24.07.2020

To,
The Controller of Patents,
Patent Office,
Delhi

Re: Representation by way of opposition under Section 25(1) by Eldred Tellis and Ganesh Acharya to patent application no. 201817014361 titled "*Combination Antibacterial Composition and Short Course Antibacterial Regimen*", in the name of The Global Alliance For TB Drug Development Inc.

In reference to the above mentioned patent application number, we herein submit the following:

1. Representation for opposition on Form 7A under Section 25(1) of the Patents Act and Rule 55 of the Patents Rule, 2003
2. Exhibits: Exhibit A, Exhibit B, Exhibit C, Exhibit D, Exhibit E, Exhibit F, Exhibit G, Exhibit H, Exhibit I and Exhibit J
3. Authorization by the Opponents
4. Petition under Rule 138 for extension of time to file Form-26 with requisite stamp duty

You are kindly requested to take this opposition on record, and grant a hearing in due course.

Yours sincerely,

Priyam Lizmary Cherian
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BEFORE THE CONTROLLER OF PATENTS, THE PATENT OFFICE,

DELHI

IN THE MATTER OF A PRE- GRANT OPPOSITION UNDER SECTION 25 (1)

AND RULE 55 OF THE PATENTS RULES, 2003

IN THE MATTER OF REPRESENTATION BY WAY OF NOTICE OF OPPOSITION UNDER SECTION 25(1) OF PATENTS ACT, 1970 FILED BY ELDRED TELLIS AND GANESH ACHARYA .....OPPONENTS

And

IN THE MATTER OF PATENT APPLICATION NO. 201817014361 FILED IN INDIA ON APRIL 4, 2018 TITLED "COMBINATION ANTIBACTERIAL COMPOSITION AND SHORT COURSE ANTIBACTERIAL REGIMEN", IN THE NAME OF THE GLOBAL ALLIANCE FOR TB DRUG DEVELOPMENT INC. …..APPLICANT

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7.	**Exhibit E**- "*Clinical Access to Bedaquiline Programme for the treatment of drug-resistant tuberculosis*" S Afr Med J 2014;104(3):164-166	210-212
8.	**Exhibit F**- Garcia-Prats AJ, et al., Linezolid for the treatment of drug-resistant tuberculosis in children: A review and recommendations, Tuberculosis (2013), http://dx.doi.org/10.1016/j.tube.2013.10.003	213-224
9.	**Exhibit G**- Copy of order of the Asst. Controller of Patents & Designs in patent application no. 314/MUM/2008 dated 05.10.2012	225-233
10.	**Exhibit H**- Copy of order of the Controller in patent application no. 3725/CHENP/2006 dated 09.10.2012	234-239
11.	**Exhibit I**- Copy of order of the Controller in patent application no. 5461/DELNP/2008 dated 21.07.2015	240-250
12.	**Exhibit J**- Copy of order of the Assistant Controller of Patents and Designs in application no. 201647001874 dated 17.07.2020	251-253
16.	Authorization	

Dated this 24th day of July, 2020

Counsel for the Opponents

Priyam Lizmary Cherian
priyamlizcherian@gmail.com

TO THE CONTROLLER OF PATENTS
PATENT OFFICE, DELHI

FORM 7A

THE PATENTS ACT, 1970
& THE PATENT RULES, 2003
NOTICE OF OPPOSITION
Section 25(1) and rule 55

We, Eldred Tellis and Ganesh Acharya, Indian residents hereby give representation by way of opposition to the grant of patent in respect of Indian Patent Application **201817014361** titled **COMBINATION ANTIBACTERIAL COMPOSITION AND SHORT COURSE ANTIBACTERIAL REGIMEN** dated **16.04.2018** and published on **07.09.2018** in the name of The Global Alliance for TB Drug Development Inc., 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 is obvious and clearly does not involve any inventive step – Section 25(1)(e);
3. 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);
4. 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);
5. 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).

Our address for service in India is:

Priyam Lizmary Cherian,
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Phone: 011 4680 5555
Email: priyamlizcherian@gmail.com

We request that all communications be addressed to us at the above address.

Dated this 24th day of July, 2020
Opponents

To
The Controller,
The Patent Office, Delhi

BEFORE THE CONTROLLER OF PATENTS

THE PATENT OFFICE, DELHI

THE PATENTS ACT, 1970 AND THE PATENTS RULES, 2003

IN THE MATTER OF A PRE-GRANT OPPOSITION UNDER SECTION 25 (1) AND RULE 55 OF THE PATENTS ACT, 1970

And

IN THE MATTER OF PATENT APPLICATION NO. 201817014361 FILED IN INDIA ON APRIL 4, 2018 TITLED "COMBINATION ANTIBACTERIAL COMPOSITION AND SHORT COURSE ANTIBACTERIAL REGIMEN", IN THE NAME OF THE GLOBAL ALLIANCE FOR TB DRUG DEVELOPMENT INC. …..APPLICANT/RESPONDENT

And

IN THE MATTER OF REPRESENTATION BY WAY OF NOTICE OF OPPOSITION UNDER SECTION 25(1) OF PATENTS ACT, 1970 FILED BY ELDRED TELLIS AND GANESH ACHARYA

.....OPPONENTS/ PETITIONERS

REPRESENTATION BY WAY OF OPPOSITION U/S 25(1), PATENTS ACT, 1970

1. A pre-grant opposition under Section 25(1) of the Patents Act, 1970, is hereby submitted by Eldred Tellis and Ganesh Acharya (hereinafter the 'Opponents') against Indian Patent Application number 201817014361

(hereinafter the 'Present Application') filed by The Global Alliance for TB Drug Development Inc. (hereinafter the 'Applicant').

LOCUS STANDI

2. The Opponent, Eldred Tellis is a resident of India and is a part of a community-based organization called the Sankalp Rehabilitation Trust. He, as a part of the organisation works on providing care, treatment and rehabilitation services for injecting drug users and people living with HIV. Tuberculosis (TB) being an opportunistic infection affects occur severely those people with weakened immune systems particularly PLHIV. Therefore, the Opponent's work spans on access to medicines for opportunistic infections including TB by overcoming intellectual property barriers.
3. The Opponent, Ganesh Acharya is a resident of India and is a person living with HIV who has survived TB ailment twice. Being a TB survivor he had the living experience of the issues faced by TB patients that prompted him to work on access to treatment and medicines for persons living with TB. He works with persons living with TB, particularly drug resistant TB and civil society organisations for access of government mandated nutritional support for TB patients and overcoming barriers to access to TB drugs.
4. Section 25(1) of the Patents Act allows that *any person* to make a representation by way of an opposition against grant of patent to an application. The Opponents therefore have the *locus standi* to make the present representation by way of an opposition against the grant of patent to the Present Application.
5. On 16.4.2018, the Present Application was filed at the Patent Office, Delhi. On 07.09.2018, the Present Application was published. On 11.12.2019 a First Examination Report (FER) was issued for the Present Application and on 11.12.2019 a response to the FER was filed by the Applicant. The Present Application is pending and has not been granted a patent. Therefore,

this representation by way of an opposition against the Present Application is maintainable before the Patent Office, Delhi.

TUBERCULOSIS: BACKGROUND

6. TB is an infectious disease caused by Mycobacterium tuberculosis (MTB) bacteria. Tuberculosis generally affects the lungs, but can also affect other parts of the body. Most infections show no symptoms, in which case it is known as latent tuberculosis. 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 one fourth of the total number of infected populace.

7. The treatment for TB usually comprises anti-bacterial medicines to be taken over a period of time. Active tuberculosis, particularly if it's a drug-resistant strain, requires several drugs to be co-administered in a regime over such period. The most common medications used to treat tuberculosis include Isoniazid, Rifampin, Ethambutol, Pyrazinamide. Additionally, some drugs may be used as add-on therapy to the current drug-resistant combination treatment. These drugs include Bedaquiline, Linezolid and Pretomanid. Thus, treatment for TB for last many years has been a multiple drug cocktail regime to be taken over a period of time.

ACCESS TO MEDICINES AND STRICT INTERPRETATION OF INDIAN PATENTABILITY STANDARDS

8. To ensure the availability of affordable medicines, it is imperative to promote effective generic competition by ensuring that patents are granted in India for uninventive, incremental improvements or to subject matter that is excluded from patentability under the Indian patent law. Particularly so in the background of a study in a cohort of 2,293 pharmaceutical patents granted between 2009 and 2016 reporting that about 72 per cent of patents granted for pharmaceuticals are secondary patents, granted for marginal improvements over previously known drugs for which primary patents exist.

(See Dr. Feroz Ali *et al, Pharmaceutical Patents Granted in India: How our safeguards against ever-greening have failed, and why the system must be Reformed*, Accessibsa, 2018)

9. It is submitted that the Hon'ble Patent Controller, while considering the present pre-grant opposition, must bear in mind the intent of Parliament in enacting the Patents (Amendment) Act to safeguard the right to health by introducing strict standards of patentability including Sections 3(d), 3(e) and 3(i).

10. Therefore, it is requested the Present Application be examined and scrutinized strictly keeping in mind the strict patentability standards in India as the decision would have an impact on the availability of life-saving regimen to thousands of TB affected persons not only in India but also worldwide.

PRESENT APPLICATION

11. The Present Application was filed in India on 16.04.2018 with 16 claims. At the time of filing, the claims were amended and brought down to 10 claims. On 05.10.2016, the PCT phase application for the Present Application was filed and was assigned application no. PCT/US2016/055414. The Present Application claims a priority date of 14.10.2015 from the US patent application no. 62/241,280. The Present Application was published on 07.09.2018 and on 11.12.2019, an FER for the Present Application was issued. The Applicant filed a response to the FER on 04.06.2020. In order to overcome the objections in the FER, the Applicant amended the claims, bringing the total number of claims to 6.

THE CLAIMS

12. Subsequent to the amendment of claims, there are currently pending claims. The claims are reproduced below:

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.

Claim 2: The pharmaceutical composition as claimed in claim 1, wherein linezolid is at a dosage of 100 mg/kg.

Claim 3: The pharmaceutical composition as claimed in claim 1, wherein linezolid is at a dosage of 50 mg/kg.

Claim 4: The pharmaceutical composition as claimed in claim 1, wherein the pharmaceutical composition is in the form of a plurality of unit dosages, the plurality of unit dosages collectively comprising the therapeutically effective amount of each of linezolid, bedaquiline and pretomanid, and optionally pyrazinamide or a pharmaceutically acceptable salt of each thereof.

Claim 5: The pharmaceutical composition as claimed in claim 1, wherein the pharmaceutical composition is in the form of a plurality of unit dosages, the plurality of unit dosages collectively comprising 600 mg or 1200 mg linezolid, 200 to 400 mg bedaquiline, 100 to 200 mg pretomanid, and optionally pyrazinamide, or a pharmaceutically acceptable salt of each thereof.

Claim 6: The pharmaceutical composition as claimed in claim 1, wherein the pharmaceutical composition is in the form of a single unit dosage, the single unit dosage comprising the therapeutically effective amount of each of linezolid, bedaquiline and pretomanid, and optionally pyrazinamide, or a pharmaceutically acceptable salt of each thereof.

13. The claims of the Present Application were initially framed as method of treatment claims. On amendment of these claims in response to the FER these claims were tweaked as composition claims suggesting claim on a substance.

THE SPECIFICATION & THE ALLEGED INVENTION

14. The Applicant suggests in the specification that the alleged invention is related to, "…*a pharmaceutical <u>composition</u>, 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.*" (emphasis supplied, See internal page 3 of the complete specification, Summary of the Invention)

15. Further, the Applicant in the specification admits that the Present Application provides, "*…a <u>method for the treatment</u> 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.*" (emphasis supplied, see complete specification, internal page 3, Summary of Invention)

16. The Applicant further admits that it was known that addition of Linezolid to Bedaquiline+Pretomanid+Sutezolid significantly increased bactericidal and sterilizing activity of the regime (see complete specification, internal page 4, para 3, lines 3-4). Thereby suggesting that combination of these drugs in a regimen to achieve bactericidal activity was known.

17. Further, the Applicant admits that Linezolid, Bedaquiline, Pretomanid and Pyrazinamide were known in the art on the date of priority of the Present Application (see complete specification, internal pages 5-7). In fact, the Applicant specifically acknowledges that a regimen of 'bedaquiline + pretomanid + linezolid' was already initiated in the NiX-TB Trial.(see complete specification, internal page 4, para 4, lines 13)

18. Further, it is submitted that some portions of the complete specification of the Present Application are verbatim reproduction from earlier published patent specifications. For instance, at page 15 of the Present Application has content which appears to be reproduced from a previously published patent

specification relating to a HIV drug- WO2004064846 (hereinafter "WO '846" and annexed herewith as **Exhibit-A).**

Below is a tabular comparison of the text in the Present Application and that in WO2004064846.

Present Specification (internal pages 15, 16)	WO2004064846 (internal page 34, lines 31-32 Internal page 35, lines 1-23)
Page 15 onwards: *'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-*	**Please refer to the 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.* *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. *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.'*	*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 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.'*

19. Similarly, the "Definitions and Certain Components of the Invention" section of the Present Application (complete specification, internal pages 8-11) has text identical to that disclosed in WO'486 at internal pages 5-9.

SUMMARY OF GROUNDS OF OPPOSITION

20. The Opponents bring this representation by way of opposition under the following grounds, each of which is without prejudice to the other:

i) Claims 1-6 of the Present Application are not novel as the composition claimed therein have been published before the priority date of the Present Application. Therefore, the Opponents bring this Opposition under Section **25(1)(b)(ii)**- that the invention as claimed in the complete specification has been published before the priority date of the claim in India or elsewhere in any other document;

ii) Claims 1-6, of the Present Application lack inventive step, and therefore fail under Sections 2(1)(j) and 2(1)(ja) of the Patents Act. Therefore, the Opponents bring this opposition under **Section 25(1)(e)**-that the invention so far as claimed in any claim of the complete specification is obvious and clearly does not involve any inventive step, having regard to the matter published before the priority date in India or elsewhere in any document;

iii) Claims 1-6 do not satisfy the test of Section 3(d) of the Patents Act as the subject matter does not exhibit enhancement of the known efficacy of known substance. Therefore, the Opponents bring this opposition under **Section 25(1)(f)** -that the subject of any claim of the complete specification is not an invention within the meaning of the Patents Act and is not patentable under the Patents Act;

iv) Claims 1-6 do not satisfy the test of Section 3(e) of the Patents Act as the subject matter does not exhibit any synergistic effect. Therefore, the Opponents bring this opposition under **Section 25(1)(f)** -that the subject of any claim of the complete specification is not an invention within the meaning of the Patents Act and is not patentable under the Patents Act;

v) Claims 1-6, claim a process for treatment of human being and hence should be disallowed under Section 3(i) of the Patents Act. Therefore, the Opponents bring this opposition under **Section 25(1)(f)** -that the subject of any claim of the complete specification is not an invention within the meaning of the Patents Act and is not patentable under the Patents Act;

vi) The Opponents bring this Opposition under **Section 25(1)(g)** - that the complete specification does not sufficiently and clearly describe the invention.

vii) The Opponents bring this Opposition under **Section 25(1)(h)**- that the Applicant failed to disclose information required by Section 8.

DETAILED GROUNDS OF OPPOSITION

CLAIMS 1-6 ARE NOT NOVEL, AND ARE CHALLENGED UNDER SECTION 25(1)(b) OF THE PATENTS ACT

21. Section 2(1)(j) of the Patents Act defines an 'invention' as one that is a '*new product or process involving an inventive step and capable of industrial application*.' (emphasis supplied) Further, the Patents Act in Section 25(1) (b) (ii) allows opposition to a patent application if the alleged invention, as claimed in any claim of the complete specification has been published before the priority date of the claim, in India or elsewhere, in any other document other than a specification filed in pursuance of an application for a patent made in India. That is, disclosure of the claims of a patent application on a date prior to the date of priority must result in rejection of the claims. Such disclosure of the alleged invention may be determined by comparing the claims of the patent application in question and the prior art keeping in mind the general knowledge available to a Person Ordinarily Skilled in the Art (POSITA).

22. It is submitted that there are two documents dated before the priority date of the Present Application that disclose the invention of claims 1-6 of the Present Application.

Archive History for NCT02333799(Submitted: 06.01.2015)

23. The Opponent relies on submission made by the Applicant in the Archive History for NCT02333799 (annexed herewith as **Exhibit-B**). The Archive History NCT02333799 is a Phase 3 study assessing the safety and efficacy of Bedaquiline plus PA-824 plus Linezolid in subjects with drug resistant Pulmonary Tuberculosis. Given that the dossier for the same was submitted on 06.01.2015 (and posted on 07.01.2015), which is much before the priority date of the Present Application viz. 14.10. 2015, the same can be relied on as a prior art document.
24. PA-824 mentioned in the NCT02333799 study is simply Pretomanid.
25. The Archive History for NCT02333799 is a dataset on a clinical trial bearing the official title, '*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).*' 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.'*
26. That is the dosage of each of the three drugs to be used in combination within a single regimen has also been disclosed, which has been claimed in claim 5 of the Present Application.
27. Further, the summary of the NCT02333799 discloses that the purpose of the trial, "…*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).*" A simple reading of this indicates that the

Applicant's assertion in its response to FER stating, "*Archive History for NCT02333799 does not disclose or suggest a multi-agent pharmaceutical composition of, bedaquiline, pretomanid and linezolid which does not need to be continued for 6 months for effective tuberculosis treatment.*" Is incorrect and misleading.

28. Further, it may also be pointed out that the Applicant's response to the FER states that that the shorter time duration of administration and discontinuation of Linezolid is the distinguishing feature of the alleged invention. It is submitted that this argument is not sustainable as the discontinuation of linezolid is **not** a part of the independent claim 1. The novelty of an invention is to be determined on the basis of the claims and not on the basis of subsequent assertions of the patent applicant.

29. A reading of the details of NCT02333799 makes it evident that is the use of combination of the drugs Bedaquiline, Linezolid and Pretomanid for treatment of XDR-TB and MDR-TB was known and disclosed before the date of priority of the Present Application.

30. Claim 1 of the Present Application is a pharmaceutical composition, comprising a therapeutically effective amount of each of linezolid, bedaquiline, and pretomanid with optionally pyrazinamide, thereby suggesting that pyrazinamide is not the core component of the claimed composition. As seen from the disclosure in NCT02333799, pharmaceutical composition of the Present Application, comprising a therapeutically effective amount of each of linezolid, bedaquiline, and pretomanid is not new and must be rejected for lack of novelty.

31. Given claim 1 of the Present Application has been shown to be anticipated by prior disclosure, the dependent claims 2-6 must also be rejected for lack of novelty.

"Nix-TB: Testing a New Potential Treatment for XDR-TB" (Published: 06.05.2015)

32. The Opponents rely on the "Nix-TB: Testing a New Potential Treatment for XDR-TB" (hereinafter "Nix-TB" and annexed herewith as **Exhibit-C**) is a factsheet about a treatment regime discussing a new study viz. the '*Nix-TB trial*' published on 06.05.2015. Given that this document was published much before the priority date of the Present Application viz. 14.10. 2015, the same can be relied on as a prior art document.

33. Nix-TB states, "*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*" (emphasis supplied, see internal page 2, LHS, para 4)

34. It is submitted that the Nix-TB clearly discloses the use of Bedaquiline, Pretomanid and Linezolid in combination as a TB drug regimen. Hence, the composition of claim 1 of the Present Application has been unambiguously disclosed and must be rejected for lack of novelty.

35. It is further submitted that the response of the Applicant to the FER on Nix-TB document stating, "*D2 fails to disclose a composition which is in the form of single or a plurality of unit dosages, the unit dosages collectively comprising the therapeutically effective amount of each of linezolid, bedaquiline and pretomanid.*" Is misleading. On one hand, the Applicant here is suggesting that the claimed composition of the Present Application is a unit dosage collectively comprising Linezolid, Bedaquiline and Pretomanid and on the other hand in the disclosed examples (see complete specification at internal pages 19-26) discloses data of a composition which is not a unit dosage.

36. Given claims 2-6 are dependent on claim 1, they also must be rejected given claim 1 is not new by virtue of prior disclosure.

CLAIMS 1-6 OF THE PRESENT APPLICATION LACK INVENTIVE STEP AND ARE CHALLENGED UNDER SECTION 25(1)(e) OF THE PATENTS ACT

37. The Patents Act under Section 2(1)(j), provides that an invention should be a new product or process involving an inventive step and capable of industrial application. 'Inventive step' as defined in Section 2(1)(ja) is '*a feature of an invention that involves technical advance as compared to the existing knowledge or having economic significance or both and that makes the invention not obvious to a person skilled in the art'*.

38. Without prejudice to other grounds raised hereinabove, the Opponents submit that claims 1-6 of the Present Application lack an inventive step and therefore should be rejected.

39. It is submitted at the outset that the Hon'ble Supreme Court in *Biswanath Prasad Radhey Shyam v/s Hindustan Metal Industries* (1979) 2SCC 511 on inventive step had noted, *"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…*

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."

40. It is submitted that it has been admitted by the Applicant that on the priority date of the Present Application, it was known that Bedaquiline, Linezolid, Pretomanid and Pyrazinamide show anti-bacterial activity and specially are useful in the treatment of TB. In fact, each of the identified drugs has been disclosed before the date of priority of the Present Application.

41. Additionally these drugs have been patented. For instance, US5688792 granted in 1997 claims Linezolid, US5668127 granted in 1997 claims Pretomanid, another patent US6087358 granted in 2000 also covers

Pretomanid, US7498343 granted in 2009 covers Bedaquiline and US2675385 covers Pyrazinamide.

42. It is submitted that on the basis of the prior art cited in the following paragraph, it would be clear that on the priority date of the Present Application a regiment for drug-resistant TB comprising Bedaquiline, Pretomanid, Linezolid and Pyrazinamide was obvious to a POSITA.

Archive History for NCT02333799 (Submitted: 06.01.2015)

43. Assuming without admitting that Archive History for NCT02333799 does not completely disclose the subject matter of the claims of the Present Application, without prejudice to the ground of novelty raised above, the Opponents again rely on Archive History for NCT02333799 (Exhibit-B). As the archival history is dated 06.01.2015, i.e. before the priority date of the Present Application, the same can be relied on as prior art.

44. It is submitted that Archive History for NCT02333799 gives information about a clinical study analysing a 3 drug regimen comprising Bedaquiline, Linezolid and Pretomanid to patients with Tuberculosis. It also disclosed the drug dosages for the 3 drugs with Bedaquiline at 400mg, Pretomanid at 200 mg and Linezolid at 200 mg.

45. On reading Archive History for NCT02333799 a POSITA working on anti-TB treatment would be motivated to use Bedaquiline, Pretomanid and Linezolid in treatment of TB for developing shorter regimens, particularly for drug-resistant TB at specific disclosed dosages for these compounds.

Diacon *et al* (Published: 26.01.2015)

46. The Opponents rely on publication titled "*Bactericidal Activity of Pyrazinamide and Clofazimine Alone and in Combinations with Pretomanid and Bedaquiline*" authored by Andreas H. Diacon *et al* (hereinafter, "Diacoan *et al*" and annexed herewith as **Exhibit-D**) published on 26.01.2015. Diacon *et al* was published before the priority date of the Present Application viz. 14.10.2015 and therefore can be relied on as prior

art document. It is pertinent to note that this study was supported by the Applicant of the Present Application.

47. Diacon *et al* recognise that there is an urgent need for new regimens to shorten tuberculosis treatment and manage patients with drug-resistant tuberculosis who are infected with HIV. They also note that there is experimental and clinical evidence suggesting that the new drugs Bedaquiline and pretomanid, combined with an existing drug, pyrazinamide, and a repurposed drug, clofazimine, may assist treatment shortening of drug-susceptible and drug-resistant tuberculosis. (See Diacan *et al*, internal page 943 at abstract, rationale)

48. Diacon *et al* in their study evaluate the 14-day bactericidal activity of clofazamine(C) and pyrazinamide(Z) in monotherapy and in combinations with Pretomanid(P) and Bedaquiline(Z).(see Diacon *et al*, internal page 943, abstract, objectives, and internal page 945 at column 2, para 1)

49. It also reported the various doses that were used in the study. For instance for the combination of Bedaquiline, Pretomanid and Pyrazinamide, they used, "*B-Pa-Z: Bedaquiline 400 mg on Day 1, 300 mg on day 2, 200 mg on Days 3–14; pretomanid 200 mg; pyrazinamide 1,500 mg*." (see Diacon *et al*, internal page 952, Box 1, Treatment Groups)

50. Diacon *et al* further reported that, "*Treatment-emergent adverse events were experienced by 65 (61.9%) patients, but in only 29 (27.6%) were these considered treatment-related.*" (See Diacon *et al*, internal page 948, column 3, para 3 at safety and tolerability)

51. Further, they reported that, "*This study has shown the combination of B-Pa-Z to have activity similar to that of the current standard anti-TB regimen over the first 14 treatment days*." (See Diacon *et al*, internal page 952, column 1 at Conclusions). The authors further add that the results indicate, "*The B-Pa-Z combination can now be taken forward to longer clinical studies assessing its activity in larger patient numbers with due attention to continued close observation of the QT interval. The suitability of this regimen for patients with multidrugresistant TB, who have relatively high*

reported rates of phenotypical Z resistance in many areas, should be studied only in the setting of Z resistance testing." (See Diacon *et al*, internal page 952, columns 2 and 3)

52. Therefore, a POSITA on reading Diacon *et al* would be taught that use of Bedaquiline, Pretomanid and Pyrazinamide in treatment of TB has shown safe results. Further, a POSITA on reading Diacon *et al* would also be motivated to take forward the study related to the identified combination of these drugs. That is, a POSITA working on an anti-TB regimen would be motivated to work on a regimen comprising a combination of Bedaquiline, Pretomanid and Pyrazinamide.

53. Hence, a POSITA on reading the Archive History for NCT02333799 with Diacon *et al*, would be motivated to explore the combinations of Bedaquiline, Delamanid, Pretomanid and Pyrazinamide for the treatment of drug-resistant TB.

Conradie *et al* (Published: March, 2014)

54. The Opponents rely on publication titled "*Clinical Access to Bedaquiline Programme for the treatment of drug-resistant tuberculosis*" S Afr Med J 2014;104(3):164-166 authored by F Conradie *et al* (hereinafter, "Conradie *et al*" and annexed herewith as **Exhibit-E**) published in March, 2014. Conradie *et al* was published before the priority date of the Present Application viz. 14.10.2015 and therefore can be relied on as prior art document.

55. Conradie *et al* documents the process of implementation of the Clinical Access to Bedaquiline Programme in South Africa. Conradie *et al* emphasize that, "*Bedaquiline must form part of a long-term strategy aimed at combatting DR-TB. Other drugs that might include linezolid – an oxazalidinone that is also used for the treatment of resistant Gram-positive infections*…" (See Conradie *et al,* internal page 166 at conclusions)

56. Hence, a POSITA on reading Conradie *et al* would be motivated to use linezolid in the regimen being considered for drug-resistant TB.

57. Therefore, a POSITA on reading Conradie *et al* with Diacon *et al* and Archive History for NCT02333799 would be motivated to use Linezolid in combination with Bedaquiline, Pretomanid and Pyrazinamide in treatment of TB, particularly drug-resistant TB.

Prats *et al* (Published: 2013)

58. The Opponents rely on publication titled "*Linezolid for the treatment of drug-resistant tuberculosis in children: A review and recommendations*" (http://dx.doi.org/10.1016/j.tube.2013.10.003) authored by Anthony J. Garcia-Prats*et al* (hereinafter, "Prats *et al*" and annexed herewith as **Exhibit-F**) published in 2013. Prats *et al* was published before the priority date of the Present Application viz. 14.10.2015 and therefore can be relied on as prior art document.

59. Prats *et al*, "…*identified 8 reports of 18 children receiving linezolid for difficult to treat DR-TB. All 18 had culture conversion and 15 of 18 had successful long-term treatment outcomes*" (See Prats *et al*, internal page 1, Summary)

60. Further, Prats *et al* point out that, "*The WHO 2008 guidelines recommend the use of Group 5 drugs, including linezolid, only when a regimen containing 4 drugs with likely activity cannot be created from Groups 1-4, though no other specific recommendations regarding linezolid were made. The recommended dosage is 600 mg twice daily for 4-6 weeks, then 600 mg once daily*" (See Prats *et al*, internal page 9, para 3). On dosage, the authors further add that, "*Generally children 12 years of age should receive the same dose as adults, and we have had success using a dose of 10 mg/kg once daily up to 300 mg for children 12 years of age, as in our cases included in this report [62,63]. For children 3 months to 12 years we recommend a dose of 10 mg/kg twice daily. For children with extensive disease or TB meningitis it may be advisable to use up to a higher total daily dose of 600 mg, at least initially*." (see Prats *et al*, internal page 9,

RHS, para 3). That is, Linezoline was recommended to be one of the add-on drugs in regimens for drug-resistant TB with at least 600mg.

61. The authors further state, "*We recommend linezolid for use in children with XDR-TB or for those who have failed treatment for MDR-TB with or without additional drug resistance. Linezolid is likely to be the most active drug for such children and could make the difference between treatment success and failure. Linezolid should be considered for children with MDR-TB with additional fluoroquinolone or second-line injectable resistance (Pre-XDR-TB), especially those who have extensive disease or meningitis. Linezolid should also be considered for children with MDR-TB meningitis, especially those who have had a slow or poor response to standard treatment. The good CSF penetration of linezolid makes it particularly useful for DR-TB meningitis, as there are few second-line agents with potent antituberculosis activity and good CSF penetration.*" (see Prats *et al*, internal page 9, LHS, para 4, and RHS, para 1)

62. Prats *et al* also suggest future course of study and state, "*Considering what appears to be potent activity of linezolid in difficult DR-TB cases, exploration of treatment intensification with a short course of linezolid in children with severe DR-TB disease may be warranted.*" (Prats *et al*, internal page 10, LHS at para 5)

63. The authors conclude that, "*Despite modest activity of linezolid against Mtb in vitro and in animal models, emerging data in adults have shown it to be effective in difficult cases of DR-TB. These benefits are currently offset by its high cost, and frequent and often severe time- and dose-dependent toxicity. Though data are limited, the efficacy and adverse effects of linezolid in treatment of children with DR-TB reported to date are similar to adults. For children with MDR-TB with additional resistance or with XDR-TB, linezolid may however make the difference between a successful or poor outcome, as demonstrated in many of the paediatric cases described to date. Because of its good CSF penetration, linezolid may also be an important option for children with MDR-TB meningitis, for which outcomes are often*

poor and other drugs with potent antituberculosis activity and good CSF penetration are limited." (internal page 10, LHS, para 8 and RHS at para 1)

64. Hence, a POSITA on reading Prats *et al* would be motivated to use Linezolid in DR-TB regimens for developing shorter regimens and would also be taught that the dose for the same could a daily dose of at least 600 mg.

65. On reading Prats *et al* with Conradie *et al,* Archive History for NCT02333799 and Diacon *et al,* a POSITA would be motivated to use Linezolid in combination with Bedaquiline, Pretomanid and Pyrazinamide in treatment of TB for developing shorter regimens, particularly for drug-resistant TB at specific disclosed dosage.

"Nix-TB: Testing a New Potential Treatment for XDR-TB" (Published: 06.05.2015)

66. Without prejudice to the ground of novelty raised above, the Opponents rely on the "Nix-TB: Testing a New Potential Treatment for XDR-TB" ("Nix-TB", **Exhibit-C**) which is a factsheet about a treatment regime discussing a new study viz. the '*Nix-TB trial*' published on 06.05.2015. Given that this document was published much before the priority date of the Present Application viz. 14.10. 2015, the same can be relied on as a prior art document.

67. Nix-TB tests a three-drug regimen consisting of bedaquiline, pretomanid; and linezolid (See Exhibit- C, see internal page 2, LHS, para 4). It further, discloses the use of Bedaquiline, Pretomanid and Linezolid in combination as a TB drug regimen.

68. Therefore, a POSITA on reading Nix-TB test would be motivated to look at the combination of Bedaquiline, Pretomanid and Linezolid while trying to develop a shorter regimen for drug-resistant TB.

69. On reading Nix-TB with Archive History for NCT02333799, Prats *et al,* Conradie *et al* and Diacon *et al,* a POSITA would be motivated to use Linezolid in combination with Bedaquiline, Pretomanid and Pyrazinamide

in treatment of TB for developing shorter regimens. Particularly the POSITA would know the preferred dosage for each of the drugs with Bedaquiline at 400mg, Pretomanid at 200 mg, Linezolid at 200 mg/600mg (pediatric and adult) at Pyrazinamide at about 1200 mg.

70. Hence a POSITA skilled in the art on reading the above disclosed prior art documents would arrive at the pharmaceutical composition of claim 1 of the Present Application comprising therapeutically effective amount of linezolid, bedaquiline, and pretomanid, and optionally pyrazinamide.

71. Therefore, claims 1-6 of the Present Application are obvious, lacking an inventive step and should be rejected for failure to meet the test of Section 2(1)(ja) of the Patents Act.

THAT CLAIMS OF THE PRESENT APPLICATION DO NOT SATISFY THE TEST OF SECTION 3(d) AND SECTION 3(e) AND SECTION 3(i), THEREFORE ARE OBJECTED TO UNDER SECTION 25(1)(f)

72. It is submitted that a representation of opposition may be filed under Section 25(1)(f) of the Patents Act, on the ground of the claimed invention not being an invention within the meaning of the Patents Act, 1970.

73. It is the Opponents' case the claimed invention of the Present Application is not an invention within the meaning of Section 3(d), Section 3(e) and Section 3(i) of the Patents Act.

Claims of Present Application not an invention under Section 3(d)

74. Without prejudice to other grounds raised herein, the Opponents raise objection under Section 25(1)(f) as the claims of the Present Application fail under Section 3(d).

75. It is submitted that the test of Section 3(d) has to be satisfied independent of Section 2(1)(j) and S. 2(1)(ja) [*see Novartis AG versus Union of India and Others* (2013) 6 SCC 1]. Further, under Section 3(d) the patent applicant has the burden of showing enhanced (therapeutic) efficacy of modified known

substance. Further, such data of enhanced therapeutic efficacy has to be provided by the Applicant (as laid down by the Hon'ble IPAB in *Novartis AG versus Union of India*, MIPR 2009 (2) 0345, para 9(xvii)) and the burden is the on the Applicant to do so.

76. It is submitted that the Archival History for NCT02333799 discloses drug regimen comprising Bedaquiline, Linezolid and Pretomanid. The complete specification of the Present Application has failed to disclose any clinical data / efficacy / efficiency data pertaining to the composition of the 6 claims showing enhanced efficacy of the known composition.

77. It is further submitted that the Applicant has failed to submit any data to indicate any sort of enhanced therapeutic efficacy of the claimed composition over the known composition in the Archival History for NCT02333799.

78. Hence, in absence of any data suggesting enhanced therapeutic efficacy of the composition claimed in claims 1-6 of the Present Application, the same ought to be rejected under Section 3(d) of the Patents Act.

Claims of Present Application not an invention under Section 3(e)

79. Without prejudice to other grounds raised herein, the Opponents raise objection on the ground that the alleged invention of claims 1-6 of the Present Application fail the test of Section 3(e). The Patents Act excludes patentability of 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.

80. Further, it is a settled principle that, "*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.*" (See order of the Asst. Controller of Patents & Designs in patent application no. 314/MUM/2008 dated 05.10.2012, at lines 3-5 at internal page 7 annexed herewith as **Exhibit-G**).

81. Further the burden on the Applicant to show synergism and the burden of the same is not discharged by merely indicating the weight of each of the ingredients of the composition. The Asst. Controller of Patents & Designs, while rejecting application no. 3725/CHENP/2006, on grounds of Section 3(e) noted, "*Applicant doesn't provide any supportive experimental data or comparative examples highlighting the surprising and or synergistic effect of the claimed formulation over the prior art compositions. Instead examples 1, 2 and 3 provide only the amount of individual components in grams.*" (See the order of the Controller in patent application no. 3725/CHENP/2006 dated 09.10.2012, herewith annexed as **Exhibit-H** at internal page 4. Para 8)

82. It is further submitted that Deputy Controller of Patents and Designs in the application 5461/DELNP/2008 had noted, *"... claims as claimed in impugned application is a mere admixture resulting only in the aggregation of the properties of the components thereof. A synergistic composition should show unexpectedly new property or better efficacy than a mere aggregation of the properties of its components. There is no other essential component in the claimed composition that could justify a synergistic effect to validate a composition claim…I am of the opinion that the claimed invention is not only lacking inventive step but also falling within the provisions of section 3(e)"* (See the order of the Controller in patent application no. 5461/DELNP/2008 dated 21.07.2015, herewith annexed as **Exhibit-I** at internal page 11)

83. Further, it is submitted that 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".

84. It is submitted that claims 1-6 are not patentable under S. 3(e). The complete specification of the Present Application does not disclose any specific 'composition' or any process to make any such specific 'composition' as has been claimed. The Applicant has not disclosed details for even a single, specific composition that displays synergistic relation between the drug components and the excipients. At the best the composition claimed by the Applicant is mere combination of 'integers' and does not show any synergistic effect beyond the individual characteristic of each of the drugs.

85. In light of lack of any data indicating synergistic effect of the combination of the integers of the composition claimed in claims 1-6, the same must be rejected for failing the test under Section 3(e).

Claims of Present Application not an invention under Section 3(i)

86. Without prejudice to other grounds raised herein, the Opponents raise objection on the ground that the alleged invention of claims 1-6 of the Present Application fail the test of Section 3(i). The provision excludes, "*any process for the medicinal, surgical, curative, prophylactic diagnostic, therapeutic or other treatment of human beings or any process for a similar treatment of animals to render them free of disease or to increase their economic value or that of their products.*"

87. That is, the Patents Act excludes from patentability the subject matter which is a method for treatment of human beings. In the context of the Present Application, the claims were modified from method of use to composition claims. It is pertinent to note that it has been recognized in the 'Guidelines for Examination of Patent Applications in the Field of Pharmaceuticals' that: *"In the field of pharmaceuticals, it is noticed that method of treatments are often claimed in the guise of composition claims."* (see para Paragraph 10.20)

88. Further, it is further submitted that Assistant Controller of Patents and Designs in the application no. 201647001874 has disallowed method of treatment claims and amended as composition claims and noted, "'3. *Claims 1-12 although refers to a pharmaceutical composition but actually trying to claim the treatment by which the compounds are administered. This is not only vague but also appears to be method of treatment in disguised form…*
6. Claims 1-12 although refers to a pharmaceutical composition but actually trying to claim the treatment by which the compounds are administered. This is not only vague but also appears to be method of treatment in disguised form…." (See order of the Assistant Controller of Patents and Designs in application no. 201647001874 dated 17.07.2020 herewith annexed as **Exhibit-J**)

89. It is submitted that the present claims 1-6 though appear to be 'composition' claims, are actually a method of treatment. This is supported by the fact that the complete specification does not disclose of composition with requisite weight of each of the ingredients and the complete specification also admits that it discloses a method of treatment. (see complete specification, internal page 3, Summary of Invention)

90. The claims 1-6 if granted in the current claims 1-6 are granted, it would block third parties from using a drug regimen comprising the drugs identified in claim 1.

91. As a method of treatment claim is *per se* cannot be considered as an invention under section 3(i) of the Patents Act claims 1-6 of the Present Application should be rejected.

THAT CLAIMS OF THE PRESENT APPLICATION MUST BE REJECTED AS THE COMPLETE SPECIFICATION DOES NOT SUFFICIENTLY AND CLEARLY DESCRIBE THE INVENTION

92. Without prejudice to the grounds raised above, the Opponents raise an objection under Section 25(1)(g). It is the case of the Opponents that the

Present Application does not sufficiently and clearly describe the invention claimed.

93. It is submitted that claim 1 of the Present Application covers a composition however the complete specification of the Present Application does not indicate what kind of composition it is. There are no suggestions about the weight of each of the ingredients. There are no references of what kind of composition is being claimed such as a capsule, injection or tablet.

94. Further, there is no disclosure in the complete specification showcasing how the claimed composition is to be developed.

95. Further, claim 4 of the Present Application claims, "*The pharmaceutical composition as claimed in claim 1, wherein the pharmaceutical composition is in the form of a plurality of unit dosages, the plurality of unit dosages collectively comprising the therapeutically effective amount of each of linezolid, bedaquiline and pretomanid, and optionally pyrazinamide or a pharmaceutically acceptable salt of each thereof.*" However, the complete specification nowhere mentions when single or plurality of unit dosage applies. Further, there is no clarity in the complete specification on what *plurality of unit dosages of each of linezolid, bedaquiline and Pretomanid* means.

96. Further, claim 1 does not lay out the dosage of each of the identified drugs, does not specify the pharmaceutically acceptable salt and the pharmaceutically acceptable carrier which is a part of the claimed composition.

97. In absence of disclosure in the complete specification detailing each component of the complete specification the complete specification fails to fully and particularly describe the invention as required under Section 10 of the Patents Act. Therefore, claim 1 and its dependent claims 1-6 should be rejected.

THAT THE APPLICANT FAILED TO DISCLOSE INFORMATION REQUIRED BY SECTION 8, HENCE THE OPPOSITION IS RAISED UNDER SECTION 25(1)(h)

98. Section 25(1) (h) of the Patents Act provides a ground for opposition if the patent applicant has not furnished information required under Section 8 of the Patents Act, within the time prescribed by law.

99. The Opponent raises an objection under Section 25(1)(h) without prejudice to the grounds raised above. It is submitted that the Applicant has not complied with the mandatory requirements of Section 8 of the Patents Act.

100. It is submitted that the Applicant has failed to provide details of the status of the application where it has received negative office action. The details of the applications not disclosed are provided below:

US20180280401	Non-final rejection dated 31/Dec/2018, 28/June/2019 and final rejection dated 08/Jan/2020	None of the 3 have been disclosed to IPO
EP3362068	Negative 'Supplementary Search Report' dated 16/May/2019	Not disclosed.

101. Given that complete information related to the corresponding applications in other jurisdictions has not been disclosed the claims of the Present Application must be rejected.

PRAYERS

In view of the above said references Opponent prays as follows:

a) To take this representation on record, grant a hearing and be allowed to lead evidence (documentary and oral) before any order is passed;

b) To reject the claims 1-6 of Application No. 201817014361;

c) To allow amendment of the Opposition as and when the need may arise;

d) To allow the Opponent to make further submissions in case the Applicant amends the claims;

e) For costs in this matter;

f) For any further and other relief in the facts and circumstances that may be granted in favour of the Opponent in the interest of justice.

Dated this the 24th day of July 2020.

Priyam Cherian

Counsel for the Opponents

To
The Controller,
The Patent Office
DELHI

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(54) Title: COMPOSITIONS AND METHODS FOR COMBINATION ANTIVIRAL THERAPY

(57) Abstract: The present invention relates to therapeutic combinations of [9-[R-2-[[(S)-[[(S)-1-(isopropoxycarbonyl)ethyl]amino]-phenoxyphosphinyl]methoxy]propyl]adenine(GS-7340) and (2R, 5S, cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine,(-)-cis FTC, Emtriva™ and their physiologically functional derivatives. The combinations may be useful in the treatment of HIV infections, including infections with HIV mutants bearing resistance to nucleoside and/or non-nucleoside inhibitors. The present invention is also concerned with pharmaceutical compositions and formulations of said combinations of GS-7340 and emtricitabine, and their physiologically functional derivatives, as well as therapeutic methods of use of those compositions and formulations.

WO 2004/064846 A1

COMPOSITIONS AND METHODS FOR COMBINATION ANTIVIRAL THERAPY

This non-provisional application claims the benefit of Provisional Application Nos. 60/440,308 and 60/440,246, both filed January 14, 2003, which is incorporated herein by reference.

FIELD OF THE INVENTION

The invention relates generally to combinations of compounds with antiviral activity and more specifically with anti-HIV properties. In particular, it relates to chemically stable combinations of structurally diverse anti-viral agents.

BACKGROUND OF THE INVENTION

Human immunodeficiency virus (HIV) infection and related diseases are a major public health problem worldwide. Human immunodeficiency virus type 1 (HIV-1) encodes at least three enzymes which are required for viral replication: reverse transcriptase (RT), protease (Prt), and integrase (Int). Although drugs targeting reverse transcriptase and protease are in wide use and have shown effectiveness, particularly when employed in combination, toxicity and development of resistant strains have limited their usefulness (Palella, et al *N. Engl. J. Med.* (1998) 338:853-860; Richman, D. D. *Nature* (2001) 410:995-1001). Human immunodeficiency virus type 1 (HIV-1) protease (Prt) is essential for viral replication and is an effective target for approved

antiviral drugs. The HIV Prt cleaves the viral Gag and Gag-Pol polyproteins to produce viral structural proteins (p17, p24, p7 and p6) and the three viral enzymes. Combination therapy with RT inhibitors has proven to be highly effective in suppressing viral replication to unquantifiable levels for a sustained period of time. Also, combination therapy with RT and Prt inhibitors (PI) have shown synergistic effects in suppressing HIV replication. Unfortunately, a high percentage, typically 30 to 50% of patients currently fail combination therapy due to the development of drug resistance, non-compliance with complicated dosing regimens, pharmacokinetic interactions, toxicity, and lack of potency. Therefore, there is a need for new HIV-1 inhibitors that are active against mutant HIV strains, have distinct resistance profiles, fewer side effects, less complicated dosing schedules, and are orally active. In particular, there is a need for a less onerous dosage regimen, such as once per day oral dosing, optimally with as few pills as possible.

The use of combinations of compounds can yield an equivalent antiviral effect with reduced toxicity, or an increase in drug efficacy. Lower overall drug doses can reduce the frequency of occurrence of drug-resistant variants of HIV. Many different methods have been used to examine the effects of combinations of compounds acting together in different assay systems (Furman WO 02/068058). Lower doses predict better patient compliance when pill burden decreases, dosing schedules are simplified and, optionally if synergy between compounds occurs (Loveday, C. "Nucleoside reverse transcriptase inhibitor resistance" (2001) *JAIDS Journal of Acquired Immune Deficiency Syndromes* 26:S10-S24). AZT (zidovudine™, 3'-azido, 3'-deoxythymidine) demonstrates synergistic antiviral activity *in vitro* in combination with agents that act at HIV-1 replicative steps other than reverse transcription, including recombinant soluble CD4 castanospermine and recombinant interferon-α. However, it must be noted that combinations of compounds can give rise to increased cytotoxicity. For example, AZT and recombinant interferon-α have an increased cytotoxic effect on normal human bone marrow progenitor cells.

Chemical stability of combinations of antiviral agents is an important aspect of co-formulation success and the present invention provides examples of such combinations.

There is a need for new combinations of orally-active drugs for the treatment of patients infected with certain viruses, e.g. HIV, that provide enhanced therapeutic safety and efficacy, impart lower resistance, and predict higher patient compliance.

SUMMARY OF THE INVENTION

The present invention provides combinations of antiviral compounds, in particular compositions and methods for inhibition of HIV. In an exemplary aspect, the invention includes a combination of GS-7340 and emtricitabine which has anti-HIV activity. The combination of GS-7340 and emtricitabine is both chemically stable and either synergistic and/or reduces the side effects of one or both of GS-7340 and emtricitabine. Increased patient compliance is likely in view of the lower pill burden and simplified dosing schedule.

The present invention relates to therapeutic combinations of 9-[*R*-2-[[(*S*)-[[(*S*)-1-(isopropoxycarbonyl)ethyl]amino]-phenoxyphosphinyl]methoxy]propyl]adenine (GS-7340) and (2*R*, 5*S*, cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine), and their use in the treatment of HIV infections including infections with HIV mutants bearing resistance to nucleoside and/or non-nucleoside inhibitors. The present invention is also concerned with pharmaceutical compositions and formulations of said combinations of GS-7340 and emtricitabine. Another aspect of the invention is a pharmaceutical formulation comprising a physiologically functional derivative of GS-7340 or a physiologically functional derivative of emtricitabine, including FTC and 3TC.

FTC is 4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one and includes all diastereomers, enantiomers, and mixtures thereof, in any proportion. For example, FTC includes the single enantiomer emtricitabine.

Therapeutic combinations and pharmaceutical compositions and formulations of the invention include the combination of phosphonamidate PMEA or PMPA compounds with FTC or (2*R*,5*S*,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (3TC, Lamivudine, Epivir™), and their use in the treatment of HIV infections.

One aspect of the invention is a method for the treatment or prevention of the symptoms or effects of an HIV infection in an infected animal which comprises

administering to, i.e. treating, said animal with a therapeutically effective amount of a formulation comprising 9-[*R*-2-[[(*S*)-[[(*S*)-1-(isopropoxycarbonyl)ethyl]amino]-phenoxyphosphinyl]methoxy]propyl]adenine (GS-7340) or a physiologically functional derivative thereof, and (2*R*,5*S*,cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine) or a physiologically functional derivative thereof.

Another aspect of the invention is a unit dosage form of a therapeutic combination comprising GS-7340 and emtricitabine, or physiological functional derivatives thereof. The unit dosage form may be formulated for administration by oral or other routes and is unexpectedly chemically stable in view of the properties of the structurally diverse components.

Another aspect of the invention is directed to a chemically stable combination antiviral compositions comprising GS-7340 and emtricitabine. In a further aspect of the invention, the chemically stable combinations of GS-7340 and emtricitabine further comprise a third antiviral agent. In this three-component mixture, the unique chemical stability of GS-7340 and emtricitabine is taken advantage of in order to enable the combination with the third antiviral agent. Particularly useful third agents include, by way of example and not limitation, those of Table A. Preferably, the third component is an agent approved for antiviral use in humans, more preferably, it is an NNRTI or a protease inhibitor (PI), more preferably yet, it is an NNRTI. In a particularly preferred embodiment, the invention is directed to a combination of the chemically stable mixture of GS-7340 and emtricitabine together with efavirenz.

Another aspect of the invention is a patient pack comprising at least one, typically two, and optionally, three active ingredients selected from GS-7340, emtricitabine, and other antiviral agents, and an information insert containing directions on the use of GS-7340 and emtricitabine together in combination.

Another aspect of the invention is a process for preparing the combinations hereinbefore described, which comprises bringing into association GS-7340 and emtricitabine of the combination in a medicament to provide an antiviral effect. In a further aspect of the present invention, there is provided the use of a combination of the present invention in the manufacture of a medicament for the treatment of any of the aforementioned viral infections or conditions.

DETAILED DESCRIPTION OF THE INVENTION

While the invention will be described in conjunction with the enumerated embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the present invention as defined by the claims.

DEFINITIONS

Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

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 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. By way of example and not limitation, in one aspect of the invention, "chemically stable" means that GS-7340 is not substantially degraded by the acidity of emtricitabine. "Substantially" in this context means at least about less than about 10%, preferably less than about 1%, more preferably less than about 0.1%, more preferably yet, less than about 0.01% acid degradation of GS-7340 over a 24-hour period when the products are in a pharmaceutical dosage form.

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 anti-viral effect denotes an antiviral effect which is greater than the predicted purely additive effects of the individual compounds of the combination.

The term "physiologically functional derivative" means a pharmaceutically active compound with equivalent or near equivalent physiological functionality to GS-7340 or emtricitabine when administered in combination with another pharmaceutically active compound in a combination of the invention. As used herein, the term "physiologically functional derivative" includes any physiologically acceptable salt, ether, ester, prodrug, solvate, stereoisomer including enantiomer, diastereomer or stereoisomerically enriched or racemic mixture, and any other compound which upon administration to the recipient, is capable of providing (directly or indirectly) such a compound or an antivirally active metabolite or residue thereof.

"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.

"Alkyl" means a saturated or unsaturated, branched, straight-chain, branched, or cyclic hydrocarbon radical derived by the removal of one hydrogen atom from a single carbon atom of a parent alkane, alkene, or alkyne. Typical alkyl groups consist of 1-18 saturated and/or unsaturated carbons, such as normal, secondary, tertiary or cyclic carbon atoms. Examples include, but are not limited to: methyl, Me ($-CH_3$), ethyl, Et ($-CH_2CH_3$), acetylenic ($-C\equiv CH$), ethylene, vinyl ($-CH=CH_2$), 1-propyl, n-Pr, n-propyl ($-CH_2CH_2CH_3$), 2-propyl, i-Pr, i-propyl ($-CH(CH_3)_2$), allyl ($-CH_2CH=CH_2$), propargyl ($-CH_2C\equiv CH$), cyclopropyl ($-C_3H_5$), 1-butyl, n-Bu, n-butyl ($-CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl, i-Bu, i-butyl ($-CH_2CH(CH_3)_2$), 2-butyl, s-Bu, s-butyl ($-CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl, t-Bu, t-butyl ($-C(CH_3)_3$), 1-pentyl, n-pentyl, ($-CH_2CH_2CH_2CH_2CH_3$), 2-pentyl ($-CH(CH_3)CH_2CH_2CH_3$), 3-pentyl ($-CH(CH_2CH_3)_2$), 2-methyl-2-butyl ($-C(CH_3)_2CH_2CH_3$), cyclopentyl ($-C_5H_9$), 3-methyl-2-butyl ($-CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl ($-CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl ($-CH_2CH(CH_3)CH_2CH_3$), 1-hexyl ($-CH_2CH_2CH_2CH_2CH_2CH_3$), 5-hexenyl ($-CH_2\ CH_2CH_2CH_2CH=CH_2$) 1-hexyl ($-CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl ($-CH(CH_2CH_3)(CH_2CH_2CH_3)$), cyclohexyl ($-C_6H_{11}$), 2-methyl-2-pentyl ($-C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl ($-CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl ($-CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl ($-C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl ($-CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl ($-C(CH_3)_2CH(CH_3)_2$), and 3,3-dimethyl-2-butyl ($-CH(CH_3)C(CH_3)_3$.

"Aryl" means a monovalent aromatic hydrocarbon radical of 6-20 carbon atoms derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Typical aryl groups include, but are not limited to, radicals derived from benzene, substituted benzene, naphthalene, anthracene, biphenyl, and the like.

"Arylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced

with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. The arylalkyl group 6 to 20 carbon atoms e.g., the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the arylalkyl group is 1 to 6 carbon atoms and the aryl moiety is 5 to 14 carbon atoms.

"Substituted alkyl", "substituted aryl", and "substituted arylalkyl" mean alkyl, aryl, and arylalkyl respectively, in which one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents include, but are not limited to, -X, -R, $-O^-$, -OR, -SR, $-S^-$, $-NR_2$, $-NR_3$, =NR, $-CX_3$, -CN, -OCN, -SCN, -N=C=O, -NCS, -NO, $-NO_2$, $=N_2$, $-N_3$, NC(=O)R, -C(=O)R, -C(=O)NRR $-S(=O)_2O^-$, $-S(=O)_2OH$, $-S(=O)_2R$, $-OS(=O)_2OR$, $-S(=O)_2NR$, -S(=O)R, $-OP(=O)O_2RR$, $-P(=O)O_2RR$ $-P(=O)(O^-)_2$, $-P(=O)(OH)_2$, -C(=O)R, -C(=O)X, -C(S)R, -C(O)OR, $-C(O)O^-$, -C(S)OR, -C(O)SR, -C(S)SR, -C(O)NRR, -C(S)NRR, -C(NR)NRR, where each X is independently a halogen: F, Cl, Br, or I; and each R is independently -H, alkyl, aryl, heterocycle, or prodrug moiety.

"Heteroaryl" and "Heterocycle" refer to a ring system in which one or more ring atoms is a heteroatom, e.g. nitrogen, oxygen, and sulfur. Heterocycles are described in: Katritzky, Alan R., Rees, C.W., and Scriven, E. Comprehensive Heterocyclic Chemistry (1996) Pergamon Press; Paquette, Leo A.; Principles of Modern Heterocyclic Chemistry W.A. Benjamin, New York, (1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28. Exemplary heterocycles include but are not limited to substituents, i.e. radicals, derived from pyrrole, indole, furan, benzofuran, thiophene, benzothiophene, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-quinolyl, 3-quinolyl, 4-quinolyl, 2-imidazole, 4-imidazole, 3-pyrazole, 4-pyrazole, pyridazine, pyrimidine, pyrazine, purine, cinnoline, pthalazine, quinazoline, quinoxaline, 3-(1,2,4-*N*)-triazolyl, 5-(1,2,4-*N*)-triazolyl, 5-tetrazolyl, 4-(1-*O*, 3-*N*)-oxazole, 5-(1-*O*, 3-*N*)-oxazole, 4-(1-*S*, 3-*N*)-thiazole, 5-(1-*S*, 3-*N*)-thiazole, 2-benzoxazole, 2-benzothiazole, 4-(1,2,3-*N*)-benzotriazole, and benzimidazole.

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 R and S, d and l 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 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.

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.

The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

"Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

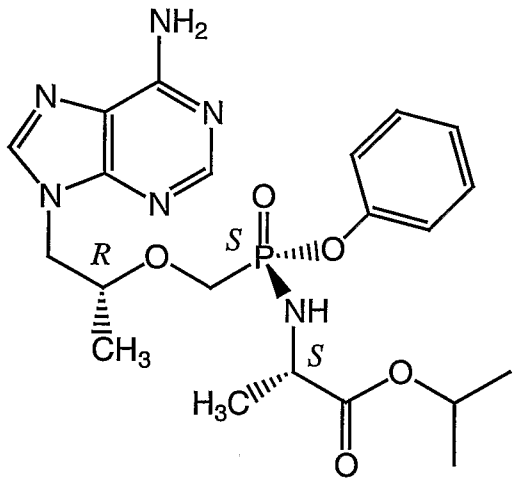
"Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

ACTIVE INGREDIENTS OF THE COMBINATIONS

The present invention provides novel combinations of two or more active ingredients being employed together. In some embodiments, a synergistic antiviral effect is achieved. In other embodiments, a chemically stable combination is obtained. The combinations include at least one active ingredient selected from (1) GS-7340 and physiologically functional derivatives, and at least one active ingredient selected from

(2) emtricitabine and physiologically functional derivatives. The term "synergistic antiviral effect" is used herein to denote an antiviral effect which is greater than the predicted purely additive effects of the individual components (a) and (b) of the combination.

GS-7340 is an antiviral prodrug known as: 9-[(*R*)-2-[[(*S*)-[[(*S*)-1-(isopropoxycarbonyl)ethyl]amino]phenoxyphosphinyl]methoxy] propyl] adenine, and has the structure:



CAS Registry Numbers for GS-7340 include: 379270-37-8 and for GS-7340 fumarate include: 379270-38-9.

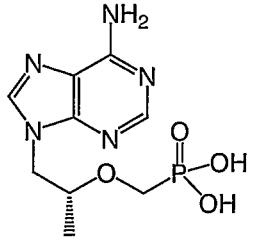
The prodrug GS-7340 is the subject of commonly owned, pending application, US Serial No. 09/909,560, filed July 20, 2001 and Becker et al WO 02/08241. GS-7340 is an isopropyl alanyl phosphonamidate, phenyl ester prodrug of tenofovir (PMPA). *In vivo* and *in vitro* characterization shows that selective intracellular activation of GS 7340 leads to preferential distribution in lymphatic tissues (Lee W, He G, Mulato A, Delaney W, Eisenberg E, Cihlar T, Xiong S, Miller M, Gill S, Shibata R, Gibbs C *Internatiional Conference on Retroviruses and Opportunistic Infections* 2002, 9th Conf:February 24-28, Abs 384-T; "Evaluation of Cidofovir, Adefovir, Tenofovir and Related Phosphonate Analogs for Inhibition of Orthopoxvirus Replication." Keith KA, Hitchcock MJM, Lee WA, Holy A, Kern ER (2002) *Antiviral Research*, 53:3, Abs 95; "Structure and activity relationship for tenofovir amidates, novel intracellular prodrugs for tenofovir" He GX, Eisenberg EJ, Cihlar T, Chapman H, Lee WA *Antiviral Research* 2001, 50:1, Abs 123)

Preclinical data on tenofovir prodrugs were presented at the 15th ICAR meeting in Prague, Czech Republic. It was demonstrated that GS-7340 is active against both cowpox and vaccinia viruses at concentrations of 20 to 100 μM. GS-7340 is 1000 times more effective than tenofovir against HIV in culture. GS-7340 has an *S*-configuration at the phosphorus. This *S*-configuration diastereomer is 10-fold more effective than the diastereomer with the *R*-configuration (Nucleosides, Nucleotides and Their Biological Applications - XIV International Roundtable (Part II), San Francisco, CA, USA). The large-scale separation of GS-7340 diastereomers and enantiomers has been achieved ("Practical synthesis, separation, and stereochemical assignment of the PMPA prodrug GS-7340" Chapman et al (2001) *Nucleosides, Nucleotides & Nucleic Acids*, 20(4-7):621-628.

In vitro data showed that GS-7340 was effective against HIV and HBV in a variety of cell types. *In vivo* studies in dogs and rhesus monkeys revealed that the compound is orally bioavailable (20%), stable in plasma and selectively hydrolyzed inside lymphatic tissue. *In vitro*, the most potent compounds displayed an EC50 value of 0.0008 μM against HIV, and a half-life of 103 min in plasma. The prodrugs have demonstrated oral bioavailability, stability and ability to rapidly convert to tenofovir inside lymphatic cells. GS-7340 is 100-fold more effective than tenofovir against HIV in culture, and the diastereomer with an *S*-configuration at the P group is 10-fold more effective than that with an *R*-configuration. GS-7340 is in phase I/II trials for the potential treatment of HIV and other viral infections.

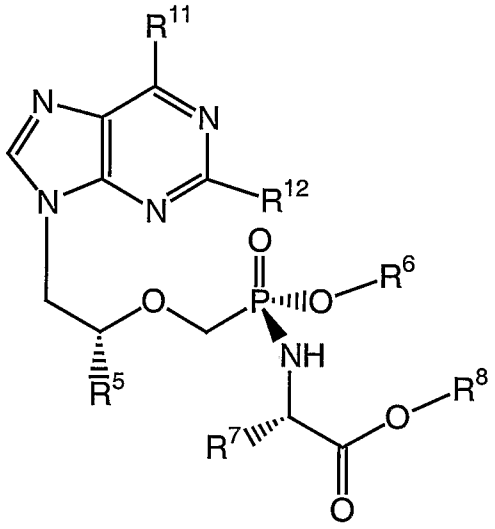
The term "GS-7340" includes all combinations of stereochemistry at the three designated centers as well as all diasteromeric and racemic mixtures. Examples include R,R,R; R,R,S; R,S,R; S,R,R; R,S.S; S,R.S; S,S,R; and S,S,S compounds and their racemic, enantiomerically enriched and partially racemic mixtures.

PMPA (US Patent Nos. 4808716, 5733788, 6057305) has the structure:



The chemical names of PMPA include: (*R*)-9-(2-phosphonylmethoxypropyl)adenine; and phosphonic acid, [[(1*R*)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]. The CAS Registry number is 147127-20-6.

Physiologically functional derivatives of GS-7340 include phosphonamidate PMEA (adefovir, 9-((*R*)-2-(phosphonomethoxy)ethyl)adenine) and PMPA compounds. Exemplary combinations include a phosphonamidate PMEA or PMPA compound in combination with emtricitabine or a physiologically functional derivative. Phosphonamidate PMEA and PMPA compounds have the structures:

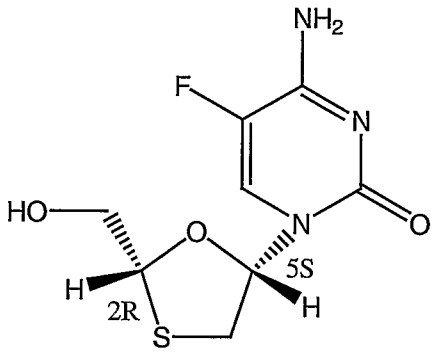


PMEA (R^5 = H) and PMPA (R^5 = CH_3). R^6 and R^8 are independently selected from H, C_1–C_6 alkyl, C_1–C_6 substituted alkyl, C_6–C_{20} aryl, C_6–C_{20} substituted aryl, C_6–C_{20} arylalkyl, C_6–C_{20} substituted arylalkyl. R^7 is the side chain of any naturally-occurring or pharmaceutically acceptable amino acid and which, if the side chain comprises carboxyl, the carboxyl group is optionally esterified with an alkyl or aryl group. For example, R^7 may be H, CH_3 or $CH(CH_3)_2$. R^{11} is amino, alkylamino, oxo, or dialkylamino. R^{12} is amino or H. Exemplary embodiments include where R^5 is methyl, R^6 is phenyl, and R^8 is methyl, ethyl, or isopropyl.

Phosphonamidate PMEA and PMPA compounds may be prepared from the corresponding dialkyl phosphonates which may be prepared according to the methods of: Quast et al (1974) *Synthesis* 490; Stowell et al (1990) *Tetrahedron Lett.* 3261; US Patent No. 5663159.

The phosphonamidate PMEA and PMPA compound may be enantiomerically-enriched or purified (single stereoisomer) where the carbon atom bearing R^5 may be the *R* or *S* enantiomer when R^5 is not H. The phosphonamidate PMEA and PMPA compound may be a racemate, i.e. a mixture of *R* and *S* stereoisomers. The invention includes all enantiomers, diastereomers, racemates, and enriched stereoisomer mixtures of phosphonamidate PMEA and PMPA compounds.

Emtricitabine ((-)-cis-FTC, Emtriva™), a single enantiomer of FTC, is a potent nucleoside reverse transcriptase inhibitor approved for the treatment of HIV (US Patent Nos. 5047407, 5179104, 5204466, 5210085, 5486520, 5538975, 5587480, 5618820, 5763606, 5814639, 5914331, 6114343, 6180639, 6215004; WO 02/070518). The single enantiomer emtricitabine has the structure:

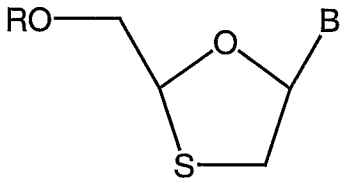


The chemical names for emtricitabine include: (-)-cis-FTC; β-L-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; (2R,5S)-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine; and 4-amino-5-fluoro-1-(2-hydroxymethyl-[1,3]-(2R,5S)-oxathiolan-5-yl)-1H-pyrimidin-2-one. The CAS Registry numbers include: 143491-57-0; 143491-54-7. It should be noted that FTC contains two chiral centers, at the 2 and 5 positions of the oxathiolane ring, and therefore can exist in the form of two pairs of optical isomers (i.e. enantiomers) and diastereomeric and racemic mixtures thereof. Thus, FTC may be either a cis or a trans isomer or mixtures thereof. Mixtures of cis and trans isomers are diastereomers with different physical properties. Each cis and trans isomer can exist as one of two enantiomers or mixtures thereof including racemic mixtures. The invention includes all enantiomers, diastereomers, racemates, and enriched stereoisomer mixtures of emtricitabine, and physiologically functional derivatives thereof. For example, the

invention includes physiological functional derivatives such as the 1:1 racemic mixture of the enantiomers (2*R*, 5*S*, cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine) and its mirror image (2*S*, 5*R*, cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one, or mixtures of the two enantiomers in any relative amount. The invention also includes mixtures of cis and trans forms of FTC.

It will be appreciated that GS-7340 and emtricitabine may exist in keto or enol tautomeric forms and the use of any tautomeric form is within the scope of this invention. GS-7340 and emtricitabine will normally be utilized in the combinations of the invention substantially free of the corresponding enantiomer, that is to say no more than about 5% w/w of the corresponding enantiomer will be present.

Physiologically functional derivatives of emtricitabine include 1,3 oxathiolane nucleosides having the structure:



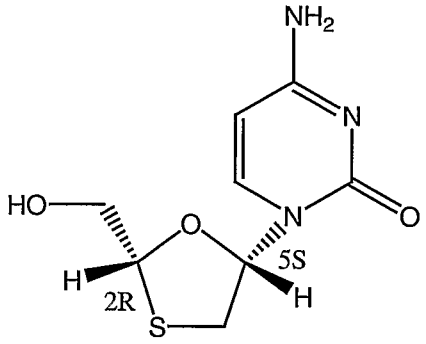
In the 1,3 oxathiolane nucleoside structure above, B is a nucleobase including any nitrogen-containing heterocyclic moiety capable of forming Watson-Crick hydrogen bonds in pairing with a complementary nucleobase or nucleobase analog, e.g. a purine, a 7-deazapurine, or a pyrimidine. Examples of B include the naturally occurring nucleobases: adenine, guanine, cytosine, uracil, thymine, and minor constituents and analogs of the naturally occurring nucleobases, e.g. 7-deazaadenine, 7-deazaguanine, 7-deaza-8-azaguanine, 7-deaza-8-azaadenine, inosine, nebularine, nitropyrrole, nitroindole, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, pseudouridine, 5-fluorocytosine, 5-chlorocytosine, 5-bromocytosine, 5-iodocytosine, 5-alkylcytosine, e.g. 5-methylcytosine, pseudocytosine, pseudoisocytosine, 5-propynylcytosine, isocytosine, isoguanine, 7-deazaguanine, 2-thiopyrimidine, 6-thioguanine, 4-thiothymine, 4-thiouracil, O^6-methylguanine, N^6-methyladenine, O^4-methylthymine, 5,6-dihydrothymine, 5,6-dihydrouracil, 4-methylindole, pyrazolo[3,4-D]pyrimidines (US Patent Nos. 6,143,877 and 6,127,121; WO 01/38584), and

ethenoadenine (Fasman (1989) in *Practical Handbook of Biochemistry and Molecular Biology*, pp. 385-394, CRC Press, Boca Raton, Fl).

Nucleobases B may be attached in the configurations of naturally-occurring nucleic acids to the 1,3 oxathiolane moiety through a covalent bond between the N-9 of purines, e.g. adenin-9-yl and guanin-9-yl, or N-1 of pyrimidines, e.g. thymin-1-yl and cytosin-1-yl (Blackburn, G. and Gait, M. Eds. "DNA and RNA structure" in Nucleic Acids in Chemistry and Biology, 2nd Edition, (1996) Oxford University Press, pp. 15-81).

Also in the 1,3 oxathiolane nucleoside structure above, R is H, C_1–C_{18} alkyl, C_1–C_{18} substituted alkyl, C_2–C_{18} alkenyl, C_2–C_{18} substituted alkenyl, C_2–C_{18} alkynyl, C_2–C_{18} substituted alkynyl, C_6–C_{20} aryl, C_6–C_{20} substituted aryl, C_2–C_{20} heterocycle, C_2–C_{20} substituted heterocycle, phosphonate, phosphophosphonate, diphosphophosphonate, phosphate, diphosphate, triphosphate, polyethyleneoxy, or a prodrug moiety

Physiologically functional derivatives of emtricitabine also include 3TC (lamivudine, Epivir®), a reverse transcriptase inhibitor approved in the United States for the treatment of HIV-1 infection in combination with AZT (Zidovudine) as Combivir® (GlaxoSmithKline). US Patent Nos. 5859021; 5905082; 6177435; 5627186; 6417191. 3TC (US Patent Nos. 5587480, 5696254, 5618820, 5756706, 5744596, 568164, 5466806, 5151426) has the structure:



For example and for some therapeutic uses, 3TC may be a physiologically functional derivative of emtricitabine in combination with GS-7340 or a physiologically functional derivative of GS-7340.

PRODRUGS

The invention includes all prodrugs of GS-7340 and emtricitabine. Whereas GS-7340 is a prodrug form of a PMPA compound, GS-7340 may bear additional prodrug moieties which confer advantageous properties. A large number of structurally-diverse prodrugs have been described for phosphonic acids (Freeman and Ross in Progress in Medicinal Chemistry 34: 112-147 (1997). A commonly used prodrug class is the acyloxyalkyl ester, which was first used as a prodrug strategy for carboxylic acids and then applied to phosphates and phosphonates by Farquhar et al (1983) *J. Pharm. Sci.* 72: 324; also US Patent Nos. 4816570, 4968788, 5663159 and 5792756. Subsequently, the acyloxyalkyl ester was used to deliver phosphonic acids across cell membranes and to enhance oral bioavailability. A close variant of the acyloxyalkyl ester strategy, the alkoxycarbonyloxyalkyl ester, may also enhance oral bioavailability as a prodrug moiety in the compounds of the combinations of the invention. Aryl esters of phosphorus groups, especially phenyl esters, are reported to enhance oral bioavailability (DeLambert et al (1994) *J. Med. Chem.* 37: 498). Phenyl esters containing a carboxylic ester ortho to the phosphate have also been described (Khamnei and Torrence, (1996) *J. Med. Chem.* 39:4109-4115). Benzyl esters are reported to generate the parent phosphonic acid. In some cases, substituents at the ortho-or para-position may accelerate the hydrolysis. Benzyl analogs with an acylated phenol or an alkylated phenol may generate the phenolic compound through the action of enzymes, e.g. esterases, oxidases, etc., which in turn undergoes cleavage at the benzylic C–O bond to generate the phosphoric acid and the quinone methide intermediate. Examples of this class of prodrugs are described by Mitchell et al (1992) *J. Chem. Soc. Perkin Trans. I* 2345; Brook et al WO 91/19721. Still other benzylic prodrugs have been described containing a carboxylic ester-containing group attached to the benzylic methylene (Glazier et al WO 91/19721). Thio-containing prodrugs are reported to be useful for the intracellular delivery of phosphonate drugs. These proesters contain an ethylthio group in which the thiol group is either esterified with an acyl group or combined with another thiol group to form a disulfide. Deesterification or reduction of the disulfide generates the free thio intermediate which subsequently breaks down to the phosphoric acid and episulfide (Puech et al (1993) *Antiviral Res.*, 22: 155-174; Benzaria et al (1996) *J. Med. Chem.* 39: 4958). Cyclic phosphonate esters have also been described as prodrugs of phosphorus-containing compounds.

Prodrug esters in accordance with the invention are independently selected from the following groups: (1) mono-, di-, and tri-phosphate esters of GS-7340 or emtricitabine or any other compound which upon administration to a human subject is capable of providing (directly or indirectly) said mono-, di, or triphosphate ester; (2) carboxylic acid esters (3) sulfonate esters, such as alkyl- or aralkylsulfonyl- (for example, methanesulfonyl); (4) amino acid esters (for example, alanine, L-valyl or L-isoleucyl); (5) phosphonate; and (6) phosphonamidate esters.

Ester groups (1)-(6) may be substituted with; straight or branched chain C_1–C_{18} alkyl (for example, methyl, *n*-propyl, *t*-butyl, or *n*-butyl); C_3–C_{12} cycloalkyl; alkoxyalkyl (for example, methoxymethyl); arylalkyl (for example, benzyl); aryloxyalkyl (for example, phenoxymethyl); C_5–C_{20} aryl (for example, phenyl optionally substituted by, for example, halogen, C_1–C_4 alkyl, C_1–C_4 alkoxy, or amino; acyloxymethyl esters $-CH_2OC(=O)R^9$ (e.g. POM) or acyloxymethyl carbonates $-CH_2OC(=O)OR^9$ (e.g. POC) where R^9 is C_1–C_6 alkyl, C_1–C_6 substituted alkyl, C_6–C_{20} aryl or C_6–C_{20} substituted aryl. For example, ester groups may be pivaloyloxymethoxy, POM, $-CH_2OC(=O)C(CH_3)_3$; $-CH_2OC(=O)OC(CH_3)_3$; or POC, $-CH_2OC(=O)OCH(CH_3)_2$.

An exemplary aryl moiety present in such esters comprises a phenyl or substituted phenyl group. Many phosphate prodrug moieties are described in US Patent No. 6312662; Jones et al (1995) *Antiviral Research* 27:1-17; Kucera et al (1990) *AIDS Res. Hum. Retro Viruses* 6:491-501; Piantadosi et al (1991) *J. Med. Chem.* 34:1408-14; Hosteller et al (1992) *Antimicrob. Agents Chemother.* 36:2025-29; Hostetler et al (1990) *J. Biol. Chem.* 265:611127; and Siddiqui et al (1999) *J. Med. Chem.* 42:4122-28.

Pharmaceutically acceptable prodrugs refer to a compound that is metabolized in the host, for example hydrolyzed or oxidized, by either enzymatic action or by general acid or base solvolysis, to form an active ingredient. Typical examples of prodrugs of the active ingredients of the combinations of the invention have biologically labile protecting groups on a functional moiety of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, esterified, deesterified, alkylated, dealkylated, acylated, deacylated, phosphorylated,

dephosphorylated, or other functional group change or conversion involving forming or breaking chemical bonds on the prodrug.

CHEMICAL STABILITY OF A PHARMACEUTICAL FORMULATION

The chemical stability of the active ingredients in a pharmaceutical formulation is of concern to minimize the generation of impurities and ensure adequate shelf-life. The active ingredients, GS-7340 and emtricitabine, in the pharmaceutical formulations of the invention have relatively low pKa values, indicative of the potential to cause acidic hydrolysis of the active ingredients. Emtricitabine, with a pKa of 2.65 (Emtriva™ Product Insert, Gilead Sciences, Inc. 2003, available at gilead.com) is subject to hydrolytic deamination of the 5-fluoro cytosine nucleobase to form the 5-fluoro uridine nucleobase. Tenofovir, with a pKa of 3.8 (Yuan L. et al "Degradation Kinetics of Oxycarbonyloxymethyl Prodrugs of Phosphonates in Solution", *Pharmaceutical Research* (2001) Vol. 18, No. 2, 234-237), is subject also to hydrolytic deamination of the exocyclic amine of the adenine nucleobase, and to hydrolysis of one or both of the amidate and ester groups (US Patent No. 5922695). It is desirable to formulate a therapeutic combination of GS-7340 and emtricitabine, and the physiological functional derivatives thereof, with a minimum of impurities and adequate stability.

The combinations of the present invention provide combination pharmaceutical dosage forms which are chemically stable to acid degradation of: (1) a first component (such as GS-7340, and physiological functional derivatives; (2) a second component (such as emtricitabine, and physiological functional derivatives; and (3) optionally a third component having antiviral activity. The third component includes anti-HIV agents and include: protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and integrase inhibitors. Exemplary third active ingredients to be administered in combination with first and second components are shown in Table A. First and second components are as defined in the above section entitled: ACTIVE INGREDIENTS OF THE COMBINATIONS.

SALTS

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_4^+ (wherein X is C_1–C_4 alkyl). 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.

ADMINISTRATION OF THE FORMULATIONS

While it is possible for the active ingredients of the combination to be administered alone and separately as monotherapies, it is preferable to administer them as a pharmaceutical co-formulation. A two-part or three-part combination may be administered simultaneously or sequentially. When administered sequentially, the combination may be administered in one, two, or three administrations.

Preferably, two-part or three-part combinations are administered in a single pharmaceutical dosage form. More preferably, a two-part combination is administered as a single oral dosage form and a three-part combination is administered as two identical oral dosage forms. Examples include a single tablet of GS-7340 and emtricitabine, or two tablets of GS-7340, emtricitabine, and efavirenz.

It will be appreciated that the compounds of the combination may be administered: (1) simultaneously by combination of the compounds in a co-formulation or (2) by alternation, i.e. delivering the compounds serially, sequentially, in parallel or simultaneously in separate pharmaceutical formulations. In alternation therapy, the delay in administering the second, and optionally a third active ingredient, should not be such as to lose the benefit of a synergistic therapeutic effect of the combination of the active ingredients. By either method of administration (1) or (2), ideally the combination should be administered to achieve peak plasma concentrations of each of the active ingredients. A one pill once-per-day regimen by administration of a combination co-formulation may be feasible for some HIV-positive patients. Effective peak plasma concentrations of the active ingredients of the combination will be in the range of approximately 0.001 to 100 μM. Optimal peak plasma concentrations may be achieved by a formulation and dosing regimen prescribed for a particular patient. It will also be understood that GS-7340 and emtricitabine, or the physiologically functional derivatives of either thereof, whether presented simultaneously or sequentially, may be administered individually, in multiples, or in any combination thereof. In general, during alternation therapy (2), an effective dosage of each compound is administered serially, where in co-formulation therapy (1), effective dosages of two or more compounds are administered together.

FORMULATION OF THE COMBINATIONS

When the individual components of the combination are administered separately they are generally each presented as a pharmaceutical formulation. Techniques and formulations generally are found in Remington's Pharmaceutical Sciences (Mack Publishing Co., Easton, PA). The references hereinafter to formulations refer unless otherwise stated to formulations containing either the combination or a component compound thereof. It will be understood that the administration of the combination of the invention by means of a single patient pack, or patient packs of each formulation, within a package insert diverting the patient to the correct use of the invention is a desirable additional feature of this invention. The invention also includes a double pack comprising in association for separate administration, formulations of GS-7340 and emtricitabine, or a physiologically functional derivative of either or both thereof.

The combination therapies of the invention include: (1) a combination of GS-7340 and emtricitabine or (2) a combination containing a physiologically functional derivative of either or both thereof.

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. 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 which are suitable for manufacture of tablets are acceptable. These excipients may be, for example, inert diluents, such as calcium or sodium carbonate, lactose, lactose monohydrate, croscarmellose sodium, povidone, calcium or sodium phosphate; granulating and disintegrating agents, such as maize starch, or alginic acid; binding agents, such as cellulose, microcrystalline cellulose, starch, gelatin or acacia; and lubricating agents, such as magnesium stearate, stearic acid or talc. Tablets may be uncoated or may be coated by known techniques including microencapsulation to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

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.

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 pharmaceutical compositions of the invention may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butane-diol or prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables.

The pharmaceutical compositions of the invention may be injected parenterally, for example, intravenously, intraperitoneally, intrathecally, intraventricularly, intrastemally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion techniques. They are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

The pharmaceutical compositions of the invention may also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurized container or a nebuliser with

the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFC 134a), carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container or nebuliser may contain a solution or suspension of the composition, e.g. using a mixture of ethanol and the propellant as the solvent, which may additional contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of a compound of the formula (I) and a suitable powder base such as lactose or starch. Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains from 20 μg to 20 mg of a composition for delivery to the patient. The overall daily dose with an aerosol will be in the range of from 20 μg to 20 mg which may be administered in a single dose or, more usually, in divided doses throughout the day.

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 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. For example, an aqueous solution intended for intravenous infusion may contain from about 3 to 500 μg of the active ingredient per milliliter of solution in order that infusion of a suitable volume at a rate of about 30 mL/hr can occur. 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 active ingredient may also be administered as a bolus, electuary or paste.

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. The synergistic effects of GS-7340 in combination with emtricitabine may be realized over a wide ratio, for example 1:50 to 50:1 (GS-7340: emtricitabine). In one embodiment, the ratio may range from about 1:10 to 10:1. In another embodiment, the weight/weight ratio of GS-7340 to emtricitabine in a co-formulated combination dosage form, such as a pill, tablet, caplet or capsule will be about 1, i.e. an approximately equal amount of GS-7340 and emtricitabine. In other exemplary co-formulations, there may be more or less GS-7340 than emtricitabine. Conveniently each compound will be employed in the combination in an amount at which it exhibits antiviral activity when used alone. For example, 150 mg GS-7340 and 200 mg emtricitabine can be co-formulated in a ratio of 0.75:1 (GS-7340: emtricitabine). In one embodiment, each compound will be employed in the combination in an amount at which it exhibits antiviral activity when used alone. Exemplary Formulations A, B, C, D, E, and F (Examples) have ratios of 0.125:1 to 1.5:1 (GS-7340: emtricitabine). Exemplary Formulations A, B, C, D, E, and F use amounts of GS-7340 and emtricitabine ranging from 25 mg to 200 mg. Other ratios and amounts of the compounds of said combinations are contemplated within the scope of the invention.

A unitary dosage form may further comprise GS-7340 and emtricitabine, or physiologically functional derivatives of either thereof, and a pharmaceutically acceptable carrier.

It will be appreciated by those skilled in the art that the amount of active ingredients in the combinations of the invention required for use in treatment will vary according to a variety of factors, including the nature of the condition being treated and the age and condition of the patient, and will ultimately be at the discretion of the attending physician or health care practitioner. The factors to be considered include the route of administration and nature of the formulation, the animal's body weight, age and general condition and the nature and severity of the disease to be treated. For example, in a Phase I/II monotherapy study of emtricitabine, patients received doses ranging from 25 mg to 200 mg twice-a-day for two weeks. At each dose regimen greater or equal to 200 mg, a 98-percent (1.75 log10) or greater viral suppression was observed. A once-a-day dose of 200 mg of emtricitabine reduced the viral load by an average of

99 percent (1.92 log10). Emtriva™ (emtricitabine) has been approved by the FDA for the treatment of HIV as a 200 mg oral tablet.

It is also possible to combine any two of the active ingredients in a unitary dosage form for simultaneous or sequential administration with a third active ingredient. The three-part combination may be administered simultaneously or sequentially. When administered sequentially, the combination may be administered in two or three administrations. Third active ingredients have anti-HIV activity and include protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and integrase inhibitors. Exemplary third active ingredients to be administered in combination with GS-7340, emtricitabine, and their physiological functional derivatives are shown in Table A.

Table A

5,6 dihydro-5-azacytidine

5-aza 2'deoxycytidine

5-azacytidine

5-yl-carbocyclic 2'-deoxyguanosine (BMS200,475)

9 (arabinofuranosyl)guanine; 9-(2' deoxyribofuranosyl)guanine

9-(2'-deoxy 2'fluororibofuranosyl)-2,6-diaminopurine

9-(2'-deoxy 2'fluororibofuranosyl)guanine

9-(2'-deoxyribofuranosyl)-2,6 diaminopurine

9-(arabinofuranosyl)-2,6 diaminopurine

Abacavir, Ziagen®

Acyclovir, ACV; 9-(2-hydroxyethoxylmethyl)guanine

Adefovir dipivoxil, Hepsera®

amdoxivir, DAPD

Amprenavir, Agenerase®

araA; 9-β-D-arabinofuranosyladenine (Vidarabine)

atazanivir sulfate (Reyataz®)

AZT; 3'-azido-2',3'-dideoxythymdine, Zidovudine, (Retrovir®)

BHCG; (.+-.)-(1a,2b,3a)-9-[2,3-bis(hydroxymethyl)cyclobutyl]guanine

BMS200,475; 5-yl-carbocyclic 2'-deoxyguanosine

Buciclovir; (*R*) 9-(3,4-dihydroxybutyl)guanine

BvaraU; 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil (Sorivudine)

Calanolide A

Capravirine

CDG; carbocyclic 2'-deoxyguanosine

Cidofovir, HPMPC; (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine

Clevudine, L-FMAU; 2'-Fluoro-5-methyl-β-L-arabino-furanosyluracil

Combivir® (lamivudine/zidovudine)

Cytallene; [1-(4'-hydroxy-1',2'-butadienyl)cytosine]

d4C; 3'-deoxy-2',3'-didehydrocytidine

DAPD; (-)-β-D-2,6-diaminopurine dioxolane

ddA; 2',3'-dideoxyadenosine

ddAPR; 2,6-diaminopurine-2',3'-dideoxyriboside

ddC; 2',3'-dideoxycytidine (Zalcitabine)

ddI; 2',3'-dideoxyinosine, didanosine, (Videx®, Videx® EC)

Delavirdine, Rescriptor®

Didanosine, ddI, Videx®; 2',3'-dideoxyinosine

DXG; dioxolane guanosine

E-5-(2-bromovinyl)-2'-deoxyuridine

Efavirenz, Sustiva®

Enfuvirtide, Fuzeon®

F-ara-A; fluoroarabinosyladenosine (Fludarabine)

FDOC; (-)-β-D-5-fluoro-1-[2-(hydroxymethyl)-1,3-dioxolane]cytosine

FEAU; 2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl-5-ethyluracil

FIAC; 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine

FIAU; 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouridine

FLG; 2',3'-dideoxy-3'-fluoroguanosine

FLT; 3'-deoxy-3'-fluorothymidine

Fludarabine; F-ara-A; fluoroarabinosyladenosine

FMAU; 2'-Fluoro-5-methyl-β-L-arabino-furanosyluracil

FMdC

Foscarnet; phosphonoformic acid, PFA

FPMPA; 9-(3-fluoro-2-phosphonylmethoxypropyl)adenine

Gancyclovir, GCV; 9-(1,3-dihydroxy-2-propoxymethyl)guanine

GS-7340; 9-[*R*-2-[[(*S*)-[[(*S*)-1-(isopropoxycarbonyl)ethyl]amino]-phenoxyphosphinyl]methoxy]propyl]adenine

HPMPA; (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine

HPMPC; (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (Cidofovir)

Hydroxyurea, Droxia®

Indinavir, Crixivan®

Kaletra® (lopinavir/ritonavir)

Lamivudine, 3TC, Epivir™; (2*R*, 5*S*, cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one

L-d4C; L-3'-deoxy-2',3'-didehydrocytidine

L-ddC; L-2',3'-dideoxycytidine

L-Fd4C; L-3'-deoxy-2',3'-didehydro-5-fluorocytidine

L-FddC; L-2',3'-dideoxy-5-fluorocytidine

Lopinavir

Nelfinavir, Viracept®

Nevirapine, Viramune®

Oxetanocin A; 9-(2-deoxy-2-hydroxymethyl-β-D-erythro-oxetanosyl)adenine

Oxetanocin G; 9-(2-deoxy-2-hydroxymethyl-β-D-erythro-oxetanosyl)guanine

Penciclovir

PMEDAP; 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine

PMPA, tenofovir; (*R*)-9-(2-phosphonylmethoxypropyl)adenine

PPA; phosphonoacetic acid

Ribavirin; 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide

Ritonavir, Norvir®

Saquinavir, Invirase®, Fortovase®

Sorivudine, BvaraU; 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil

Stavudine, d4T, Zerit®; 2',3'-didehydro-3'-deoxythymidine

Trifluorothymidine, TFT; Trifluorothymidine

Trizivir® (abacavir sulfate/lamivudine/zidovudine)

Vidarabine, araA; 9-β-D-arabinofuranosyladenine

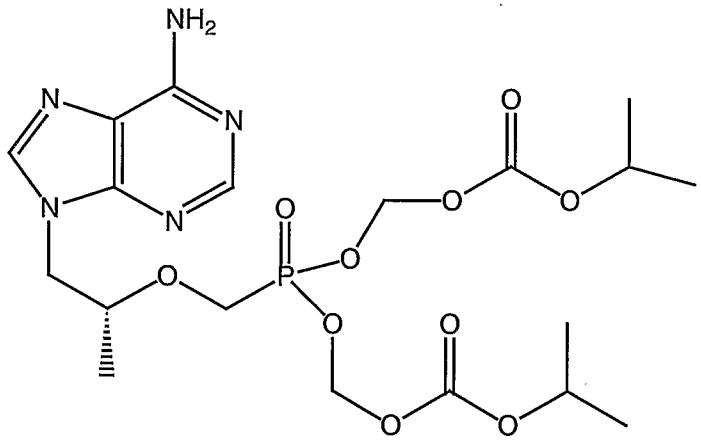
Viread®, tenofovir disoproxil fumarate (DF), Bis POC PMPA, TDF; 2,4,6,8-Tetraoxa-5-phosphanonanedioic acid, 5-[[(1*R*)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]-, bis(1-methylethyl) ester, 5-oxide, (2E)-2-butenedioate (1:1)

Zalcitabine, Hivid®, ddC; 2',3'-dideoxycytidine

Zidovudine, AZT, Retrovir®; 3'-azido-2',3'-dideoxythymdine

Zonavir; 5-propynyl-1-arabinosyluracil

Another aspect of the present invention is a three-part combination comprising GS-7340, emtricitabine, and tenofovir. Tenofovir DF (also known as Viread®, Tenofovir disoproxil fumarate, Tenofovir disoproxil, Tenofovir, TDF, Bis-POC-PMPA (US Patent Nos. 5935946, 5922695, 5977089, 6043230, 6069249) has the structure:



The chemical names for Tenofovir disoproxil (DF) include: [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester; and 2,4,6,8-tetraoxa-5-phosphanonanedioic acid, 5-[[(1*R*)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]-, bis(1-methylethyl) ester, 5-oxide. The CAS Registry numbers include: 201341-05-1; 202138-50-9; 206184-49-8. It should be noted that the ethoxymethyl unit of tenofovir (PMPA) has a chiral center. The *R* (rectus, right handed configuration) enantiomer is shown. However, the invention includes the *S* isomer, as well. The invention includes all enantiomers, diastereomers, racemates, and enriched stereoisomer mixtures of tenofovir and physiologically functional derivatives thereof.

Tenofovir DF is a new nucleotide reverse transcriptase inhibitor recently approved in the United States for the treatment of HIV-1 infection in combination with other antiretroviral agents. Tenofovir disoproxil fumarate or Viread® (Gilead Science, Inc.) is the fumarate salt of tenofovir disoproxil. Viread® may be named as: 2,4,6,8-Tetraoxa-5-phosphanonanedioic acid, 5-[[(1*R*)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]-, bis(1-methylethyl) ester, 5-oxide, (2E)-2-butenedioate (1:1). The CAS Registry number is 202138-50-9.

For example, a typical unitary dosage may contain 1 mg to 1000 mg of GS-7340, 1 mg to 1000 mg of emtricitabine, and 1 mg to 1000 mg of the third active ingredient. A unitary dosage form may further comprise GS-7340, emtricitabine, the third active ingredient, or physiologically functional derivatives of any of the active ingredients thereof, and a pharmaceutically acceptable carrier.

Combinations of the present invention enable patients greater freedom from multiple dosage medication regimens and ease the needed diligence required in remembering and complying with complex daily dosing times and schedules. By combining GS-7340 and emtricitabine into a single dosage form, the desired daily regimen may be presented in a single dose or as two or more sub-doses per day. The combination of co-formulated GS-7340 and emtricitabine may be administered as a single pill, once per day.

A further aspect of the invention is a patient pack comprising at least one active ingredient GS-7340, emtricitabine or a physiologically functional derivative of either of the combination and an information package or product insert containing directions on the use of the combination of the invention.

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. Exemplary Tablet Formulation A has colloidal silicon dioxide (Examples). Glidants can be used to increase and aid blend composition homogeneity in formulations of anti-HIV drugs (US Patent No. 6113920). 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 present invention provides pharmaceutical formulations combining the active ingredients GS-7340 and emtricitabine, or physiologically functional derivatives thereof, in a sufficiently homogenized form, and a method for using this pharmaceutical formulation. An object of the present invention is to utilize glidants to reduce the segregation of active ingredients in pharmaceutical compositions during pre-compression material handling. Another object of the present invention is to provide a pharmaceutical formulation combining the active ingredients GS-7340 and emtricitabine, or physiologically functional derivatives thereof, with a pharmaceutically acceptable glidant, resulting in a mixture characterized by a pharmaceutically acceptable measure of homogeneity.

Formulations include those suitable for oral, rectal, nasal, topical (including transdermal, buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods represent a further feature of the present invention and include the step of bringing into association the active ingredients with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, caplets, cachets or tablets each containing a predetermined amount of the active ingredients; 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 active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatin, hydroxypropyl methylcellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycollate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Molded tablets may be made by molding a mixture of the powdered compound moistened with an inert liquid diluent in a suitable machine. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredients therein using, for example, cellulose ether derivatives (e.g., hydroxypropyl methylcellulose) or methacrylate derivatives in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredients in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier. Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylates. Topical administration may also be by means of a transdermal iontophoretic device.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for penile administration for prophylactic or therapeutic use may be presented in condoms, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art. The

suppositories may be conveniently formed by admixture of the active combination with the softened or melted carrier(s) followed by chilling and shaping in moulds.

Formulations suitable for parenteral administration include aqueous and nonaqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents; and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Exemplary unit dosage formulations are those containing a daily dose or daily subdose of the active ingredients, as hereinbefore recited, or an appropriate fraction thereof. It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include such further agents as sweeteners, thickeners and flavoring agents.

The compounds of the combination of the present invention may be obtained in a conventional manner, known to those skilled in the art. Tenofovir disoproxil fumarate can be prepared, for example, as described in US Patent No. 5977089. Methods for the preparation of FTC are described in WO 92/14743, incorporated herein by reference.

COMPOSITION USE

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. 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 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 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 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.

ASSAYS OF THE COMBINATIONS

The combinations of the inventions may be tested for *in vitro* activity against HIV and sensitivity, and for cytotoxicity in laboratory adapted cell lines, e.g. MT2 and in peripheral blood mononuclear cells (PBMC) according to standard assays developed for testing anti-HIV compounds, such as WO 02/068058 and US Patent No. 6475491. Combination assays may be performed at varying concentrations of the compounds of the combinations to determine EC_{50} by serial dilutions.

EXAMPLES

The following examples further describe and demonstrate particular embodiments within the scope of the present invention. The examples are given solely for illustration and are not to be construed as limitations as many variations are possible without departing from spirit and scope of the Invention. The following examples are intended for illustration only and are not intended to limit the scope of the invention in any way. "Active ingredient" denotes GS-7340, emtricitabine, or a physiologically functional derivative of either thereof.

Tablet Formulation

The following exemplary formulations A, B, C, D, E, and F are prepared by wet granulation of the ingredients with an aqueous solution, addition of extragranular components and then followed by addition of magnesium stearate and compression.

Formulation A:

	mg/tablet
GS-7340	150
emtricitabine	200
Microcrystalline Cellulose	200
Lactose Monohydrate	325
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Colloidal silicon dioxide	5
Magnesium Stearate	10
	1000

Formulation B:

	mg/tablet
GS-7340	150
emtricitabine	200
Microcrystalline Cellulose	200
Lactose Monohydrate	330
Croscarmellose Sodium	60
Pregelatinized Starch	50
Magnesium Stearate	10
	1000

Formulation C:

	mg/tablet
GS-7340	50
emtricitabine	200
Microcrystalline Cellulose	200
Lactose Monohydrate	330
Croscarmellose Sodium	60
Pregelatinized Starch	50
Magnesium Stearate	10
	900

Formulation D:

	mg/tablet
GS-7340	25
emtricitabine	200
Microcrystalline Cellulose	200
Lactose Monohydrate	330
Croscarmellose Sodium	60
Pregelatinized Starch	50
Magnesium Stearate	10
	875

Formulation E:

	mg/tablet
GS-7340	150
emtricitabine	100
Microcrystalline Cellulose	200
Lactose Monohydrate	330
Croscarmellose Sodium	60
Pregelatinized Starch	50
Magnesium Stearate	10
	900

Formulation F:

	mg/tablet
GS-7340	100
emtricitabine	100
Microcrystalline Cellulose	200
Lactose Monohydrate	330
Croscarmellose Sodium	60
Pregelatinized Starch	50
Magnesium Stearate	10
	850

Formulation G (Controlled Release Formulation):

This formulation is prepared by wet granulation of the ingredients with an aqueous solution, followed by the addition of magnesium stearate and compression.

	mg/tablet
GS-7340	300
emtricitabine	200
Hydroxypropyl Methylcellulose	112
Lactose B.P.	53
Pregelatinized Starch B.P.	28
Magnesium Stearate	7
total:	700

Drug release takes place over a period of about 6-8 hours and is complete after 12 hours.

Capsule Formulations

Formulation H:

A capsule formulation is prepared by admixing the ingredients and filling into a two-part hard gelatin or hydroxypropyl methylcellulose capsule.

	mg/capsule
Active Ingredient	500
Microcrystalline Cellulose	143
Sodium Starch Glycollate	25

Magnesium Stearate	2
total:	670

Formulation I (Controlled Release Capsule):

The following controlled release capsule formulation is prepared by extruding ingredients a, b, and c using an extruder, followed by spheronization of the extrudate and drying. The dried pellets are then coated with release-controlling membrane (d) and filled into a two-piece, hard gelatin or hydroxypropyl methylcellulose capsule.

	mg/capsule
(a) Active Ingredient	500
(b) Microcrystalline Cellulose	125
(c) Lactose B.P.	125
(d) Ethyl Cellulose	13
total:	763

Formulation J (Oral Suspension):

The active ingredients are admixed with the ingredients and filling them as dry powder. Purified water is added and mixed well before use.

Active Ingredient	500 mg
Confectioner's Sugar	2000 mg
Simethicone	300 mg
Methylparaben	30 mg
Propylparaben	10 mg
Flavor, Peach	500 mg
Purified Water q.s. to	5.00 ml

Formulation K (Suppository):

One-fifth of the Witepsol H15 is melted in a steam-jacketed pan at 45°C. maximum. The active ingredients are sifted through a 200 micron sieve and added to the molten base with mixing, using a Silverson fitted with a cutting head, until a smooth dispersion is achieved. Maintaining the mixture at 45°C., the remaining Witepsol H15 is added to the suspension and stirred to ensure a homogenous mix. The entire suspension is

passed through a 250 micron stainless steel screen and, with continuous stirring, is allowed to cool to 40°C. At a temperature of 38°C. to 40°C., 2.02 g of the mixture is filled into suitable, 2 mL plastic molds. The suppositories are allowed to cool to room temperature.

	mg/Suppository
Active Ingredient	500
Hard Fat, B.P. (Witepsol H15 - Dynamit Nobel)	1770
total	2270

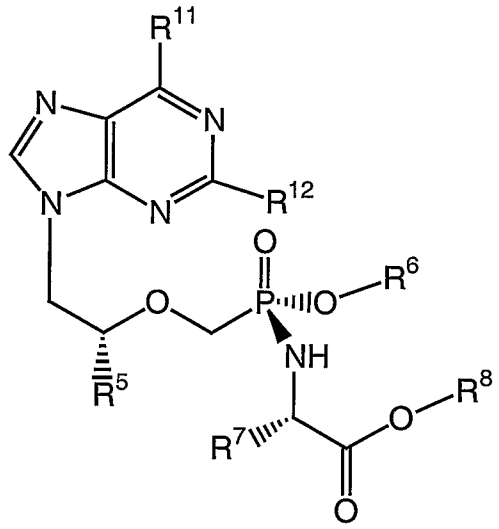
A fixed dose combination tablet of GS-7340 and emtricitabine, or their physiologically functional derivatives, may be formulated using a wet granulation/fluid-bed drying process using conventional methods. See: US Patent No. 5935946; L. Young (editor). Tableting Specification Manual 5th ed., American Pharmaceutical Association, Washington, DC, (2001); L. Lachman, H. Lieberman (editors). Pharmaceutical Dosage Forms: Tablets (Vol 2), Marcel Dekker Inc., New York, 185-202 (1981); J. T. Fell and J. M. Newton, J. Pharm. Pharmacol. 20, 657-659 (1968); US Pharmacopeia 24-National Formulary 19, "Tablet Friability", Chapter <1216>, Page 2148 (2000).

All publications and patent applications cited herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although certain embodiments are described in detail above, those having ordinary skill in the art will clearly understand that many modifications are possible in the embodiments without departing from the teachings thereof. All such modifications are intended to be encompassed within the claims of the invention.

Embodiments of the Invention:

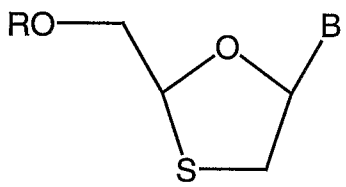
A. A pharmaceutical composition comprising an effective amount of a compound of the formula:



wherein R^5 is H or CH_3; R^6 and R^8 are independently selected from H, C_1–C_6 alkyl, C_1–C_6 substituted alkyl, C_6–C_{20} aryl, C_6–C_{20} substituted aryl, C_6–C_{20} arylalkyl, and C_6–C_{20} substituted arylalkyl; R^7 is the side chain of any naturally-occurring or pharmaceutically acceptable amino acid and where if the side chain comprises carboxyl, the carboxyl group is optionally esterified with an alkyl or aryl group; R^{11} is amino, alkylamino, oxo, or dialkylamino; and R^{12} is amino or H;

or a physiologically functional derivative thereof;

in combination with an effective amount of a compound of the formula



(2)

wherein B is selected from adenine, guanine, cytosine, uracil, thymine, 7-deazaadenine, 7-deazaguanine, 7-deaza-8-azaguanine, 7-deaza-8-azaadenine, inosine, nebularine, nitropyrrole, nitroindole, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, pseudouridine, 5-fluorocytosine, 5-chlorocytosine, 5-bromocytosine, 5-iodocytosine, pseudocytosine, pseudoisocytosine, 5-propynylcytosine, isocytosine, isoguanine, 7-deazaguanine, 2-thiopyrimidine, 6-thioguanine, 4-thiothymine, 4-thiouracil, O^6-methylguanine, N^6-methyladenine, O^4-methylthymine, 5,6-dihydrothymine, 5,6-dihydrouracil, 4-methylindole, and a pyrazolo[3,4-D]pyrimidine; and

R is selected from H, C_1–C_{18} alkyl, C_1–C_{18} substituted alkyl, C_2–C_{18} alkenyl, C_2–C_{18} substituted alkenyl, C_2–C_{18} alkynyl, C_2–C_{18} substituted alkynyl, C_6–C_{20} aryl, C_6–C_{20} substituted aryl, C_2–C_{20} heterocycle, C_2–C_{20} substituted heterocycle, phosphonate, phosphophosphonate, diphosphophosphonate, phosphate, diphosphate, triphosphate, polyethyleneoxy or a physiologically functional derivative thereof; and

a pharmaceutically acceptable carrier.

B. A composition of embodiment A wherein, in formula 1, R^7 is H, CH_3 or $CH(CH_3)_2$.

C. A composition of embodiment A wherein, in formula 1, R^6 is phenyl.

D. A composition of embodiment A wherein, in formula 1, R^8 is CH_3, CH_2CH_3, or $CH(CH_3)_2$.

E. A composition of embodiments A through D wherein, in formula 2, B is cytosine or a 5-halocytosine and R is H.

F. A composition of embodiments A through E wherein, in formula 2, B is 5-fluorocytosine and R is H.

G. A pharmaceutical formulation of embodiments A through F further comprising a third active ingredient selected from the group consisting of a protease inhibitor, a

nucleoside or nucleotide reverse transcriptase inhibitor, a non-nucleoside reverse transcriptase inhibitor, and an integrase inhibitor.

H. A pharmaceutical formulation of embodiments A through G in unit dosage form.

I. A method for the treatment or prevention of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal a pharmaceutical composition of embodiments A through G.

We claim:

1. A method for the treatment or prevention of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal a therapeutically effective amount of a combination comprising 9-[*R*-2-[[(*S*)-[[(*S*)-1-(isopropoxycarbonyl)ethyl]amino]-phenoxyphosphinyl]methoxy]propyl]adenine (GS-7340) or a physiologically functional derivative thereof, and (2*R*,5S,cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine) or a physiologically functional derivative thereof.

2. The method according to claim 1 wherein the combination comprises GS-7340 and emtricitabine.

3. The method according to claim 2 wherein the combination comprises about 150 mg of GS-7340 and about 200 mg of emtricitabine.

4. The method according to claim 1 wherein GS-7340 or a physiologically functional derivative thereof and emtricitabine or a physiologically functional derivative thereof are present in a ratio of about 1:50 to about 50:1 by weight.

5. The method according to claim 1 wherein GS-7340 or a physiologically functional derivative thereof and emtricitabine or a physiologically functional derivative thereof are present in a ratio of about 1:10 to about 10:1 by weight.

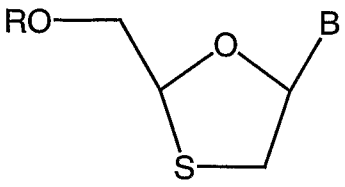
6. The method according to claim 1 wherein GS-7340 or a physiologically functional derivative thereof and emtricitabine or a physiologically functional derivative thereof are each present in an amount from about 1 mg to about 1000 mg per unit dosage form.

7. The method according to claim 1 wherein GS-7340 or a physiologically functional derivative thereof and emtricitabine or a physiologically functional derivative thereof are each present in an amount from about 100 mg to about 300 mg per unit dosage form.

8. A method according to claim 1 wherein GS-7340 is a fumarate salt.

9. A method for the treatment or prevention of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal a

therapeutically effective amount of a combination comprising 9-[*R*-2-[[(*S*)-[[(*S*)-1-(isopropoxycarbonyl)ethyl]amino]-phenoxyphosphinyl]methoxy]propyl]adenine (GS-7340) or a physiologically functional derivative thereof, and a compound of the formula:



wherein B is selected from adenine, guanine, cytosine, uracil, thymine, 7-deazaadenine, 7-deazaguanine, 7-deaza-8-azaguanine, 7-deaza-8-azaadenine, inosine, nebularine, nitropyrrole, nitroindole, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, pseudouridine, 5-fluorocytosine, 5-chlorocytosine, 5-bromocytosine, 5-iodocytosine, pseudocytosine, pseudoisocytosine, 5-propynylcytosine, isocytosine, isoguanine, 7-deazaguanine, 2-thiopyrimidine, 6-thioguanine, 4-thiothymine, 4-thiouracil, O^6-methylguanine, N^6-methyladenine, O^4-methylthymine, 5,6-dihydrothymine, 5,6-dihydrouracil, 4-methylindole, and a pyrazolo[3,4-D]pyrimidine; and

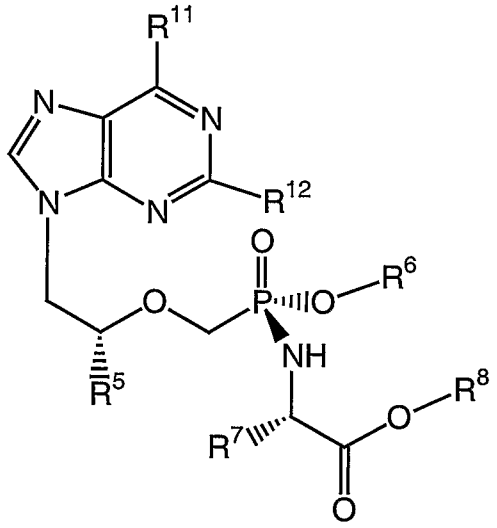
R is selected from H, C_1–C_{18} alkyl, C_1–C_{18} substituted alkyl, C_2–C_{18} alkenyl, C_2–C_{18} substituted alkenyl, C_2–C_{18} alkynyl, C_2–C_{18} substituted alkynyl, C_6–C_{20} aryl, C_6–C_{20} substituted aryl, C_2–C_{20} heterocycle, C_2–C_{20} substituted heterocycle, phosphonate, phosphophosphonate, diphosphophosphonate, phosphate, diphosphate, triphosphate, polyethyleneoxy, and a prodrug moiety.

10. The method according to claim 9 wherein the combination comprises a physiologically functional derivative of emtricitabine which is (2*R*, 5*S*, cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (3TC).

11. The method according to claim 1 wherein the combination comprises a physiologically functional derivative of emtricitabine which is a racemic mixture of the enantiomers (2*R*, 5*S*, cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-

(1H)-pyrimidin-2-one and (2*S*, 5*R*, cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one.

12. The method according to claim 1 wherein the combination comprises a physiologically functional derivative of GS-7340 which has the structure:



wherein R^5 is H or CH_3; R^6 and R^8 are independently selected from H, C_1–C_6 alkyl, C_1–C_6 substituted alkyl, C_6–C_{20} aryl, C_6–C_{20} substituted aryl, C_6–C_{20} arylalkyl, and C_6–C_{20} substituted arylalkyl; R^7 is the side chain of any naturally-occurring or pharmaceutically acceptable amino acid and where if the side chain comprises carboxyl, the carboxyl group is optionally esterified with an alkyl or aryl group; R^{11} is amino, alkylamino, oxo, or dialkylamino; and R^{12} is amino or H;

or a pharmaceutically acceptable salt or solvate thereof.

13. The method according to claim 12 wherein R^7 is H, CH_3 or $CH(CH_3)_2$.

14. The method according to claim 12 wherein R^6 is phenyl.

15. The method according to claim 12 wherein R^8 is CH_3, CH_2CH_3, or $CH(CH_3)_2$.

16. The method according to claim 1 wherein GS-7340 or a physiologically functional derivative thereof, and emtricitabine or a physiologically functional derivative thereof are administered sequentially.

17. The method according to claim 1 wherein the combination is administered as a single combined formulation.

18. The method according to claim 17 wherein the single combined formulation is administered once per day to an infected human.

19. The method according to claim 1 in which said animal is a human.

20. The method according to claim 1 wherein the combination further comprises a third active ingredient selected from a protease inhibitor (PI), a nucleoside reverse transcriptase inhibitor (NRTI), a non- nucleoside reverse transcriptase inhibitor (NNRTI), and an integrase inhibitor.

21. The method according to claim 20 wherein the third active ingredient is tenofovir disoproxil fumarate.

22. The method according to claim 1 wherein the combination further comprises a pharmaceutically acceptable glidant selected from silicon dioxide, powdered cellulose, microcrystalline cellulose, a metallic stearate, sodium aluminosilicate, sodium benzoate, calcium carbonate, calcium silicate, corn starch, magnesium carbonate, asbestos free talc, stearowet C, starch, starch 1500, magnesium lauryl sulfate, magnesium oxide, and combinations thereof.

23. The method according to claim 22 wherein the metallic stearate is selected from calcium stearate, magnesium stearate, zinc stearate, and combinations thereof.

24. A pharmaceutical formulation comprising 9-[*R*-2-[[(*S*)-[[(*S*)-1-(isopropoxycarbonyl)ethyl]amino]-phenoxyphosphinyl]methoxy]propyl]adenine (GS-7340) or a physiologically functional derivative thereof and (2*R*, 5*S*, cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine) or a physiologically functional derivative thereof.

25. The pharmaceutical formulation according to claim 24 further comprising one or more pharmaceutically acceptable carriers or excipients.

26. The pharmaceutical formulation according to claim 25 wherein the pharmaceutically acceptable carriers or excipients are selected from pregelatinized starch, croscarmellose sodium, povidone, lactose monohydrate, microcrystalline cellulose, and magnesium stearate; and combinations thereof.

27. The pharmaceutical formulation according to claim 24 wherein GS-7340 or a physiologically functional derivative thereof and emtricitabine or a physiologically functional derivative thereof are present in a ratio of 1:50 to 50:1 by weight.

28. The pharmaceutical formulation according to claim 24 wherein GS-7340 or a physiologically functional derivative thereof and emtricitabine or a physiologically functional derivative thereof are present in a ratio of 1:10 to 10:1 by weight.

29. The pharmaceutical formulation according to claim 24 in unit dosage form.

30. The pharmaceutical formulation according to claim 29 wherein GS-7340 or a physiologically functional derivative thereof and emtricitabine or a physiologically functional derivative thereof are each and individually present in an amount from 100 mg to 1000 mg per unit dosage form.

31. The pharmaceutical formulation according to claim 24 comprising GS-7340 and emtricitabine.

32. The pharmaceutical formulation according to claim 31 comprising about 150 mg of GS-7340 and about 200 mg of emtricitabine.

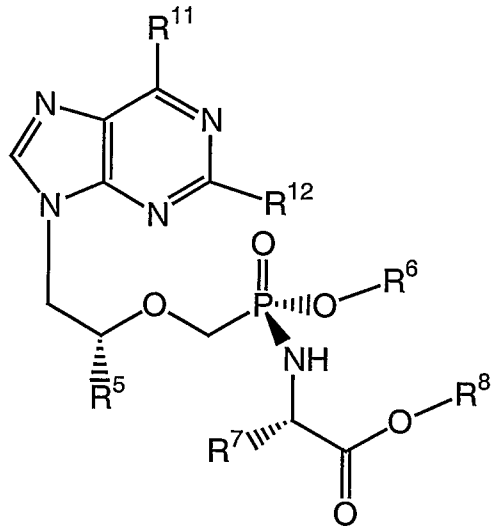
33. The pharmaceutical formulation according to claim 24 suitable for oral administration.

34. The pharmaceutical formulation according to claim 30 in the form of a tablet or capsule.

35. The pharmaceutical formulation according to claim 30 suitable for administration once per day to an infected human.

36. The pharmaceutical formulation according to claim 24 comprising a physiologically functional derivative of emtricitabine which is (2*R*, 5*S*, cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (3TC).

37. The pharmaceutical formulation according to claim 24 comprising a physiologically functional derivative of GS-7340 which has the structure:



wherein R^5 is H or CH_3; R^6 and R^8 are independently selected from H, C_1–C_6 alkyl, C_1–C_6 substituted alkyl, C_6–C_{20} aryl, C_6–C_{20} substituted aryl, C_6–C_{20} arylalkyl, and C_6–C_{20} substituted arylalkyl; R^7 is the side chain of any naturally-occurring or pharmaceutically acceptable amino acid and where if the side chain comprises carboxyl, the carboxyl group is optionally esterified with an alkyl or aryl group; R^{11} is amino, alkylamino, oxo, or dialkylamino; and R^{12} is amino or H;

or a pharmaceutically acceptable salt or solvate thereof.

38. The pharmaceutical formulation according to claim 37 wherein R^7 is H, CH_3 or $CH(CH_3)_2$.

39. The pharmaceutical formulation according to claim 37 wherein R^6 is phenyl.

40. The pharmaceutical formulation according to claim 37 wherein R^8 is CH_3, CH_2CH_3, or $CH(CH_3)_2$.

41. A patient pack comprising at least one active ingredient selected from 9-[*R*-2-[[(*S*)-[[(*S*)-1-(isopropoxycarbonyl)ethyl]amino]-phenoxyphosphinyl]methoxy]propyl]adenine (GS-7340) and (2*R* ,5*S*, cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine), and an information insert containing directions on the use of GS-7340 and emtricitabine together in combination.

42. The patient pack according to claim 41 comprising a co-formulated pill, tablet, caplet, or capsule of 100 to 1000 mg of GS-7340 and 100 to 1000 mg of emtricitabine.

43. The patient pack according to claim 41 comprising a co-formulated pill, tablet, caplet, or capsule of 300 mg of GS-7340 and 200 mg of emtricitabine.

44. The patient pack according to claim 41 comprising a separate pill, tablet, caplet, or capsule of 100 to 1000 mg of GS-7340 and 100 to 1000 mg of emtricitabine.

45. The patient pack according to claim 44 comprising a separate pill, tablet, caplet, or capsule of 150 mg of GS-7340 and 200 mg of emtricitabine.

46. A chemically stable combination of GS-7340 and emtricitabine.

47. The chemically stable combination of Claim 46 wherein the combination is a pharmaceutical dosage form.

48. The chemically stable combination of Claim 47 wherein the dosage form is oral.

49. The chemically stable combination of Claims 46-48 which further comprises a third antiviral agent.

50. The chemically stable combination of Claim 49 where in the third antiviral agent is an NNRTI or PI.

51. The chemically stable combination of Claim 50 wherein the third antiviral agent is a PI.

52. The chemically stable combination of Clam 50 wherein the third antiviral agent is an NNRTI.

53. The chemically stable combination of Claim 49 wherein the third antiviral agent is selected from Reyataz, Kaletra or Sustiva.

54. A chemically stable oral pharmaceutical dosage form comprising GS-7340 and emtricitabine.

55. A chemically stable oral pharmaceutical dosage form comprising GS-7340, emtricitabine and Reyataz.

56. A chemically stable oral pharmaceutical dosage form comprising GS-7340, emtricitabine and Kaletra.

57. A chemically stable oral pharmaceutical dosage form comprising GS-7340, emtricitabine and Sustiva.

5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/000868

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/675 A61K31/513
//(A61K31/675,31:513)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, INSPEC, CHEM ABS Data, EMBASE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RISTIG MARIA B ET AL: "Tenofovir disoproxil fumarate therapy for chronic hepatitis B in human immunodeficiency virus/hepatitis B virus-coinfected individuals for whom interferon-alpha and lamivudine therapy have failed." JOURNAL OF INFECTIOUS DISEASES, vol. 186, no. 12, 15 December 2002 (2002-12-15), pages 1844-1847, XP002284897 ISSN: 0022-1899	1,4-7, 9-11, 16-30, 33-36,41
Y	abstract page 1844, column 2, second last paragraph: "Subjects" page 1845, column 2, last paragraph before "Discussion"	2,3,8, 12-15, 31,32, 37-40, 42-57

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 June 2004	16. 07. 2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Rodriguez-Palmero, M

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MURRY, JEFFREY P. ET AL: "Reversion of the M184V mutation in simian immunodeficiency virus reverse transcriptase is selected by tenofovir , even in the presence of lamivudine" JOURNAL OF VIROLOGY , 77(2), 1120-1130 CODEN: JOVIAM; ISSN: 0022-538X, 12 January 2003 (2003-01-12), XP002284898	1,4-7, 9-11, 16-30, 33-36,41
Y	page 1126, figure 2 page 1129, column 1, paragraph 2	2,3,8, 12-15, 31,32, 37-40, 42-57

X	"Anti-HIV drug updates--three drugs on the near horizon." PROJECT INFORM PERSPECTIVE. JAN 2003, no. 35, January 2003 (2003-01), pages 4-7, XP001181983	1,4-7, 9-11, 16-30, 33-36,41
Y	page 6, column 2, paragraph 3 - page 7, column 2, paragraph 3	2,3,8, 12-15, 31,32, 37-40, 42-57

X	MULATO A S ET AL: "ANTI-HIV ACTIVITY OF ADEFOVIR (PMEA) AND PMPA IN COMBINATION WITH ANTIRETROVIRAL COMPOUNDS: IN VITRO ANALYSES" ANTIVIRAL RESEARCH, ELSEVIER SCIENCE BV., AMSTERDAM, NL, vol. 36, no. 2, November 1997 (1997-11), pages 91-97, XP000890091 ISSN: 0166-3542	1,4-7, 9-11, 16-30, 33-36
Y	page 93, column 2, paragraph 2 - page 95, column 1, paragraph 2 page 94, figure 1	2,3,8, 12-15, 31,32, 37-40, 42-57

X	WO 00/25797 A (BARRY DAVID ; ROUSSEAU FRANCK (US); FURMAN PHILLIP A (US); PAINTER GEO) 11 May 2000 (2000-05-11) page 5, line 5-12 page 15, lines 1-24 claims 5, 10 and 20	24-30, 33-36,41

Y	WO 02/08241 A (GILEAD SCIENCES INC ; BECKER MARK W (US); HE GONG XIN (US); LEE WILLIA) 31 January 2002 (2002-01-31) page 6, line 20 - page 7, line 18 page 37, line 10 - page 40, line 9	2,3,8, 12-15, 31,32, 37-40, 42-57

	-/--	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RICHMAN D D: "Antiretroviral activity of emtricitabine, a potent nucleoside reverse transcriptase inhibitor." ANTIVIRAL THERAPY. JUN 2001, vol. 6, no. 2, June 2001 (2001-06), pages 83-88, XP009032162 ISSN: 1359-6535 page 84, column 2, second last paragraph - page 85, column 1, paragraph 2 -----	1-57
A	DE CLERCQ ERIK: "New anti-HIV agents and targets" MEDICINAL RESEARCH REVIEWS, vol. 22, no. 6, November 2002 (2002-11), pages 531-565, XP002284899 ISSN: 0198-6325 page 541, paragraph 3 - page 542, paragraph 5 -----	1-57

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/000868

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-23 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US2004/000868

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0025797 A	11-05-2000	AU 1810600 A	22-05-2000
		CN 1329497 T	02-01-2002
		EP 1380303 A1	14-01-2004
		EP 1382343 A1	21-01-2004
		EP 1124562 A1	22-08-2001
		ID 29471 A	30-08-2001
		JP 2002528508 T	03-09-2002
		WO 0025797 A1	11-05-2000
		US 2003158150 A1	21-08-2003
		US 6528515 B1	04-03-2003
WO 0208241 A	31-01-2002	AU 8294101 A	05-02-2002
		BG 107572 A	28-11-2003
		BR 0112646 A	24-06-2003
		CA 2416757 A1	31-01-2002
		CN 1443189 T	17-09-2003
		CZ 20030413 A3	17-12-2003
		EP 1301519 A2	16-04-2003
		HU 0301307 A2	29-09-2003
		JP 2004504402 T	12-02-2004
		NO 20030270 A	20-03-2003
		WO 0208241 A2	31-01-2002
		US 2004018150 A1	29-01-2004
		US 2003219727 A1	27-11-2003
		US 2002119443 A1	29-08-2002
		ZA 200210271 A	28-10-2003

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

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:

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CHRU Themba Lethu Clinic - Helen Joseph Hospital

PRS Account

Global Alliance for TB Drug Development

Verification Date

April 2020

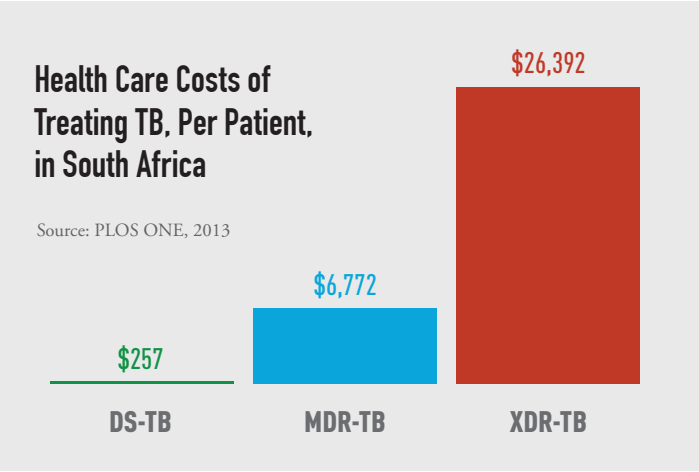
ICMJE **Data element required by the International Committee of Medical Journal Editors and the World Health Organization ICTRP**

Exhibit-C

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.

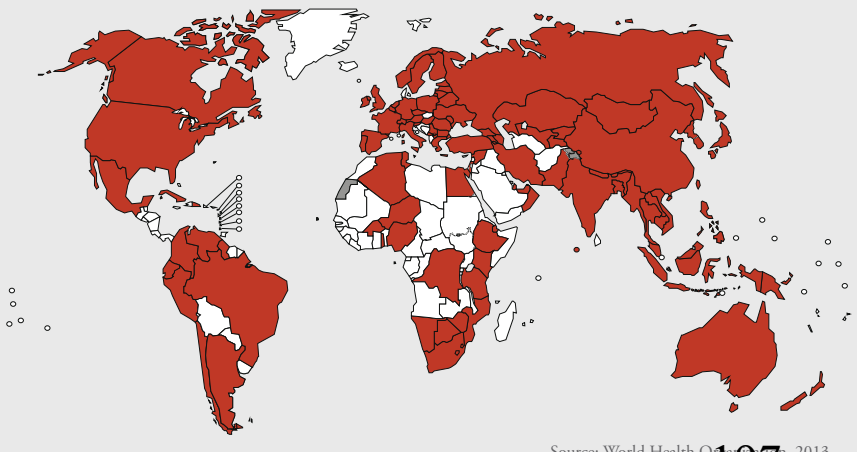


Current care and treatment for XDR-TB

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.

Bactericidal Activity of Pyrazinamide and Clofazimine Alone and in Combinations with Pretomanid and Bedaquiline

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Abstract

Rationale: New regimens to shorten tuberculosis treatment and manage patients with drug-resistant tuberculosis who are infected with HIV are urgently needed. Experimental and clinical evidence suggests that the new drugs bedaquiline (B) and pretomanid (Pa), combined with an existing drug, pyrazinamide (Z), and a repurposed drug, clofazimine (C), may assist treatment shortening of drug-susceptible and drug-resistant tuberculosis.

Objectives: To evaluate the 14-day bactericidal activity of C and Z in monotherapy and in combinations with Pa and B.

Methods: Groups of 15 treatment-naive, sputum smear–positive patients with pulmonary tuberculosis were randomized to receive combinations of B with Z-C, Pa-Z, Pa-Z-C, and Pa-C, or C or Z alone, or standard combination treatment for 14 days. The primary endpoint was the mean daily fall in $\log_{10}$ *Mycobacterium tuberculosis* CFU per milliliter sputum estimated by joint nonlinear mixed-effects Bayesian regression modeling.

Measurements and Main Results: Estimated activities were 0.167 (95% confidence interval [CI], 0.075–0.257) for B-Pa-Z, 0.151 (95% CI, 0.071–0.232) for standard treatment, 0.124 (95% CI, 0.035–0.214) for B-Z-C, 0.115 (95% CI, 0.039–0.189) for B-Pa-Z-C, and 0.076 (95% CI, 0.005–0.145) for B-Pa-C. Z alone had modest activity (0.036; 95% CI, −0.026 to 0.099). C had no activity alone (−0.017; 95% CI, −0.085 to 0.053) or in combinations. Treatments were well tolerated and safe.

Conclusions: B-Pa-Z, including two novel agents without resistance in prevalent *M. tuberculosis* strains, is a potential new tuberculosis treatment regimen. C had no measurable activity in the first 14 days of treatment.

Clinical trial registered with www.clinicaltrials.gov (NCT 01691534).

Keywords: tuberculosis; antitubercular agents; drug evaluation

Although recent World Health Organization (WHO) Global Tuberculosis Reports have, for the first time, indicated a drop in some tuberculosis (TB) indicators in most world regions, this improvement is insufficient to meet the aims of TB control bodies (1); furthermore, accompanying this decline is an increasing awareness of the need for new anti-TB drugs and regimens not only to shorten the treatment duration of fully drug-sensitive TB, but also to manage increasing numbers of patients with multidrug-resistant and extensively drug-resistant TB being identified worldwide (1). To accelerate the

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Author Contributions: A.H.D. contributed to execution of the study, interpretation, and report writing. A.H.D., R.D., F.v.G.-B., and G.S. contributed to patient management. A.H.D., P.R.D., C.v.N., J.H., D.E., and C.M.M. contributed to the study design. A.V. contributed microbiology data. D.E., C.M.M., D.A.B., and R.S. contributed to the data analysis. A.H.D., D.E., P.R.D., C.M.M., D.A.B., R.S., and C.v.N. contributed to the data interpretation. D.A.B. and R.S. contributed to the writing of the statistical concepts. P.R.D. and A.H.D. wrote the first draft.

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At a Glance Commentary

Scientific Knowledge on the Subject

Novel antituberculosis drugs and regimens are urgently needed to shorten tuberculosis treatment and manage patients with drug-resistant tuberculosis and infected with HIV. Early bactericidal activity studies are the first step in their evaluation. The activity of pyrazinamide and clofazimine alone and in combinations with pretomanid and bedaquiline over the first 14 days of treatment is largely unknown.

What This Study Adds to the Field

The 14-day bactericidal activity of several drug combinations was not significantly different from standard isoniazid-rifampin-pyrazinamide-ethambutol treatment. The combination of bedaquiline, pretomanid, and pyrazinamide seems safe to take forward to longer-term evaluations. The study confirmed the relatively low 14-day activity of pyrazinamide alone and confirmed its activity in combination with bedaquiline or pretomanid. Clofazimine had no demonstrable activity and seems not to add to the activity of other drugs in combinations.

development of much needed new drugs and regimens increasing use is made of early evaluation not only of single drugs but novel regimens constructed from new, established, or repurposed drugs based on results from murine studies and early clinical experience.

For this randomized phase 2 trial that is part of a program of evaluating promising new drug combinations early in clinical development, two new drugs, bedaquiline (B) and pretomanid (Pa; recently renamed from PA-824), were chosen for further evaluation in company with the older drug pyrazinamide (Z), which augmented the activity of other anti-TB drugs, both older first-line agents and recently introduced agents, in murine models (2–4) and clinical studies (5). Also studied was clofazimine (C), an established antileprosy agent; in open uncontrolled studies C was part of a regimen that shortened the time needed to cure patients with multidrug-resistant TB to 9 months in comparison with the 18–24 months usually recommended by WHO (6).

The objective of this trial was to evaluate antimycobacterial activity, safety, tolerability, and pharmacokinetics of different combinations of B, Pa, C, and Z to select suitable combinations for further clinical assessment in longer more comprehensive studies, and of C and Z given alone to estimate the contribution of these agents to the combination regimens. Some of the results of this study have been reported in the form of an abstract (7).

Methods

Trial Design, Patients, and Procedures

This was a phase 2A, two-center, open-label, randomized clinical trial to assess the 14-day early bactericidal activity (EBA), safety, tolerability, and pharmacokinetics of Z and C given alone or in combinations of B-Pa-Z, B-Pa-Z-C, B-Pa-C, B-Z-C, or standard anti-TB treatment (isoniazid [H], rifampin [R], Z, ethambutol [E]; HRZE) in seven parallel groups of 15 treatment-naive, sputum microscopy smear-positive (≥1+ on the WHO–IUATLD scale) (8) patients with pulmonary TB. The study was conducted between October 2012 and April 2013 and registered with clinicaltrials.gov.

Patients were consenting adults from outpatient clinics in Cape Town, South Africa, with body weight of 40–90 kg without complicating factors that might compromise safety or interpretation of endpoints. Excluded were patients infected with HIV with less than or equal to 300 CD4 cells per microliter, and patients with a history or signs of lens opacities, significant cardiac arrhythmia, or QT-prolongation. Subjects were hospitalized at the Task Clinical Research Centre, Bellville, or the Centre for Tuberculosis Research Innovation, University of Cape Town Lung Institute, for the duration of investigational treatment, and monitored daily for safety including regular laboratory assessments and 12-lead ECGs in the morning and evening before treatment and on Days 1, 2, 3, 8, and 14 after start of treatment. All ECGs were read by a central cardiology service and the QT intervals were corrected for the effect of heart rate by both the methods of Fridericia (QTcF) (9) and of Bazett (QtcB) (10). Two and 4 weeks after discharge patients were assessed for late safety signals and confirmation that they had started a full course of standard anti-TB treatment.

Eligible participants were randomized centrally by computer-generated sequence and by persons with no direct trial involvement. Sponsor staff, participants, investigators, pharmacists, and site staff were not masked to the regimens, but all laboratory staff involved in endpoint assessments were masked.

Mycobacteriology

Smear positivity and H and R susceptibility was ascertained before enrollment using auramine microscopy and GenoType MTB*DRplus* version 2 (Hain, Nehren, Germany) on a spot sputum sample. Sputum for CFU counts of *Mycobacterium tuberculosis* and determination of time to a positive signal (TTP) in liquid culture medium was collected daily for 16 hours overnight from 2 days before treatment initiation to the last treatment day; collections were completed before administration of the day's therapy and the samples transported to the laboratory at 2–8°C for processing as previously described for such studies (11, 12).

Microbiologic testing was done centrally in the Department of Medical Biochemistry, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa. Briefly, sputum was homogenized with magnetic stirring. Dithiothreitol (1:20 dilution; Sputasol; Oxoid, Cambridge, UK) was added to a maximum of 10 ml of homogenized sputum in equal volume, vortexed for 20 seconds, and left to digest at room temperature for 20 minutes. For CFU counting, 1 ml of this digested sputum was used to prepare a range of 10-fold dilutions from 10^0 to 10^{-5}. From each dilution, 100 μl was plated in quadruplicate on 7H11 agar plates (Becton Dickinson, Franklin Lakes, NJ) that contained 200 U/ml of polymyxin B, 10 μg/ml of amphotericin B, 100 μg/ml of ticarcillin, and 10 μg/ml of trimethoprim (Selectatab; Mast, Merseyside, UK). Numbers of CFU were counted after 3–4 weeks of incubation at 37°C at the dilution yielding 20–200 visible colonies.

For the TTP measurement, we used a standardized liquid culture system (Bactec Mycobacteria Growth Indicator Tube;

MGIT 960; Becton Dickinson). Briefly, homogenized sputum was decontaminated (AlphaTec NAC-PAC Red; AlphaTec, Vancouver, BC, Canada), centrifuged, resuspended, and 0.5 ml of the resulting 2 ml was used for incubation in duplicate. Cultures from baseline overnight sputum collections were used for drug susceptibility testing against first-line anti-TB agents. Z was additionally tested on cultures from the Day 14 overnight sputum sample in subjects on the Z-alone treatment (MGIT 960, Becton Dickinson).

Minimum inhibitory concentrations (MIC) using a broth microdilution method were determined for B (60 subjects), Pa (45 subjects), and C (60 subjects) on the isolates recovered at baseline, and the MIC of C repeated on the Day-14 isolates from subjects on the C-alone treatment. In summary, cultures were grown in Difco 7H9 broth (Becton Dickinson) containing 0.05% Tween 80 (Sigma-Aldrich, Johannesburg, South Africa), incubated at 37°C for 10–14 days and adjusted to 0.5 McFarland standard. Sterile Difco 7H9 broth containing 0.1% casitone (Sigma-Aldrich) and 0.5% glycerol (Sigma-Aldrich) was prepared and 100 μl dispensed into each well of a 96-well microtiter plate. Drugs were diluted in 7H9 broth to obtain a 128 μg/ml concentration of which 100 μl was added to the first row of wells, mixed, and 100 μl from the first row transferred to the second row. Twofold dilutions were made up to the 11th row to obtain a 32–0.03 μg/ml range. The 12th row was a blank control. A total of 100 μl of each bacterial inoculum was suspended into the drug-containing and control wells; final volume per well was 200 μl. Plates were sealed, incubated at 37°C for 14 days, removed to add 50 μl of diluted resazurin (Sigma-Aldrich) to each well, and incubated a further 24 hours before the results were read. The lowest concentration of the drug showing no color change was considered the MIC. *M. tuberculosis* speciation was by polymerase chain reaction (13).

Pharmacokinetics

The pharmacokinetic (PK) plasma concentrations were obtained hourly from time 0 to 5 hours after dosing and again at 10, 16, and 24 hours on Day 14 for B and its M2 metabolite, Pa, Z, and C during the administration of every treatment arm containing the relevant drug, except for standard treatment. We calculated C_{min} (plasma concentration 24 hours after dosing), C_{max} (maximum observed plasma concentration), T_{max} (time to reach C_{max}, obtained without interpolation), $AUC_{(0\text{-}t)}$ (area under the plasma concentration–time curve from zero to the last quantifiable PK plasma concentration before the subsequent dose, using the linear trapezoidal rule), and $AUC_{(0\text{–}24)}$ (area under the PK plasma concentration-time curve from 0 to 24 h). Before calculation of $AUC_{(0\text{–}24)}$, the 24-hour post-dose PK plasma concentration, if not available, was interpolated using the half-life of the PK profile. For each analyte the time above MIC (TMIC) was calculated as the percentage of time the plasma concentration was above MIC. A noncompartmental approach for fitting individual plasma concentrations at Day 14 was followed. Bioanalysis for drug plasma concentrations was conducted by PRA (Lenexa, KS) using liquid–liquid extraction and ultra performance liquid chromatography with tandem mass spectrometric detection. The limit of detection was 10.0 ng/ml for Pa, 4.0 ng/ml for C, 5.0 ng/ml for B, and 0.5 μg/ml for Z.

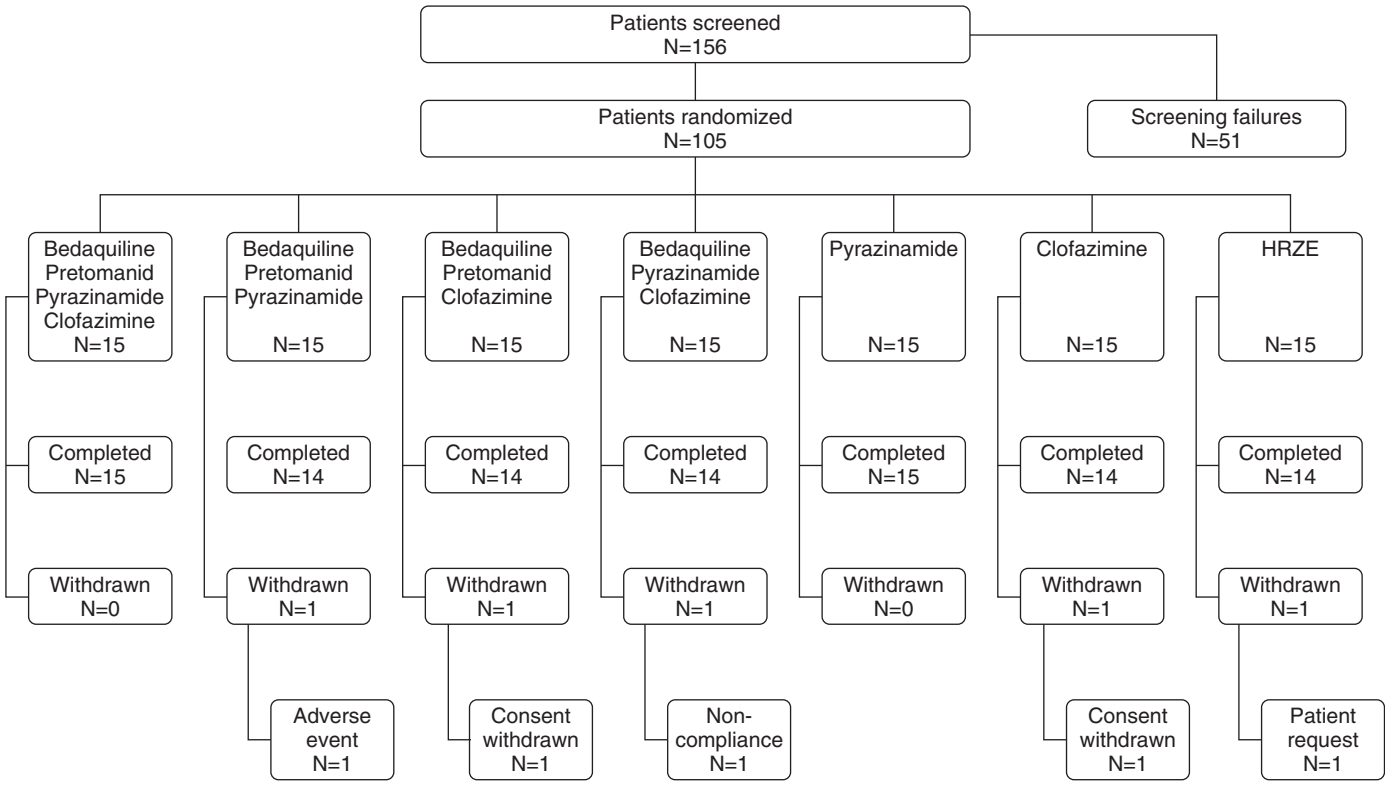

Figure 1. Patient disposition. HRZE = isoniazid (H), rifampin (R), pyrazinamide (Z), ethambutol (E).

Table 1. Baseline Characteristics

Characteristic	B-Pa-Z-C	B-Pa-Z	B-Pa-C	B-Z-C	Z	C	HRZE	All
Subjects, n	15	15	15	15	15	15	15	105
Age, yr	35.5 (8.74)	33.4 (14.22)	33.3 (11.15)	30.9 (13.12)	32.4 (11.44)	28.4 (8.77)	34.3 (10.25)	32.6 (11.15)
Males, %	12 (80.0)	12 (80.0)	7 (46.7)	9 (60.0)	9 (60.0)	9 (60)	7 (46.7)	65 (61.9)
Ethnicity*								
Black, n (%)	9 (60.0)	5 (33.3)	6 (40.0)	8 (53.3)	8 (53.3)	6 (40.0)	9 (60.0)	51 (48.6)
Mixed ethnic, n (%)	6 (40.0)	10 (66.7)	9 (60.0)	7 (46.7)	6 (40.0)	9 (60.0)	6 (40.0)	52 (49.5)
Height, cm	168.6 (7.98)	165.3 (8.64)	166.8 (11.37)	166.6 (11.86)	165.1 (7.58)	167.8 (9.05)	164.3 (8.74)	166.3 (9.28)
Weight, kg	56.4 (9.49)	50.2 (7.36)	57.38 (10.58)	58.7 (9.21)	52.4 (7.21)	53.3 (9.41)	56.1 (10.80)	55.0 (9.43)
BMI, kg/m^2	19.9 (3.66)	18.4 (1.98)	21.0 (4.64)	21.3 (3.34)	19.2 (1.86)	18.8 (2.15)	20.8 (3.29)	19.9 (3.23)
HIV-infected, %	1 (6.7)	1 (6.7)	3 (20.0)	0 (0.0)	2 (13.3)	1 (6.7)	3 (20.0)	11 (10.5)
Log_{10}CFU/ml sputum (95% CI)†	5.524 (5.244–5.814)	5.504 (5.185–5.827)	5.579 (5.244–5.950)	5.206 (4.883–5.533)	5.581 (5.259–5.930)	5.516 (5.266–5.772)	5.399 (5.083–5.718)	5.471 (5.222–5.719)
TTP, h (95% CI)†	107.4 (89.9–127.6)	98.7 (82.8–119.0)	90.8 (77.7–106.7)	118.5 (99.5–140.9)	101.4 (84.5–121.5)	100.4 (84.7–119.1)	122.1 (101.6–146.2)	105.2 (80.4–137.7)

Definition of abbreviations: B = bedaquiline; BMI = body mass index; C = clofazimine; CI = confidence interval; HRZE = isoniazid (H), rifampin (R), pyrazinamide (Z), ethambutol (E); Pa = pretomanid; TTP = time to positivity.

*One subject white (B-Pa-C) and one subject Asian (Z).

†Derived from the joint Bayesian nonlinear mixed-effects regression model.

Statistical Analyses

The primary efficacy endpoint was the EBA expressed as the rate of change in $\log_{10}$CFU counts over the 14 days of treatment ($EBA_{CFU}0–14$); secondary endpoints included the EBA determined from the change in TTP in liquid media ($EBA_{TTP}0–14$) and the EBA by both methodologies over secondary time points: $EBA_{CFU}0–2$, $EBA_{CFU}7–14$, $EBA_{TTP}0–2$, and $EBA_{TTP}7–14$.

To estimate EBA parameters a hierarchical Bayesian nonlinear mixed effects regression model was fitted to all patients' CFU and TTP, respectively, from Day 0 to 14 jointly. This technique has recently been described elsewhere in detail (14, 15). The model is suited for small datasets and can describe treatment responses over time quantitatively from CFU or TTP data even when cultures are increasingly negative as a consequence of potent treatments or longer observation times in phase 2 studies. The regression function consisted of parameters describing the intercept, two slopes characterizing the rate of decline during an initial and late phase of the treatment period, a node parameter at which transition from one slope to another occurs, and a parameter governing this transition. We used the parameters from this model to describe the EBA of each patient from the basic fits of $\log_{10}$CFU and $\log_{10}$TTP. Pairwise between-treatment comparisons were done with analysis of variance.

The sample size of 15 patients per treatment arm conformed with similar phase 2 trials and allowed for up to three dropouts per treatment arm, which, based on previous similar trials conducted at these centers, represented a conservative estimate of the expected dropout rate.

Role of the Funding Source

The Global Alliance for TB Drug Development participated in study design and data collection, and contributed to data analysis and its interpretation and the writing of this report. The corresponding author had full access to all study data and took the final responsibility regarding submission for publication. The study was sponsored by the Global Alliance for TB Drug Development with support from the Bill and Melinda Gates Foundation, the U.S. Agency for International Development, UK Department for International Development, Irish Aid, and Australia Department of Foreign Affairs and Trade.

Results

Patients

The disposition of patients and the baseline characteristics are summarized in Figure 1 and Table 1, respectively. There were no significant differences between the groups. Five patients were withdrawn from the study; two withdrew consent, one developed an adverse event and was withdrawn according to protocol requirements, one patient was noncompliant with medication, and one patient requested discharge for family reasons.

Antimycobacterial Activity

Activities estimated by the reduction of logCFU counts and extension of TTP during the 14-day study can be found in Tables 2 and 3, and are illustrated in Figures 2 and 3. The activity of the standard regimen did not show a pronounced initial fall of bacterial load as it is sometimes seen but it was within the range of previous results and validated the laboratory methodology. The highest mean $EBA_{CFU}0–14$ estimate (primary endpoint) was found with B-Pa-Z. All combinations' activity was significantly

Table 2. Bactericidal Activity of Combinations of Bedaquiline, Pretomanid, Pyrazinamide, and Clofazimine, and Pyrazinamide and Clofazimine Alone Expressed as the Daily Rate of Change in $\log_{10}$CFU of *Mycobacterium tuberculosis* per Milliliter Sputum Using Joint Bayesian Nonlinear Mixed-Effects Regression Modeling

Drug Regimen	Period		
	0–14 d	0–2 d	7–14 d
B-Pa-Z-C			
n	13	13	13
$\log_{10}$CFU/ml sputum, mean (95% CI)	0.115 (0.039 to 0.189)	0.161 (0.042 to 0.279)	0.085 (−0.013 to 0.175)
B-Pa-Z			
n	12	12	12
$\log_{10}$CFU/ml sputum, mean (95% CI)	0.167 (0.075 to 0.257)	0.196 (0.061 to 0.330)	0.146 (0.033 to 0.248)
B-Pa-C			
n	15	15	15
$\log_{10}$CFU/ml sputum, mean (95% CI)	0.076 (0.005 to 0.145)	0.062 (−0.045 to 0.161)	0.085 (−0.006 to 0.182)
B-Z-C			
n	13	13	13
$\log_{10}$CFU/ml sputum, mean (95% CI)	0.124 (0.035 to 0.214)	0.132 (0.008 to 0.262)	0.118 (−0.017 to 0.250)
Z			
n	15	15	15
$\log_{10}$CFU/ml sputum, mean (95% CI)	0.036 (−0.026 to 0.099)	0.080 (−0.028 to 0.209)	0.022 (−0.058 to 0.101)
C			
n	14	14	14
$\log_{10}$CFU/ml sputum, mean (95% CI)	−0.017 (−0.085 to 0.053)	0.018 (−0.089 to 0.125)	−0.038 (−0.130 to 0.046)
HRZE			
n	15	15	15
$\log_{10}$CFU/ml sputum, mean (95% CI)	0.151 (0.071 to 0.232)	0.141 (0.039 to 0.251)	0.157 (0.048 to 0.267)

Definition of abbreviations: B = bedaquiline; C = clofazimine; CI = confidence interval; HRZE = isoniazid (H), rifampin (R), pyrazinamide (Z), ethambutol (E); Pa = pretomanid.

Table 3. Bactericidal Activity of Combinations of Bedaquiline, Pretomanid, Pyrazinamide, and Clofazimine, and Pyrazinamide and Clofazimine Alone Expressed as the Daily Percentage Change in Time to Positive Signal in Liquid Culture for *Mycobacterium tuberculosis* over the Particular Time Period Using Joint Bayesian Nonlinear Mixed-Effects Regression Modeling

Drug Regimen	Period		
	0–14 d	0–2 d	7–14 d
B-Pa-Z-C			
n	13	13	13
% change, mean (95% CI)	6.3 (4.2 to 8.6)	10.6 (8.0 to 13.3)	3.6 (1.5 to 6.2)
B-Pa-Z			
n	14	14	14
% change, mean (95% CI)	7.0 (5.1 to 9.4)	13.2 (9.0 to 17.9)	4.5 (2.9 to 6.2)
B-Pa-C			
n	15	15	15
% change, mean (95% CI)	4.3 (2.9 to 5.7)	6.0 (4.2 to 7.8)	3.1 (1.7 to 4.7)
B-Z-C			
n	13	13	13
% change, mean (95% CI)	4.9 (3.3 to 6.8)	9.1 (6.5 to 12.2)	3.0 (1.5 to 4.9)
Z			
n	15	15	15
% change, mean (95% CI)	2.0 (0.8 to 3.4)	4.7 (2.4 to 7.5)	0.8 (−0.7 to 2.3)
C			
n	14	14	14
% change, mean (95% CI)	−0.3 (−1.5 to 1.0)	2.1 (−0.5 to 5.0)	−1.3 (−2.9 to 0.4)
HRZE			
n	15	15	15
% change, mean (95% CI)	6.3 (4.8 to 7.6)	12.9 (8.9 to 17.9)	4.4 (2.9 to 5.8)

Definition of abbreviations: B = bedaquiline; C = clofazimine; CI = confidence interval; HRZE = isoniazid (H), rifampin (R), pyrazinamide (Z), ethambutol (E); Pa = pretomanid.

different from zero and not significantly different from standard treatment. Z alone showed little activity. C alone was not active and did not add to the combinations where this could be assessed. The activity of both single drug treatments was not statistically different from zero as estimated from logCFU.

TTP measurements revealed less variation and more statistically significant findings than CFU counts. The ranking of mean $EBA_{TTP}0–14$ was similar to the ranking of mean $EBA_{CFU}0–14$. B-Pa-Z had the greatest activity followed by standard treatment. The activity of B-Pa-C was found to be lower than that of standard treatment, and Z had modest activity but significantly greater than zero. The activity of C was not significantly different from zero.

We also conducted the primary EBA calculations discussed previously with more traditional regression methods and found very similar results. The remaining secondary activity measures followed a similar pattern to the Day 0–14 activities, with B-Pa-Z having the highest bactericidal activity as determined from CFU counts with the exception of the period 7–14 days, where standard treatment had a numerically greater fall in CFU counts. C did not show significant activity in any of the secondary endpoints or measurement methods.

Pharmacokinetics

Table 4 summarizes the median (range) of C_{max} and $AUC_{0–24}$ of Pa, B and its M2 metabolite, Z, and C on Treatment Day 14. The PK properties of Pa observed in this trial were consistent across Pa-containing treatment groups. Drug concentrations were somewhat higher than those observed during phase 1 trials in healthy volunteers (16, 17) and previous EBA studies (5, 18, 19) when Pa was given fasting. This is probably the result of the concomitant drug administration with food during the current trial. B was given at a dose of 200 mg daily, but preceded by loading doses on treatment days 1–3, a dosing regimen similar to that in a previous dose-ranging monotherapy EBA study (20). The B PK findings, and those of its metabolite M2, were within the expected range for the current dose and consistent across treatment arms. The C_{max} of Z also fell within the range expected following a dose of approximately 30 mg/kg body weight (21). C concentrations varied widely between individuals but were within the ranges expected from single-dose studies in healthy volunteers (22).

Mycobacteriology

All isolates were speciated as *M. tuberculosis*. All tested isolates were susceptible to B with a MIC of less than 0.03 μg/ml. The MICs for Pa ranged from less than 0.03 to 0.06 μg/ml for isolates from group B-Pa-C and from less than 0.03 to 0.125 μg/ml for isolates from groups B-Pa-Z-C and B-Pa-Z. The median MIC of C for all tested isolates from patients receiving C was 0.125 μg/ml and ranged from less than 0.03 to 0.25 μg/ml. Of the 14 paired isolates, seven showed identical values and seven increased or decreased by one to three dilutions steps after the 14 days of treatment, but not all pairs were tested on the same batch. All isolates from patients treated with Z alone were susceptible to Z at baseline and after 14 days of treatment. Phenotypical Z resistance was detected in two isolates from patients treated with B-Pa-Z-C and B-Z-C who were susceptible to all other drugs tested. These patients were removed from the activity calculations. Phenotypical H resistance was found in three patients on cultures grown from sputum before treatment but none was in the HRZE group.

Safety and Tolerability

Treatment-emergent adverse events were experienced by 65 (61.9%) patients, but in only 29 (27.6%) were these considered treatment-related (Table 5). Grade 3 or 4 events were experienced by seven (6.7%) patients; one patient receiving B-Pa-Z had a grade 3 rise in transaminase values (maximum alanine transaminase; 263 U/L) leading to premature withdrawal according to protocol; two others receiving B-Pa-Z and B-Pa-C, respectively, experienced grade 4 increased transaminase values reported after treatment completion. One patient receiving B-Z-C had grade 3 increased creatine kinase values at study

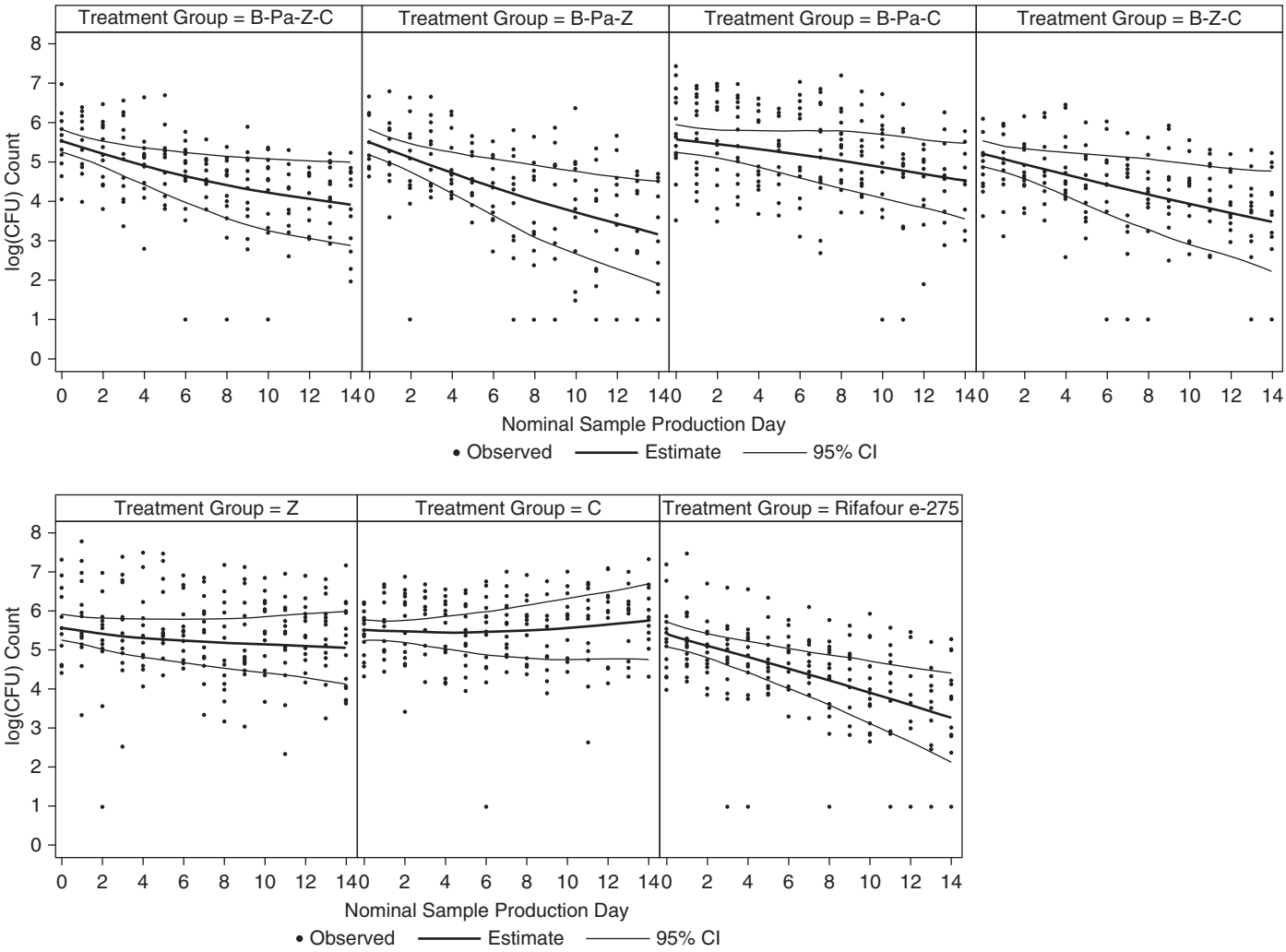

Figure 2. Mean $\log_{10}$CFU over time. Observed values (*dots*) and posterior estimates calculated from the joint Bayesian nonlinear mixed-effects regression model with 95% CIs of mean logCFU over time. B = bedaquiline; C = clofazimine; CI = confidence interval; Pa = pretomanid; Rifafour e-275 = HRZE consisting of isoniazid (H), rifampin (R), pyrazinamide (Z), ethambutol (E).

completion; two patients receiving C alone reported decreased appetite (grade 3) and gastroenteritis (grade 3). Two patients receiving C alone reported skin discoloration, as did one receiving B-Pa-Z-C.

Increased mean QTcB (Figure 4) and QTcF were found with all treatments, being lowest in patients receiving Z and standard treatment. No QTcB or QTcF greater than or equal to 500 milliseconds were reported. An increase from baseline of greater than or equal to 60 milliseconds in QTcB was reported for two patients (13.3%) in the B-Pa-C arm and for one patient (6.7%) in the C-alone arm. An increase from baseline of greater than or equal to 60 milliseconds in QTcF was reported for four patients (26.7%) in the B-Pa-C arm and for one patient (6.7%) in the C-alone arm. Correlations of PK and QT prolongation were weak and no consistent trends were observed.

Discussion

This study of anti-TB bactericidal activity measured by the fall in viable CFU of *M. tuberculosis* in sputum over the first 14 treatment days has demonstrated that the novel combination of two new drugs, B and Pa, with the established drug Z, has activity similar to that of the current standard treatment regimen of HRZE. This was true not only with regard to the primary efficacy endpoint of activity over the first 14 days of treatment but also for all secondary endpoints measured either by the fall in CFU counts or prolongation of TTP in liquid media. B-Pa-Z is a potential new anti-TB regimen that contains two novel drugs to which no resistant wild-type bacteria are expected to exist.

Not unexpectedly Z monotherapy had low activity, which was significant only when measured with the prolongation of TTP but not with the daily fall in CFU (0.036 logCFU/ml) over 14 days; in the only previous 14-day evaluation of the EBA of Z a considerably higher effect of 0.113 logCFU/ml was seen following a higher dose (2 vs. 1.5 g) and starting from a considerably higher baseline CFU count

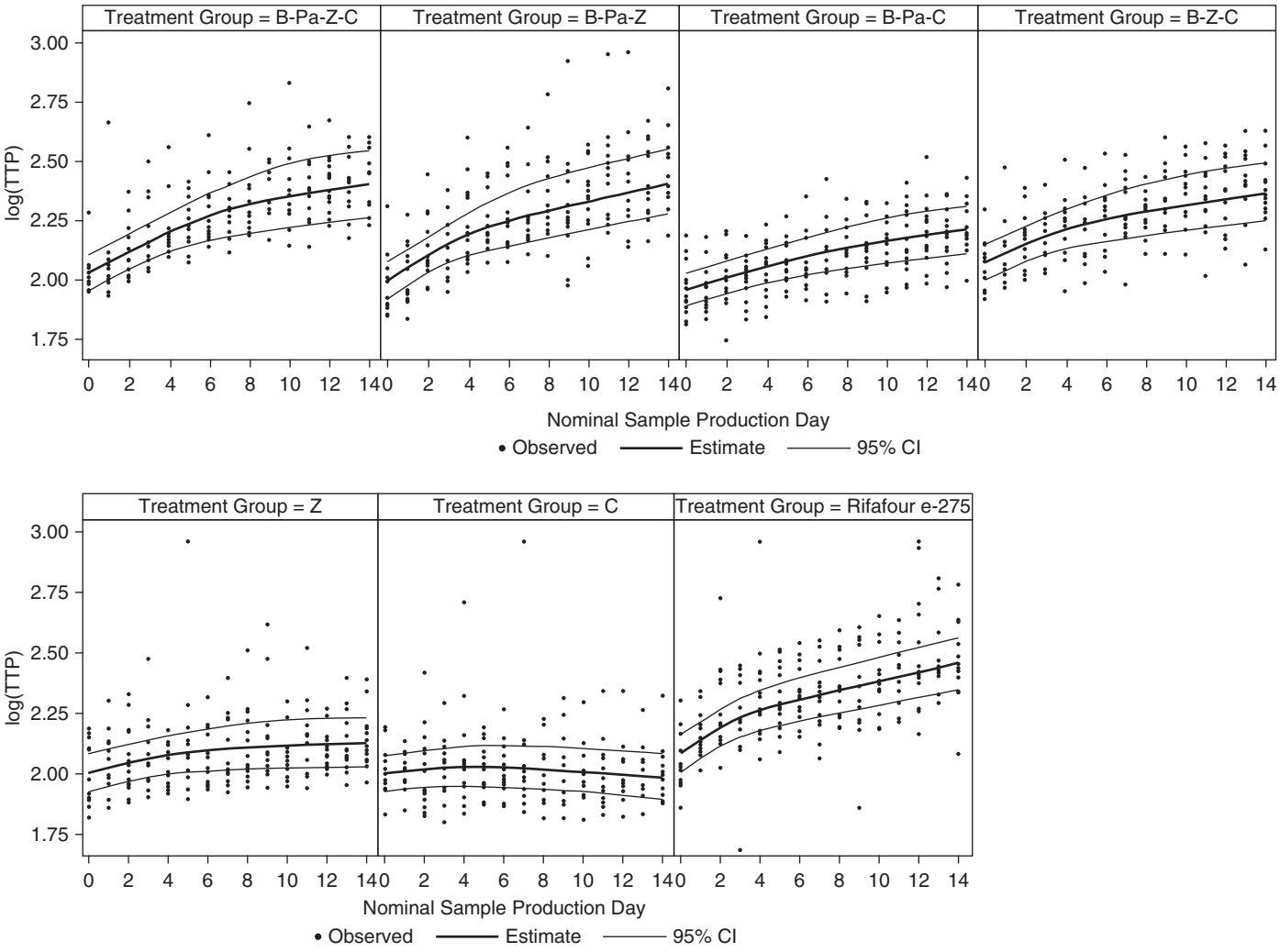

Figure 3. Mean $\log_{10}$TTP over time. Observed values (*dots*) and posterior estimates calculated from the joint Bayesian nonlinear mixed-effects regression model with 95% CIs of mean TTP over time. B = bedaquiline; C = clofazimine; CI = confidence interval; Pa = pretomanid; Rifafour e-275 = HRZE consisting of isoniazid (H), rifampin (R), pyrazinamide (Z), ethambutol (E); TTP = time to positivity.

(estimated 6.8 vs. 5.6 logCFU) (23); whether either of these factors played a role in the lower fall in CFU counts in this study is uncertain. Nonetheless, it is clear from this and previous studies that Z combined with Pa or B has potent antimycobacterial activity in murine (2, 3) and clinical TB (5). Although activity results measured by prolongation of TTP provided a very similar ranking of the drugs and regimens as logCFU it is interesting to observe that the 14-day EBA of Z was significantly different from zero when measured with TTP but not with logCFU. This is consistent with previous observations of TTP discriminating better between treatments than logCFU in EBA studies (24).

The results with C were disappointing. Not only was there no significant fall in CFU counts, nor any prolongation of TTP, but when added to other drug combinations the results tended to be poorer. Long-term murine studies have found C efficacious in the treatment of *M. tuberculosis* infections (25, 26) but *ex vivo* results have been negative (27) and clinical studies and reviews have reported anecdotal or equivocal results (6, 28, 29). Can C be adequately dosed to be active against *M. tuberculosis* at 100 mg/day? Using loading doses we predicted C_{max} levels of around 400 ng/ml at Day 14 (*see* Box 1) (30). Study subjects fell short of that with a median C_{max} of around 240 ng/ml across groups. In *post hoc* estimates we found these exposures to be comparatively adequate; the proportion of subjects with drug concentrations above MIC for the entire dosing interval was 62% following C-containing combinations and 70% following C alone, compared with 92% and 100% for Pa and B in subjects on Pa and B-containing combinations, respectively.

C and B are highly plasma protein bound. To account for this we estimated the percentage of time that B, Pa, or C plasma concentrations in individual participants were over the TMIC corrected for protein binding. This was done by multiplying MICs with a factor of 4 for Pa and a factor of 8 for B and for C to take into account the different fractions of free

Table 4. Pharmacokinetics of Bedaquiline, Bedaquiline Metabolite M2, Pretomanid, Pyrazinamide, and Clofazimine

Agent	Regimen	C_{max} (*ng/ml*)		AUC_{0-24} (*ng · h/ml*)	
		Median	Range	Median	Range
B	B-Pa-Z-C	2,100	1,020–4,860	23,468	15,020–55,107
	B-Pa-Z	2,520	1,720–3,360	29,048	19,219–37,097
	B-Pa-C	2,520	1,110–3,820	27,420	15,542–54,852
	B-Z-C	2,105	663–5,360	21,030.5	9,088–47,647
M2	B-Pa-Z-C	268.5	88.2–561	5,613.5	1,870–12,071
	B-Pa-Z	338	204–782	6,738	4,105–15,792
	B-Pa-C	170	108–327	3,669	2,366–6,342
	B-Z-C	262	135–493	5,137.5	3,028–10,271
Pa	B-Pa-Z-C	3,600	2,690–4,460	60,487	36,541–74,762
	B-Pa-Z	4,430	2,880–5,500	76,292	41,080–109,139
	B-Pa-C	3,600	2,330–6,130	61,534	35,462–119,234
Z	B-Pa-Z-C	38,300	32,200–48,700	408,851	307,960–588,811
	B-Pa-Z	42,500	30,700–54,300	455,114	301,344–521,190
	B-Z-C	36,150	26,500–61,400	389,953.5	310,595–578,335
	Z	42,800	33,000–55,200	415,693	320,530–690,000
C	B-Pa-Z-C	229	171–442	4,458.5	2,640–7,747
	B-Pa-C	243	74.8–436	4,267	1,259–8,421
	B-Z-C	268	91.9–413	3,741.5	1,728–8,633
	C	231.5	75–341	4,090.5	1,354–6,612

Definition of abbreviations: AUC_{0-24} = area under the plasma concentration–time curve from 0 to 24 hours; B = bedaquiline; C = clofazimine; C_{max} = maximum observed plasma concentration; M2 = metabolite of bedaquiline; Pa = pretomanid; Z = pyrazinamide.

compound in MIC medium and in plasma. The corrected TMIC was the percent time the plasma concentration was over that value in a given participant. Pa and B retained high proportions of individuals with TMIC over the entire dosing interval (92 and 86% of subjects, respectively), whereas the proportion of subjects with TMIC over the entire dosing interval fell to 0–7% for C-containing regimens and 0% for C alone (details of these estimates are available from the corresponding author on request). This means that sufficient free serum drug concentrations of C, which are likely relevant for drug activity against *M. tuberculosis* measured in sputum of patients with TB, were not achieved in this study.

It should also be noted that C is known to modulate certain immune functions and has displayed significant antiinflammatory characteristics in clinical (31) and *in vivo* animal studies (32). In a recent murine study, chronic administration of C led to an antiinflammatory response that might aid the actions of other anti-TB drugs without the agent having any specific mycobactericidal effect itself. This, however, is speculation and until C is evaluated in appropriately controlled long-term clinical studies uncertainty will remain. Skin discoloration, observed in a few patients even after exposure for only 2 weeks in this study, remains a concern for the acceptance of regimens containing C.

B, Pa, and C can potentially cause QT prolongation. In the present study all three agents were combined but QT prolongations remained within acceptable limits after 2 weeks of exposure. From a 24-week study mean increases in QTcF from baseline were reported to be larger in patients receiving B and C than in those receiving B but not C, although this was not associated with clinically relevant arrhythmias (33). There is no indication from this and a previous study of the combination of moxifloxacin, Pa, and Z (5) that the risk of serious arrhythmias is a limiting factor for the use of these regimens, but studies of longer duration with intensive ECG monitoring are required for firm conclusions.

Table 5. Adverse Events

Treatment	B-Pa-Z-C [*n* (%)]	B-Pa-Z [*n* (%)]	B-Pa-C [*n* (%)]	B-Z-C [*n* (%)]	Z [*n* (%)]	C [*n* (%)]	HRZE [*n* (%)]	Total [*n* (%)]
Gastrointestinal								
Diarrhea	2 (13.3)	0	1 (6.7)	0	1 (6.7)	0	0	4 (3.8)
Abdominal pain	1 (6.7)	0	0	0	0	0	1 (6.7)	2 (1.9)
Abdominal tenderness	0	1 (6.7)	0	0	0	1 (6.7)	0	2 (1.9)
Nausea	0	0	0	0	0	0	2 (13.3)	2 (1.9)
Vomiting	0	0	0	0	1 (6.7)	0	1 (6.7)	2 (1.9)
Dermatologic								
Skin discoloration	1 (6.7)	0	0	0	0	2 (13.3)	0	3 (2.9)
Pruritus	1 (6.7)	0	0	0	0	0	1 (6.7)	2 (1.9)
Papules	0	0	1 (6.7)	0	0	1 (6.7)	0	2 (1.9)
Nervous system								
Headache	0	2 (13.3)	1 (6.7)	1 (6.7)	2 (13.3)	1 (6.7)	1 (6.7)	8 (7.6)
Cardiovascular								
First-degree AV block	2 (13.3)	0	1 (6.7)	0	0	0	1 (6.7)	4 (3.8)
Prolonged QT interval	3 (20)	0	3 (20)	2 (13.3)	0	0	0	8 (7.6)
Laboratory toxicities								
Increased ALT	1 (6.7)	1 (6.7)	0	1 (6.7)	1 (6.7)	1 (6.7)	0	5 (4.8)

Definition of abbreviations: ALT = alanine aminotransferase; AV = atrioventricular; B = bedaquiline; C = clofazimine; HRZE = isoniazid (H), rifampin (R), pyrazinamide (Z), ethambutol (E); Pa = pretomanid.

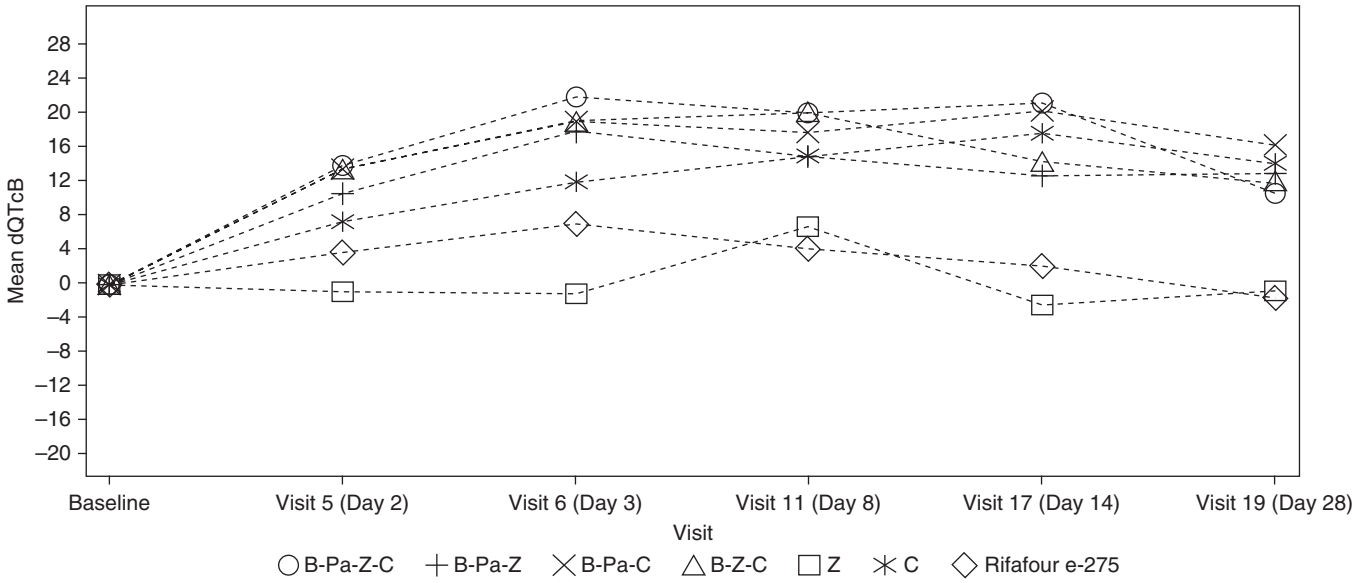

Figure 4. QTcB interval prolongation over time. Mean change from baseline in predose QTcB interval over time in milliseconds. A notably smaller mean change from baseline is observed for Z and standard treatment. B = bedaquiline; C = clofazimine; Pa = pretomanid; QTcB = QT interval corrected by Bazett method; Rifafour e-275 = HRZE consisting of isoniazid (H), rifampin (R), pyrazinamide (Z), ethambutol (E).

Conclusions

This study has shown the combination of B-Pa-Z to have activity similar to that of the current standard anti-TB regimen over the first 14 treatment days. Z had modest activity alone and was active in combination with bedaquiline or pretomanid. C displayed no activity alone and there was no indication of improved activity when it was added to other drug combinations. Pa, B, and C all have potential to lengthen the QT interval but QT interval prolongations remained within specified safety limits. The B-Pa-Z combination can now be taken forward to longer clinical studies assessing its activity in larger patient numbers with due attention to continued close observation of the QT interval. The suitability of this regimen for patients with multidrug-resistant TB, who have relatively high reported rates of phenotypical Z resistance in many areas, should be studied only in the setting of Z resistance testing. ■

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Box 1. Treatment Groups

Seven single agents or combinations were administered orally once daily for 14 consecutive days in the morning. Investigational treatments were given within 30 minutes after breakfast with 240 ml water and HRZE with a full glass of water 1 hour before or 2 hours after breakfast. For both B and C, loading doses were used to reach pharmacokinetic parameters assumed to be associated with efficacy more quickly within the study period. We modeled existing pharmacokinetic data for C to ensure that most subjects would be exposed to concentrations of C for most of the 14-day study as when given at 100 mg/day over months (30).

1. B-Pa-Z-C: Bedaquiline 400 mg on Day 1, 300 mg on Day 2, 200 mg on Days 3–14; pretomanid 200 mg; pyrazinamide 1,500 mg; clofazimine 300 mg on Days 1–3, 100 mg on Days 4–14.
2. B-Pa-Z: Bedaquiline 400 mg on Day 1, 300 mg on day 2, 200 mg on Days 3–14; pretomanid 200 mg; pyrazinamide 1,500 mg.
3. B-Pa-C: Bedaquiline 400 mg on Day 1, 300 mg on Day 2, 200 mg on Days 3–14; pretomanid 200 mg; clofazimine 300 mg on Days 1–3 and 100 mg on Days 4–14.
4. B-Z-C: Bedaquiline 400 mg on Day 1, 300 mg on Day 2, 200 mg on Days 3–14; pyrazinamide 1,500 mg; clofazimine 300 mg on Days 1–3, 100 mg on Days 4–14.
5. Z: Pyrazinamide 1,500 mg.
6. C: Clofazimine 300 mg on Days 1–3,100 mg on Days 4–14.
7. HRZE Rifafour e-275 (Sanofi, Midrand, South Africa): tablets containing rifampin 150 mg (R), isoniazid 75 mg (H), pyrazinamide 400 mg (Z), ethambutol 275 mg (E). Patients 40–54 kg, three tablets; 55–70 kg, four tablets; ⩾71 kg, five tablets.

References

1. World Health Organization. Global tuberculosis report. 2014 [accessed 2015 Feb 24]. Available from: http://www.who.int/tb/publications/global_report/en/
2. Ibrahim M, Andries K, Lounis N, Chauffour A, Truffot-Pernot C, Jarlier V, Veziris N. Synergistic activity of R207910 combined with pyrazinamide against murine tuberculosis. *Antimicrob Agents Chemother* 2007;51:1011–1015.
3. Tasneen R, Tyagi S, Williams K, Grosset J, Nuermberger E. Enhanced bactericidal activity of rifampin and/or pyrazinamide when combined with PA-824 in a murine model of tuberculosis. *Antimicrob Agents Chemother* 2008;52:3664–3668.
4. Williams K, Minkowski A, Amoabeng O, Peloquin CA, Taylor D, Andries K, Wallis RS, Mdluli KE, Nuermberger EL. Sterilizing activities of novel combinations lacking first- and second-line drugs in a murine model of tuberculosis. *Antimicrob Agents Chemother* 2012;56:3114–3120.
5. Diacon AH, Dawson R, von Groote-Bidlingmaier F, Symons G, Venter A, Donald PR, van Niekerk C, Everitt D, Winter H, Becker P, *et al*. 14-day bactericidal activity of PA-824, bedaquiline, pyrazinamide, and moxifloxacin combinations: a randomised trial. *Lancet* 2012;380:986–993.
6. Van Deun A, Maug AK, Salim MA, Das PK, Sarker MR, Daru P, Rieder HL. Short, highly effective, and inexpensive standardized treatment of multidrug-resistant tuberculosis. *Am J Respir Crit Care Med* 2010;182:684–692.
7. Diacon A, Dawson R, Van Niekerk C, Hutchings J, Murray S, Schall R, Burger D, Everitt D, Mendel C. 14 day EBA study of PA-824, bedaquiline, pyrazinamide and clofazimine in smear positive TB patients. Presented at the Conference on Retroviruses and Opportunistic Infections (CROI). March 3–6, 2014, Boston, MA.
8. International Union against Tuberculosis and Lung Disease. Laboratory diagnosis of tuberculosis by sputum microscopy: the handbook. 2013 [accessed 2015 Feb 24]. Available from: http://www.theunion.org/what-we-do/publications/technical/laboratory-diagnosis-of-tuberculosis-by-sputum-microscopy-the-handbook
9. Fridericia L. The duration of systole in the electrocardiogram of normal subjects and of patients with heart disease. *Acta Med Scand* 1920;53:469–486.
10. Bazett HC. The time relations of the blood-pressure changes after excision of the adrenal glands, with some observations on blood volume changes. *J Physiol* 1920;53:320–339.
11. Donald PR, Diacon AH. The early bactericidal activity of anti-tuberculosis drugs: a literature review. *Tuberculosis (Edinb)* 2008;88:S75–S83.
12. Diacon A, Maritz JS, Donald P. Early bactericidal activity of antituberculosis agents. In: Donald PR, Van Helden PD, editors. Antituberculosis chemotherapy. Basel: Karger; 2011. pp. 213–219.
13. Warren RM, Gey van Pittius NC, Barnard M, Hesseling A, Engelke E, de Kock M, Gutierrez MC, Chege GK, Victor TC, Hoal EG, *et al*. Differentiation of *Mycobacterium tuberculosis* complex by PCR amplification of genomic regions of difference. *Int J Tuberc Lung Dis* 2006;10:818–822.
14. Burger DA, Schall R. A Bayesian non-linear mixed effects regression model for the characterization of early bactericidal activity of tuberculosis drugs. *J Biopharm Stat* [online ahead of print] 16 Oct 2014; DOI: 10.1080/10543406.2014.971170.
15. Davies GR, Brindle R, Khoo SH, Aarons LJ. Use of nonlinear mixed-effects analysis for improved precision of early pharmacodynamic measures in tuberculosis treatment. *Antimicrob Agents Chemother* 2006;50:3154–3156.
16. Ginsberg AM, Laurenzi MW, Rouse DJ, Whitney KD, Spigelman MK. Assessment of the effects of the nitroimidazo-oxazine PA-824 on renal function in healthy subjects. *Antimicrob Agents Chemother* 2009;53:3726–3733.
17. Ginsberg AM, Laurenzi MW, Rouse DJ, Whitney KD, Spigelman MK. Safety, tolerability, and pharmacokinetics of PA-824 in healthy subjects. *Antimicrob Agents Chemother* 2009;53:3720–3725.
18. Diacon AH, Dawson R, du Bois J, Narunsky K, Venter A, Donald PR, van Niekerk C, Erondu N, Ginsberg AM, Becker P, *et al*. Phase II dose-ranging trial of the early bactericidal activity of PA-824. *Antimicrob Agents Chemother* 2012;56:3027–3031.
19. Diacon AH, Dawson R, Hanekom M, Narunsky K, Maritz SJ, Venter A, Donald PR, van Niekerk C, Whitney K, Rouse DJ, *et al*. Early bactericidal activity and pharmacokinetics of PA-824 in smear-positive tuberculosis patients. *Antimicrob Agents Chemother* 2010;54:3402–3407.
20. Diacon AH, Dawson R, Von Groote-Bidlingmaier F, Symons G, Venter A, Donald PR, Conradie A, Erondu N, Ginsberg AM, Egizi E, *et al*. Randomized dose-ranging study of the 14-day early bactericidal activity of bedaquiline (TMC207) in patients with sputum microscopy smear-positive pulmonary tuberculosis. *Antimicrob Agents Chemother* 2013;57:2199–2203.
21. Donald PR, Maritz JS, Diacon AH. Pyrazinamide pharmacokinetics and efficacy in adults and children. *Tuberculosis (Edinb)* 2012;92:1–8.
22. Nix DE, Adam RD, Auclair B, Krueger TS, Godo PG, Peloquin CA. Pharmacokinetics and relative bioavailability of clofazimine in relation to food, orange juice and antacid. *Tuberculosis (Edinb)* 2004;84:365–373.
23. Jindani A, Aber VR, Edwards EA, Mitchison DA. The early bactericidal activity of drugs in patients with pulmonary tuberculosis. *Am Rev Respir Dis* 1980;121:939–949.
24. Kayigire XA, Friedrich SO, Venter A, Dawson R, Gillespie SH, Boeree MJ, Heinrich N, Hoelscher M, Diacon AH; Pan African Consortium for the Evaluation of Anti-Tuberculosis Antibiotics. Direct comparison of Xpert MTB/RIF assay with liquid and solid mycobacterial culture for quantification of early bactericidal activity. *J Clin Microbiol* 2013;51:1894–1898.
25. Reddy VM, Nadadhur G, Daneluzzi D, O'Sullivan JF, Gangadharam PR. Antituberculosis activities of clofazimine and its new analogs B4154 and B4157. *Antimicrob Agents Chemother* 1996;40:633–636.
26. Grosset JH, Tyagi S, Almeida DV, Converse PJ, Li SY, Ammerman NC, Bishai WR, Enarson D, Trébucq A. Assessment of clofazimine activity in a second-line regimen for tuberculosis in mice. *Am J Respir Crit Care Med* 2013;188:608–612.
27. Janulionis E, Sofer C, Song HY, Wallis RS. Lack of activity of orally administered clofazimine against intracellular *Mycobacterium tuberculosis* in whole-blood culture. *Antimicrob Agents Chemother* 2004;48:3133–3135.
28. Cholo MC, Steel HC, Fourie PB, Germishuizen WA, Anderson R. Clofazimine: current status and future prospects. *J Antimicrob Chemother* 2012;67:290–298.
29. Hwang TJ, Dotsenko S, Jafarov A, Weyer K, Falzon D, Lunte K, Nunn P, Jaramillo E, Keshavjee S, Wares DF. Safety and availability of clofazimine in the treatment of multidrug and extensively drug-resistant tuberculosis: analysis of published guidance and meta-analysis of cohort studies. *BMJ Open* 2014;4:e004143.
30. Schaad-Lanyi Z, Dieterle W, Dubois JP, Theobald W, Vischer W. Pharmacokinetics of clofazimine in healthy volunteers. *Int J Lepr Other Mycobact Dis* 1987;55:9–15.
31. Bezerra EL, Vilar MJ, da Trindade Neto PB, Sato EI. Double-blind, randomized, controlled clinical trial of clofazimine compared with chloroquine in patients with systemic lupus erythematosus. *Arthritis Rheum* 2005;52:3073–3078.
32. Ren YR, Pan F, Parvez S, Fleig A, Chong CR, Xu J, Dang Y, Zhang J, Jiang H, Penner R, *et al*. Clofazimine inhibits human Kv1.3 potassium channel by perturbing calcium oscillation in T lymphocytes. *PLoS One* 2008;3:e4009.
33. Dannemann BR, Bakare N, De Marez T, Lounis N, Van Heeswijk R, Meyvisch P, Haxaire M, Andries K, Everitt D, Upton A. Corrected QT interval (QTcF) prolongation in a phase 2 open-label trial of TMC207 plus background regimen as treatment for MDR-TB: effect of coadministration with clofazimine. Presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). September 9–13, 2013 [accessed 2015 Feb 24], Denver, CO. Abstract A-1259. Available from: http://icaaconline.com/php/icaac2013abstracts/data/papers/2012/A/2012_A-1259.htm

Exhibit-E

CLINICAL PRACTICE

Clinical Access to Bedaquiline Programme for the treatment of drug-resistant tuberculosis

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While clinical disease caused by drug-sensitive *Mycobacterium tuberculosis* (MTB) can usually be treated successfully, clinical disease caused by drug-insensitive MTB is associated with a poorer prognosis. In December 2012, a new drug, bedaquiline, was approved by the US Food and Drug Administration. This article documents the process whereby the National Department of Health, Right to Care and Médecins Sans Frontières obtained access to this medication for South Africans who might benefit from subsequent implementation of the Clinical Access to Bedaquiline Programme.

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While clinical disease caused by drug-sensitive *Mycobacterium tuberculosis* (MTB) can usually be treated successfully, clinical disease caused by drug-insensitive MTB is associated with a poorer prognosis. In December 2012, a new drug, bedaquiline, was approved by the US Food and Drug Administration (FDA) for the treatment of multidrug-resistant (MDR) tuberculosis (TB). In a review paper, the four goals of a compassionate use/early access programme for new TB drugs are outlined: to protect patients; to minimise the risk of treatment failure and emergence of resistance; to exercise fairness; and to comply with regulatory guidance.[1] This article documents the process whereby the National Department of Health (NDoH), Right to Care (a US Agency for International Development-funded non-governmental organisation) and Médecins Sans Frontières (MSF) obtained access to this medication for South Africans who might benefit from subsequent implementation of the Clinical Access to Bedaquiline Programme (CAP). Attention was paid to the regulatory framework, fairness and protection of patients while being cognisant of the need to prevent emergence of resistance to bedaquiline.

Setting

In South Africa (SA), 14 161 cases of MDR-TB were documented in 2012. The proportion of MDR-TB cases with additional resistance to a quinolone and a second-line injectable, i.e. extensively drug resistant TB (XDR-TB), is estimated at 10.9% (N=1 545)[2] with an ill-defined proportion being 'pre-XDR-TB', i.e. MDR with resistance to either a quinolone or a second-line injectable. While the outcomes of patients with pre-XDR-TB have not been well documented, the outcomes of XDR-TB are poor. In a retrospective cohort study at four designated XDR-TB provincial treatment facilities in SA between August 2002 and February 2008, 195 adult patients with culture-proven XDR-TB were analysed; 21 patients died before initiation of any treatment, 174 patients (82 with HIV infection) were treated, and 62 (36%) patients died during follow-up. Sputum culture conversion was achieved in only 33/174 (19%) patients.[3]

There are several new classes of TB drugs becoming available, including bedaquiline, which is a diarylquinoline. Bedaquiline offers a new mechanism of anti-TB action by specifically inhibiting mycobacterial adenosine triphosphate synthase.[4] In December 2012, the FDA granted accelerated approval for bedaquiline based on two phase 2 studies involving 440 people with drug-resistant TB (DR-TB). The first trial was a randomised, double-blind, placebo-controlled trial with bedaquiline and an optimised background regimen. The second was an open-label trial. Bedaquiline, when given with other existing MDR-TB drugs, increased the proportion of people whose sputum cultures converted to negative after 2 and 6 months of treatment. Bedaquiline, when given with other existing MDR-TB drugs, also reduced the time to sputum culture conversion, offering the possibility of a shorter treatment duration in the future. Bedaquiline is currently only commercially available in the US and is not yet registered by the SA Medicines Control Council (MCC). In the interim, some patients with few other treatment options, and before the drug's approval in their countries, have been offered bedaquiline through an early access programme put in place by the manufacturer, Janssen Pharmaceutica.

Under controlled compassionate use programmes and early access trials, several countries including SA have made bedaquiline available for patients with XDR- or pre-XDR-TB. In an expanded access programme (EAP) model, a clinician requests a drug for a named individual patient based on a specific clinical access guidance document. In an EAP, patients can be enrolled and offered access to the medication if they meet specific eligibility criteria. Based on this model, MDR-TB patients who have limited treatment options have been allowed access to bedaquiline as part of an individually tailored treatment regimen in a CAP in SA.

Table 1. MDR-TB patients, 2004 - 2010*

	Patients, *n*									
Province	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**Total, *N***
Eastern Cape	379	545	836	1 092	1 501	1 858	1 782	2 178	2 205	12 376
Free State	116	151	198	179	381	253	267	412	390	2 347
Gauteng	537	676	732	986	1 028	1 307	934	1 643	1 198	9 041
KwaZulu-Natal	583	1 024	2 200	2 208	1 573	1 773	2 032	1 825	6 630	19 848
Limpopo	59	40	77	91	185	204	126	290	266	1 338
Mpumalanga	162	134	139	506	657	446	312	824	760	3 940
Northern Cape	168	155	188	199	290	631	353	427	373	2 784
North West	130	203	225	397	363	520	158	473	267	2 736
Western Cape	1 085	1 192	1 179	1 771	2 220	2 078	1 422	2 013	2 072	15 032
Total, *N*	3 219	4 120	5 774	7 429	8 198	9 070	7 386	10 085	14 161	69 442

MDR-TB = multidrug-resistant tuberculosis.
*Laboratory diagnosis from the National Health Laboratory Service.

Key aspects of the CAP

Exercise of fairness

SA has nine provinces, all having MDR-/XDR-TB treatment facilities but with differing MDR-/XDR-TB burdens (Table 1). According to the National Health Laboratory Service, in KwaZulu-Natal, 11 393 diagnoses of MDR-TB were made between 2004 and 2010 and in the same time period 782 diagnoses were made in Limpopo.

To ensure equitable access to bedaquiline by selected XDR- or pre-XDR-TB patients, there should ideally be a clinical site in each province offering the programme.

Initially, four sites were approved by the MCC to begin the programme, based on results of clinical research. However, this excluded other, less well-resourced provinces. Additional sites in all the remaining provinces have since been identified for expansion of the project.

To prepare all future sites, good clinical practice training was provided to at least two members of staff from the selected MDR-/XDR-TB facilities. Strict adherence to the guidance document was stressed as the newer sites were not all experienced in conducting research. Once trained, the TB directorate of the NDoH set up an official start-up meeting at each of the sites.

Protection of patients

The principle underpinning protection of participating patients is their ability and capacity to make autonomous decisions and to give informed consent. Both the CAP and the informed consent document were approved by each site's research ethics committee prior to the start of the programme and enrolment of participants.

A potential safety risk identified in the development of bedaquiline, in common with moxifloxacin and clofazimine that are included in MDR-TB treatment regimens, was the prolongation of the QTc interval on electrocardiogram (ECG) with associated risks of life-threatening ventricular arrhythmias and sudden death. Thus, built into the CAP is rigorous ECG monitoring with only sites capable of adhering to this being permitted to enrol patients.

Regulatory guidance

Clinical research sites that were involved in the phase 2 clinical trials of bedaquiline were approached first to implement a compassionate use programme for bedaquiline. A requirement to participate was that the patients who accessed the drug were treated within the national TB programme, thus ensuring that the new drug is supported by other quality assured and approved second-line TB drugs.

In November 2011, the compassionate use programme was presented to the MCC by the MDR-TB directorate of the NDoH. The MCC was concerned at that time that the drug was not registered by any other regulatory authorities. They requested that the protocol be amended and implemented as a clinical trial, with appropriate safety monitoring, and with the TB directorate of the NDoH being responsible for sponsorship.

In collaboration with Right to Care and MSF, a CAP for SA was drafted by the NDoH based on the compassionate use framework. In December 2012, the CAP was approved by the MCC.

Minimise the risk of treatment failure

Bedaquiline is a new TB drug with a novel mode of action. The old adage of never adding a single drug to a failing regimen is important to reduce the risk of developing acquired resistance. An SA clinical advisory committee was therefore established, comprising a number of experts in the treatment of MDR-/XDR-TB. This virtual committee operates by e-mail consensus, with all new cases being discussed, and three members, other than the proposing responsible clinician, approving the use of bedaquiline as part of an appropriate regimen. Regimens for individual patients accessing bedaquiline through the programme are individualised and tailored according to the patient's TB susceptibility pattern, treatment history and exposure to other TB drugs, and other individual factors. Key roles of the committee are to ensure that bedaquiline is used only when other TB drugs known to be effective, or are likely to be effective, are available to be used in the patient's regimen. The committee advises on an optimal treatment regimen for each case.

Once approval from the local team is obtained, the responsible physician submits a patient summary to the Janssen Global Programme Manager who then co-ordinates clinical approval by Janssen, at the hands of a panel of clinical experts, and communicates the decision back to the responsible physician for each site. In parallel, approval is obtained from the MCC on a Section 21 or named-patient basis.

Discussion

This initiative has had a number of far-reaching consequences. Firstly, patients with DR-TB that has a poor prognosis are being

offered expedited access to a novel drug in a safe and controlled environment. Secondly, the model that has been established may prove useful in the future for other new TB agents.

While much public attention has been paid to MDR-TB in the last 5 years, it remains under-researched. The programme has expanded the capacity for research that does not exist currently in most MDR-TB facilities.

It bears noting that evidence for the regimen used in the current national TB treatment programme is based on expert opinion and not on clinical trial data. Cohort data from Van Deun *et al.*[5] demonstrated the efficacy of a seven-drug combination, 9-month course of treatment for MDR-TB. This so called 'Bangladesh' regimen consists of high-dose isoniazid, high-dose gatifloxacin, kanamycin, prothionamide, ethambutol, pyrazinamide and clofazimine given for only 9 months and is now being compared with the standard 18 - 24-month regimen in The Evaluation of a Standardised Treatment Regimen of Anti-Tuberculosis Drugs for Patients with Multidrug-Resistant Tuberculosis (STREAM), an International Union Against Tuberculosis and Lung Disease sponsored, non-inferiority clinical trial. This is the first of a number of trials aimed at establishing more effective, safer and shorter MDR-TB regimens using newly available drugs. The CAP in SA has provided the initial training and back-up for such clinical trials in the future.

Finally, HIV co-infection with TB is very common in SA. National HIV guidelines mandate the expedited initiation of antiretroviral therapy in any patient with MDR-/XDR-TB. Data on concomitant antiretroviral use with bedaquiline will emerge following implemenation of the programme.

Conclusion

There remains much work to be done to find a new effective, safe and evidence-based treatment regimen for MDR-/XDR-TB. Bedaquiline must form part of a long-term strategy aimed at combatting DR-TB. Other drugs that might include linezolid – an oxazalidinone that is also used for the treatment of resistant Gram-positive infections – and clofazimine – a drug used for the treatment of leprosy – must be added to develop regimens for the treatment of XDR- and pre-XDR-TB. The CAP in SA has resulted in both access to treatment with a novel drug for patients with DR-TB and enhancement of research capacity.

1. Horsburgh CR, Haxaire-Theeuwes M, Wingfield C, et al. Compassionate use of and expanded access to new drugs for drug-resistant tuberculosis. Int J Tuberc Lung Dis 2013;17(2):146-152. [http://dx.doi.org/10.5588/ijtld.12.0017]
2. World Health Organization. Global Tuberculosis Report 2013. Geneva: WHO, 2013. http://www.who.int/tb/publications/global_report/en/ (accessed 10 July 2013).
3. Dheda K, Shean K, Zumla A, et al. Early treatment outcomes and HIV status of patients with extensively drug-resistant tuberculosis in South Africa: A retrospective cohort study. Lancet 2010;375(9728):1798-1807. [http://dx.doi.org/10.1016/S0140-6736(10)60492-8]
4. Diacon AH, Pym AS, Grobusch M, et al. The diarylquinolone TMC 207 for the treatment of multidrug-resistant tuberculosis. N Engl J Med 2009;369(23):2397-2405. [http://dx.doi.org/10.1056/NEJMoa0808427]
5. Van Deun A, Maug AK, Salim MA, et al. Short, highly effective, and inexpensive standardized treatment of multidrug-resistant tuberculosis. Am J Respir Crit Care Med 2010;182(5):684-692. [http://dx.doi.org/10.1164/rccm.201001-0077OC]

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Exhibit-F

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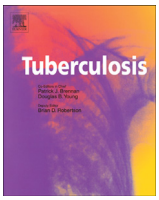

REVIEW

Linezolid for the treatment of drug-resistant tuberculosis in children: A review and recommendations

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SUMMARY

Options for the treatment of children with drug-resistant tuberculosis (DR-TB) are limited. Emerging evidence in adults from systematic reviews and a randomized trial has shown good efficacy of linezolid in difficult cases of DR-TB but with frequent serious adverse effects. Published data in children are limited and we are unaware of formal guidelines for linezolid in treatment of paediatric DR-TB, though it will likely be an important component of DR-TB treatment for a growing number of children. We performed a structured review of existing literature on the efficacy, adverse effects, pharmacokinetics and pharmacodynamics of linezolid in DR-TB, highlighting the key evidence from the adult literature and systematically evaluating published paediatric data. Our search identified 8 reports of 18 children receiving linezolid for difficult to treat DR-TB. All 18 had culture conversion and 15 of 18 had successful long-term treatment outcomes. Adverse events were reported in 9 of 18; a linezolid dose reduction was required in 5 of 18, and 2 of 18 permanently discontinued linezolid because of adverse events. We make specific recommendations for the use of linezolid in children with DR-TB, and identify priority questions for future research. For children with multidrug-resistant (MDR)-TB with additional resistance or with extensively drug-resistant (XDR)-TB, linezolid may make the difference between a successful or poor outcome, and until newer antituberculosis agents with better efficacy and safety become available in children, linezolid will be an important component of treatment for children with the worst forms of DR-TB.

1. Introduction

Children account for an estimated 10–15% of the global burden of disease caused by *Mycobacterium tuberculosis* (*Mtb*) with conservative estimates of 490,000 reported cases and 64,000 deaths among HIV-negative children in 2011 [1,2]. Multidrug-resistant tuberculosis [MDR-TB; i.e. *Mtb* resistant to at least both rifampicin (R; RMP) and isoniazid (H; INH)] is increasing worldwide, with an estimated 630,000 prevalent cases in 2011 [2]. There is a growing recognition of the importance of drug-resistant TB (DR-TB) in younger ages. A recent systematic review identified 8 cohorts with 318 children with MDR-TB and reported a pooled estimate for treatment success of 81.7% [3]. Extensively drug-resistant TB (XDR-TB; i.e. resistance to isoniazid, rifampicin, a fluoroquinolone, and one of the second-line injectable drugs) has been identified in 84 countries and accounts for 9.0% of MDR-TB cases globally [2,4]. A systematic review reported successful outcomes in only 43.7% of adults with XDR-TB [5]. There is little published data or guidance on best management of children with XDR-TB.

The World Health Organization (WHO) categorizes linezolid in Group 5, an antituberculosis agent with unclear efficacy or concerns regarding usage [6]. There is an increased interest in linezolid for DR-TB treatment, especially in XDR-TB, and recent systematic reviews and a randomized controlled trial have added substantially to the adult literature. There is little published data about linezolid use in children with DR-TB, though it will likely be an important component of DR-TB treatment for a growing number of children given the lack of availability of new TB drugs in children. This paper reviews the existing knowledge about the efficacy, adverse effects, pharmacokinetics and pharmacodynamics of linezolid in DR-TB, highlighting the key evidence from the adult literature and systematically evaluating published paediatric data. We also make

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Table 1
Minimum inhibitory concentrations (MICs) (in μg/ml)*, epidemiologic cut-off (ECOFF), and proposed critical concentrations (in μg/ml) for linezolid against *Mycobacterium tuberculosis*.

	Mtb strains	Middlebrook 7H10	Middlebrook 7H11	Bactec460	MGIT960
Published reports of MICs					
Zurenko GE et al., 1996 [20]	Clinical isolates, DS	0.5†	–	–	–
	Clinical isolates, DR	0.5–2.0‡	–	–	–
Rodriguez JC et al., 2002 [19]	Clinical isolates, mostly DS	–	0.5, 1.0	–	–
Alcala L et al., 2003 [21]	Clinical isolates, DS and DR	0.5, 1.0	–	–	–
Erturan Z et al., 2005 [22]	Clinical isolates, DR	–	–	4.0, 8.0	–
Sood R et al., 2005 [23]	Clinical isolates, DR	–	1.0, 32.0	–	–
Tato M et al., 2006 [24]	Clinical isolates, DS and DR	0.25, 0.5	–	–	–
Yang C et al., 2011 [26]	Clinical isolates, DS and DR	0.125, 0.5	–	–	–
Epidemiologic cut-off					
Schon T et al., 2011 [30]	–	–	0.5	–	–
Proposed critical concentrations					
Rusch-Gerdes S et al., 2006 [25]	–	–	–	1.0	1.0
WHO 2008 [28]	–	–	–	1.0	1.0
WHO 2012 [29]	–	–	–	–	1.0

Mtb = *Mycobacterium tuberculosis*; DS = drug-susceptible; DR = drug-resistant, to at least isoniazid or rifampicin, or both; WHO = World Health Organization; MGIT = Mycobacterial Growth Indicator Tubes.
* Expressed as MIC_{50}, MIC_{90}, respectively, unless otherwise specified.
† Inhibited all strains.
‡ Range of MICs.

recommendations for the use of linezolid in children with DR-TB, and identify specific questions for future study.

2. Methods

2.1. Structure of review

Because the evaluation of antituberculosis drug efficacy relies on microbiologic endpoints, which are challenging to evaluate in children, there are few trials of antituberculosis drug efficacy in children with TB disease, but no reason to presume that agents efficacious in adults will not also be in children, who typically have paucibacillary TB [7,8]. Clinicians managing children with DR-TB should be aware of the adult literature for drug efficacy. There may be considerable age-related variation in severity and frequency of adverse effects of drugs, so the safety profile of drugs should be specifically evaluated in children [7–9]. We summarize the key evidence on the efficacy and safety of linezolid in adults receiving the prolonged courses of linezolid used in DR-TB treatment, and report all the paediatric experience identified in our search.

The pharmacokinetics of many antituberculosis drugs differ in children and adults [10–12] due mostly to age-related changes in drug absorption, distribution, metabolism, and excretion [9,13,14]. Paediatric antituberculosis drug doses should be used that result in the same drug exposure as that of efficacious recommended doses in adults [7,9]. We therefore also present studies of the pharmacokinetics of linezolid in adults with TB, and in children with TB or other conditions.

2.2. Search

We searched Pubmed through December 31, 2012, using a broad search strategy and a second specific search for pharmacokinetic information, using the terms described in Supplemental Table 1. We also reviewed the bibliographies of key articles and reviews, and surveyed experts in the field. We systematically extracted information on the outcomes and adverse effects for all children treated with linezolid for DR-TB. We used key articles from our search to describe the efficacy, adverse effects, and pharmacokinetics of linezolid for DR-TB treatment in adults and children.

3. Overview

Linezolid belongs to the oxazolidinone class of antibiotics, which bind to the 50S ribosomal subunit, inhibiting formation of the initiation complex and preventing translation and protein synthesis [15–17]. This novel mechanism of action limits cross-resistance with other protein-synthesis inhibitors and makes it attractive for drug-resistant infections [15]. Linezolid has been approved by the U.S. Food and Drug Administration for treatment of susceptible strains of some microorganisms, most commonly resistant Gram-positive bacteria, for nosocomial pneumonia, and for skin and skin structure infections, but is used off-label for drug-resistant TB [18]. Patent coverage of linezolid in the U.S. and other countries, along with a lack of quality-assured alternative producers, has resulted in prohibitively high costs of linezolid in many settings [18]. Linezolid is available as 600 mg tablets and as 100 mg/5 ml powder for suspension.

4. Efficacy of linezolid against *M. tuberculosis*

4.1. Activity in vitro and in animals

The *in vitro* activity of linezolid against *Mtb* has been consistently demonstrated, and minimum inhibitory concentrations (MIC) from published studies are listed in Table 1 [19–26]. The critical concentration of a drug is defined as the 'lowest concentration of drug that will inhibit 95% of wild strains of *M. tuberculosis* that have never been exposed to drugs, while at the same time not inhibiting clinical strains of *M. tuberculosis* that are considered to be resistant' [6,27], and the epidemiological cut-off (ECOFF) is defined as the highest MIC among the wild-type MIC distribution [27]. Currently proposed [25] and WHO recommended critical concentrations [28,29], and a proposed ECOFF for linezolid are listed in Table 1 [30]. A single study showed a trend towards higher linezolid MICs in MDR isolates over 10 years despite a lack of linezolid exposure, which was associated with resistance to the fluoroquinolones (except levofloxacin) and to kanamycin; the explanation for these findings is not clear [31]. Using a test concentration of 6 μg/ml linezolid on 295 MDR clinical isolates including 9 which were XDR, only 2 isolates were found to be resistant [32]; however the clinical relevance of that breakpoint is not clear.

In a study assessing *in vitro* combinations of drugs against *Mtb*, linezolid showed synergistic activity with rifampicin but not the fluoroquinolones [33]. Linezolid had intracellular activity against *Mtb* in a murine macrophage model [23], but against non-replicating *Mtb* in a latent growth phase only the highest concentrations showed any bactericidal activity, suggesting limited sterilizing ability [34].

In one of the first *in vivo* evaluations, linezolid showed dose-dependent activity in a murine model of *Mtb*, based on lung and spleen colony forming units (CFUs) in comparison to untreated controls [35]. Subsequent studies in mice were less encouraging, showing limited bactericidal activity at doses approximating the clinically relevant exposure in humans [36], antagonistic activity when it was added to isoniazid, rifampicin, and pyrazinamide, [37] and no increased activity of linezolid and moxifloxacin over moxifloxacin alone [38]. We found no reports evaluating the combination of linezolid with pyrazinamide, although the related compound PNU-100480 showed augmented activity when combined with pyrazinamide [39].

4.2. Activity in adults

A single study reported a modest early bactericidal activity (EBA) for linezolid at doses of 600 mg once and twice daily [40]. The EBA for days 0–2 (EBA_{0-2}) was 0.26 for linezolid 600 mg twice daily and 0.18 for 600 mg once daily, compared to 0.67 for INH 300 mg [40]. The values for the extended EBA for days 2–7 (EBA_{2-7}) were 0.09 for twice daily and 0.04 for once daily linezolid, and 0.16 for isoniazid [40]. The differences in EBA of linezolid 600 mg once and 600 mg twice daily were small and not statistically significant [40]. There was no correlation between area under the concentration time curve (AUC)/MIC or %T > MIC with linezolid EBA in this study, which may be related to the relatively favourable pharmacodynamics at both doses [40]. These data provide some evidence for the effectiveness of once daily dosing, though the small sample size limited the ability to detect small differences. The low EBA_{2-7} may suggest minimal sterilizing activity, though this is an imperfect marker of sterilizing activity, and pyrazinamide, which is known to have potent sterilizing activity, also has a limited EBA_{2-7}.

In one of the first clinical studies of linezolid in DR-TB, three adults with MDR-TB and resistance to other second-line agents had successful outcomes with linezolid use [41]. Multiple other small case series and observational studies reported similar results, with good outcomes in patients with substantial drug resistance and limited treatment options, but with frequent adverse effects [42–56]. These and other reports were synthesized in two systematic reviews published in 2012 evaluating the safety and efficacy of linezolid for DR-TB in adults [57,58]. The first included 11 studies representing 148 patients [57]. The pooled percentage of patients with treatment success was 68.0% (95% CI 58.0–79.0) and culture conversion was 97.9% (95% CI 95.2–100%) [57]. There was no significant difference in pooled treatment success in studies with a mean duration of treatment >7 months versus ≤7 months, or for studies that used >600 mg daily versus ≤600 mg daily [57]. The second systematic review included 207 patients in 12 studies, including many but not all the same studies as the first review, and reported similar findings [58]. Of 121 patients with definite treatment outcomes, 82% (95% CI 74–88%) had successful treatment outcomes, with 93% (95% CI 87–97%) having culture conversion [58]. A subgroup analysis found no significant differences in outcomes between those receiving ≤600 mg daily versus >600 mg [58].

A single clinical trial evaluated linezolid in 39 highly treatment-experienced patients with chronic XDR-TB in which patients were randomized to immediate versus delayed addition of linezolid to their existing failed background regimen [59]. By 4 months, 79% in the immediate group compared to 35% in the delayed group had culture conversion ($p = 0.001$), and by 6 months 87% of all the patients had culture converted [59]. At the time of study publication, 8/38 patients had withdrawn from the study due to treatment failure ($n = 4$), personal reasons ($n = 1$), and adverse events ($n = 3$), while 17/38 were still receiving the study treatment [59]. Thirteen had successfully completed treatment with no relapse to date, suggesting sterilizing potential for linezolid [59]. Although the numbers are small, these results are much better than existing reported outcomes for XDR-TB and provide evidence for linezolid efficacy in these patients [59]. Of the 4 patients who did not have culture conversion, all acquired linezolid resistance, with increased MICs by a factor of 8–32 from baseline and known mutations identified by gene sequencing [59]. This demonstrates that resistance can emerge during treatment, despite a low mutant-prevention concentration ($MPC_{90} = 1.2$ μg/ml) comparable to that of moxifloxacin [60] and *in vitro* evidence that it is difficult to induce linezolid resistance in *Mtb* [34].

Only 5% [57] and 8.7% [58] of patients were HIV-infected in the two systematic reviews, and HIV infection was an exclusion criteria in the above clinical trial [59], so caution should be taken in extrapolating these results to HIV-infected persons.

4.3. Activity in children

There is substantial evidence of the effectiveness of short courses (less than 28 days) of linezolid in children for complicated bacterial skin and soft tissue infections, nosocomial and community-acquired pneumonia, and resistant Gram-positive infections, including four clinical trials [61]. Experience with linezolid in children with DR-TB is limited, and our search identified 8 reports including 18 children [one patient was included in two reports [62,63]] treated with linezolid for DR-TB [43,54,62–67], with results summarized in Table 2. All 18 patients had culture conversion, most within 1–3 months, and 15 of 18 (83%) had a successful long-term outcome, with 1 lost-to-follow-up and 2 deaths. The deaths were due to respiratory failure in one, and Stage 3 tuberculous meningitis and liver failure in a second, and both patients were culture-negative at the time of death [43,67]. In many of these patients, the good outcomes were despite extensive disease, substantial drug resistance, and prolonged culture positivity and failed treatment with other second-line drugs prior to linezolid use for periods as long as 9 months [54], 7 months [62], and 6–12 months [63].

Despite the small numbers and all patients were identified from case reports or small series, the outcomes described in children on linezolid are good. The proportions of children with culture conversion and successful treatment are similar to those reported for adults. This provides some evidence for the utility of linezolid in children with DR-TB, including those with XDR-TB.

4.4. Safety and tolerability

Although well tolerated in short courses, linezolid is associated with important dose- and time-dependent adverse effects [68,69]. In general, adverse effects are reported less in linezolid treated children than adults [61,70]. Inhibition of mitochondrial protein synthesis by linezolid may be the cause of many of these adverse effects [68].

4.5. Gastrointestinal adverse effects

Gastrointestinal adverse effects are commonly associated with linezolid, but rarely require alteration or discontinuation of the

Table 2
Demographics and treatment outcomes for children (<18 years) treated with linezolid for drug-resistant tuberculosis.

Published report	Age (yrs) and gender	HIV	TB resistance profile	Dose and duration of linezolid treatment	Culture conversion	Treatment outcome
Park IN et al., 2006 [43]	17 F	Neg	H, R, E, CS, KM, OFX, PAS, PTH	600 mg OD, 8 months	Yes, 147 days	Death (respiratory failure)
Condos R et al., 2008 [54]	10 F	Pos	H, R, E, Z, S, CIP, AM, AUG, RB, PAS, CAP	600 mg OD, 25 months	Yes, 29 days	Successful
Schaaf HS et al., 2009 [62] and Rose PC et al., 2012 [63]	0.9 F	Neg	H, R, E, OFX, AM	10–12 mg/kg BD, 19 months	Yes, 23 days	Successful
Pinon M et al., 2010 [64]	1.9 F	Neg†	(H, R, E, Z, S, KM)*	10 mg/kg BD, 13 months	Yes, 1 month	Successful
	0.9 M	–	(H, R, E, Z, S, ETH, PAS, CS)*	10 mg/kg BD, 3 months	Yes, 2 months	Lost-to-follow-up
Dauby N et al., 2011 [65]	14 F	Neg	H, R, RB, E, OFX, Z, AM, CS, PTH	600 mg OD, 8 months	Yes, 11 weeks	Successful
Kjollerstrom P et al., 2011 [66]	14 M	Neg	H, R, Z, E, S, RB, ETH, CAP, AM	600 mg BD, 9 months	Yes, 12 weeks	Successful
	12 F	Neg	H, R, Z, S, RB, ETH, CS, PAS, KM, OFX	600 mg BD, 4 months; 300 mg OD 2 months	Yes, 6 weeks	Successful
	4 F	Neg	H, R, S, ETH	10 mg/kg BD, 1 month; half dose for 5 months	Yes, 12 weeks	Successful
	17 M	Pos	H, R, Z, E, S	600 mg BD, 11 months	Yes, 12 weeks	Successful
Rose PC et al., 2012 [63]	13 M	Neg	H, R, AM	300 mg OD, 23 months	Yes, 3 months	Successful
	10 M	Pos	H, R, E, AM, OFX	300 mg OD, 20 months	Yes, 4 months	Successful
	13 F	Neg	H, R, E, AM, ETH, OFX	300 mg OD, 15 months	Yes, 2.5 months	Successful
	0.6 M	Neg	H, R, E, AM, OFX	10 mg/kg BD, 15 months‡	Yes, 3 months	Successful
	10 F	Pos	H, R, E, ETH, KM, S	300 mg BD, 24 months; 200 mg BD, 3 months‡	Yes, 18 months	Successful
	5 F	Pos	H, R, E, KM, S, OFX	300 mg OD, 7 months	NA (negative prior to linezolid)	Successful
Katragkou A et al., 2013 [67]	2.5 F	Neg	H, R, E, Z, LFX, AM, CAP	10 mg/kg TD, 7 months, 7 mg/kg TD 3 months‡	NA (negative prior to linezolid)†	Successful
	1.5 M	Neg†	H, R, Z, E, AM	10 mg/kg TD, 6 months	Yes, 1 month	Death (Stage 3 TBM, liver failure)

H = isoniazid, R = rifampicin, E = ethambutol, Z = pyrazinamide, ETH = ethionamide, PTH = prothionamide, PAS = para-aminosalicylic acid, KM = kanamycin, AM = amikacin, CAP = capreomycin, OFX = ofloxacin, LFX = levofloxacin, CIP = ciprofloxacin, RB = rifabutin, AUG = augmentin, CS = cycloserine, OD = once daily, BD = twice daily; TD = thrice daily; F = female; M = male; NA = not applicable; TBM = tuberculous meningitis.

* Resistance profile of source case reported.

† not in original publication, but provided by authors.

‡ Treatment ongoing at time of report.

Table 3
Adverse events among children (<18 years) treated with linezolid for drug-resistant tuberculosis.

Published report	Age (yrs) and gender	HIV	Dose and duration of linezolid treatment	Adverse event/s	Action and outcome
Park IN et al., 2006 [43]	17 F	Neg	600 mg OD, 8 months	None	
Condos R et al., 2008 [54]	10 F	Pos	600 mg OD 25 months	None	
Schaaf HS et al., 2009 [62] and Rose PC et al., 2012 [63]	0.9 F	Neg	10–12 mg/kg BD, 19 months	None	
Pinon M et al., 2010 [64]	1.9 F	Neg†	10 mg/kg BD, 13 months	None	
	0.9 M	–	10 mg/kg BD, 3 months	None	
Dauby N et al., 2011 [65]	14 F	Neg	600 mg OD, 4 months, 300 mg OD, 4 months	Moderate peripheral neuropathy after 4 months	Improved with dose reduction to 300 mg once daily
Kjollerstrom P et al., 2011 [66]	14 M	Neg	600 mg BD, 9 months	Severe progressive peripheral neuropathy after 9 months	Completely resolved after discontinuation of linezolid
	12 F	Neg	600 mg BD, 4 months; 300 mg OD 2 months	Peripheral neuropathy after 4 months	Responded to dose reduction to 300 mg once daily
				Severe anaemia requiring transfusion	Anaemia attributed to linezolid and comorbid sickle cell disease; linezolid continued
	4 F	Neg	10 mg/kg BD, 1 month; half dose for 5 months	Urticarial rash	Attributed to linezolid hypersensitivity; resolved after dose reduced to half
	17 M	Pos	600 mg BD, 11 months	None	
Rose PC et al., 2012 [63]	13 M	Neg	300 mg OD, 23 months	None	
	10 M	Pos	300 mg OD, 20 months	Pancreatitis at 8 months	Attributed to d4T, anticonvulsant, high-fat diet, and possibly linezolid; linezolid continued
	13 F	Neg	300 mg OD, 15 months	None	
	0.6 M	Neg	10 mg/kg BD, 15 months*	None	
	10 F	Pos	300 mg BD, 24 months, 200 mg BD, 3 months*	Peripheral neuropathy at 24 months	Linezolid dose reduced, d4T changed to ABC, terizidone dose reduced, pyridoxine increased; symptoms resolved
				Mild anaemia and leukopaenia at 25 months,	Anaemia, leukopaenia attributed to HIV
	5 F	Pos	300 mg once daily, 7 months	Severe pancreatitis and lactic acidosis requiring ICU admission at 7 months	Attributed to linezolid which was discontinued, fully recovered
Katragkou A et al. (2013) [67]	2.5 F	Neg	10 mg/kg TD, 7 months, 7 mg/kg TD 3mnths*	Mild neutropaenia after 7 m of linezolid	Attributed to linezolid, but did not improve after reduction of linezolid dose
	1.5 M	Neg†	10 mg/kg TD, 6 months	Liver failure, resulting in death	Cause unknown, unlikely related to linezolid

OD = once daily, BD = twice daily; TD = thrice daily; F = female; M = male; NA = not applicable.
* Treatment ongoing at time of report.
† Not in original publication, but provided by authors.

drug [69]. In phase III clinical trials in adults, the most common drug-related adverse events were nausea (3.4%) and diarrhoea (4.3%) [69]. In a review of clinical trials of short durations of linezolid in children, diarrhoea (3.8–9.1%) and vomiting (1.2–4.2%) were the most common adverse effects, though there was no difference in frequency between linezolid and the comparators (cefadroxil and vancomycin) [71].

4.6. Hematologic adverse effects

Both dose and time-dependent myelosuppression were noted in pre-clinical evaluations of linezolid in animals [69]. A review of adult clinical trial data of linezolid courses <28 days showed no statistical difference in haematologic adverse effects between linezolid and comparator groups, although there was a trend towards increased mild anaemia and thrombocytopaenia in the linezolid group for those treated for more than 2 weeks [69,72]. Anaemia is more frequent in longer courses of linezolid, thought to be related to a bone marrow suppression due to inhibition of mitochondrial protein synthesis [69]. Studies have been variable in adults, but suggest a slight risk of thrombocytopaenia that is increased with longer duration of linezolid, but reversible with drug cessation [69]. The exact mechanism of thrombocytopaenia is unknown, but an immune-mediated phenomenon has been proposed [69]. Reversible leukopaenia and pancytopaenia have been described but are rare [69]. A single report of two adult patients suggested that linezolid-associated cytopaenias may respond to vitamin B6 (pyridoxine) supplementation [73], but was followed by other observational studies in adults which showed no effect of pyridoxine 125 mg daily [74] or 200 mg daily [75] on the risk of anaemia or thrombocytopaenia. Pyridoxine supplementation would not be expected to impact on the proposed mechanisms for cytopaenias described above. The risk of cytopaenias with prolonged linezolid treatment in DR-TB is discussed below.

Paediatric data from clinical trials of short courses of linezolid showed a trend towards mild reversible thrombocytopaenia in children treated >14 days but no statistical difference in hematologic adverse events between the linezolid and comparator groups [76].

4.7. Neurologic adverse effects

Peripheral neuropathy was not noted in clinical trials of linezolid, but has been well described during prolonged courses [69,77]. It usually presents as paraesthesia and numbness in distal extremities in a "stocking and glove" distribution, with lower extremities affected more commonly than upper [69]. In adults, peripheral neuropathy is not responsive to vitamin B6 [73], and is usually not reversible, but may improve slowly in some cases after linezolid discontinuation [69,77]. Linezolid also causes toxic optic neuropathy, with painless, bilateral central vision loss, often of sudden onset, and gradual progressive loss of colour vision and visual acuity [69]. Onset of symptoms is from 3 to 12 months, and existing evidence suggests optic neuropathy will improve with discontinuation of linezolid, but can result in permanent visual deficits [69]. The risk of neuropathy with prolonged treatment is discussed below.

In addition to the cases of peripheral neuropathy among linezolid-treated children with DR-TB described below, a recent review identified 8 cases of neuropathy in children − 5 with peripheral neuropathy alone, 1 with optic neuropathy, and 2 with both peripheral and optic neuropathy [78]. Seven of 8 were on prolonged courses with a range of 4 weeks to 7 months at the time of onset [78]. As opposed to adults, 5 of 5 in which the outcome was reported had improvement or resolution of symptoms after discontinuation of linezolid [78]. A single case of possible auditory nerve neuropathy has been described in a neonate [79].

There is little information on the impact of co-treatment of linezolid with isoniazid or cycloserine/terizidone on peripheral neuropathy. High-dose isoniazid causes neuropathy due to Vitamin B6 depletion, and pyridoxine supplementation greatly reduces this risk [80]. Cycloserine and terizidone may also cause peripheral neuropathy by a Vitamin B6 related mechanism, though this is controversial [81,82]. Even though the likely mechanism of linezolid-induced neuropathy by mitochondrial protein synthesis inhibition is distinct from that of isoniazid or cyloserine and terizidone, close monitoring of co-treated patients is warranted. The nucleoside reverse transcriptase inhibitor (NRTI) class of antiretrovirals (ARVs) also causes peripheral neuropathy by mitochondrial protein synthesis inhibition [83] and there is a potential for increased risk of neuropathy when used concomitantly with linezolid in HIV-infected children, but little data reported to date. With the exception of symptomatic management, the lack of effective treatments for ARV-induced neuropathy makes it less likely that pyridoxine or other existing medications will be effective for linezolid-induced neuropathy [84]. Additional evidence is needed and close monitoring of such patients is indicated.

4.7.1. Other

Linezolid-associated hyperlactatemia and lactic acidosis have been described, with a 2009 review identifying 9 adult cases [69]. Patients may be asymptomatic or have non-specific symptoms, with nausea and vomiting commonly reported [69]. Hyperlactatemia resolves over the course of 1–2 weeks after linezolid discontinuation [69]. Metabolic acidosis was reported in 2 of 79 (2.5%) children receiving linezolid in a randomized trial, though both had other comorbidities [85]. Three additional cases were described in children with liver disease and other comorbid illnesses [86], and more recently a case was described in an HIV-infected child receiving ARVs and long-term linezolid for DR-TB [63].

Rhabdomyolysis has been reported in an adult on linezolid for DR-TB [87]. Linezolid is a weak monoamine oxidase inhibitor (MAOI), and in combination with other drugs such as selective serotonin reuptake inhibitors (SSRIs) may rarely precipitate serotonin syndrome [69]. A single suspected case has been described in a child [88].

4.8. Adverse events in DR-TB treatment regimens

In the first systematic review of linezolid for DR-TB, the pooled percentage of adverse events was 61.5% (95% CI 40.2–80.8%), with pooled percentages of neuropathy of 36.1% (95%CI 19.1–53.2) and bone marrow suppression of 28.5% (95%CI 14.8–42.1), and with 36.2% (95%CI 20.7–51.8) stopping linezolid because of adverse events [57]. There was a trend towards increased risk of adverse events for linezolid doses >600 mg [49.9% (37.3–62.4)] versus ≤600 mg [34.4% (95%CI 23.0–45.8)] ($p = 0.07$), and a statistically significant difference in those discontinuing linezolid because of adverse events for doses >600 mg [60.8% (95%CI 42.7–78.8)] versus ≤600 mg [29.5% (95%CI 3.2–55.7)] ($p = 0.05$) [57].

In the second systematic review, 59% (95% CI 49–68%) had an adverse event, of which 69% (95% CI 58–79%) required linezolid discontinuation or dose adjustment [58]. The most common adverse events were anaemia (38.1%), peripheral neuropathy (47.1%), gastrointestinal disorder (16.7%), optic neuritis (13.2%), and thrombocytopaenia (11.8%) [58]. There was a statistically increased risk of adverse events for those receiving >600 mg daily (74.5%) versus those receiving ≤600 mg daily (46.7%) [58]. The higher dose was also associated with statistically increased risk of some specific adverse events, including anaemia (60% vs. 2.5%), leukopaenia (17.1% vs. 2.0%), and gastrointestinal symptoms (29.4% vs. 8.0%) despite a much shorter duration of treatment in the higher dose group [58]. In the clinical trial of linezolid for chronic XDR-TB, 33 of 38 (87%) of the patients had a clinically significant adverse event, of which 31 were possibly or probably related to linezolid [59]. After a second randomization in this study to continuation with 300 mg versus 600 mg linezolid, the 600 mg group was 2.7 times (95% CI 1.1–6.5) more likely to experience an adverse event compared to the 300 mg group, though adverse events were still common in the 300 mg group [59]. The lack of HIV-infected persons in these studies makes extrapolation of these results to this important subgroup difficult.

Table 3 lists the adverse events among published reports of children on linezolid for DR-TB. At least one adverse event was reported for 9 of 18 children (50%) with 5 of 18 (28%) requiring a linezolid dose reduction, and 2 of 18 (11%) permanently discontinuing linezolid. Peripheral neuropathy was the most common, occurring in 4 of 18 (22%), but was reported to resolve after dose reduction or discontinuation of linezolid in each case. The association of linezolid with anaemia reported in 2 of 18 (11%) is unclear, as one episode was attributed to linezolid and a vaso-occlusive crisis in a child with comorbid sickle cell disease, and in the second a bone marrow biopsy showed dyserythropoeisis possibly due to HIV. The single life-threatening adverse event was a case of severe pancreatitis and lactic acidosis [63]. Three of 5 (60%) known HIV-infected children experienced adverse events, compared to 5 of 12 (42%) known HIV-uninfected. In our limited personal clinical experience, 3/3 HIV-infected children had adverse events but 0/4 HIV-uninfected children [63]. As the NRTI class of ARVs also can inhibit mitochondrial DNA, there is a theoretical basis for increased risk of toxicity in HIV-infected persons taking NRTIs [89]. These numbers are too small to draw any robust conclusions about different risk between the two groups, but very close monitoring of

Table 4
Results of pharmacokinetic studies of linezolid in adults with tuberculosis, and children (concentrations in μg/mL, area under the concentration time curve (AUC) in μg h/mL, time in h).

Study	Methods	Age (in years or specified)	*N*	Dosage	T_{max}	*t*1/2	C_{max}	C_{min}	AUC 0–24
Adults with tuberculosis (none known HIV-infected)									
Dietze R et al., 2008 [40] *	HPLC	45.0 (39.0–48.0)	9	600 mg twice daily	1.0 (1.0–4.0)	4.56 (2.1–7.0)	19.4 (11.8–24.9)	–	232.9 (100.8–394.4)
		33.5 (23.0–42.0)	10	600 mg once daily	1.5 (1.0–4.0)	3.20 (1.5–5.0)	15.0 (11.9–21.3)	–	96.9 (47.8–143.7)
Koh WJ et al., 2009 [44] †	HPLC	–	10	300 mg once daily	–	–	11.6 (4.4)	2.1 (1.3)	–
Alffenaar JW et al., 2010 [99] *	LCMS/MS assay	28 (26–38)*	8	300 mg twice daily	1.2 (0.5–1.2)	5.6 (3.0–6.4)	9.5 (7.7–10.1)	1.9 (0.6–2.2)	115.2 (77.0–128.4)¶
			8	600 mg twice daily	1.4 (0.8–1.4)	5.8 (4.7–6.0)	20.4 (16.3–21.9)	5.8 (2.7–6.8)	291.6 (202.4–321.8)¶
Children without tuberculosis (none known HIV-infected)									
Jungbluth GL et al., 2003 [91] †	HPLC	Newborn, preterm,§ <1 week of age	9	10 mg/kg	–	5.6 (2.4–9.8)	12.7 (9.6–22.2)	–	108 (41–191)‡
		Newborn, full term, <1 week of age	10	10 mg/kg	–	3.0 (1.3–6.1)	11.5 (8.0–18.3)	–	55 (19–103)‡
		Newborn, full term ≥1week ≤28 days	10	10 mg/kg	–	1.5 (1.2–1.9)	12.9 (7.7021.6)	–	34 (23–50)‡
		Infants >28 days to <3 months	12	10 mg/kg	–	1.8 (1.2–2.8)	11.0 (7.2–18.0)	–	33 (17–48)‡
		Young children, 3 months to 11 years	59	10 mg/kg	–	2.9 (0.9–8.0)	15.1 (6.8–36.7)	–	58 (19–153)‡
		Adolescents 12–17 years	36	10 mg/kg or 600 mg	–	4.1 (1.3–8.1)	16.7 (9.9–28.9)	–	95 (32–178)‡

T_{max} = Time to reach maximum concentration; $t_{1/2}$ = elimination half-life; C_{max} = maximum serum concentration; C_{min} = minimum serum concentration; AUC = area under the concentration–time curve; HPLC = high-performance liquid chromatography; LCMS/MS = liquid chromatography-tandem mass spectrometry; TB = tuberculosis; MDR = multidrug-resistant; XDR = extensively drug-resistant.

* All values in this study reported as median and interquartile range.

† All values in this study reported as mean and range.

‡ AUC 0 to ∞.

§ Preterm considered<34 weeks gestation.

¶ Originally reported as AUC 0–12, but values doubled here to generate AUC 0–24 to facilitate comparisons between studies.

Table 5
Recommendations for the use of linezolid in children with drug-resistant tuberculosis.

Indications	
XDR-TB	Should be used routinely in all cases
Pre-XDR-TB, failed treatment with second-line drugs	Should be used routinely in all cases
Pre-XDR-TB, meningitis	Consider, depending on severity of illness, extent of disease, other available drugs, response to treatment
Pre-XDR-TB, standard cases	Consider, depending on severity of illness, extent of disease, other available drugs, response to treatment
MDR-TB, failed treatment with second-line drugs	Should be used routinely in all cases
MDR-TB, meningitis	Consider, depending on severity of illness, extent of disease and other available drugs
MDR-TB, standard cases	Not routinely recommended
Dosing	
<12 years of age	10 mg/kg twice daily
≥12 years of age	10 mg/kg once daily up to 300 mg
Monitoring	
Full blood picture – monthly	Dose reduction for cytopaenias
Active clinical monitoring for peripheral neuropathy	Dose reduction for peripheral neuropathy; discontinuation if no improvement
Monitoring visual acuity where able; challenge with such monitoring in young children should not limit linezolid use when otherwise indicated	Discontinuation if any signs of optic neuropathy
Monitoring for lactic acidosis, rhabdomyolysis, other rare adverse effects only if clinically indicated	Dose reduction or discontinuation depending on severity

XDR-TB = extensively drug-resistant tuberculosis; Pre-XDR-TB = multidrug-resistant tuberculosis with additional resistance to either a fluoroquinolone or a second-line injectable drug; MDR-TB = multidrug-resistant tuberculosis.

HIV-infected persons on linezolid is warranted until additional data are available.

These data show a substantial number of children treated with linezolid for DR-TB will have adverse effects. Though this appears to be less than in adult reports, the small number of paediatric cases makes it difficult to say with certainty. The majority of adverse effects responded to dose reduction, including neuropathy. Children on long-term linezolid should have close monitoring for any toxicity, with a dose reduction for any non-life-threatening adverse effects.

5. Pharmacokinetics and pharmacodynamics

5.1. Pharmacokinetics

Linezolid is well absorbed in both the oral suspension and tablet formulation, with oral availability approaching 100% [15,90]. In healthy volunteers the time to maximum concentration (T_{max}) is 0.5–2 h. Co-administration with a high fat meal may delay the T_{max} and slightly reduce the maximum plasma concentration (C_{max}), but does not affect the (AUC) [90]. Protein binding is reported to be 31% [15,90]. Linezolid has complex metabolism with two primary and multiple minor metabolites [90]. The rate-limiting step in linezolid clearance is the non-enzymic formation of the primary metabolite, and both renal and non-renal routes are involved in elimination [90], with non-renal elimination accounting for roughly 65% [91]. In healthy volunteers the mean C_{max} after steady state dosing with 600 mg varies from 16.3 to 21 μg/ml and the mean AUC_{0-12} from 107 to 138 μg h/ml [90]. Increased clearance, decreased AUC, and substantial inter-patient variability have been noted in ill patients relative to healthy volunteers [92,93]. Linezolid has good tissue penetration [90,94], including into lung and epithelial lining fluid [95,96]. Penetration into cerebrospinal fluid (CSF) is good, with reported CSF-to-plasma ratios of 0.7 [90] and 0.66 [97] and PK parameters in the CSF of adult neurosurgery patients [97] and ventricular fluid of children and adolescents [98] suggesting excellent pharmacodynamics. Meningeal inflammation did not appear to influence CSF penetration.

Our search identified 3 studies of linezolid pharmacokinetics in adults with TB, with results reported in Table 4 [40,44,99,100]. The trial of linezolid for chronic XDR reported mean AUC_{0-24} of 91.1 μg h/ml for 300 mg once daily, and 180.4 μg h/ml for 600 mg once daily [59]. In the same study, in all those taking 600 mg daily the serum concentration exceeded the MIC for the entire dosing period, but the trough was below the MIC for 9 of 16 taking 300 mg once daily, including 2 patients who developed linezolid resistance [59].

Our search did not identify any studies of linezolid pharmacokinetics in children with TB. A review summarized the paediatric pharmacokinetic data on linezolid from four clinical trials including over 180 children (Table 4) [91]. In newborns linezolid clearance approximates that in adults, but increases to 2–3 times adult values by the first week of life, gradually declining over time until around 12 years of age when it and other PK parameters approximate that of adults [91]. The increased clearance results in shorter serum half-life ($t_{1/2}$) and smaller AUCs relative to adults [91]. It was recommended that in order to approximate the adult dose of 600 mg twice daily for Gram-positive infections, to give a dose of 10 mg/kg 8 hourly in children <12 years of age, and for adolescents ≥ 12 years of age to give adult doses [91].

Based on published pharmacokinetics, a dose of 10 mg/kg in children 3 months to <12 years of age will approximate the C_{max} of a 600 mg dose in adults. Because of the increased clearance, the exposure (AUC) of a 10 mg/kg dose in the same age group will approximate that of a 300 mg dose in adults, so twice daily dosing would be expected to provide similar coverage as a 600 mg adult dose. A dose of 10 mg/kg twice daily for those <10 years, and 10 mg/kg once daily for those ≥10 years has been suggested [63], and is the dose most commonly used in published linezolid-treated paediatric DR-TB cases to date.

Clinicians should be aware of drug interactions with clarithromycin, also a WHO Group 5 antituberculosis drug which may be given as part of a treatment regimen for XDR-TB patients. In adults, co-administration with 500 mg clarithromycin increased linezolid exposure by 44% [101], which theoretically could increase the risk of adverse effects.

5.2. Pharmacodynamics

Linezolid appears to have both time and concentration dependent killing, with both the AUC/MIC ratio and percent time above MIC (%T > MIC) correlated with linezolid activity against Gram-positive bacteria [102,103]. Suggested targets for Gram-positive bacteria are AUC/MICs>80–120 and %T > MIC of 100% [100,102]. Specific targets for *Mtb* have not been established, though its much slower doubling time relative to Gram-positive bacteria means lower targets may still be effective [104]. A moderate post-antibiotic effect for linezolid, reported to be 4 h in a single study [105], would support maintaining concentrations above the MIC throughout the entire treatment period, though the clinical importance of this in *Mtb* is not known.

Excellent values have been reported for both free AUC/MIC and %T > MIC for 600 mg once and twice daily dosing, though there was no correlation between either of these measures and the EBA_{0-2} or EBA_{2-7} in the study [40]. Favourable pharmacodynamic parameters were also described for both linezolid 600 mg twice daily (AUC_{0-24}/MIC 243.2, and %T > MIC 100.0), and 600 mg once daily (AUC_{0-24}/MIC 116.2, and %T > MIC 62.8) [100]. A linezolid dose of 300 mg twice daily resulted in an AUC_{0-24}/MIC from 167 to 667 for 7 of 8 patients with a ratio >100 and %T > MIC of 100% for all patients, suggesting that lower doses may maintain efficacy while hopefully limiting toxicity [99]. A higher %T > MIC of 100% for a 300 mg twice daily dose [99] compared to 62.8% for a 600 mg once daily dose [100] may reflect differences between the two studies in both the MICs of the *Mtb* isolates and in the reported linezolid pharmacokinetics. The 300 mg twice daily dose resulted in higher exposures, which may be related to differences in the pharmacokinetic assay methodology between studies or to individual participant variability in these small samples, though real differences due to dose-related alterations in linezolid elimination cannot be excluded. In the single linezolid clinical trial for XDR-TB, neither C_{max} nor trough concentration was associated with time to culture conversion [59].

6. Recommendations for the use of linezolid in children with DR-TB

The WHO 2008 guidelines recommend the use of Group 5 drugs, including linezolid, only when a regimen containing 4 drugs with likely activity cannot be created from Groups 1–4, though no other specific recommendations regarding linezolid were made [6]. The recommended dosage is 600 mg twice daily for 4–6 weeks, then 600 mg once daily [6]. The WHO 2011 guidelines update did not specifically address linezolid [106]. We are unaware of any other formal recommendations for the use of linezolid in children with DR-TB, in these or other documents [6,106].

In the absence of existing recommendations, Table 5 summarizes our working recommendations for the use of linezolid in children with DR-TB.

6.1. Indications for use in children with DR-TB

Because of the high cost, considerable toxicity, and good outcomes with current treatment regimens, existing evidence does not support the routine use of linezolid for children with MDR-TB. We recommend linezolid for use in children with XDR-TB or for those who have failed treatment for MDR-TB with or without additional drug resistance. Linezolid is likely to be the most active drug for such children and could make the difference between treatment success and failure. Linezolid should be considered for children with MDR-TB with additional fluoroquinolone or second-line injectable resistance (Pre-XDR-TB), especially those who have extensive disease or meningitis. Linezolid should also be considered for children with MDR-TB meningitis, especially those who have had a slow or poor response to standard treatment. The good CSF penetration of linezolid makes it particularly useful for DR-TB meningitis, as there are few second-line agents with potent antituberculosis activity and good CSF penetration.

6.2. Dosage

There remains uncertainty about the optimal dose of linezolid in adults with DR-TB, which balances efficacy and toxicity [107–109]. Currently most adults will start with a dose of linezolid 600 mg once daily for the intensive phase of treatment, though some would advocate for a 300 mg dose. In the continuation phase adults will complete their treatment with either a dose of 600 mg or 300 mg once daily, though many of those using 600 mg will switch to 300 mg due to adverse effects.

Generally children ≥12 years of age should receive the same dose as adults, and we have had success using a dose of 10 mg/kg once daily up to 300 mg for children ≥12 years of age, as in our cases included in this report [62,63]. For children 3 months to 12 years we recommend a dose of 10 mg/kg twice daily. For children with extensive disease or TB meningitis it may be advisable to use up to a higher total daily dose of 600 mg, at least initially.

6.3. Monitoring

For children on linezolid we recommend monitoring of full blood counts monthly. We also recommend active monitoring for signs of peripheral neuropathy. Children who develop signs of peripheral neuropathy should initially have a linezolid dose reduction, as many will respond to this. The decision to reduce the linezolid dose should be made considering the severity of the adverse effects, severity of the TB disease, and other available treatment options. Adults using 600 mg once daily usually reduce the dose to 300 mg once daily when necessary. In children we recommend reducing the dose by 1/3 or 1/2, however there is little evidence for this, and close monitoring for persistence or worsening of the adverse effects, or recrudescence or worsening of the TB disease is important. Thrice weekly dosing of linezolid seemed to reduce adverse effects in a small number of adult patients [110]. This is a potential approach for those with few other treatment options, and further evaluation of this strategy in adults and children would be useful. If peripheral neuropathy persists then linezolid may need to be discontinued. The cumulative evidence, though of low quality, suggests no effect of vitamin B6 (pyridoxine) supplementation on the risk of linezolid-related adverse effects, and routine supplementation is not warranted; however patients receiving high-dose INH or cycloserine/terizidone should receive pyridoxine supplementation as currently recommended.

In settings where resources and expertise for ophthalmologic assessments are available, routine eye exams in children on long-term linezolid are warranted. Considering the challenges of ophthalmologic assessments in young children, this is unlikely to be feasible in resource-limited settings, and referral for ophthalmologic examination or discontinuation of linezolid for possible optic neuropathy may be best indicated by any signs of decreasing visual acuity. Because of the reported rarity of optic neuropathy, the limited treatment options and the importance of linezolid to the antituberculosis drug regimen in children with extensive resistance, we strongly recommend that the inability to perform routine eye exams in young children should not limit the use of linezolid when it is otherwise indicated. Any signs of deteriorating visual acuity without other explanation should prompt a thorough ophthalmologic examination and discontinuation of linezolid. Though routine monitoring for rare adverse effects such as lactic

acidosis or rhabdomyolysis is unnecessary, clinicians should be aware of the potential for these effects should patients develop consistent signs or symptoms.

7. Questions for future study

The optimal dosing of linezolid in adults and children remains unclear. Once an adult dose has been established, a more formal recommendation can be made for paediatric dosing that gives a similar drug exposure. We are unaware of published linezolid pharmacokinetic data in children with TB, though such data would be important for guiding appropriate dosing for this indication. An ongoing study in Cape Town, South Africa is evaluating the pharmacokinetics, safety, and tolerability of second-line antituberculosis drugs in children, including linezolid, when used in children with DR-TB.

Little data exist on linezolid use in HIV-infected adults or children with DR-TB. Because of a potential increased risk of toxicity related to co-administration with ARVs and limited published data to date, additional information about the efficacy and safety in HIV-infected persons is needed. Additional evidence on the impact of linezolid co-treatment with high-dose INH and cycloserine/terizidone, as well as the impact of pyridoxine supplementation on adverse effects in this context would be useful.

Biomarkers for treatment response or other improved surrogate endpoints for trials in both adults and children with drug-susceptible and drug-resistant tuberculosis are urgently needed and would greatly facilitate individualized management of children with drug-resistant tuberculosis and the rational use of drugs like linezolid.

Considering what appears to be potent activity of linezolid in difficult DR-TB cases, exploration of treatment intensification with a short course of linezolid in children with severe DR-TB disease may be warranted. The second-line injectable agents (amikacin, kanamycin, and capreomycin) are considered key drugs for DR-TB treatment, but must be given by painful intramuscular injections and are associated with permanent sensorineural hearing loss in as many as 24% of children when given long term [111]. Substitution of the second-line injectables by linezolid in the intensive phase of treatment is an approach that warrants study.

A search of www.clinicaltrials.gov and the WHO International Clinical Trials Registry Platform (http://www.who.int/ictrp/en/) revealed no registered studies of linezolid in children with TB, and future large trials of linezolid in children with DR-TB are unlikely. We would encourage clinicians using linezolid for DR-TB in children to systematically record key information about each case, documenting degree of drug resistance, dosing, treatment response including culture conversion and outcome, and any adverse effects, and to report these cases as widely as possible.

The oxazolidinone antibiotic PNU-100480 has shown better efficacy against *Mtb* than linezolid in pre-clinical studies [37], and further study and development of it and other novel agents will be important to improving treatment options for adults and children with DR-TB. The inclusion of children with DR-TB in such trials is of critical importance.

8. Conclusion

Despite modest activity of linezolid against *Mtb in vitro* and in animal models, emerging data in adults have shown it to be effective in difficult cases of DR-TB. These benefits are currently offset by its high cost, and frequent and often severe time- and dose-dependent toxicity. Though data are limited, the efficacy and adverse effects of linezolid in treatment of children with DR-TB reported to date are similar to adults. For children with MDR-TB with additional resistance or with XDR-TB, linezolid may however make the difference between a successful or poor outcome, as demonstrated in many of the paediatric cases described to date. Because of its good CSF penetration, linezolid may also be an important option for children with MDR-TB meningitis, for which outcomes are often poor and other drugs with potent antituberculosis activity and good CSF penetration are limited. We would support calls for lowering linezolid costs and making it available, including in suspension form, for children with these indications [18]. Until newer antituberculosis agents with better efficacy and safety become available, linezolid will be an important component of treatment for children with the worst forms of drug-resistant tuberculosis.

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Appendix A. Supplementary data

Supplementary data related to this article can be found in the online version at http://dx.doi.org/10.1016/j.tube.2013.10.003.

References

[1] Nelson LJ, Wells CD. Global epidemiology of childhood tuberculosis. Int J Tuberc Lung Dis 2004;8:636–47.
[2] World Health Organization. Global tuberculosis report 2012. Geneva, Switzerland: WHO; 2012.
[3] Ettehad D, Schaaf HS, Seddon JA, Cooke GS, Ford N. Treatment outcomes for children with multidrug-resistant tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis 2012;12:449–56.
[4] Extensively drug-resistant tuberculosis (XDR-TB): recommendations for prevention and control. Wkly Epidemiol Rec 2006;81:430–2.
[5] Jacobson KR, Tierney DB, Jeon CY, Mitnick CD, Murray MB. Treatment outcomes among patients with extensively drug-resistant tuberculosis: systematic review and meta-analysis. Clin Infect Dis 2010;51:6–14.
[6] World Health Organization. Guidelines for the programmatic management of drug-resistant tuberculosis: emergency update 2008; 2008.
[7] Burman WJ, Cotton MF, Gibb DM, Walker AS, Vernon AA, Donald PR. Ensuring the involvement of children in the evaluation of new tuberculosis treatment regimens. PLoS Med 2008;5:e176.
[8] Donald PR, Ahmed A, Burman WJ, Cotton MF, Graham SM, Mendel C, McIlleron H, Mac Kenzie WR, Nachman S, Schaaf HS, Starke JR, Wingfield C, Hesseling AC. Requirements for the clinical evaluation of new anti-tuberculosis agents in children. Int J Tuberculosis Lung Dis 2013;17:794–9.
[9] Donald PR. The assessment of new anti-tuberculosis drugs for a paediatric indication. Int J Tuberc Lung Dis 2007;11:1162–5.
[10] Thee S, Detjen AA, Wahn U, Magdorf K. Isoniazid pharmacokinetic studies of the 1960s: considering a higher isoniazid dose in childhood tuberculosis. Scand J Infect Dis 2010;42:294–8.
[11] Thee S, Seddon JA, Donald PR, Seifart HI, Werely CJ, Hesseling AC, Rosenkranz B, Roll S, Magdorf K, Schaaf HS. Pharmacokinetics of isoniazid, rifampin, and pyrazinamide in children younger than two years of age with tuberculosis: evidence for implementation of revised World Health Organization recommendations. Antimicrob Agents Chemother 2011;55:5560–7.
[12] Donald PR, Maritz JS, Diacon AH. The pharmacokinetics and pharmacodynamics of rifampicin in adults and children in relation to the dosage recommended for children. Tuberculosis (Edinb) 2011;91:196–207.

[13] Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology — drug disposition, action, and therapy in infants and children. New Engl J Med 2003;349:1157–67.
[14] Butler DR, Kuhn RJ, Chandler MH. Pharmacokinetics of anti-infective agents in paediatric patients. Clin Pharmacokinet 1994;26:374–95.
[15] Diekema DJ, Jones RN. Oxazolidinone antibiotics. The Lancet 2001;358:1975–82.
[16] Shinabarger DL, Marotti KR, Murray RW, Lin AH, Melchior EP, Swaney SM, Dunyak DS, Demyan WF, Buysse JM. Mechanism of action of oxazolidinones: effects of linezolid and eperezolid on translation reactions. Antimicrob Agents Chemother 1997;41:2132–6.
[17] Lin AH, Murray RW, Vidmar TJ, Marotti KR. The oxazolidinone eperezolid binds to the 50S ribosomal subunit and competes with binding of chloramphenicol and lincomycin. Antimicrob Agents Chemother 1997;41:2127–31.
[18] The MSF Access Campaign, The International Union Against Tuberculosis and Lung Disease. DR-TB drugs under the microscope: sources and prices for drug-resistant tuberculosis medicines. 2nd ed.; November 2012.
[19] Rodriguez JC, Ruiz M, Lopez M, Royo G. In vitro activity of moxifloxacin, levofloxacin, gatifloxacin and linezolid against *Mycobacterium tuberculosis*. Int J Antimicrob Agents 2002;20:464–7.
[20] Zurenko GE, Yagi BH, Schaadt RD, Allison JW, Kilburn JO, Glickman SE, Hutchinson DK, Barbachyn MR, Brickner SJ. In vitro activities of U-100592 and U-100766, novel oxazolidinone antibacterial agents. Antimicrob Agents Chemother 1996;40:839–45.
[21] Alcala L, Ruiz-Serrano MJ, Perez-Fernandez Turegano C, Garcia De Viedma D, Diaz-Infantes M, Marin-Arriaza M, Bouza E. In vitro activities of linezolid against clinical isolates of *Mycobacterium tuberculosis* that are susceptible or resistant to first-line antituberculous drugs. Antimicrob Agents Chemother 2003;47:416–7.
[22] Erturan Z, Uzun M. In vitro activity of linezolid against multidrug-resistant *Mycobacterium tuberculosis* isolates. Int J Antimicrob Agents 2005;26:78–80.
[23] Sood R, Rao M, Singhal S, Rattan A. Activity of RBx 7644 and RBx 8700, new investigational oxazolidinones, against *Mycobacterium tuberculosis* infected murine macrophages. Int J Antimicrob Agents 2005;25:464–8.
[24] Tato M, de la Pedrosa EG, Canton R, Gomez-Garcia I, Fortun J, Martin-Davila P, Baquero F, Gomez-Mampaso E. In vitro activity of linezolid against *Mycobacterium tuberculosis* complex, including multidrug-resistant *Mycobacterium bovis* isolates. Int J Antimicrob Agents 2006;28:75–8.
[25] Rusch-Gerdes S, Pfyffer GE, Casal M, Chadwick M, Siddiqi S. Multicenter laboratory validation of the BACTEC MGIT 960 technique for testing susceptibilities of *Mycobacterium tuberculosis* to classical second-line drugs and newer antimicrobials. J Clin Microbiol 2006;44:688–92.
[26] Yang C, Lei H, Wang D, Meng X, He J, Tong A, Zhu L, Jiang Y, Dong M. In vitro activity of linezolid against clinical isolates of *Mycobacterium tuberculosis*, including multidrug-resistant and extensively drug-resistant strains from Beijing, China. Jpn J Infect Dis 2012;65:240–2.
[27] Angeby K, Jureen P, Kahlmeter G, Hoffner SE, Schon T. Challenging a dogma: antimicrobial susceptibility testing breakpoints for *Mycobacterium tuberculosis*. Bull World Health Organ 2012;90:693–8.
[28] World Health Organization. Policy guidance on drug-susceptibility testing (DST) of second-line antituberculosis drugs. Geneva, Switzerland: WHO; 2008.
[29] World Health Organization prepared by Fuad Mirzayev. Summary of outcomes from who expert group meeting on drug susceptibility testing – PRELIMINARY. In: Fourth annual GLI meeting 17 April 2012 2012.
[30] Schon T, Jureen P, Chryssanthou E, Giske CG, Sturegard E, Kahlmeter G, Hoffner S, Angeby KA. Wild-type distributions of seven oral second-line drugs against *Mycobacterium tuberculosis*. Int J Tuberc Lung Dis 2011;15:502–9.
[31] Huang TS, Liu YC, Sy CL, Chen YS, Tu HZ, Chen BC. In vitro activities of linezolid against clinical isolates of *Mycobacterium tuberculosis* complex isolated in Taiwan over 10 years. Antimicrob Agents Chemother 2008;52:2226–7.
[32] Prammananan T, Chaiprasert A, Leechawengwongs M. In vitro activity of linezolid against multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant (XDR)-TB isolates. Int J Antimicrob Agents 2009;33:190–1.
[33] Rodriguez Diaz JC, Ruiz M, Lopez M, Royo G. Synergic activity of fluoroquinolones and linezolid against *Mycobacterium tuberculosis*. Int J Antimicrob Agents 2003;21:354–6.
[34] Cremades R, Rodriguez JC, Garcia-Pachon E, Galiana A, Ruiz-Garcia M, Lopez P, Royo G. Interaction between linezolid and *Mycobacterium tuberculosis* in an experimental in vitro model. APMIS 2011;119:304–8.
[35] Cynamon MH, Klemens SP, Sharpe CA, Chase S. Activities of several novel oxazolidinones against *Mycobacterium tuberculosis* in a murine model. Antimicrob Agents Chemother 1999;43:1189–91.
[36] Williams KN, Stover CK, Zhu T, Tasneen R, Tyagi S, Grosset JH, Nuermberger E. Promising antituberculosis activity of the oxazolidinone PNU-100480 relative to that of linezolid in a murine model. Antimicrob Agents Chemother 2009;53:1314–9.
[37] Williams KN, Brickner SJ, Stover CK, Zhu T, Ogden A, Tasneen R, Tyagi S, Grosset JH, Nuermberger EL. Addition of PNU-100480 to first-line drugs shortens the time needed to cure murine tuberculosis. Am J Respir Crit Care Med 2009;180:371–6.
[38] Fattorini L, Tan D, Iona E, Mattei M, Giannoni F, Brunori L, Recchia S, Orefici G. Activities of moxifloxacin alone and in combination with other antimicrobial agents against multidrug-resistant *Mycobacterium tuberculosis* infection in BALB/c mice. Antimicrob Agents Chemother 2003;47:360–2.
[39] Wallis RS, Jakubiec W, Kumar V, Bedarida G, Silvia A, Paige D, Zhu T, Mitton-Fry M, Ladutko L, Campbell S, Miller PF. Biomarker-assisted dose selection for safety and efficacy in early development of PNU-100480 for tuberculosis. Antimicrob Agents Chemother 2011;55:567–74.
[40] Dietze R, Hadad DJ, McGee B, Molino LP, Maciel EL, Peloquin CA, Johnson DF, Debanne SM, Eisenach K, Boom WH, Palaci M, Johnson JL. Early and extended early bactericidal activity of linezolid in pulmonary tuberculosis. Am J Respir Crit Care Med 2008;178:1180–5.
[41] Fortun J, Martin-Davila P, Navas E, Perez-Elias MJ, Cobo J, Tato M, De la Pedrosa EG, Gomez-Mampaso E, Moreno S. Linezolid for the treatment of multidrug-resistant tuberculosis. J Antimicrob Chemother 2005;56:180–5.
[42] von der Lippe B, Sandven P, Brubakk O. Efficacy and safety of linezolid in multidrug resistant tuberculosis (MDR-TB) – a report of ten cases. J Infect 2006;52:92–6.
[43] Park IN, Hong SB, Oh YM, Kim MN, Lim CM, Lee SD, Koh Y, Kim WS, Kim DS, Kim WD, Shim TS. Efficacy and tolerability of daily-half dose linezolid in patients with intractable multidrug-resistant tuberculosis. J Antimicrob Chemother 2006;58:701–4.
[44] Koh WJ, Kwon OJ, Gwak H, Chung JW, Cho SN, Kim WS, Shim TS. Daily 300 mg dose of linezolid for the treatment of intractable multidrug-resistant and extensively drug-resistant tuberculosis. J Antimicrob Chemother 2009;64:388–91.
[45] Yew WW, Chau CH, Wen KH. Linezolid in the treatment of 'difficult' multidrug-resistant tuberculosis. Int J Tuberc Lung Dis 2008;12:345–6.
[46] Migliori GB, Eker B, Richardson MD, Sotgiu G, Zellweger JP, Skrahina A, Ortmann J, Girardi E, Hoffmann H, Besozzi G, Bevilacqua N, Kirsten D, Centis R, Lange C. A retrospective TBNET assessment of linezolid safety, tolerability and efficacy in multidrug-resistant tuberculosis. Eur Respir J 2009;34:387–93.
[47] Nam HS, Koh WJ, Kwon OJ, Cho SN, Shim TS. Daily half-dose linezolid for the treatment of intractable multidrug-resistant tuberculosis. Int J Antimicrob Agents 2009;33:92–3.
[48] Udwadia ZF, Sen T, Moharil G. Assessment of linezolid efficacy and safety in MDR- and XDR-TB: an Indian perspective. Eur Respir J 2010;35:936–8. author reply 938–940.
[49] Anger HA, Dworkin F, Sharma S, Munsiff SS, Nilsen DM, Ahuja SD. Linezolid use for treatment of multidrug-resistant and extensively drug-resistant tuberculosis, New York City, 2000–06. J Antimicrob Chemother 2010;65:775–83.
[50] Schecter GF, Scott C, True L, Raftery A, Flood J, Mase S. Linezolid in the treatment of multidrug-resistant tuberculosis. Clin Infect Dis 2010;50:49–55.
[51] De Lorenzo S, Centis R, D'Ambrosio L, Sotgiu G, Migliori GB. On linezolid efficacy and tolerability. Eur Respir J 2012;39:770–2.
[52] Singla R, Caminero JA, Jaiswal A, Singla N, Gupta S, Bali RK, Behera D. Linezolid: an effective, safe and cheap drug for patients failing multidrug-resistant tuberculosis treatment in India. Eur Respir J 2012;39:956–62.
[53] Villar M, Sotgiu G, D'Ambrosio L, Raymundo E, Fernandes L, Barbedo J, Diogo N, Lange C, Centis R, Migliori GB. Linezolid safety, tolerability and efficacy to treat multidrug- and extensively drug-resistant tuberculosis. Eur Respir J 2011;38:730–3.
[54] Condos R, Hadgiangelis N, Leibert E, Jacquette G, Harkin T, Rom WN. Case series report of a linezolid-containing regimen for extensively drug-resistant tuberculosis. Chest 2008;134:187–92.
[55] Tangg SJ, Zhang Q, Zheng LH, Sun H, Gu J, Hao XH, Liu YD, Yao L, Xiao HP. Efficacy and safety of linezolid in the treatment of extensively drug-resistant tuberculosis. Jpn J Infect Dis 2011;64:509–12.
[56] Xu HB, Jiang RH, Li L, Xiao HP. Linezolid in the treatment of MDR-TB: a retrospective clinical study. Int J Tuberc Lung Dis 2012;16:358–63.
[57] Cox H, Ford N. Linezolid for the treatment of complicated drug-resistant tuberculosis: a systematic review and meta-analysis. Int J Tuberc Lung Dis 2012;16:447–54.
[58] Sotgiu G, Centis R, D'Ambrosio L, Alffenaar JW, Anger HA, Caminero JA, Castiglia P, De Lorenzo S, Ferrara G, Koh WJ, Schecter GF, Shim TS, Singla R, Skrahina A, Spanevello A, Udwadia ZF, Villar M, Zampogna E, Zellweger JP, Zumla A, Migliori GB. Efficacy, safety and tolerability of linezolid containing regimens in treating MDR-TB and XDR-TB: systematic review and meta-analysis. Eur Respir J 2012;40:1430–42.
[59] Lee M, Lee J, Carroll MW, Choi H, Min S, Song T, Via LE, Goldfeder LC, Kang E, Jin B, Park H, Kwak H, Kim H, Jeon HS, Jeong I, Joh JS, Chen RY, Olivier KN, Shaw PA, Follmann D, Song SD, Lee JK, Lee D, Kim CT, Dartois V, Park SK, Cho SN, Barry 3rd CE. Linezolid for treatment of chronic extensively drug-resistant tuberculosis. N Engl J Med 2012;367:1508–18.
[60] Rodriguez JC, Cebrian L, Lopez M, Ruiz M, Jimenez I, Royo G. Mutant prevention concentration: comparison of fluoroquinolones and linezolid with *Mycobacterium tuberculosis*. J Antimicrob Chemother 2004;53:441–4.
[61] Garazzino S, Tovo PA. Clinical experience with linezolid in infants and children. J Antimicrob Chemother 2011;66(Suppl. 4):iv23–41.
[62] Schaaf HS, Willemse M, Donald PR. Long-term linezolid treatment in a young child with extensively drug-resistant tuberculosis. Pediatr Infect Dis J 2009;28:748–50.

[63] Rose PC, Hallbauer UM, Seddon JA, Hesseling AC, Schaaf HS. Linezolid-containing regimens for the treatment of drug-resistant tuberculosis in South African children. Int J Tuberc Lung Dis 2012;16:1588–93.
[64] Pinon M, Scolfaro C, Bignamini E, Cordola G, Esposito I, Milano R, Mignone F, Bertaina C, Tovo PA. Two pediatric cases of multidrug-resistant tuberculosis treated with linezolid and moxifloxacin. Pediatrics 2010;126:e1253–6.
[65] Dauby N, Muylle I, Mouchet F, Sergysels R, Payen MC. Meropenem/clavulanate and linezolid treatment for extensively drug-resistant tuberculosis. Pediatr Infect Dis J 2011;30:812–3.
[66] Kjollerstrom P, Brito MJ, Gouveia C, Ferreira G, Varandas L. Linezolid in the treatment of multidrug-resistant/extensively drug-resistant tuberculosis in paediatric patients: experience of a paediatric infectious diseases unit. Scand J Infect Dis 2011;43:556–9.
[67] Katragkou A, Antachopoulos C, Hatziagorou E, Sdougka M, Roilides E, Tsanakas J. Drug-resistant tuberculosis in two children in Greece: report of the first extensively drug-resistant case. Eur J Pediatr 2012.
[68] De Vriese AS, Coster RV, Smet J, Seneca S, Lovering A, Van Haute LL, Vanopdenbosch LJ, Martin JJ, Groote CC, Vandecasteele S, Boelaert JR. Linezolid-induced inhibition of mitochondrial protein synthesis. Clin Infect Dis 2006;42:1111–7.
[69] Vinh DC, Rubinstein E. Linezolid: a review of safety and tolerability. J Infect 2009;59(Suppl. 1):S59–74.
[70] Chiappini E, Conti C, Galli L, de Martino M. Clinical efficacy and tolerability of linezolid in pediatric patients: a systematic review. Clin Ther 2010;32:66–88.
[71] Saiman L, Goldfarb J, Kaplan SA, Wible K, Edge-Padbury B, Naberhuis-Stehouwer S, Bruss JB. Safety and tolerability of linezolid in children. Pediatr Infect Dis J 2003;22:S193–200.
[72] Gerson SL, Kaplan SL, Bruss JB, Le V, Arellano FM, Hafkin B, Kuter DJ. Hematologic effects of linezolid: summary of clinical experience. Antimicrob Agents Chemother 2002;46:2723–6.
[73] Spellberg B, Yoo T, Bayer AS. Reversal of linezolid-associated cytopenias, but not peripheral neuropathy, by administration of vitamin B6. J Antimicrob Chemother 2004;54:832–5.
[74] Plachouras D, Giannitsioti E, Athanassia S, Kontopidou F, Papadopoulos A, Kanellakopoulou K, Giamarellou H. No effect of pyridoxine on the incidence of myelosuppression during prolonged linezolid treatment. Clin Infect Dis 2006;43:e89–91.
[75] Soriano A, Ortega M, Garcia S, Penarroja G, Bove A, Marcos M, Martinez JC, Martinez JA, Mensa J. Comparative study of the effects of pyridoxine, rifampin, and renal function on hematological adverse events induced by linezolid. Antimicrob Agents Chemother 2007;51:2559–63.
[76] Meissner HC, Townsend T, Wenman W, Kaplan SL, Morfin MR, Edge-Padbury B, Naberhuis-Stehouwer S, Bruss JB. Hematologic effects of linezolid in young children. Pediatr Infect Dis J 2003;22:S186–92.
[77] Bressler AM, Zimmer SM, Gilmore JL, Somani J. Peripheral neuropathy associated with prolonged use of linezolid. Lancet Infect Dis 2004;4:528–31.
[78] Nambiar S, Rellosa N, Wassel RT, Borders-Hemphill V, Bradley JS. Linezolid-associated peripheral and optic neuropathy in children. Pediatrics 2011;127:e1528–32.
[79] Brennan K, Jones BL, Jackson L. Auditory nerve neuropathy in a neonate after linezolid treatment. Pediatr Infect Dis J 2009;28:169.
[80] van der Watt JJ, Harrison TB, Benatar M, Heckmann JM. Polyneuropathy, anti-tuberculosis treatment and the role of pyridoxine in the HIV/AIDS era: a systematic review. Int J Tuberc Lung Dis 2011;15:722–8.
[81] Nair S, Maguire W, Baron H, Imbruce R. The effect of cycloserine on pyridoxine-dependent metabolism in tuberculosis. J Clin Pharmacol 1976;16:439–43.
[82] Kass JS, Shandera WX. Nervous system effects of antituberculosis therapy. CNS Drugs 2010;24:655–67.
[83] McComsey G, Lonergan JT. Mitochondrial dysfunction: patient monitoring and toxicity management. J Acquir Immune Defic Syndr 2004;37(Suppl. 1):S30–5.
[84] Youle M, Osio M. A double-blind, parallel-group, placebo-controlled, multicentre study of acetyl L-carnitine in the symptomatic treatment of antiretroviral toxic neuropathy in patients with HIV-1 infection. HIV Med 2007;8:241–50.
[85] Yogev R, Patterson LE, Kaplan SL, Adler S, Morfin MR, Martin A, Edge-Padbury B, Naberhuis-Stehouwer S, Bruss JB. Linezolid for the treatment of complicated skin and skin structure infections in children. Pediatr Infect Dis J 2003;22:S172–7.
[86] Su E, Crowley K, Carcillo JA, Michaels MG. Linezolid and lactic acidosis: a role for lactate monitoring with long-term linezolid use in children. Pediatr Infect Dis J 2011;30:804–6.
[87] Carroll MW, Choi H, Min S, Hwang S, Park H, Song T, Park Y, Jeon HS, Goldfeder LC, Via LE, Lebron J, Jin B, Cai Y, Barry 3rd CE, Lee M. Rhabdomyolysis in a patient treated with linezolid for extensively drug-resistant tuberculosis. Clin Infect Dis 2012;54:1624–7.
[88] Thomas CR, Rosenberg M, Blythe V, Meyer 3rd WJ. Serotonin syndrome and linezolid. J Am Acad Child Adolesc Psychiatry 2004;43:790.
[89] Madariaga MG, Swindells S, McKee EE. Oxazolidinones and human immunodeficiency virus. Antimicrob Agents Chemother 2007;51:1130. author reply 1130.
[90] MacGowan AP. Pharmacokinetic and pharmacodynamic profile of linezolid in healthy volunteers and patients with Gram-positive infections. J Antimicrob Chemother 2003;51(Suppl. 2):ii17–25.
[91] Jungbluth GL, Welshman IR, Hopkins NK. Linezolid pharmacokinetics in pediatric patients: an overview. Pediatr Infect Dis J 2003;22:S153–7.
[92] Meagher AK, Forrest A, Rayner CR, Birmingham MC, Schentag JJ. Population pharmacokinetics of linezolid in patients treated in a compassionate-use program. Antimicrob Agents Chemother 2003;47:548–53.
[93] Dong H, Wang X, Dong Y, Lei J, Li H, You H, Wang M, Xing J, Sun J, Zhu H. Clinical pharmacokinetic/pharmacodynamic profile of linezolid in severely ill intensive care unit patients. Int J Antimicrob Agents 2011;38:296–300.
[94] Gee T, Ellis R, Marshall G, Andrews J, Ashby J, Wise R. Pharmacokinetics and tissue penetration of linezolid following multiple oral doses. Antimicrob Agents Chemother 2001;45:1843–6.
[95] Conte JE, Golden JA, Kipps J, Zurlinden E. Intrapulmonary pharmacokinetics of linezolid. Antimicrob Agents Chemother 2002;46:1475–80.
[96] Honeybourne D, Tobin C, Jevons G, Andrews J, Wise R. Intrapulmonary penetration of linezolid. J Antimicrob Chemother 2003;51:1431–4.
[97] Myrianthefs P, Markantonis SL, Vlachos K, Anagnostaki M, Boutzouka E, Panidis D, Baltopoulos G. Serum and cerebrospinal fluid concentrations of linezolid in neurosurgical patients. Antimicrob Agents Chemother 2006;50:3971–6.
[98] Yogev R, Damle B, Levy G, Nachman S. Pharmacokinetics and distribution of linezolid in cerebrospinal fluid in children and adolescents. Pediatr Infect Dis J 2010;29:827–30.
[99] Alffenaar JW, van Altena R, Harmelink IM, Filguera P, Molenaar E, Wessels AM, van Soolingen D, Kosterink JG, Uges DR, van der Werf TS. Comparison of the pharmacokinetics of two dosage regimens of linezolid in multidrug-resistant and extensively drug-resistant tuberculosis patients. Clin Pharmacokinet 2010;49:559–65.
[100] McGee B, Dietze R, Hadad DJ, Molino LP, Maciel EL, Boom WH, Palaci M, Johnson JL, Peloquin CA. Population pharmacokinetics of linezolid in adults with pulmonary tuberculosis. Antimicrob Agents Chemother 2009;53:3981–4.
[101] Bolhuis MS, Altena RV, Soolingen DV, Lange WC, Uges DR, Werf TS, Kosterink JG, Alffenaar JW. Clarithromycin increases linezolid exposure in multidrug-resistant tuberculosis patients. Eur Respir J 2013.
[102] Rayner CR, Forrest A, Meagher AK, Birmingham MC, Schentag JJ. Clinical pharmacodynamics of linezolid in seriously ill patients treated in a compassionate use programme. Clin Pharmacokinet 2003;42:1411–23.
[103] Andes D, van Ogtrop ML, Peng J, Craig WA. In vivo pharmacodynamics of a new oxazolidinone (linezolid). Antimicrob Agents Chemother 2002;46:3484–9.
[104] Mitnick CD, McGee B, Peloquin CA. Tuberculosis pharmacotherapy: strategies to optimize patient care. Expert Opin Pharmacother 2009;10:381–401.
[105] Hui M, Au-Yeang C, Wong KT, Chan CY, Yew WW, Leung CC. Post-antibiotic effects of linezolid and other agents against *Mycobacterium tuberculosis*. Int J Antimicrob Agents 2008;31:395–6.
[106] World Health Organization. Guidelines for the programmatic management of drug-resistant tuberculosis – 2011 update. Geneva, Switzerland: WHO; 2011.
[107] Koh WJ, Kang YR, Jeon K, Kwon OJ, Lyu J, Kim WS, Shim TS. Daily 300 mg dose of linezolid for multidrug-resistant and extensively drug-resistant tuberculosis: updated analysis of 51 patients. J Antimicrob Chemother 2012;67:1503–7.
[108] Bolhuis MS, van Altena R, Alffenaar JW. Comment on: daily 300 mg dose of linezolid for multidrug-resistant and extensively drug-resistant tuberculosis: updated analysis of 51 patients. J Antimicrob Chemother 2012;67:2055–6. author reply 2056–2057.
[109] Yew WW, Chang KC, Chau CH. Comment on: daily 300 mg dose of linezolid for the treatment of intractable multidrug-resistant and extensively drug-resistant tuberculosis. J Antimicrob Chemother 2009;64:1119. author reply 1119–1120.
[110] Chang KC, Yew WW, Cheung SW, Leung CC, Tam CM, Chau CH, Wen PK, Chan RC. Can intermittent dosing optimize prolonged linezolid treatment of difficult multidrug-resistant tuberculosis? Antimicrob Agents Chemother 2013;57:3445–9.
[111] Seddon JA, Thee S, Jacobs K, Ebrahim A, Hesseling AC, Schaaf HS. Hearing loss in children treated for multidrug-resistant tuberculosis. J Infect 2012.

Exhibit-G

THE PATENTS ACT 1970

(AMENDED BY THE PATENTS ACT 2005)

AND

THE PATENT RULES , 2003

(AMENDED BY THE PATENT RULES 2006)

In the matter of Patent application No.

314/MUM/2008 **Application Date** 13/02/2008

AND

In the matter of Section (14)

& (15)of the Patents Act

RAJEEV M. HUZURBAZAR.**……The Applicant**

Present: RAJEEV M. HUZURBAZAR.

DECISION

The instant Patent Application filed as ORDINARY APPLICATION filed on 13/02/2008 entitled 'ORAL FOOD SUPPLEMENT POWDER FOR DIARRHOEA IN PAEDIATRICS' . The initially filed claims in its Complete Specification were examined in accordance with the Patents Act 1970 and consequently numbers of objections comprising of both formal and technical were conveyed by the Patent office, Mumbai to the applicant as per First Examination Report dated 28th April 2010 which is the part of file of the instant case. The main technical objections were u/s 10(4) & u/s 3(e) of the Patents Act..

The initially filed set of claims are stated as follows:

1 .An Oral Food Supplement compositon, for Diarrhea for paediatric patients, comprising,

Mixed Fruit Powder	1 to 50%
Potato Powder	15 to 30 %
Rice Powder	11 to 15 %
Soyabeen Powder	1.5 to 10 %
Sago Powder	.5 to 10 %
Lentil Powder	1 to 5 %
Tur Powder	1 to 5 %

2. A process of making the food supplement composition as claimed in claim 1, where in the all the powder are spray Dried,Powder are weighed and added to mixer and process is continued for 30 min.mixing mass is then transferred to stainless steel vessel , powder is then transferred to filling machine and packed in containers.

The applicants had filed their reply to the First Examination Report (FER) 11th January 2011 ie within the prescribed period enclosing therewith the desired Forms, revised retyped papers etc. They simultaneously made some rewording of the said claims . The said reply & the set of claims is also part of file of the instant case . Further Second Examination Report has been issued by the office maintaining the main requirements which has been replied by the applicant.

The last date to put the application in order for grant has been expired on 28/04/2011 . As the Patent office was still not satisfied with the said compliance, the above objections were maintained & stated that the revised set of claims are not allowable under section 3(e) of the Act. For the sake of natural justice the applicant has been offered an hearing on 04/07/2011.

The main requirements of the First Examination Report (FER) the claims fall within the scope of section 3(e) of the Act.

Applicant Sri Rajeev Huzurbazar appeared for hearing before me on 04/07/2011.

The set of claims on record are stated as follows.

1.An Oral Food Supplement compositon, for Diarrhea for paediatric patients, comprising ,

Apple, Banana, Guava Powder	1 to 50%
Potato Powder	15 to 30 %
Rice Powder	11 to 15 %
Soyabeen Powder	1.5 to 10 %
Sago Powder	0.5 to 10 %
Lentil Powder	1 to 5 %
Tur Powder	1 to 5 %

2. An Oral Food Supplement composition has combine / synergistic effect as given in next pages. Chart presentation of Various combination is given from page one to four along with synergistic effect of Oral Food Composition on first page .

The applicant submitted that the present Invention relate to application of Oral Food Supplement Powder in case of diarrhea in pediatric patient. Diarrhea is too frequent passage of poorly formed stools i.e passage of excessive water in faces. Diarrheal diseases constitute a major cause of morbidity and mortality worldwide.More than five million children under age of five years die every year of diarrhea. Diarrhea has been shown to have significant impact on. nutrition. Child with multiple episodes of diarrhea suffers most severely from protein energy malnutrition . A considerable quantity of nutritients is lost in diarrheal stool. Protein energy malnutrition develops. A vicious cycle of diarrhea-malnutrition-diarrhea sets in. Significant death occur as a result of malnutrition, unnecessary starvation, consequent series of diarrhea. Pediatric diarrheal patients by administering an effective daily amount of Food Supplement Powder of composition comprising mixture of Mixed Fruit Powder, Rice, Potato, Soyabeen, Sago, when given in definite proportion and dose with other drugs controls the diarrhea , formation of normal stool very fast as compared to drugs given alone to

paediatric patients.. Food Supplement powder not only reduces the fluidity and frequency of loose stool which is necessary to alter the picture of diarrhea , but also prevents malnutrition. The formulation may be available in biscuit form.

The applicant merely repeated the same thing which they have submitted during the reply to the FER that the claims has been revised & it meets the requirement of Section 3(e) & it is a synergistic composition & not a admixture. The Applicant stated that they submitted the clinical data while filing reply to first examination repot & further data in view of their reply to hearing . They stated that it is not the individual effect of the ingredients but due to the combined effect of all the ingredients that they are using in the food supplement composition & that help to control the diarrhea . They stated that if using the hundred percent of one the ingredient in the composition it has a adverse effect on body & not effective to control diarrhea. Further , they stated that indifferent proportion of different ingredients will also not give result & will not be effective in controlling diarrhea.

So , they requested you to waive the objection.

The above submission to the hearing is also part of the instant case. Their submissions have been considered carefully but it does not fulfill the requirement of Section 3(e) of the Act .

I had gone through the specification & their submission .The instant application relates to an oral food supplement in the form of compositon, for controlling diarrhea particularly for paediatric patients, infants & babies. It comprising mainly different fruit powder, potato powder ,rice powder,soyabeen powder,sago powder ,lentil powder ,tur dal powder in a proportion mentioned in the specification & claimed in the claims.

Section 3(e) of the Act says ***"...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."***

So, the question of 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.

Claims as stated above of the applicant claimed a composition comprising fruit powder, potato powder ,rice powder,soyabeen powder, sago powder,lentil powder ,tur dal powder in a different proportion . 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. There is not a single example in the entire specification which demonstrates that the said combination provides surprising results apart from being a mere collocation of the properties of the individual ingredients. What applicant claiming as a synergy has not been demonstrated at all in the complete specification . What applicant tries to show in the form of clinical data & other data at the time filing reply to the first examination report & reply to the hearing has not filed as a part of description in the specification.

So, it is pertinent to mention here that , at the time of filing of instant application for patent no where mentioned in the specification that how the components or ingredients of the composition act together and is responsible for controlling the diarrhea. No comparative results/data on the controlling in respect diarrhea of the claimed composition is disclosed. A mere statement at the time of hearing & filing reply on enhanced property

of the composition regarding controlling diarrhea in absence of experimental/technical evidences in the specification itself is not credible. The specification is silent on unexpected effect/synergism of the claimed composition. 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 . Actually applicant has to study the ingredients used in the composition individually and need to see whether these ingredients possess property towards controlling the diarrhea individually & how effective in controlling diarrhea when these have mixed together in particular proportion. So, the applicant failed to demonstrate the data of individual ingredients and when these have mixed together, need to be mentioned in the description of specification.

In the absence of such evidence, it is evident that the claims cannot be patented under Section 3(e) of the Act and ought to be rejected.

It is not uncommon for the effect of two or more chemicals/ingredients on an organism to be greater than the effect of each chemical/ingredient individually, or the sum of the individual effects. The presence of different ingredient together enhances the effects of the composition as a whole. This is called a synergistic effect or synergy .The applicant has to define the a synergy ie how different entities cooperate advantageously for a final outcome shall be defined in the specification which applicant failed to define.

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.

So, considering the clause (e) of Section 3 of the Act & in the absence of synergistic data in the specification as stated above , the composition claimed herein in claims is a mere admixture.

Further the revised set of claims which applicant has filed after the hearing , claim 2 is not the same claim which applicant has filed at the time of filing of the application. What applicant claimed in the initially file set claims in claim 2 is process of making he composition . However, it has been converted to the product claim & now claiming composition with synergistic effect which they have not demonstrated in the specification and claimed in such a way that it lacks clarity. Further , applicant has added the the matter regarding the fruit powder . They are now claiming fruit powder used in the composition is made from fruits apple ,banana & guava are not fully supported by the description of the initially filed specification. Applicant has carried out the voluntary amendments without following the prescribed procedure under the Act. Though applicant has explained in their submission of first examination report regarding the steps used for making the composition , they have not taken the care to explain these each of the steps in the complete specification filed initially. So, the complete specification does not meet the requirement of 10 (4) of the Patents Act, 1970.

As per Section 10 (4) of the Patents Act every complete specification shall –

a) fully & particularly describe the invention & its operation or use & the method by which it is to be performed ;

b) disclose the best method of performing the the invention which is known to the applicant & for which he is entitled to claim the protection ; and

c) end with a claim or claims defining the scope of the invention for which protection is claimed ;

d) be accompanied by an abstract to provide the technical information on the invention .

The Complete Specification describing the invention is a techno-legal document. It should disclose the invention completely to meet the requirement of the Patents Act and should also enable a person possessing average skill in the art to work the invention without assistance of the patentee . This is possible when the complete specification describes the invention fully and particularly and describes its operation and/or method by which it is to be performed. It is also essential that the best method for performing the invention, which is known to the applicant is disclosed in the Complete Specification . The complete specification must describe an embodiement of the invention claimed in claims & that description must be sufficient to enable those in the industry concerned to carry it into effect without their to making further invention. The ordinary skilled person in the art must be able to perform the invention which satisfies the requirement of disclosure. Further as stated above, applicant failed to demonstrate the synergy with required data in the specification.

Having considered all the circumstances, reply , submission made by the agent for the applicant, I hereby refuse the application on the grounds as stated above.

Accordingly the instant Patent Application No. 314/MUM/2008 is refused u/s 15 of the Patents Act 1970.

Dated: 05.10.2012. (A. T.PATRE)

Place: Mumbai Asstt. Controller of Patents & Designs

Exhibit-H

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No. POC/DECISION/SEC.15 /2012/

Dated: 09/10/2012

To

Depenning & Depenning
31 South Bank Road
Chennai 600 028

Sub:Patent Application No. 3725/Chenp/2006- Reg.

Sir,

The above referred patent application filed by you has been refused U/S 15 of Patents Act, 1970 for not meeting requirements of Act. A copy of the order is enclosed herewith.

Thanking you,

Yours faithfully,

Priyadharsini Rajanbabu

(PRIYADHARSINI RAJANBABU)

Asst. Controller of Patents & Designs.

Encl.: Copy of decision.

THE PATENTS ACT, 1970
&
THE PATENTS RULES, 2003

In the matter of Application for Patent bearing the number as 3725/CHENP/2006
Filed by NOVARTIS AG

And

In the matter of Section 15 of the Patents Act, 1970

ORDER

1. A PCT national phase application for patent titled "AN ETHANOL FREE PHRMACEUTICAL COMPOSITION COMPRISING PIMECROLIMUS" was filed by NOVARTIS AG on 09.10.2006 at Patent office Chennai.

2. The said application was the national phase application of PCT international application PCT/EP05/03669 filed on 07.04.2005, which claimed priority from GB application 04-08070.1 and GB 0408076.8. A request for examination for this application was filed on 13.03.2008.

3. A FER was sent on 02.09.11, a reply was refiled on 29.05.12. Again a second examination report sent on 28.06.2012, a reply was refiled on 2.08.2012. As on the last date for putting the application in order for grant, the application was found to be not complying with certain requirements of act. Accordingly a hearing notice was issued with the following objections:

a. Objection no.5 of FER, dated 2 September 2011 and Objection no.01 of Examination report dated 28 June 2012 stands maintained.

b. Claims 1-2[amended] do not meet the requirements of Section 10(5)(c) of the Patents Act, 1970 ; as claims are not succinct.

4. Hearing has been fixed on 24/08/12 at 11.00Am. Agent for the applicant attended the hearing. And they refiled the documents on 31/08/12.

5. Objection number 5 of FER dated 2nd sep 2011 talks about section 3(e) of the patents act 1970 and objection no.1 of examination report dated 28th june 2012 speaks about novelty and inventive step.

6. Amended claims 1 and 2 are not allowable U/S 3(e) and 10(5)(c) of the patents act 1970.

Amended claims

1. A pharmaceutical foam composition substantially free of ethanol and comprising pimecrolimus in a carrier vehicle comprising a mixture of oily solvents amounting to at atleast 40% of the total weight of the composition and consisting of

i. Hexylene glycol in the range of 1% to10%
ii. Optionally Oleyl alcohol in the range of 1% to20% and
iii. Dimethyllisosorbide in the range of 35% to90% and medium chain triglycerides in the range of 5% to20% and additionally:
iv. Hydroxypropyl cellulose and /or stearyl alcohol in the range of 0.1% to5%
v. p-hydroxybenzoic acid ester with ethyleneglycol phenylether in the range of 0.1% to 0.5% and
vi. glyceryl monostearate in the range of 1% to3% and non-ionic sugar esters and butane/propane 80/20 as propellant gas for foaming.

2. A pharmaceutical foam composition substantially free of ethanol and comprising pimecrolimus in a carrier vehicle comprising a mixture of oily

solvents amounting to at atleast 40% of the total weight of the composition and consisting of

i. Hexylene glycol in the range of 2% to20%
ii. medium chain triglycerides in the range of 50% to 80% and optionally Dimethyl lisosorbide in the range of 0% to 20% and additionally:
iii. Water in an amount less than 25%
iv. polyvinylpyrrolidine and stearyl alcohol in the range of 1% to 10%
v. p-hydroxybenzoic acid ester with ethyleneglycol phenylether in the range of 0.1% to 0.5% and
vi. glyceryl monostearate in the range of 1% to 3% and lecithin; and butane/propane 80/20 as propellant gas for foaming.

7. Amended claims is now limited to pharmaceutical foam composition with specific ingredients based on the formulation described in examples 1, 2 and 3. The pharmaceutical form of the present invention shows improved penetration properties and is particularly convenient in terms of ease of administration and patient compliance. Hence objection no.1of examination report dated 28th Jun '12 is waived.

8. Claims 1 and 2 are not succinct and it doesn't fall under single inventive concept, because the components range and components as claimed in claim 1and claim 2 are different. And hence they are not allowable u/s 10(5) (c) of the patents act 1970.

9. Applicant doesn't provide any supportive experimental data or comparative examples highlighting the surprising and or synergistic effect of the claimed formulation over the prior art compositions. Instead, examples 1, 2 and 3 provide only the amount of individual components in grams.

10. Hence in view of the above findings I hereby conclude that the application do not meet the requirements of section 3(e) and 10(5) (c) of the Patent Act. Therefore I refuse the application for patent 3725/CHENP/2006 U/S 15 of the Patents Act, 1970.

Dated 9th October, 2012

(PRIYADHARSINI RAJANBABU)
Assistant controller of Patents & Designs.

The Patents Act, 1970 (As Amended in 2005)

(Section 15)

In the matter of Application no. 5461/DELNP/2008 filed in India on 24/06/2008 for Grant of Patent; Corresponding International Patent Application No. PCT/EP2007/050516, dated 19/01/2007,
Claiming Priority Date 20/01/2006, EP;
Applicants:- M/S TIBOTEC PHARMACEUTICALS LTD., IRELAND
Applicants Attorneys: M/S REMFRY& SAGAR, GURGAON, INDIA
ATTORNEY'S PRESENT FOR ARGUMENT: MR. AMIT SAINI
EXAMINER: DR RAJENDRA KUMAR LOHIA, EXAMINER, PATENT OFFICE, NEW DELHI, INDIA

Date of Hearing: 20/04/2015

Decision

[A] An application titled as "LONG TERM TREATMENT OF HIV-INFECTION WITH TMC278" " was filed in the Patent office, New Delhi on 24/06/2008 for Grant of the Patent. The details of the application are mentioned herein below:

S.NO.	Detail of the application	Dates of activity
1	Application No 5461/DELNP/2008	filed on 24/06/2008
2	International application no PCT/EP2007/050516	filed on 19/01/2007
3	Priority countryEP	Date of priority 20/01/2006
4	Publication U/S 11(A)	24/10/2008
5	Form 18 filing done by……APPLICANT HIMSELF	19/11/2009
6	FER Last Date for compliance of objection	23/10/2013 23/10/2014
7	Date of reply to the FER	22/10/2014
8	Notice of Hearing U/s 14	17/03/2015
9	Final Date of hearing U/S-14	20/04/2015

[B] Instant application was examined by the patent office and a First Examination report (FER) thereof was issued on 23/10/2013. The reply to FER was filed by the Applicant's agent on 22/10/2014 alongwith amended set of claims which are as follows:-

1. A parenteral formulation comprising an anti-virally effective amount of (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile or a pharmaceutically acceptable acid-addition salt thereof, a surfactant and an aqueous carrier, for the treatment of a subject infected with HIV, wherein the formulation is to be administered by subcutaneous or intramuscular administration intermittently at a time interval that is in the range of month to three months, wherein the effective amount of (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile ranges from 0.5 mg to 50 mg/day.
2. The formulation as claimed in claim 1, wherein the formulation is administered once every two months.
3. The formulation as claimed in claim 1, wherein the formulation is administered once every month.
4. The formulation as claimed in claim 1 wherein the effective amount of (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile in the parenteral formulation ranges from 1 mg/day to 20 mg/day
5. The formulation as claimed in claim 1, wherein the effective amount of (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile in the parenteral formulation is 3 mg/day to 7 mg/day.
6. The formulation as claimed in claim 1, wherein the effective amount of (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile in the parenteral formulation is 5 mg/day.
7. The formulation as claimed in claim 1, wherein the effective amount of (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile in the parenteral formulation ranges from 1mg/day to 10 mg/day.
8. The formulation as claimed in claim 1, wherein the formulation is to be administered by intramuscular administration.
9. The formulation as claimed in any one of the preceding claim comprising (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile base.

[C] On further examination based on the submissions given, the examiner found that the submissions and the observations given by the agents are not satisfactory to meet the requirements of the Act. This application was further re-examined by the Examiner and the following objections maintained:

1. Claim 1 (and thus dependent claims) are not clear and succinct and sufficiently definitive to the scope of allegedinvention in the absence of mention of any/all significant elements/components of composition, like constituentsand their proportions, percentage etc. that reflects technological contribution to establish the novelty and inventivestep and define the scope of alleged invention. [Requirements of Sec. 2(1) (j) and Sec. 10]
2. Claims 1 to 9 fall u/s 3(e) of the Patents (Amended) Act, 2005 as the said claims defines a mere admixture resulting only in the aggregation of the properties of the components thereof. It is not clear if the combined agents acttogether to provide a

technical effect that is greater than just the sum of the two or more agents alone, or whetherthe combination is in fact a mere juxtaposition with no interaction of the agents.
3. Claim 1 and its dependent claims does not constitute an invention under section 2[1(j)] of Patents Act 1970 (as amended in 2005) as the claims are lacking in inventive step in the view of cited Patent documents WO03016306(D1), WO2005021001(D2) and document no (D3) Journal of Medicinal Chemistry, 20041106 American Chemical Society, US ISSN00222623by JANSSEN P A; ET AL , Vol:48, Nr:6, Page(s):1901 1909.
4. Claim 1 and its dependent claims do anticipated by prior claiming in the view of cited Patent documents WO03016306 (D1), WO2005021001(D2) and document no (D3) Journal of Medicinal Chemistry, 20041106 American Chemical Society, US ISSN 00222623by JANSSEN P A; ET AL , Vol:48, Nr:6, Page(s):1901 1909.
5. Claims 1-9 can not be allowed under section 3(i) of the Patent 1970 as amended in 2005.
6. Endorsement by or assignment from inventor or applicant in convention country or authority in favor of legal representative should be filed with original documents.
7. Petition should be filed for delay in form 3 filed dated 27/03/2014.

[D] In view of the above said final objection and nature of the objection the attorney were given an opportunity of being heard and to submit their arguments in favour of their application U/S 14. The date of hearing U/S 14 was fixed on 20/04/2015. MR. AMIT SAINI appeared for hearing and submitted arguments in favour of their case. The finally revised claims (total eight) were also given during the hearing by the applicants agent, the same are reproduced herein below:

1. A parenteral formulation comprising an anti-virally effective amount of (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile or a pharmaceutically acceptable acid-addition salt thereof, a poloxamer and sterile water as aqueous carrier, for the treatment of a subject infected with HIV, wherein the formulation is to be administered by intramuscular administration intermittently at a time interval that is in the range of two to three weeks, or three to four weeks, or one month to three months, wherein the effective amount of (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile in the formulation ranges from 7 mg to 4500 mg.
2. The formulation as claimed in claim 1 wherein the effective amount of (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile in the parenteral formulation ranges from 14 mg to 1800 mg .
3. The formulation as claimed in claim 1, wherein the effective amount of (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile in the parenteral formulation ranges from 42 mg to 630 mg.
4. The formulation as claimed in claim 1, wherein the effective amount of (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile in the parenteral formulation ranges from 70 mg to 450 mg.
5. The formulation as claimed in claim 1, wherein the effective amount of (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile in the parenteral formulation ranges from 14 mg to 900 mg.

6. The formulation as claimed in claim 1, wherein the formulation is to be administered by intramuscular administration.
7. The formulation as claimed in any one of the preceding claim comprising (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino] benzonitrile base.
8. The formulation as claimed in any one of the preceding claims wherein the poloxamer is poloxamer 338.

[E]The written submission in response to hearing given by the agent on 11/05/2015 is being reproduced herein below:-

Regarding paragraph 1, the applicant submitted that the amended claims have been suitably amended. All the essential constituents of the claimed formulation have been defined in claim 1. The amount of API (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile (TMC 278) present in the formulation has also been recited in claim 1.

Further, the applicant submitted that it is common knowledge for the skilled man that a carrier is added up to 100 %. Therefore, it is clear that the sterile water as the aqueous carrier makes up the formulation ad 100%. Since the amount of the API is expressed as mg this would mean ad 100% (w/v).

The applicant submits that the expression “intramuscular administration” is clear to a person skilled in the art. Further, the expression “intermittent administration” is clearly described in the specification. In this regard, the learned Controller’s attention is respectfully invited to page 6 of the specification “The parenteral formulations of TMC278 are administered intermittently at a time interval of at least one week, or in particular at a time interval mentioned herein, meaning that the parenteral formulation is administered without any interjacent additional administrations of TMC278. Or with other words, TMC278 is administered at particular points in time separated from one another by a time period of at least one week, or in particular at a time interval mentioned herein, during which no TMC278 is administered. Hence the administration schedule is simple, requiring few administrations, and therefore dramatically reduces the problem of “pill burden” faced with standard HIV medication. This in turn will improve the patient’s compliance to the prescribed medication.”

The above expressions are essential to sufficiently define the invention as the crux of the invention is to provide a parenteral formulation of TMC 278 which is suitable for IM administration for the long term treatment of HIV infection.

Having said that, it is to be noted the claimed invention pertains to a parental formulation of TMC 278 and not to method of administration or period of the administration.

Accordingly, the amended claims are clear and sufficiently define the invention.

With regard to objection relating section 3(e), they submitted that claims have been suitably amended and the amended claims do not fall under the prohibition of Section 3(e).

Regarding section 3(e), Applicant argued that the claimed formulation is not a mere admixture and the formulation of TMC 278 with sterile water and poloxamer makes it suitable for IM administration for long term treatment of HIV infection.

It is clear that when providing a formulation with only poloxamer, no anti-HIV activity would be obtained. It is also clear that when providing a formulation with only sterile water, no anti-HIV activity would be obtained.

Further, it is clear that a pharmaceutical formulation is not only an active ingredient as such, but that it needs to be formulated in order to be able to be administered to a patient. In cited document D3, it is disclosed that TMC278 is a novel anti-HIV agent for oral once daily administration.

The combined effect of TMC 278, sterile water and poloxamer results in a formulation which is suitable for IM administration and which has anti HIV activity. With the present formulation, it is possible to treat HIV infection over a long term which certainly provides advantages for the patient for therapy compliance and hence drug efficacy.

Regarding paragraphs 3 and 4, the applicant submitted that the invention claimed in the amended claims is novel and inventive over the cited prior art documents. In this regard, following arguments were submitted by the applicant:

Novelty

The formulation comprises TMC 278, poloxamer and sterile water. This combination of features, and certainly not together with the other features of claim 1, is not disclosed as such in the cited prior art documents and hence the claimed invention is novel over the cited prior art documents.

Inventive step

The problem underlying the present invention is the provision of a means to treat a subject infected with HIV, said means providing for good compliance of the infected individual.

It is needless to say that there is a long felt need for an effective and convenient means to tackle HIV infection. In order to be effective and convenient, such means must be such that it provides for good compliance of the HIV infected individual.

One way to increase compliance is to provide means that require a reduced number of administrations, in other words means for long term HIV treatment upon administration. Thus administration of said means provides for treatment of HIV infection over a prolonged period of time. In this way, the so-called pill burden is reduced and this is advantageous for therapy adherence.

The problem underlying the present invention is solved by the parenteral formulation as laid down in claim 1, namely TMC278 in a parenteral formulation to be administered subcutaneously or intramuscularly intermittently at a time interval that is in the range of two weeks to three

weeks, or three weeks to four weeks, or one month to three months, and comprises a poloxamer and sterile water.

A skilled person could not deduce from the cited prior art that a formulation that combines the following features :

1) TMC278 as anti-HIV agent
2) Intramuscular (IM) administration
3) Intermittent administration at a time interval that is in the range of two weeks to three weeks, or three weeks to four weeks, or one month to three months
4) Comprising a poloxamer and sterile water as aqueous carrier
5) Comprising an amount of TMC278 ranging from 7 mg to 4500 mg

would be able to achieve treatment of HIV infection for a prolonged period, and hence provide a treatment means with good compliance.

There is no suggestion in the cited prior art that directly and unambiguously directs the skilled person to the present formulation for long term HIV infection treatment and therefore the present claims must not only be found novel, but also inventive.

The learned Controller has referred to D1 (WO03/016306).In this respect, it is to be noted that this prior art document indeed discloses TMC278 to treat HIV infection, but there is no direct and unambiguous disclosure of a long term treatment as such, in particular treatment of HIV infection over a prolonged period of time, said prolonged period of time being bridged by administering a formulation intermittently at intervals of such a prolonged period of time. There is no direct and unambiguous disclosure in WO03/016306 that this could be obtained.

D1 is also silent as such on a formulation comprising a poloxamer and sterile water.

The overall disclosure of D2 (WO 2005/021001) is directed to a once daily combination therapy, opposed to for instance twice daily administration, of TMC278 in combination with other antiretroviral agents, but there is no direct hint towards a long term treatment, in particular treatment of HIV infection over a prolonged period of time, said prolonged period of time being bridged by administering a formulation intermittently at intervals of such a prolonged period of time (opposed to once daily administration). There is no direct and unambiguous disclosure in D2 that this could be obtained. Described are parenteral formulations of the combinations but only in general terms. This reference does not disclose the present parenteral formulation as defined in amended claim 1. D2 is also silent on a formulation comprising a poloxamer and sterile water. So the present formulation cannot directly and unambiguously be derived from D2.

Also reference D3 does not disclose the present invention. D3 is completely silent on an IM or SC composition for TMC278. This prior art document is mainly focused on oral formulations. D3 describes TMC278 or R278474 as a new NNRTI suitable for high compliance oral treatment for HIV infection (please refer to abstract). It is noted in D3 that desirable features to be met for an anti-HIV drug are high oral bioavailability allowing once daily administration (please refer to abstract and page 1904).

It further describes an IV and oral formulation in PEG400 (page 1905). D3 is also silent on a formulation comprising a poloxamer and sterile water. So the present formulation cannot directly and unambiguously be derived from D3.

So based on the above, it is clear that D3 does not disclose or directs towards the long term treatment of HIV with a formulation for IM or SC administration intermittently at a time interval that is in the range of two weeks to three weeks, or three weeks to four weeks, or one month to three months. D3 does not offer any suggestion towards the development of an IM or SC composition for the long term treatment of HIV infection, does not offer any suggestion towards the development of an IM or SC composition for the long term treatment of HIV infection and comprising a poloxamer and sterile water.

It is to be note that with hindsight the present invention relating to a formulation for the long term treatment of HIV infection with TMC278 when administered intramuscularly and intermittently at a time interval that is in the range of two weeks to three weeks, or three weeks to four weeks, or one month to three months might seem obvious and straightforward, but at the time of filing the present application this was certainly not the case.

One has to realize that for IM or SC administration, one is confronted with a very limited volume that might be administered in one injection which is as little as a few ml. This means that, if one wants to obtain a long term treatment wherein effective plasma levels of the HIV drug have to be maintained for a period in the range of two weeks to three weeks, or three weeks to four weeks, or one month to three months, the IM or SC formulation needs to contain enough drug to bridge this time period and that it should be possible to formulate this amount of drug in the very limited volume that can be administered by IM or SC injection.

One also has to realise that a long term HIV infection treatment medication needs to have a favourable adverse event profile.

In view of the fact that many of the available HIV drugs are known to show side-effects and in view of the fact that HIV drugs often require administration of a substantial dose in order to be effective (please note that, as indicated above, long term HIV infection treatment requires the administration of such a dose as to obtain effective plasma levels over a prolonged period of time), it is clear that turning to a long term HIV infection treatment formulation is not straightforward nor obvious for the skilled person.

The learned Controller did not cite any prior art document or combination of prior art documents wherein this concept is unambiguously disclosed or can directly be deduced.

It can only be assumed that it was the general assumption that such an approach would simply not be possible.

With the present invention however, it is shown that with the presently claimed formulation of TMC278 as anti HIV drug it is possible to obtain effective plasma levels over a prolonged period of time. This makes long term HIV infection treatment with administration of TMC278 at a time

interval that is in the range of two weeks to three weeks, or three weeks to four weeks, or one month to three months possible. This is a huge finding which can tremendously affect the battle against HIV. It can have a tremendous impact on therapy compliance.

One could not predict with a reasonable expectation of success that TMC278 would be a drug making long term HIV infection treatment possible via a formulation that does not put a daily pill burden on patients.

It is also to be noted that TMC278 has a favourable adverse event profile, in particular a favourable profile for neuropsychiatric and metabolic events. Reference therefore is made to the enclosed Medscape abstracts (also submitted along with response to First Examination Report). Also this could not be deduced from the cited prior art documents.

Thus, TMC278 as a drug suitable for long term HIV infection treatment could not have been deduced directly and unambiguously from the prior art.

A further feature of the present claims is that the formulation is for administration by IM injection.

It is to be noted that IM depot formulations on the market are typically for drugs requiring a low daily dose, in the range of up to only a few mg, such as hormones or anti-psychotic drugs. There is no clear and direct guidance in the prior art that IM or SC administration would have potential as administration route in order to obtain effective plasma levels over a prolonged period of time for an HIV drug, which is typically dosed much higher than hormones or anti-psychotics. There is certainly no clear and direct guidance towards the fact that this way of administration would be an option for a long term HIV infection treatment when administered at a time interval that is in the range of two weeks to three weeks, or three weeks to four weeks, or one month to three months.

In conclusion, the skilled person is not unambiguously directed towards the presently claimed formulation for obtaining long term HIV infection treatment by IM administration of TMC278 intermittently at a time interval that is in the range of one month to three months. The combination of these features of the formulation cannot simply be deduced from the prior art. There is no real indication of a reasonable expectation of success. Therefore, the present claims must be found inventive over the cited prior art.

Further, it is emphasized that the novelty and inventive step reside in the claimed parenteral formulation per se and not merely its use (i.e. method of administration/period of administration).

Regarding paragraph 5, the applicant submitted that the claimed invention pertains to a parenteral formulation and not a method of treatment of a disease or method of administration of a drug.Therefore, the present invention does not fall under the prohibition of Section 3(i).

Regarding paragraph 6, it is submitted that a copy of declaration as to applicant's entitlement to apply for a patent (copy enclosed), as published by International Bureau has been submitted along with response to First Examination Report.

Regarding paragraph 7, it is submitted that a petition of obviating the irregularity in filing Form 3 has been submitted along with the response to First Examination Report. A copy of CBR is enclosed for the ready reference.

[F] OBSERVATION:

I shall now deal with objections of Ld. Examiner in the light of amendments in the claim in the hearing and arguments placed before me by the applicant's attorney in favor of their case.

Regarding objection no. 1, the arguments of Ld. Attorney is agreeable as the claims have been amended and all the essential constituents of the claimed formulation have been defined in claim 1. The amount of API (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile (TMC 278) present in the formulation has also been recited in claim 1. Hence objection no. 1 is not maintainable.

Regarding objection no. 3 in respect of inventive step, following prior art documents were cited by the Ld. Examiner:-
D1-WO03016306
D2-WO2005021001
D3-Journal of Medicinal Chemistry, 20041106 American Chemical Society, US ISSN 00222623by JANSSEN P A; ET AL, Vol: 48, Nr:6, Page(s):1901 1909.

Document D1 specifically discloses more than 250 pyrimidine derivative having HIV replication inhibiting properties that act as non-nucleoside RT inhibitors (NNRTIs) having the ability to inhibit the replication both wild-type and of mutant strains. One of said NNRTIs is 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl] amino] -benzonitrile (TMC278). Document D1 further teaches compositions for treating viral infections comprising a therapeutically effective amount of a compound of formula (I) or pharmaceutically acceptable addition salts and a pharmaceutically acceptable carrier or diluents and the carrier may take a wide variety of forms depending on the form of preparation desired for administration. For parenteral compositions, the carrier will usually comprise sterile water(D1, page 45, para 5). Though D1 does not specifically teach the poloxamers but at page 48 para 35 teaches water soluble polymer which includes copolymers of ethylene oxide and propylene oxide.

Document D2 discloses the use of a combination of 4-[[4-[[4-(2-cyanoetlιenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl] amino]-benzonitrile (TMC278) and a NRTI for the treatment of HIV infection at a dose that can be administered once daily (p. 3, line 36- p. 5,

line 9; p. 15, lines 11-15; claim 1). The compositions may be administered via parenteral injection (p. 13, line 37- p. 14, line 5; p. 16, line 37- p. 17, line 19).

Document D3 teaches TMC278 or R278474 as a new NNRTI suitable for high compliance oral treatment for HIV infection. This document is not considered to be of particular relevance for assessing the inventive step.

Document D1 and D2 teach all the features of present invention except the intermittent administration of the formulation at a time interval of at least one week to one year with amount of TMC278 ranging from 7 mg to 4500 mg.

In view of aforesaid teaching, it is observed that if the features of D1 is combined with D2, the combination provides clear motivation and reasonable expectation of success for a person skilled in the art to prepare the claimed formulation comprising TMC278 as an active ingredient, poloxamer and sterile water, wherein the formulation is administered by intramuscular administration intermittently at a time interval in the range of two to three weeks, or three to four weeks, or one month to three months and the effective amount of (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile in the formulation ranges from 7 mg to 4500 mg.

Moreover, the composition of cited documents is the same formulation comprising the same ingredients as applicants claimed invention it will inherently have the same properties. Therefore, the invention as claimed in the instant application is obvious to a person skilled in the art and do not show any technical advancement and unexpected improvement of the properties over the known prior art. The applicant's submission that the novelty and inventive step reside in the claimed parenteral formulation per se and not merely its use (i.e. method of administration/period of administration) are not acceptable in respect of inventive step since the pharmaceutical composition comprising TMC278 is well known in the art and the difference lies only in the particular administration condition of the formulation and it would be obvious to a person skilled in the art to optimize the administration conditions of TMC278.

As regards to objection no. 2 in respect of section 3(e), it is observed that the claims as claimed in impugned application is a mere admixture resulting only in the aggregation of the properties of the components thereof. A synergistic composition should show unexpectedly new property or better efficacy than a mere aggregation of the properties of its components. There is no other essential component in the claimed composition that could justify a synergistic effect to validate a composition claim. Though the submission of applicant claimed that the combined effect of TMC 278, sterile water and poloxamer results in a formulation which is suitable for IM administration and which has anti HIV activity and with the present formulation, it is possible to treat HIV infection over a long term which certainly provides advantages for the patient for therapy compliance and hence drug efficacy but the submission is insufficient and only based on the administration of TMC278 in the light of the documents cited i.e. D1 or D2, wherein the TMC278 has already been used as anti HIV agent. Hence the claimed composition appears to be incapable of proving any sort of synergism

and is therefore a mere admixture of ingredients which by virtue of their own anti HIV property. Any unexpected properties or improvement are not established by any example or data in the description in the claimed formulation.

With regard to objection no. 5 relating section 3(i), the applicant's argument that the claimed invention pertains to a parenteral formulation and not a method of treatment of a disease or method of administration of a drug does not appear convincing. It is observed that though the claims preamble corresponds to the formulation but the claims have same effect as claims over methods for medical treatment, as the subject matter is not a product or process but the way in which a product is therapeutically used and the invention would only have effects on the body and not technical effects.

Since the cited documents have not been filed in Indian Patent Office, the objection regarding prior claiming stands moot.

Objections 6 and 7 are formal in nature which were complied by applicant's end hence are not further maintainable.

In the light of above, I am of the opinion that the claimed invention is not only lacking inventive step but also falling within the provisions of section 3(e) and 3(i) of the Patents Act 1970. Therefore, I refuse to proceed for grant of patent for application no. 5461/DELNP/2008.

Date – 21/07/2015

(N.R.MEENA)
Deputy Controller of Patents and Designs Patent Office, Delhi

Copy to:-M/S. Remfry & Sager

THE PATENTS ACT, 1970

SECTION 15

IN THE MATTER OF AN APPLICATION FOR PATENT

Application number: 201647001874

DECISION

In view of the outstanding objections after the response to FER was received, a hearing was offered on 17th July, 2020 with a list of objections as follows.

1. The claim amendments appears to be not allowable as the amended set of claims do not appear to meet the restrains of section 59 in that the scope of claims after the amendment sought, do not fall wholly within the scope of the claims before amendment. The amended claims 1-12 do not meet the requirements of Section 59(1) of The Patents Act, 1970 as the claimed subject matter falls beyond the scope of matter in substance disclosed or shown in the specification as originally filed because not any one of embodiment of description and/or examples describe as such a pharmaceutical composition for use in a method for treating a subject having or at risk of developing cancer, and further Section 59(1) also provides that when any amendments are made to the claims, such amendments should fall within the scope of the originally filed claims.
Claims 1-12 of the alleged invention do not meet the requirements of Section 10(4) and 10(5) of The Patents Act, 1970 as the claimed subject-matter does not support by the description as that is not described as one of the embodiment and also not exemplified in the specification. Hence, the said claims are not allowable.

Claims do not clearly define the invention: The expression "according to" as used in the dependent claims 2-12 should be replaced appropriately.

2. It is well settled principle in the field of Patent law that –"WHAT IS NOT CLAIMED IS DISCLAIMED". Hence it is ample clear that if something is not claimed initially is actually disclaimed. Accordingly the amended claims 1-12 are not allowable under Section 57 & Section 59 of the Act.

3. Claims 1-12 although refers to a pharmaceutical composition but actually trying to claim the treatment by which the compounds are administered. This is not only vague but also appears to be method of treatment in disguised form. The said claims are also trying to claim dosage form and accordingly also attract the section 3(d) of the Act.

4. Drawings should be filed in the prescribed manner as per Rule 15 of the Patents Rules. The numbering of pages should be serially numbered started from the cover page and mentioned at the bottom of each page in the complete specification. Blank space should be scored out in the complete specification.

5. Applicant has not given proper reply to FER for the cited documents D1-D4. D5: Eisai Highlights New Research on Melanoma, Breast and Endometrial Cancer at ASCO Annual Meeting - May 16, 2013 (http://eisai.mediaroom.com/2013-05-16-Eisai-Highlights-New-Research-On-Melanoma-Breast-and-EndometrialCancer-at-ASCO-Annual-Meeting). D5 also discloses the composition of the claimed drugs for the treatment of cancer. So at the time of filing of this application, it would have been obvious to a person skilled in the art to combine the teachings of D1-D5 to arrive at the claimed invention, thus for the present application no inventive step can be acknowledged and hence the claims are not allowable under Section 2(1)(j) of the Act.

6. Claims 1-12 are not allowed U/s 3(d) of the Act, as the subject matter of claims tells about new use of known substance. Claims 1-12 although refers to a pharmaceutical composition but actually trying to claim the treatment by which the compounds are administered. This is not only vague but also appears to be method of treatment in disguised form.

The said claims are also trying to claim dosage form and accordingly also attract the section 3(d) of the Act. The subject matter of claims falls under section 3 of the Patent Act, 1970 and are not inventions under the said section/clause:

a) Claims 1-12 falls within the scope of such clause (e) of section 3 of the Patents Act, 1970- these Claims are not clear and sufficiently definitive to the scope of alleged invention in the absence of mention of any significant elements/components of composition, like constituents and their proportions, percentage etc. that reflects technological contribution to establish the novelty and inventive step and define the scope of alleged invention.

b) Claims 1-12 falls within the scope of such clause (i) of section 3 of the Patents Act, 1970- method of treatment claims are not allowed under the said clause.

7. Subject matter of claims falls under section 2(1)(j) of the Patent Act, 1970 and are not inventions under the said section/clause:

Claims 1-12 do not constitute an invention under section 2(1)(j) of the Patent Act, as the said use claims neither relates to any product nor any process and accordingly are not considered as an invention.

The claims 1-12 are not clearly worded and it related to a composition for the use of cancer treatment, which is merely a method of medical treatment in the guise of known composition and it is not within the scope of the patentable under Section 2(1)(j) of the Patents Act.

The applicant's agent informed by a letter dated 16th July 2020 that the applicant has lost interest in the subject application and does not wish to proceed further with the same.

In view of the outstanding objections which have not been compiled with and the fact that applicant has lost interest in the subject application. Hence I hereby refuse the instant application 201647001874 under section 15 of the Act.

Dated this 17th July, 2020.

Anjaneyulu Reddi

Assistant Controller of Patents & Designs

Gmail Priyam Liz <priyamlizcherian@gmail.com>

Re: Authorization

1 message

Eldred Tellis <[redacted]> Thu, Jul 23, 2020 at 4:56 PM
To: Priyam Lizmary Cherian <priyamlizcherian@gmail.com>

1. I, Eldred Tellis, Indian Inhabitant, with office address at 1st floor, SS Bengali Municipal School, Thakurdwar Road, Charni Road (E), Mumbai-400002, India hereby authorise Advocates- Ms. Priyam Lizmary Cherian and Ms. Shruti Jain, having their office at A-13, First Floor, Nizammudin West, New Delhi 110013, to act on my behalf in connection with the pre-grant opposition under section 25(1) against Patent Application No. 201817014361, titled " COMBINATION ANTIBACTERIAL COMPOSITION AND SHORT COURSE ANTIBACTERIAL REGIMEN", in the name of The Global Alliance for TB Drug Development Inc. filed on 04.04.2018, and request that all notices, requisitions and communication relating thereto may be sent to such persons at the above address unless otherwise specified.

2. I hereby revoke all previous authorisations, if any made, in respect of the same matter or proceeding.

3. I hereby assent to the action already taken by the said persons in the above matter.

Dated this the 23rd day of July 2020

Eldred Tellis

Gmail Priyam Liz <priyamlizcherian@gmail.com>

Authorization

1 message

Ganesh Acharya <[redacted]> Thu, Jul 23, 2020 at 4:55 PM
To: Priyam Lizmary Cherian <priyamlizcherian@gmail.com>

I, Mr. Ganesh Acharya, about 40 years of age, Indian Inhabitant, with address at Flat No.101,Mahakavi Bamamdada Karkad Palace, Belawali, Badlapur East, District-Thane,Maharashtra-421503, India, hereby authorise Advocates- Ms. Priyam Lizmary Cherian and Ms. Shruti Jain, having their office at A-13, First Floor, Nizammudin West, New Delhi 110013, to act on my behalf in connection with the pre-grant opposition under section 25(1) against Patent Application No. 201817014361, titled "COMBINATION ANTIBACTERIAL COMPOSITION AND SHORT COURSE ANTIBACTERIAL REGIMEN", in the name of The Global Alliance for TB Drug Development Inc. filed on 04.04.2018, and request that all notices, requisitions and communication relating thereto may be sent to such persons at the above address unless otherwise specified.

I hereby revoke all previous authorisations, if any made, in respect of the same matter or proceeding.

I hereby assent to the action already taken by the said persons in the above matter.

Dated this the 23rd day of July 2020

Ganesh Acharya

IN THE MATTER OF THE PATENTS ACT, 1970
AND
IN THE MATTER OF THE PATENTS RULES, 2003
AND
In the matter of Indian Patent Application No**.** 201817014361 filed on 04.04.2018
AND
In the matter of representation by way of opposition opposition under Section 25(1) by Eldred Tellis and Ganesh Acharya

REQUEST FOR EXTENSION OF TIME UNDER RULE 138 OF THE PATENTS RULES, 2003 FOR FILING FORM-26 WITH APPROPRIATE STAMP DUTY

On behalf of the Opponents – Eldred Tellis and Ganesh Acharya, it is submitted:

1. That Eldred Tellis and Ganesh Acharya have filed a representation by way of opposition under Section 25(1) against patent application no. 201817014361.
2. That given the restrictions due to COVID-19 related measures, it is difficult to receive hard copies of documentation with the signature of the opponent for the purposes of filing.
3. That an email authorizing the opponent's counsel is being filed in the interim.
4. That the Opponents undertake to file the Form-26 with requisite stamp duty at a later stage, as and when directed by the Hon'ble Controller.
5. That the Hon'ble Controller may allow an extension for filing Form-26 by the Opponents in the matter of pre-grant opposition filed against patent application no. 201817014361 by Eldred Tellis and Ganesh Acharya.

Priyam Lizmary Cherian
Counsel for Eldred Tellis and Ganesh Acharya
A-13, Third Floor,
Nizamuddin West, Delhi
Phone: 011 46805555
Email:priyamlizcherian@gmail.com

Dated this 24th day of July, 2020

To
The Controller of Patents
The Patent Office, DELHI