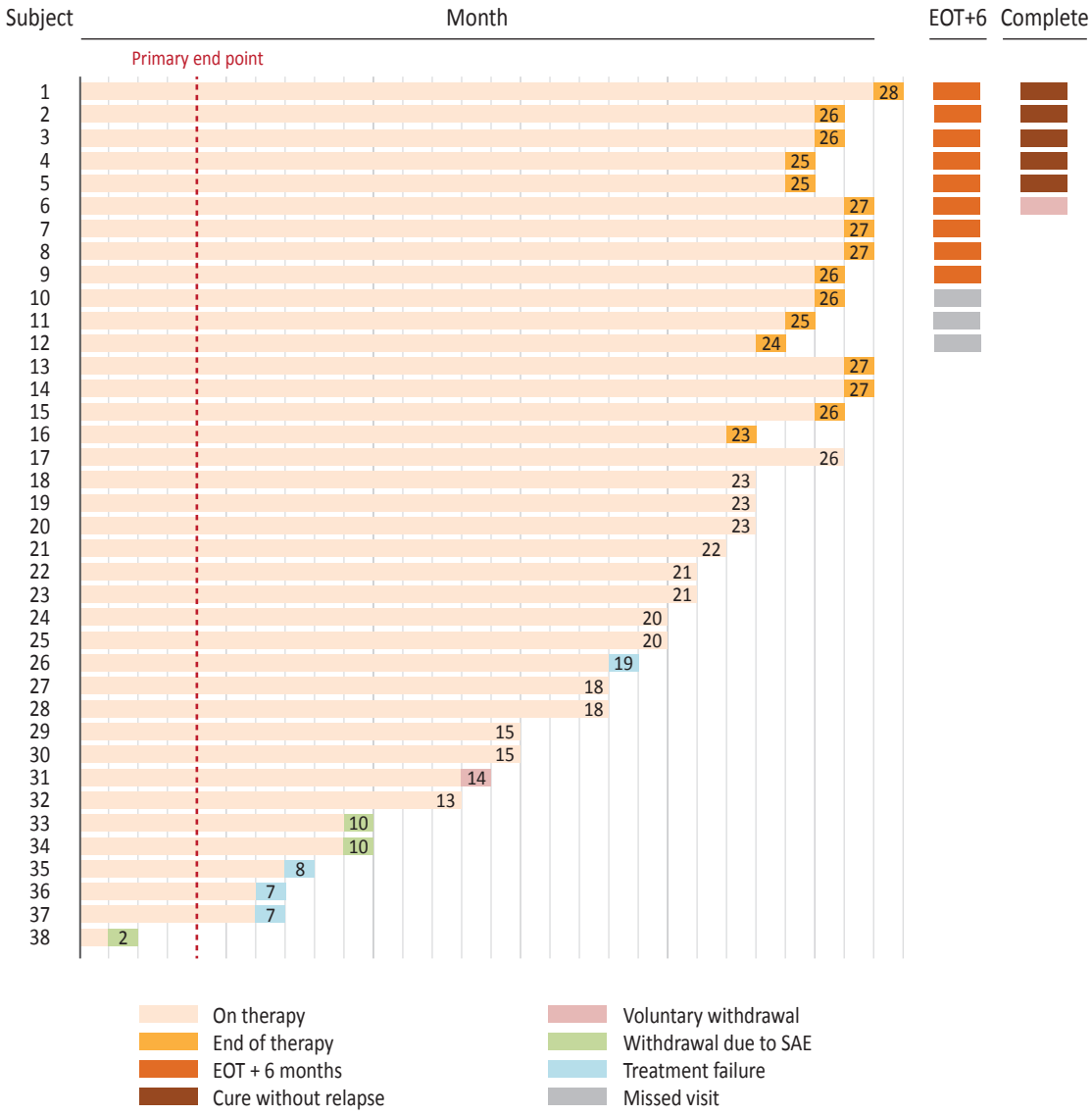
Supplemental Table 4: Linezolid stoppage and dose adjustments*

Subject ID	Stoppage period (days)	Reason	Relationship to LZD	Dose adjustment
F101	2	Hepatic enzyme elevation	Possibly related	Re-challenge with 600mg
F103	–	Anemia	Probably related	Permanently stopped
F104	1	Joint pain	Unrelated	Re-challenge with 600mg
	18	Neutropenia	Probably related	De-escalation to 300mg
F106	18	Peripheral neuropathy	Probably related	Re-challenge with 300mg
F110	11	Peripheral neuropathy	Probably related	De-escalation to 300mg
F112	–	Optic neuropathy	Possibly related	Permanently stopped
F113	21	Peripheral neuropathy	Probably related	De-escalation to 300mg
F114	5	Hepatic enzyme elevation	Possibly related	Re-challenge with 300mg
	20	Rhabdomyolysis	Possibly related	Re-challenge with 300mg
F117	23	Hepatic enzyme elevation	Possibly related	Re-challenge with 300mg
F119	17	Optic neuropathy	Probably related	De-escalation to 300mg
F121	34	Peripheral neuropathy	Probably related	De-escalation to 300mg
F122	22	Anemia	Probably related	De-escalation to 300mg
	12	Peripheral neuropathy	Probably related	Re-challenge with 300mg
F123	14	Peripheral neuropathy	Probably related	De-escalation to 300mg
	33	Optic neuropathy	Probably related	Re-challenge with 300mg
F124	20	Anemia	Probably related	De-escalation to 300mg
F126	21	Peripheral neuropathy	Probably related	De-escalation to 300mg
F201	8	Gastrointestinal bleeding	Unrelated	Re-challenge with 600mg
	14	Peripheral neuropathy	Probably related	De-escalation to 300mg
F202	25	Neurologic reaction to psychological medicines	Unlikely related	Re-challenge with 600mg
F203	10	Peripheral neuropathy	Probably related	De-escalation to 300mg
	13	Pneumonia	Unlikely related	Re-challenge with 300mg
F205	28	Optic neuropathy	Possibly related	Re-challenge with 300mg
F208	16	Peripheral neuropathy	Probably related	De-escalation to 300mg
F209	25	Optic neuropathy	Possibly related	De-escalation to 300mg
F210	26	Optic neuropathy	Probably related	Re-challenge with 300mg

	-	Optic neuropathy	Probably related	Permanently stopped
F212	25	Anemia	Probably related	De-escalation to 300mg
F213	26	Peripheral neuropathy	Possibly related	De-escalation to 300mg
	14	Peripheral neuropathy	Possibly related	Re-challenge with 300mg
F215	11	Anemia	Probably related	De-escalation to 300mg
	3	Total bilirubin elevation	Possibly related	Re-challenge with 300mg

*For this table 3 cases of brief drug stoppages for personal reasons (unrelated to the study) were excluded

Supplemental Figure 4: Current status of study subjects



Supplemental Material 2: Subjective Peripheral Neuropathy Score (SPNS)

1. Instruction for recording; ask the patient to rate the severity of each symptom (a-f) on a s cale of 1 (mild)-10 (most severe). Enter the score for each symptom in the column marke d Presence/Severity. If symptom has been present in the past, but not since the last visit, enter"00"/Currently Absent. If the symptom has never been present, enter "11"/Always Been Normal.

Always been normal	Currently absent	Mild …… Severe									
11	00	01	02	03	04	05	06	07	08	09	10

2. Symptom(s) Presence/Severity
   a. Pain, aching or burning in hands, arms …… _____
   b. Pain, aching or burning in feet, legs …….. _____
   c. "Pins and needles" in hands, arms …… _____
   d. "Pins and needles" in feet, legs …….. _____
   e. Numbness (lack of feeling) in hands, arms …….. _____
   f. Numbness (lack of feeling) in feet, legs …… _____

3. Instructions for grading subjective patient-elicited symptoms:
   Use highest severity score recorded in (a-f) above to obtain a subjective peripheral neuropathy grade :

   Presence/Severity Score of
   01-03 = Grade of 1
   04-06 = Grade of 2
   07-10 = Grade of 3
   11 or 00 = Grade of 0

4. Subjective peripheral neuropathy grade?…… _____