25 June 2020
EMA/200048/2020
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Pretomanid FGK

International non-proprietary name: pretomanid

Procedure No. EMEA/H/C/005167/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

ALT	alanine aminotransferase
ANC	absolute neutrophil count
AREDS2	Age-Related Eye Disease Study 2
AS	Active substance
AST	aspartate aminotransferase
B	bedaquiline (formerly J, TMC207)
BCS	Biopharmaceutics Classification System
BPaL	bedaquiline, pretomanid, and linezolid regimen
C	Clofazimine
CHMP	Committee for Medicinal Products for Human use
CSR	clinical study report
DoE	Design of experiments
DR	drug resistant
DS	drug susceptible
E	Ethambutol
EBA	early bactericidal activity
eDISH	evaluation of drug-induced serious hepatotoxicity
GC	gas chromatography
GGT	gamma-glutamyltransferase
H	Isoniazid
HPLC	high performance liquid chromatography
HRZE	isoniazid, rifampicin, pyrazinamide, and ethambutol regimen
HDTMA	Hexadecyltrimethyl-Ammonium Bromide
HM LDPE	high molecular low density polyethylene
ICH	International Conference on Harmonisation
IR	infra red spectroscopy
KF	Karl Fischer titration
L	Linezolid
LDPE	low density polyethylene
M	Moxifloxacin
MDR-TB	multidrug-resistant TB (resistance to H and R)
MGIT	mycobacterial growth indicator tube

MS	mass spectrometry
MTB	Mycobacterium tuberculosis
NLT	not less than
NMR	nuclear magnetic resonance
NMT	not more than
Pa	Pretomanid
Ph. Eur.	European Pharmacopoeia
pre-XDR-TB	pre-extensively DR TB (resistance to H + R + quinolone or injectable)
PVC	Polyvinyl chloride
PVDC	Polyvinylidene chloride
QbD	Quality by design
QTc	QT interval corrected for heart rate
QTcF	QT interval corrected for heart rate using Fridericia's method
QTPP	Quality target product profile
R	Rifampicin
RH	relative humidity
SmPC	Summary of Product Characteristics
TB	Tuberculosis
TB Alliance	Global Alliance for TB Drug Development
TEAE	treatment-emergent adverse event
TI MDR-TB	treatment-intolerant multidrug-resistant tuberculosis
TI/NR MDR-TB	treatment-intolerant/nonresponsive tuberculosis
TSE	Transmissible Spongiform Encephalopathy
UV	ultra violet spectrometry
XR(P)D	X-Ray (Powder) Diffraction
XDR-TB	extensively drug-resistant (resistance to H + R + fluoroquinolone + injectable)
Z	Pyrazinamide

1. Background information on the procedure

1.1. Submission of the dossier

The applicant FGK Representative Service GmbH submitted on 11 March 2019 an application for marketing authorisation to the European Medicines Agency (EMA) for Pretomanid FGK, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 20 September 2018.

Pretomanid FGK, was designated as an orphan medicinal product EU/3/07/513 on 29/11/2007 in the following condition: Treatment of tuberculosis.

The applicant applied for the following indication: Pretomanid is indicated as part of a combination regimen with bedaquiline and linezolid, in adults, for the treatment of pulmonary extensively drug resistant (XDR), or treatment-intolerant or nonresponsive multidrug-resistant (MDR) tuberculosis (TB).

Consideration should be given to official guidance on the appropriate use of antibacterial agents

The claimed indication concerns the specific regimen used in the pivotal study (Nix-TB), namely, pretomanid in combination with bedaquiline and linezolid.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Pretomanid FGK as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the Orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: ema.europa.eu/en/medicines/human/EPAR/pretomanid-fgk.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is

composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0058/2019 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0058/2019 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No

847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

Applicant's request(s) for consideration

Conditional marketing authorisation

The applicant requested a Full Marketing Authorisation. During the procedure, the CHMP proposed a Conditional Marketing Authorisation in accordance with Article 14a of the above-mentioned Regulation in case of an approval.

New active Substance status

The applicant requested the active substance pretomanid contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

Protocol assistance

The applicant did seek Protocol assistance from the CHMP.

Several CHMP advices were sought for the preclinical part of the program and on quality aspects. The advice on quality aspects concerned designation of starting materials, control of impurities and the stability programs.

A CHMP advice on clinical issues were sought in 2014; however, this advice addressed specifically a phase 3 study (NC-006), addressing another Pa-containing regimen and a different indication, and does not apply to the present application.

The clinical program presented so far is considered compliant with the CHMP guidance. Studies undertaken in patients with DS-TB include the proper control regimen (HRZE). The pivotal study, Nix-TB, does not include a control regimen, which is acceptable since no valid standard control regimen exists.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Pretomanid FGK as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the Orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: ema.europa.eu/en/medicines/human/EPAR/pretomanid-fgk

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Filip Josephson Co-Rapporteur: Ingrid Wang

The application was received by the EMA on	11 March 2019
The procedure started on	28 March 2019

The Rapporteur's first Assessment Report was circulated to all CHMP members on	17 June 2019
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	17 June 2019
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	1 July 2019
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	25 July 2019
The applicant submitted the responses to the CHMP consolidated List of Questions on	28 November 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	08 January 2020
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	16 January 2020
The CHMP agreed on a list of outstanding issues in writing and to be sent to the applicant on	30 January 2020
The applicant submitted the responses to the CHMP List of Outstanding Issues on	24 February 2020
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	11 March 2020
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	N/A
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive Opinion for granting a conditional marketing authorisation to Pretomanid FGK on	26 March 2020
The EC returned the Opinion to the CHMP, requesting an assessment of the impact on the conclusion of the CHMP similarity assessment of new information related to the similarity of Pretomanid FGK vis-à-vis authorised orphan medicinal products.	20 May 2020
The CHMP, in the light of the scientific data available and the scientific discussion within the Committee, reconsidered the benefit-risk of Pretomanid FGK, adopted a revised positive Opinion which concluded that the application satisfied the criteria for authorisation and recommended the granting of the marketing authorisation.	25 June 2020

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Tuberculosis in humans is an infectious disease caused by *M. tuberculosis*, which typically affects the lungs (pulmonary tuberculosis) but can affect other sites as well (extra-pulmonary tuberculosis). While incidence rates and mortality for tuberculosis have been falling, the disease remains one of the world's major causes of illness and death – prompting the WHO in 1993 to declare tuberculosis to be a global health emergency. While tuberculosis is found in every country in the world, it disproportionately affects people in resource-poor settings, particularly those in Asia and Africa. Nevertheless, tuberculosis outbreaks still occur in industrialized nations. The WHO estimates that 1.7 billion people—approximately one quarter of the world's population—are infected with *M. tuberculosis* (WHO 2018). Worldwide, in 2017, there were an estimated 10 million incident cases of tuberculosis (9% co-infected with HIV), and 1.6 million people died from tuberculosis. Worldwide, tuberculosis is 1 of the top 10 causes of death and the leading cause from a single infectious agent. Globally in 2017, there were an estimated 330,000 MDR-TB or rifampicin-resistant tuberculosis cases among notified tuberculosis patients. In a survey of 113 countries and 5 territories, the average proportion of MDR-TB cases with XDR-TB was 8.5% (95% CI: 6.2%, 11%), an increase from the 6.2% reported in 2016 (WHO 2017).

2.1.2. Management

MDR-TB refers to disease caused by bacteria resistant to rifampicin and isoniazid. XDR-TB is resistant to at least 4 of the major drugs or drug classes used in tuberculosis therapy, that is, rifampicin, isoniazid, fluoroquinolones, and at least 1 of the injectable aminoglycosides. Consequently, the treatment of XDR-TB is very challenging. Before the very recent introduction in the XDR-TB therapeutic armamentarium of new (bedaquiline) or repurposed (linezolid) drugs for which there is little or no pre-existing resistance, the treatment success rate in XDR-TB patients was extremely poor. Rates of treatment success across the South African studies averaged 14%, with a range of 2% to 22%; outside of South Africa, rates of treatment success were more varied, ranging from 15% to 60%, with only 2 studies reporting rates above 50%.

The success rate for treating XDR-TB has very recently improved due to 1) the use of bedaquiline, which is approved by the EMA and FDA as part of combination regimens to treat adults with MDR-TB, and 2) the increasingly frequent off-label use of linezolid for the treatment of drug-resistant tuberculosis (both drugs used as "add-ons" to pre-existing or modified background regimens). Cure rates of 70-80% with such drug combinations have been reported (Lee 2015, Olayanju 2018). However, in these studies, treatment was very long (around 2 years) and background regimens were complex, including > 10 drugs, oral and injectables. Hence, new shorter and standardized regimens are highly warranted.

About the product

Pretomanid is a new chemical entity and a member of the nitroimidazooxazine class (whereas delamanid is a member of the nitroimidazooxazole class). The mechanism of action is complex and has not been fully elucidated. Under aerobic conditions, pretomanid appears to inhibit MTB cell wall

biosynthesis by inhibiting the synthesis of mycolic acid, while under anaerobic conditions, it generates reactive nitrogen species. Pretomanid is active both to actively replicating MTB and to dormant bacilli (both bactericidal and sterilizing activity).

The claimed indication is for the treatment of adults with pulmonary XDR-TB, or MDR-TB patients non-responsive/intolerant to the MDR-TB regimen (such as an injectable). The claimed indication concerns the specific regimen used in the pivotal study (Nix-TB), namely, pretomanid in combination with bedaquiline and linezolid.

Type of Application and aspects on development

The applicant requested a Full Marketing Authorisation. The CHMP Rapporteurs, and the CHMP following discussions during the procedure, support a Conditional Marketing Authorisation (refer to the benefit/risk section).

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as an immediate release tablet containing 200 mg of pretomanid as active substance.

Other ingredients are: lactose monohydrate, microcrystalline cellulose, sodium starch glycolate, magnesium stearate, colloidal anhydrous silica, sodium lauryl sulfate and povidone.

The product is available in high-density polyethylene (HDPE) bottles with polypropylene closure and PVC/PVdC-Aluminium foil blisters packs

2.2.2. Active Substance

General information

The chemical name of pretomanid is (6S)-2-Nitro-6-{[4-(trifluoromethoxy)phenyl]methoxy}-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine. It corresponds to the molecular formula $C_{14}H_{12}F_3N_3O_5$, its relative molecular mass is 359.26 and it has the structure shown in Figure 1.

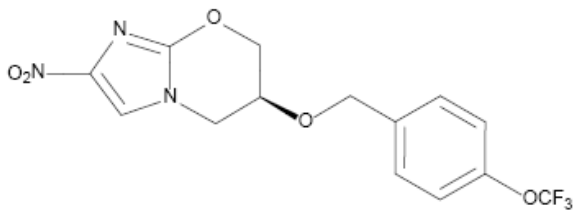


Figure 1. Structure of pretomanid.

The structure of the active substance (AS) was elucidated by a combination of by infrared (IR) spectroscopy, fluorine (19F), proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), and single crystal x-ray structure determination.

In addition, characteristics of the active substance were also determined with thermal analysis, elemental analysis, and UV spectrometry.

Pretomanid is a white to off-white to yellow colour non-hygroscopic crystalline powder. It is practically insoluble in water and its solubility in buffers (pH 1.0-9.0) is in the range of 0.01-0.02 mg/mL and in the range of 0.01-0.09 mg/mL even in simulated physiological media pH 1.5, 5.0 and 6.5 at 37°C. Its partition coefficient (LogP) is 2.42.

The molecule contains one chiral center (6S). The S-enantiomer is the desired isomer. The chiral centre of pretomanid originates from a starting material. The undesired isomer (R-enantiomer) is controlled in the active substance specification.

Different solid-state forms of pretomanid have been identified and characterised. Form I is the most thermodynamically stable polymorphic form at ambient temperature and pressure and is the one synthesised and used for the finished product.

Changes in polymorphic form during processing and on storage are extremely unlikely to occur.

Manufacture, characterisation and process controls

The synthesis of pretomanid consists of three chemical transformations and a recrystallisation process. The manufacturing process has been described in sufficient detail from the starting materials. The choice of these substances as starting materials has been justified and is in accordance with CHMP scientific advice. Intermediates are isolated and controlled as part of the acceptable control strategy. The formation and removal of impurities in all steps are discussed and controlled.

The manufacturing process development work has been described in sufficient detail and has resulted in a manufacturing process giving reproducible active substance with high purity. An enhanced approach to process development was taken in line with ICH Q11. While Design of Experiments (DoE) studies of process parameters and risk assessments were performed on each step to review and identify the possibilities of critical process parameters for that step, no design space is claimed. The critical process parameters and their control for the manufacture of pretomanid have been identified, presented and are considered acceptable.

The characterisation of the AS and its impurities are in accordance with the EU guideline on chemistry of new active substances. Several impurities are associated with the manufacturing process, some of which are present in substantial amounts and some are potentially mutagenic. The control of the impurities was not fully satisfactory in the initial submission and the issue was identified by the CHMP as a Major Objection. Updated information was provided and included an adequate discussion on the characterisation of potential impurities including genotoxic impurities, their origin, control and carry-over. The control of impurities, including potentially mutagenic impurities is considered acceptable and in line with the CHMP scientific advice. Results from spiking studies have been presented to support the controls proposed for impurities in starting materials, intermediates and pretomanid.

Residual solvents used in the different steps during the synthesis were discussed. No class 1 solvents or metal catalysts are used in the manufacture of the starting materials or the active substance. The control strategy to control all class 2 and class 3 solvents in the release specification is endorsed. Also, benzene, as a potential contaminant in some of the solvents, is controlled in the solvents' specifications, this approach is acceptable.

The active substance is packaged in white antistatic low-density polyethylene (LDPE) bag that is twist tied. The LDPE inner bag is placed in an outer high molecular low-density polyethylene (HM LDPE) bag that is heat sealed. To further protect the active substance, the HM LDPE bag is placed in a triple laminated aluminium bag that is heat sealed. This bag is placed in a high-density polyethylene (HDPE) container that is closed with plastic lid having rubber gasket followed by locking ring and metal seal

and labelled. The LDPE bags are certified to comply with regulations for food contact. Specifications and certificates are provided for the container closure systems.

Specification

Pretomanid active substance specification includes appropriate tests and limits for description (visual), identification (IR, HPLC), crystal form (XRPD), water content (KF), sulfated ash (Ph. Eur.), related substances (HPLC), assay (HPLC), content of enantiomer (chiral HPLC), residual solvents (GC) and particle size distribution (laser diffraction).

The proposed specifications and limits are acceptable. Limits for impurities and solvents have been set in accordance with ICH Q3A and Q3C. The absence of tests for elemental impurities and microbial testing has been acceptably justified by the applicant.

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data were presented for three validation/stability batches manufactured by the proposed commercial manufacturing sites. Results were also presented for 21 batches used in non-clinical studies, clinical studies, validation and stability studies and manufactured by previous manufacturing processes and manufacturers. The results comply with specification in place at the time of analysis; consistency of active substance has been demonstrated.

Stability

Stability data on three commercial scale batches of active substance stored in the intended commercial packaging for up to 18 months under long term conditions (25 °C/ 60% RH) and intermediate conditions (30 °C/ 75% RH) and for up to 6 months under accelerated stability conditions (40 °C/ 75% RH) was provided according to the ICH guidelines.

Parameters investigated: description, assay, related substances, content of enantiomer, water content and crystal form. The analytical methods used correspond to the release methods and are stability indicating. There were no significant changes in any of the studied attributes nor clear trends.

In addition, long-term (25 °C/ 60% RH) and accelerated (40 °C/ 75% RH) stability data have been presented for 12 batches manufactured by previous active substance manufacturer and used for manufacturing finished product clinical batches. Up to 48 months long-term data is available for these batches; results also comply with the specification at the time of analysis.

Photostability study was conducted in accordance with ICH Q1B, Option 2 on one commercial scale batch of pretomanid. No significant degradation of was observed.

Forced degradation studies under different conditions have also been performed as part of the related substances HPLC method validation. The results showed that pretomanid rapidly degrades when treated with base, while no significant degradation of pretomanid was observed by other standard treatments: high humidity, heat, solution state degradation study by acid and by peroxide.

Based on the presented data the proposed retest period of 24 months for the active substance with no special storage conditions can be accepted.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

The finished product is a white to off-white, oval shaped, uncoated immediate release tablets for oral use packed in blister or plastic bottles containing 200 mg pretomanid. The tablet is debossed with M on one side and P200 on the other side of tablet; its dimensions are 18 × 9 mm.
The qualitative composition of Pretomanid FGK include pretomanid, lactose monohydrate, microcrystalline cellulose, sodium starch glycolate, magnesium stearate, colloidal anhydrous silica, sodium lauryl sulfate and povidone.

The excipients used are commonly known and have well-established use in solid oral dosage forms. Their choice has been justified considering the very low solubility of pretomanid. As pretomanid is a neutral molecule, with a high resistance to degradation under normal conditions, a formal compatibility study with excipients was not considered necessary. Subsequent stability testing under both long term and accelerated test conditions has shown the product to be, chemically stable, with no evidence of degradation (please refer to "Stability of the product"). These data confirm the compatibility of pretomanid with the various excipients in the tablet formulation.

Pretomanid is considered BCS class II, having low aqueous solubility and high permeability. It has a long needle-like crystal shape, which results in poor flow and agglomeration during handling. Micronised material was introduced very early in the development process to achieve acceptable blend uniformity. Dissolution was also facilitated by the micronised active substance.

The development of the dissolution method was described in detail. The optimal amount of surfactant and the optimal paddle speed were investigated by the applicant.

The discriminating power of the dissolution method was also investigated. It has been shown that the dissolution method is sufficiently discriminatory. The dissolution properties of tablets manufactured by different manufacturers involved in the development phase were provided. All results complied with the proposed specification limit for dissolution.

An acceptable tablet manufactured by a wet granulation process was finally developed taking into account the physicochemical and pharmacokinetic properties of the active substance.

Possible relationships between excipient particle size and granular flow have been investigated during pharmaceutical development. The manufacturing process was developed at different sites before the process was transferred and optimised at the commercial manufacturing site. For optimisation of the manufacturing process, a risk assessment was carried out on manufacturing parameters that might have an impact on the CQAs. The applicant has applied quality by Design (QbD) principles in the development of the finished product and their manufacturing process. DoE was used to investigate the wet granulation process, the lubrication step and tablet compression. However, no design spaces were claimed for the manufacturing process of the finished product.

The proposed formulation and manufacturing processes for commercial product is the same as used in Phase 3 clinical studies as well as the primary stability batches.

The tablets are packaged in PVC/PVdC/aluminium blisters and HDPE bottles, which is standard type of packaging for this formulation type and was demonstrated through stability studies to provide adequate protection of the product during storage. HDPE bottles are capped with polypropylene screw cap with a pulp liner and absorbent cotton is placed inside the bottles. Acceptable specifications were provided for the packaging materials. The packaging materials in contact with the tablet comply with the Ph. Eur. and relevant EU regulations and directives.

Manufacture of the product and process controls

The finished product is manufactured by wet granulation process. The manufacturing process is standard and consists of dry sifting, dry blending, high shear granulation, wet milling, fluid bed drying, dry milling, sifting of extragranular material, blending, lubrication and tablet compression. The tablet bulk is packed in double LDPE bags with silica gel bags between the two PE bags, which are kept in triple laminated aluminium bags. Holding times have been stated.

In order to achieve the required batch size, and in line with the equipment available, the granulation step is carried out as equal sub-lots. Once dried and screened, the lots of granules are combined for blending of the extragranular materials to prepare a single batch for compression.

Extensive process investigations were conducted and have resulted in appropriate controls and limits for the granulation, blending, and compression steps, assigned accordingly.

The manufacturing process has been adequately validated on three production scale batches. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner.

Product specification

The finished product release and shelf life specifications include appropriate tests and limits for description (visual), identification (HPLC, UV), assay (HPLC), related substances (HPLC), uniformity of dosage units (Ph. Eur.), dissolution (Ph. Eur.- UV) and microbial quality.

The omission of test for elemental impurities is justified and a satisfactory risk assessment in line with ICHQ3D has been provided.

The applicant has thoroughly investigated and discussed the formation and origin of nitrosamine related impurities. The possible formation of the nitrosamine carcinogenic impurities in products was also discussed including all currently identified root causes for presence of nitrosamine impurities listed in section 12 "What are the currently identified root causes for presence of nitrosamines?" (UPDATED) of "Questions and answers on "Information on nitrosamines for marketing authorisation holders". 20 December 2019 EMA/CHMP/428592/2019 Rev. 2. No risks of formation of nitrosamines during the manufacturing and packaging of the products were identified by the applicant.

The analytical methods used have been adequately described and validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used in the routine analysis of finished product has been presented.

Acceptable batch data for three production scale batches were provided. Batch analyses have been presented for a total of three validation/stability batches, five phase 3 clinical batches and for further 6 small clinical batches. Additional information on batches with 50 and 100 mg tablet strengths which were used in phase 1, 2a and 3 clinical studies has been presented in the dossier. All the commercial formulation batches comply with the specification that applied at the time. The results indicate consistency and uniformity of the product, and that the process is under control.

Stability of the product

Formal stability data has been provided on three production scale batches (validation batches manufactured at the commercial manufacturing site and were packed in both commercial packaging

configuration and stored under long term 30±2°C/ 75±5% RH for up to 12 months and under accelerated conditions at 40±2°C/ 75±5% RH for 6 months in accordance with the ICH guidelines.

The following parameters have been investigated: description, HPLC-assay, related substances, dissolution, microbiological attributes. The stability results showed no clear trends or significant changes in any of the tested parameters at both storage conditions.

In addition, stability data from five batches manufactured at the two development sites were presented as supportive data. These batches were stored at the same storage conditions as described above for up to 36 months under long term and six months under accelerated conditions and presented.

Dedicated in-use stability studies for this solid oral dosage form in multi-dose container (bottle) were not considered necessary because according to ICH Q1A (R2) and EMA question and answer *"Claims for in-use shelf-life for solid oral dosage forms in multi-dose containers",* there is no indication from the presented stability and/or stress studies that the product is susceptible to deterioration.

Results of a formal photostability study as per ICH 1QB on one commercial scale batch showed results confirming that the finished product not photosensitive and precautionary measures to protect finished product from light are not needed.

A forced degradation study was performed on Pretomanid FGK tablet as a part of the related substances HPLC method validation. The results showed that pretomanid was rapidly degraded by base treatment at elevated temperature and no significant degradation was observed by any other standard treatments.

Based on the presented stability data the claimed shelf-life of 2 years without any special temperature storage conditions is acceptable (SmPC sections 6.3 and 6.4).

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that from a quality perspective the product should have a satisfactory and uniform clinical performance.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable and consistent. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.2.6. Recommendations for future quality development

None.

2.3. Non-clinical aspects

2.3.1. Introduction

Pretomanid (PA-824, Pa) is a new chemical entity and a member of the antimycobacterial nitroimidazooxazines drug class. The mechanism of action is complex and has not been fully

established. Under aerobic conditions, pretomanid appears to inhibit MTB cell wall biosynthesis by inhibiting the synthesis of mycolic acid, while under anaerobic conditions it generates reactive nitrogen species and a concomitant drop in intracellular ATP levels. The activities of pretomanid require nitro-reduction within the mycobacterial cell by the deazaflavin-dependent nitroreductase, Ddn. Pretomanid displays activity against drug-susceptible as well as drug-resistant (including multi-drug resistant and extensively drug resistant) strains of Mycobacterium tuberculosis with a minimal inhibitory concentration (MIC) generally in the range of ≤ 0.015 to 2 µg/mL.

2.3.2. Pharmacology

Primary pharmacodynamic studies

The applicant has conducted several in vitro and in vivo studies to address the primary pharmacodynamics of pretomanid. The primary pharmacodynamics studies are only briefly summarised in the NC section. Please see the Clinical section for further details.

In vitro

Pretomanid's anti-bacterial activity is considered narrow in spectrum. Published effect studies in vitro using different assays, indicate that the MIC-range was fully comparable in these studies, ranging from 0.005 to 0.48 µg/ml regardless of resistance classification (i.e. DS-, MDR- and XDR-TB isolates). Of note, isolates with mutations known to yield resistance to pretomanid have MIC values of ≥16 µg/ml. Among tested mycobacterial species besides MTB, the MTBC subspecies *M. bovis, M. africanum*, and *M. pinnipedi* were susceptible, with MICs from ≤ 0.0312 to 0.125 µg/mL. MICs were not measurable against *M. avium, M. phlei, M. smegmatis, M. fortuitum* or *M. kansasii. M. leprae* is naturally resistant as well (it lacks a functional ddn (deazaflavin-dependent Nitroreductase) gene, needed for activation of pretomanid). Pretomanid lacks activity against other aerobic bacteria and yeast including E. coli, S. aureus, and C. albicans.

In vivo

In vivo activity of pretomanid was evaluated in acute, chronic and latent TB murine models as monotherapy and in combination with a variety of first- and second-line TB drugs, including the compounds in clinical development. In the published studies, efficacy was generally assessed by CFU counts in lungs and/or spleens, either at the end of the treatment period (assessment of bactericidal activity), or 12 weeks after completion of treatment, to assess relapse (sterilizing capacity). In general, pretomanid dosed at 25 mg/kg yielded similar effect in an acute TB model as isoniazid control (25 mg/kg). The minimal effective dose (the lowest dose that prevents development of gross lung lesions and splenomegaly) and minimal bactericidal dose (the lowest dose that reduces lung CFU burdens by 99% compared to pretreatment) was found to be 12.5 and100 mg/kg, respectively, in aerosol infected mice.

Exposure response studies in acute mouse models with various dosing schedules over 24 days, with log CFU reduction in lung tissue as the end point, showed that free drug time over MIC had the best correlation to effect (R2 = 0.87).

In summary, the nonclinical in vitro and in vivo studies demonstrated that pretomanid was active against actively growing drug-sensitive and drug-resistant *M.tb.* strains.

Secondary pharmacodynamic studies

The applicant conducted a radioligand binding assay to evaluate the potential of pretomanid to inhibit the binding of a radiolabeled reference ligand to six selected hormone receptors including estrogen, glucocorticoids, progesterone, testosterone, and thyrotropin-releasing hormone. These results, with IC_{50} values >30 µM (10.8 µg/mL), indicate that pretomanid shows no affinity for binding to these receptors at therapeutic concentrations (clinical C_{max} = 3.1 µg/mL). The scientific rationale for screening against the selected hormone receptors was not presented. A broad off-target screen against common drug target classes (e.g. receptors, ion channels, transporters, and enzymes) was not provided initially. However, in the response to this issue the applicant performed a receptor off-target screen against 86 common pharmacological targets in vitro. Pretomanid (10 µM = 3.6 µg/mL; Clinical Cmax= 3.1 µg/mL at 200 mg dose) showed no significant interaction, defined as inhibition or stimulation >50%, for any of the human targets tested.

Furthermore, based on the observation of pretomanid-associated testicular effects in toxicology studies, the applicant studied the effect of pretomanid on cholesterol biosynthesis in cultured rat hepatocytes. In this study pretomanid, at 10 and 30 µM, had no apparent effect on cholesterol biosynthesis.

Safety pharmacology programme

The safety pharmacology of pretomanid was investigated in the core battery of the cardiovascular, respiratory, and central nervous systems studies in general accordance with ICH guidelines S7A and S7B. These included in vitro cardiovascular electrophysiology studies performed on cells expressing the hERG gene and in vivo evaluation of cardiovascular, central nervous system, and respiratory function in rats, dogs and nonhuman primates. Additional cardiovascular studies were conducted in dogs to evaluate the potential for QT prolongation associated with combined administration of bedaquiline, and a similar study examined potential QT prolongation in monkeys associated with combined administration of pretomanid and moxifloxacin (see Pharmacodynamic drug interactions below).

Cardiovascular

The results of three separate in vitro cardiovascular (hERG) studies of pretomanid demonstrated inhibition of potassium channel current with a half maximal inhibitory concentration (IC_{50}) of approximately 17 to 20 µM (6.1 to 7.2 µg/mL), providing an exposure margin of ~ 2x above clinical C_{max} (3.1 µg/mL, unbound plasma concentration = 2.5 µg/mL), indicating a potential for pretomanid to induce QT prolongation. It is noted that none of the three hERG studies were conducted in accordance with GLP regulations. However, the different hERG assays included vehicle controls, sufficient number of pretomanid test concentrations and yielded similar IC50 values, and the positive controls performed as expected, supporting the validity of the assays. In addition, there was no QT prolongation observed with pretomanid in the clinical thorough QT study in man (see below). Thus, the lack of formal GLP compliance of the hERG studies is considered acceptable.

In vivo cardiac safety pharmacology (telemetry) studies in dogs did not demonstrate any signal for QT prolongation at the dose tested (100 mg/kg, SC). However, in this study the dog plasma concentration of pretomanid was below the clinical Cmax, which in turn may preclude the interpretation of study data. In subsequent in vivo studies, a significant QT prolongation was observed in all dose groups in monkeys given single oral doses of pretomanid (50, 150, 450 mg/kg, PO). Compared to vehicle, mean heart rate corrected QTc interval (Fridericia's Formula) increased by 5 to 8%, 6 to 10%, 7 to 11%, (+15 to 23, + 19 to 30, +20 to 35 msec) at 50, 150, 450 mg/kg, respectively. In contrast, no increased QT prolongation or any other ECG changes were observed in monkeys administered repeated daily oral doses of pretomanid (50, 150 or 450/300 mg/kg/day; 450 mg/kg dose decreased to 300

mg/kg on Day 37) for 3 months or at 25, 50 or 100 mg/kg/day for 9 months (see also toxicology section). In the clinical thorough QT/QTc study performed in healthy subjects (single doses of 400 and 1000 mg, study DMID-10-0058) revealed no QT prolongation or concerns for the cardiac safety profile of pretomanid. In conclusion, pretomanid monotherapy appears to have low risk to cause clinically meaningful effects on the rate of ventricular repolarization in patients at the MRHD (200 mg/day).

Respiratory

The effects of pretomanid on the respiratory system was investigated in male Sprague Dawley rats administered 0, 50, 150, 450 mg/kg. A single oral dose of pretomanid at 450 mg/kg (but not at ≤ 150 mg/kg) caused significant decreases in respiration rate and minute volume (21% to 25% lower than control) that did not resolve within 24 hours of dosing. Although no pharmacokinetic (PK) data were generated in this study, an estimated exposure based on single dose PK studies in rat, indicate that the exposure margins (C_{max} = ~ 10 µg/mL) in rats at NOEL (150 mg/kg) exceeded clinical C_{max} by >3 fold.

Central nervous system

The CNS effects were investigated by means of an Irwin screen in male Sprague Dawley rats administered 0, 50, 150, and 450 mg/kg pretomanid. After a single oral dose of pretomanid in the Irwin study, depressed neural function (decreased touch response, body tone and grooming) was observed at ≥ 150 mg/kg but not at 50 mg/kg. The effects at 450 mg/kg did not resolve within 24 hours after dosing. The estimated plasma exposure of pretomanid at the NOEL of 50 mg/kg in the rat Irwin study, based on single dose rat PK (50 mg/kg, Covance 7504-101), was C_{max} = 9.4-11 µg/mL, which is approximately 3x the predicted exposure in patients at the maximum recommended human dose (MRHD) of 200 mg/day (3.1 µg/mL).

Following repeated daily administration of pretomanid in toxicology studies in mice, rats, and monkeys, clinical signs of an effect on nervous system function were observed in all species. In 4-week studies in mice, clinical signs recorded as spasm, jump and tumble, and abnormal spontaneous motor activity were seen at 500 mg/kg/day (but not at ≤ 150 mg/kg/day), and convulsions were observed at ≥ 250 mg/kg/day (but not at ≤150 mg/kg/day). In rats, effects on nervous system function included hypersensitivity to touch at ≥100 mg/kg/day (but not at ≤30 mg/kg/day) in a three-month study and convulsions in male and female rats as observed in several studies ≥ 100 – 300 mg/kg. Exposure margins for rats, the most sensitive species for CNS effects (NOEL = 30 mg/kg/day), at the threshold for CNS effects compared with human exposure (C_{max} = 3.1 µg/mL) at the MRHD was 2-fold. Exposure multiple for mice and monkeys at the NOEL for CNS effects were only slightly higher, in the range of 3- to 5-fold.

In summary, the applicant has investigated the safety pharmacology of pretomanid in general accordance with the current guidelines. Pretomanid plasma exposures in animals at the NOAELs for effects on the function of the nervous, cardiovascular, and respiratory systems were similar to or slightly higher (2-3-fold) than the anticipated steady-state exposure at the maximum recommended human dose of 200 mg/day.

Pharmacodynamic drug interactions

The applicant has conducted PD interaction studies evaluating cardiovascular effects when pretomanid was combined with bedaquiline in dogs or with moxifloxacin in monkeys. There was no apparent evidence of an interactive effect on QT prolongation when pretomanid was administered at a single dose of 100 mg/kg with bedaquiline (100 mg/kg) to dogs or at a dose of 50 mg/kg with moxifloxacin (30 or 100 mg/kg) to monkeys. However, it should be noted that the exposure to pretomanid in dogs

following subcutaneous administration of 100 mg/kg in the combination study with bedaquiline was unexpectedly low (C_{max} = 2.26 µg/mL) and was below clinical C_{max} (3.1 µg/mL), which is an inherent methodological limitation of this study, and the dog appears to be an inappropriate species for safety studies with pretomanid. In the PD interaction study performed n monkeys, there was a lack of a response of 3/4 animals to the 100 mg/kg moxifloxacin dose ("positive control" for assessing QT prolongation potential). Therefore, the submitted PD drug interaction studies have several deficiencies to allow any conclusions on an interactive effect.

While it's of importance to understand potential CV effects for different drug combinations with pretomanid, QT prolongation is known to occur with bedaquiline in patients and thus for the B-Pa-L (bedaquiline-pretomanid-linezolid) regimen, therefore data from further combination studies with bedaquiline and pretomanid in e.g. monkeys, at clinically relevant pretomanid exposure, are not considered necessary. In fact, a moderate QTcF increase of <10 msec was, as expected, reported for the B-Pa-L regimen in the clinical Nix-TB study. However, there was a surprisingly high increase in the QTcF (about 20 msec) in the clinical studies NC-002 and NC-005 with the Pa-M-Z regimen (pretomanid-moxifloxacin-pyrazinamide), where no relevant increase is expected by Pa and Z. Since moxifloxacin is well recognised to produce a predictable small prolongation of the corrected QT (QTc) interval (4-7msec), these findings may be a consequence of a yet uncharacterised Pa-Z/M drug interaction (see clinical section for details).

2.3.3. Pharmacokinetics

Studies have been performed to characterize the absorption, distribution, metabolism, and excretion (ADME) of pretomanid, using the intended clinical route of administration (oral), and the species selected for non-clinical safety testing, i.e. rats and monkeys as the main non-clinical species but also mice and rabbits***.***

Methods of analysis

Good Laboratory Practice (GLP) methods used for analyses of pretomanid in plasma in non-clinical GLP studies were based on protein precipitation or liquid-liquid extraction followed by liquid chromatographic separation and tandem mass spectrometric (LC-MS/MS). Metabolites in plasma, urine, faeces, hepatocyte incubations and liver microsomes from mouse, rat, monkey and human ADME studies were separated by HPLC, detected by LSC and identified and structurally elucidated by LC-MS/MS. The bioanalytical methods are considered adequate.-

Absorption

In rats the oral 0.4% sodium CMC formulation resulted in higher plasma exposure to pretomanid than the HPβCD formulation, probably due to higher uptake, and was therefore used in the rat toxicology studies. Even though the plasma exposure was higher following the tablet, capsule, and HPβCD formulations, a CMC formulation (0.5% CMC) was used also for the pivotal toxicological studies in monkey.

The rate of absorption of pretomanid to plasma was in general moderate to slow after a single oral dose in 0.4 to 0.5% CMC, with mean T_{max} values of 1 to 4 h in mice, 5.3 to 8.0 h in rats, 1.3 h in dogs, 3.7 to 6.7 h in rabbits and 2.3 to 6.0 h in monkeys, which is in line with the mean T_{max} of 4 to 5 h in humans. Mean oral bioavailability for pretomanid in CMC was low in monkeys (19%) and not determined in mice, rats (52% for pretomanid in HPβCD) and dogs. Absolute oral bioavailability was not determined in humans.

Exposure to pretomanid (AUC) following a single oral dose in CMC increased approximately in proportion with dose in mice (100 to 600 mg/kg), rabbits (10 to 500 mg/kg) and monkeys (25 to 100 mg/kg) and

less than dose proportional in rats (50 to 500 mg/kg). In rats the exposure to pretomanid was slightly higher in females than in males (approximately 2-fold) at the higher dose levels (250 and 500 mg/kg), which is reflected in the plasma elimination half-lives.

Fed conditions did not appear to have any impact on the bioavailability in monkeys. Oral bioavailability (AUC) following the tablet formulation was slightly higher (24%) in fasted monkeys as compared with monkeys fed with standard diet whereas FDA high fat/calorie breakfast had no consistent effect.

Elimination of pretomanid from plasma following oral administration in CMC was in general rather rapid with mean plasma elimination half-lives ranging from 2 to 10 h in female mice, 5.0 (male) to 24.3 (female) h rats, 3.0 to 4.9 h in female rabbits and 3.3 to 5.4 h in male monkey. In rat and monkey, the terminal elimination half-life in plasma was markedly longer for total ^{14}C-pretomanid-derived radioactivity (40 to 71 h in rats and 149 to 156 h in monkeys) than for pretomanid, indicating that metabolites with long plasma half-lives and potential for accumulation at repeated dosing are formed in both toxicological species.

Distribution

Tissue distribution in albino (Spraque Dawley) and pigmented (Long Evans) rats following oral administration of cold or [benzyl or imidazooxazine ^{14}C]-labelled pretomanid was evaluated by liquid scintillation counting or LC/MS/MS and quantitative whole-body autoradiography (QWBA).

The results were in general similar between pigmented and non-pigmented rats and showed that pretomanid-derived radioactivity was widely and rapidly distributed to all evaluated tissues at the first sampling 0.25 h post dose with a maximum of tissue/plasma ratios above 1 at 4 h which decreased to below 1 at the last sampling (96 h for pigmented and 48 h for unpigmented rats). Highest concentrations of radioactivity, excluding the GI tract, were observed in harderian gland, liver, adrenal gland, renal cortex, kidney, renal medulla, pancreas, exorbital lacrimal gland and spinal cord.

There were slight differences between the radioactivity elimination rates of the two different labels. For [imidazooxazine-^{14}C]-pretomanid, clearance of radioactivity from most tissues was complete by 96 hours postdose. In contrast, for [benzyl-CH_2-^{14}C]-pretomanid, clearance was incomplete from most tissues by 96 hours postdose, with a mean of 15.3% of the administered dose remaining in the carcasses at 96 hours postdose.

Tissue/plasma ratios above 1 in the brain at 0.25 to 12 hours post dose indicated passage through the blood-brain barrier. There were however no indications of selective or persistent distribution to melanin-containing tissues of pigmented male rats.

Protein binding in mouse, rat, dog, monkey and human plasma was moderate to high and similar between species (average protein binding ranged from 80.1% in rats to 87.1% in dogs; 86.4% in humans) and did not change over the concentration range tested (0.5 to 12 µg/mL). Mean blood/plasma concentration ratios determined for pretomanid at 2.5-10 µg/ml did not show any significant concentration-dependency (up to 0.83 in humans, 0.98 in monkeys, 1.47 in rats and 1.72 in mice) and indicated a low potential for partitioning into erythrocytes of humans and monkeys and a moderate potential in mice and rats.

In pregnant rats and rabbits, pretomanid was readily transferred through the placenta with similar or higher concentrations observed in rat foetal plasma as compared to maternal plasma (up to 1.8-fold at 24 h post dose), whereas the foetal plasma concentrations in rabbits were approximately 59% to 110 % of those observed in maternal plasma at 1 to 24 h post dose.

Metabolism

The metabolism of pretomanid was investigated *in vitro* in liver microsomes of mouse, monkey and humans, in hepatocytes of mouse, rat, rabbit, monkey and human and *in vivo* in rat, monkey and human.

In vitro

Pretomanid was moderately to relatively stable in liver microsomes and hepatocytes across species with a metabolic turnover from <10% to 51% in humans and from <10% in rats up to 63% in monkeys at 0.5 to 50 µM substrate concentration.

Twelve metabolites variably distributed among all species were detected in hepatocytes (**M36, M37**, M38, M39, M40, M41, **M42, M10**, M8, **M11, M43** and **M20**; the 7 bolded metabolites represented ≥ 1% of total radioactivity). In human hepatocytes 11 metabolites were detected (M8, **M10, M11**, M19, **M20**, M36, **M37**, M38, M40, **M42**, and **M43**; the 6 bolded metabolites represented ≥ 1% of total radioactivity), of which all were detected in laboratory animal hepatocytes.

In addition, metabolite, M50 was detected in liver microsomes of humans, mice and monkeys at 2-4%, 3-11% and 5%, respectively, of initial pretomanid concentration.

CYP3A4 seems to be involved in the metabolism of pretomanid *in vitro* with 80% of parent compound remaining after incubation with human rCYP3A4.

In vivo

Pretomanid was extensively metabolised. Unchanged drug and 21 pretomanid-derived metabolites were characterized in rat plasma, urine, and faeces of rats and monkeys following a single oral dose of 15 mg/kg [benzyl or imidazooxazine ^{14}C]-pretomanid (M1, M6, M7, M12, M13, M15, M18, M19, M20, M21, M23, M10, M11, M24A, M26, M27, M31, M48, M49, M50, M52). Metabolism occurred via oxidation at multiple sites, the reduction of the nitro group to an amine, oxidative deamination, and oxidative-cleavage to form hydroxy imidazole and 4-trifluoromethoxybenzoic acid. Subsequent conjugation of some of the primary metabolites with glucuronic acid, glycine (monkeys only), or cysteine was also observed. In rat plasma, principal metabolites included trifluoromethoxybenzoic acid (M19), trifluoromethoxyphenyl-methoxy-hydroxypyranpretomanid (M31), pyran ring-opened pretomanid-carboxylic acid (M49), ethyl-trifluoromethoxybenzoic acid (M21) and hydroxy-imidazole (M50). In monkey plasma, the principal metabolites included M19, M49, M31 and M50 observed in rat plasma, plus four additional metabolites (M24, M27, M48, including one conjugated metabolite (M11)).

In humans, 14 metabolites (M10, M13, M19, M20, M24, M26, M27, M28, M31, M48, M49, M50, M51 and M52) were detected in plasma following a a single oral dose [benzyl or imidazooxazine ^{14}C]-pretomanid (873 to 1100 mg). Highest levels were noted for M19, which represented 35% of the exposure to parent [benzyl ^{14}C]-pretomanid] based on $AUC_{0\text{-inf}}$, and M27 (hydroxy-dihydro-pyran ring opened-amino), M50 (hydroxy imidazole) and M52 (hydroxyl aminooxazine), which represented less than 10% of the exposure to parent [imidazooxazine ^{14}C]-pretomanid] based on $AUC_{0\text{-t}}$. Two of the human circulating metabolites, M28 and M51, were not detected in rat or monkey plasma but in rat and monkey urine and rat faeces (M28), i.e. all human circulating metabolites detected following a single oral dose of pretomanid were formed in the toxicological animal species.

The terminal elimination half-life in plasma of humans, rats and monkeys was markedly longer for total ^{14}C-pretomanide-derived radioactivity (approximately 18 days in humans, 40 to 71 h in rats and 149 to 156 h in monkeys) than for pretomanid (16 h in humans, 5.0 to 9.1 h in rats and 3.9 to 5.4 h in monkeys at comparable doses). The long plasma half-lives for total radioactivity were shown to be due to unextractable radioactivity and to represent at least 3 metabolites in humans, M44, M45 and M46, which were all indicated to be present in plasma of rats and monkeys. Based on the differences in half-

lives between species, accumulation of unextractable bound metabolites at repeated dosing are expected to be higher in humans than in rats or monkeys.

Excretion

Mass balance data were obtained from rats and monkeys. Overall, the results indicate that elimination pathways for pretomanid in rats, monkeys and humans are comparable. Urinary excretion of metabolites is the major clearance route for absorbed drug (in humans, rats and monkeys, approximately 55-65%, 45-56% and 30-32%, respectively, of the dose are excreted in urine) with metabolism and excretion into faeces playing a smaller role in elimination (in humans, rats and monkeys, approximately 25-40%, 26% and 26-28%, respectively, of the dose are excreted in faeces).

In lactating rats pretomanid was excreted in milk with greater (1.4-1.6-fold) concentrations of compound observed in milk at 6 hours after the maternal dose as compared to the 4-hour post-dose maternal plasma concentration. The plasma exposure in suckling pups was ⩽1/20 of maternal plasma, but the concentrations detected in the high dose group were still more than 10% of the C_{max} in patients administered 200 mg pretomanid/day (3.1 µg/ml in study NC-005)**.** This is adequately reflected in the SmPC.

Pharmacokinetic drug interactions

See the Clinical Pharmacokinetics Assessment.

2.3.4. Toxicology

Repeat dose toxicity

GLP-compliant repeat-dose toxicity studies of 14 days, 3 months, and 6 months duration were conducted in rats and studies of 2 weeks, 3 months, and 9 months duration were conducted in cynomolgus monkeys. In all studies, doses were administered daily by oral or nasogastric gavage corresponding to the proposed clinical oral dosing regimen. In the chronic studies, post-treatment assessments continued for 3 months to evaluate reversibility, progression, or delayed toxicity.

Rat

Oral administration of pretomanid to male and female rats for 14 days caused clinical signs of toxicity at ≥ 100 mg/kg/day, including ataxia, hyperactivity, and mortality in females at ≥ 500 mg/kg/day, and convulsions and mortality in both males and females at 1000 mg/kg/day. Body weight gains were lower in both sexes at ≥ 100 mg/kg/day and weight loss occurred at ≥ 500 mg/kg/day. Treatment-related microscopic findings included minimal to moderate degeneration/inflammation of cardiac myocytes in the heart (1000 mg/kg/day); lymphoid depletion in spleen, thymus, Peyer's patches, and bronchus-associated lymphoid tissue (≥ 500 mg/kg/day); bone marrow fatty infiltration (≥ 100 mg/kg/day) and cell depletion (1000 mg/kg/day); testicular, epididymal, and prostate degeneration and cell debris (≥ 500 mg/kg/day males); minimal to slight uterine atrophy (≥ 250 mg/kg/day females); and centrilobular hepatocellular hypertrophy in liver (≥ 100 mg/kg/day). Based on adverse clinical observations, reduced body weight gains, and bone marrow microscopic findings, a dose of 50 mg/kg/day was considered the NOAEL.

Oral administration of pretomanid to male and female rats for 3 months followed by a 3-month recovery period caused marked reversible decreases in food consumption and body weight gain at 100 ≥ mg/kg/day. Irreversible testicular atrophy was observed at ≥ 100 mg/kg/day, hepatocellular hypertrophy at ≥ 100 mg/kg/day, and reversible splenic and thymic lymphoid depletion at 300

mg/kg/day males. Irreversible cataracts were observed at 300 mg/kg/day. Delayed onset cataracts were observed in one high dose recovery male that did not have cataracts at the end of dosing. The NOAEL was 30 mg/kg/day based on adverse clinical observations, decreased body weight and testicular degeneration/atrophy observed at 100 mg/kg/day.

Oral administration of pretomanid to male and female rats for 26 weeks caused reduction in body weight gain and food consumption at 30, 100, or 300/200 mg/kg/day. Pretomanid caused irreversible, late onset, cataracts at 100 mg/kg/day and testicular atrophy/degeneration at doses of 30 mg/kg/day and higher. The observed testicular toxicity was associated with changes in endocrinological and semenological clinical biomarkers, however, testosterone levels were not affected. Renal tubular degeneration was observed in 100 and 300/200 mg/kg males and in all treated females. These findings reversed completely in males given 300/200 mg/kg/day and reduced in incidence but not severity in females at 300/200 mg/kg/day. The renal changes were not associated with any functional changes as indicated by renal clinical pathology parameters. Based on the severity and adversity of clinical and histologic findings in this study, the NOAEL was determined to be 10 mg/kg/day.

Cynomolgus monkey

In the 2-week oral toxicity study in the cynomolgus monkey the main findings were CNS-related clinical signs included ataxia and convulsions followed by lateral recumbency and ataxia, or front limb tremors at a dose of 1000 mg/kg/day and hypoactivity at ≥ 450 mg/kg/day. Decreased heart rate and prolonged QT interval was observed at ≥ 450 mg/kg/day. Reduced body weight and body weight loss were observed at ≥ 450 mg/kg/day and was accompanied by reduced food consumption at ≥ 150 mg/kg/day. Decreases in thymic weights correlated with minimal to moderate lymphoid depletion in the thymus at ≥ 450 mg/kg/day. The no-observable-effect level (NOEL) was 50 mg/kg/day based on the reduced food consumption and mild QT interval prolongation observed at 150 mg/kg/day. Drug-related changes completely resolved or were very nearly resolved in the 450 mg/kg/day monkeys after 16 days of recovery.

In the 3-month oral toxicity study with a 3-month recovery period in the cynomolgus monkey the high dose (450 mg/kg/day) was associated with ataxia and convulsions in two animals and marked body weight loss in most animals causing a reduction in dose. These monkeys exhibited inappetence, watery faeces, and decreased activity; histologically, they exhibited reversible hepatocellular hypertrophy and reversible mild to severe thymic lymphoid depletion. At the end of the recovery period, in the 450/300 mg/kg/day group, one male and one female had bilateral cataracts not observed during pre-treatment or during the dosing period. Recovery was not evaluated in the low- and mid-dose groups precluding observations on possible late onset cataracts at these doses. At 150 mg/kg/day, females also lost body weight and males had only a slight gain. Mild to moderate thymic lymphoid depletion was observed in males and females of this group. No significant adverse findings were observed for males or females at 50 mg/kg/day; hence, this dose was concluded to be the NOAEL.

Oral administration of pretomanid to cynomolgus monkeys for 9 months at doses of 0, 25, 50, and 100 mg/kg/day was generally well tolerated. Loss of appetite and corresponding weight loss was observed during the first 3 months of the study, though animals gradually acclimated to the drug and treatment procedures with corresponding improvements in food consumption and body weight. The only gross pathologic finding at necropsy related to pretomanid was thickening of the wall of the stomach and/or small intestine in most monkeys at all dose levels. In the small intestine, thickening was segmental. There were no histopathologic findings to account for the macroscopic thickening, and no histopathologic findings in any tissue that were considered to be related to pretomanid. Thickening showed evidence of resolving during the recovery period, as it was present only in the stomach of one high-dose male at the end of the recovery period. In the final report, the Study Director concluded that

"Gastrointestinal lesions were seen at the lowest dose level; therefore, a no observed adverse effect level (NOAEL) was not determined for this study but is considered to be less than 25 mg/kg/day."

The applicant does not consider the thickened appearance of the stomach and/or small intestine to be an adverse effect without correlating data. The macroscopic thickening was not associated with any in-life or post-mortem evidence of an effect on gastrointestinal tract function or general health. The applicant does consider the lower weight gain in females at ≥ 50 mg/kg/day to be adverse, and therefore would identify 25 mg/kg/day as the NOAEL in females. The applicant also believes that the weight loss in 2 males at 100 mg/kg/day, which resulted in a decreased weight gain in that group, is adverse; however, it is noted that 3 of the 6 males (50%) in this group had weight gains comparable to those in control males. Therefore, the applicant identified 50 mg/kg/day as the NOAEL in males. The applicant's position is agreed and the NOAEL in this study is considered to be 25 mg/kg/day in females and 50 mg/kg/day in males.

Testicular Toxicity

Testicular toxicity was observed in male mice and rats in all repeat-dose studies but was not observed in male monkeys in any repeat-dose study.

Testicular toxicity in rodents was dose- and time dependent. In mice, the NOAELs for testicular effects produced exposures (C_{max} and AUC_{0-24}) anticipated to be lower than maximum recommended human dose (200 mg/day). In male rats, testicular toxicity showed evidence of being partially reversible with shorter duration of administration or at dose levels near the threshold. At higher dose levels, testicular toxicity was irreversible, associated with lack of fertility, and accompanied by reduced serum inhibin-B concentration and increased serum follicle-stimulating hormone (FSH) concentration. In a 13-week study, exposures at the NOAEL were similar to or slightly higher than anticipated human exposures at the proposed dose. In a 26-week study, exposures at the NOAEL were lower than predicted human exposures.

In contrast to rodents, testicular toxicity was not observed in male cynomolgus monkeys, even with prolonged administration at plasma exposures that greatly exceeded the threshold for testicular toxicity in male rats. No testicular findings were identified in male monkeys given high daily oral doses of pretomanid for 9 months. Small decreases in sperm count and motility together with an increased ratio of abnormal to normal sperm were noted at high doses in a 13-week monkey study and the applicant considered this secondary to poor physical condition. However, this is not agreed. It cannot be ruled out that the observed effects on sperm motility, sperm count and sperm morphology is a direct effect of pretomanid exposure, especially when taking into account the consistent testicular toxicity observed in the rat and mice repeat-dose toxicity studies. At the NOAEL of 50 mg/kg/day the C_{max} and AUC_{0-24} on Day 87/88 was 5.814 µg/mL and 70.395 µg*h/mL, respectively resulting in exposure margins of 1.9 and 1.2, respectively, to the maximum human exposure.

Cataracts

In rats, cataracts developed in about 20% of the animals administered a high dose of pretomanid for 13 weeks or a lower (but still high) dose for 26 weeks. In monkeys, cataracts developed in 2 of 4 animals given a high dose of pretomanid for 16 weeks. In that study, the cataracts were not present in 8 monkeys necropsied at the end of dosing but arose during a 13-week recovery period.

By contrast, in a second 13-week study in which monkeys were given high daily oral doses of pretomanid for 13 weeks and then maintained for an additional 20-week recovery period, frequent eye exams were conducted, but no cataracts developed. Cataracts also did not develop in monkeys given high daily oral doses of pretomanid for 39 weeks.

Because of cataract findings in non-clinical studies the applicant has included slit-lamp examinations with careful age-related eye disease study 2 (AREDS2) scoring of lens opacities in clinical studies. These studies have shown no association of concern between pretomanid exposure and cataracts in humans. The safety of pretomanid with respect to cataract development was also evaluated by searching for TEAEs in the SMQ for lens disorders. It was concluded that the extensive evaluation of lens opacities in the clinical development program did not raise any concern in humans that pretomanid may cause cataracts in the proposed regimen.

CNS Effects

Central nervous system (CNS)-related clinical signs, including convulsions, were observed in mice, rats, and monkeys following administration of pretomanid at higher dose levels. These were not associated with gross pathologic or histopathologic findings in the brain in any study.

In mice given daily oral doses of pretomanid for 4 weeks, CNS-related clinical signs (spasm, jump-and-tumble, and abnormal spontaneous motor activity) were observed at high doses that exceeded the maximum tolerated dose (MTD). In male rats, high single doses of pretomanid caused depressed neural function (decreased touch response, body tone and grooming). In repeat dose studies in rats, high doses were associated with clinical signs of hyperactivity, sensitivity, ataxia, and convulsions. In monkeys, CNS-related clinical signs included ataxia and convulsions followed by lateral recumbency and ataxia, or front limb tremors at a high dose of pretomanid given for two weeks. In longer repeat dose studies in which monkeys were given lower, but still high doses of pretomanid, ataxia and convulsions were rare but observed in 2 studies.

The incidence of treatment-emergent adverse events (TEAEs) in the standardized MedDRA query (SMQ) for convulsions was low across the clinical development program, and it was concluded that the available clinical data does not raise concern that pretomanid may cause convulsion in the proposed regimen.

Liver Effects

Hepatocellular hypertrophy was seen in mice, rats, and monkeys given daily oral doses of pretomanid at higher dose levels and was considered an adaptive response associated with increased metabolism. There were a few instances of minimally increased serum transaminase activity in rats and one instance of single-cell hepatocellular necrosis in a mouse; however, these occurred at dose levels that exceeded the maximum tolerated dose and were not the major cause of animal morbidity.

Genotoxicity

The genotoxicity of pretomanid has been studied with respect to gene mutations in bacteria and mammalian cells and chromosomal aberrations *in vitro* using rat S9 mix and *in vivo* in the mouse with negative results. It is concluded that pretomanid itself is not genotoxic.

However, one metabolite (M50 = hydroxy imidazole) was further studied and was found positive in the bacterial reverse mutation assay both with and without metabolic activation (rat S9) despite negative results when testing pretomanid itself. The Applicant states that these tests were conducted since M50 was not quantified in the *in vitro* genotoxicity studies using pretomanid and therefore it was unclear if the potential genotoxic hazard posed by the metabolite had been evaluated. The applicant suggests that it is likely that M50 is formed by rat S9 based on the in vivo detection at 24% of the AUC. M50 was formed when mouse liver microsomes and mouse hepatocytes were incubated with pretomanid. It is however unclear to what extent M50 (or any other metabolite) is formed by rat microsomes, data is lacking, and the use of rat S9 instead of mouse microsomes in the in vitro studies could be questioned.

However, in humans M50 is considered as a minor metabolite and there is no requirement to study it separately unless accumulation in humans is demonstrated. In any case, the mutagenicity potential of M50 has been adequately tested *in vitro* and *in vivo*. The M50 metabolite was confirmed to be present in plasma of mice exposed to pretomanid (in the 28-day repeat-dose toxicity study and in the carcinogenicity study in transgenic mice), indicating that also the clastogenic potential of M50 was sufficiently studied *in vivo* in the mouse bone marrow micronucleus studies performed with pretomanid. Overall, the M50 metabolite is considered to be mutagenic *in vitro* and the possible risk for carcinogenicity cannot be excluded and need to be predicted from in vivo carcinogenicity studies.

Carcinogenicity

A 26-week oral study was conducted to evaluate the carcinogenic potential of pretomanid in Tg.rasH2 transgenic mice. This study also evaluated the toxicokinetic profile of pretomanid and M50 in wild-type Tg.rasH2 litter mates following 2 weeks administration. There were no pretomanid-induced early deaths, tumors, non-neoplastic microscopic findings, or gross necropsy findings when male and female TG.rasH2 mice were administered pretomanid once daily by oral gavage for 26 weeks at doses of 5, 15 and 40 mg/kg/day in males and 10, 30 and 80 mg/kg/day in females. Oral administration of pretomanid produced plasma exposure to both pretomanid and its metabolite M50 at all doses, with exposure to pretomanid more than 10 times the exposure to the M50 metabolite at all doses in both sexes. The exposure to pretomanid at the high dose was in the range 2 to 5 times the maximum human exposure based on C_{max} and AUC_{0-24}.

Chronic toxicity studies conducted with pretomanid in rats and monkeys have not raised any cause for concern about carcinogenic hazard. There was no increase in the incidence of pre-neoplastic or neoplastic findings in rats given daily oral doses of pretomanid for 26 weeks at up to the maximum tolerated dose level of 100 mg/kg or monkeys given daily oral doses of pretomanid for 39 weeks at up to the maximum tolerated dose level of 100 mg/kg.

Reproduction Toxicity

Administration of pretomanid to male rats for 13 weeks reduced fertility at 30 mg/kg/day and produced complete infertility at 100 mg/kg/day. Infertility was associated with testicular atrophy, lower sperm counts, reduced sperm motility, lower serum inhibin B concentration, and higher serum FSH concentration. After a 10 week recovery period, effects on fertility at 30 mg/kg/day showed partial improvement but effects at 100 mg/kg/day were unchanged. The NOAEL for male fertility is considered to be 10 mg/kg/day. Toxicokinetic data were not generated in this study but C_{max} and AUC_{0-24} values in male rats at 10 mg/kg/day were approximately 2.3 µg/mL and 27.5 µg*h/mL, respectively, in the 26-week repeat-dose toxicity study (Covance 7504-170), indicating no exposure margin to the clinical exposure. Based on these findings together with testicular toxicity findings in repeat-dose studies in the rat and the mouse and effects on sperm in a limited number of cynomolgus monkeys adverse effects on human male fertility cannot be ruled out.

Pretomanid did not directly affect reproductive function in female rats given daily oral doses for two weeks at up to 100 mg/kg/day.

In the rat EFD study the maternal NOAEL for pretomanid was 10 mg/kg/day, based on reduced maternal body weight and/or body weight gain and feed consumption during the entire dosage period at ≥ 30 mg/kg/day. At 100 mg/kg/day, dams also showed clinical signs of ill health. Pretomanid was not teratogenic and did not affect fetal survival, growth, or development at ≥ 30 mg/kg/day. At 100 mg/kg/day, postimplantation loss was increased, litters were smaller, the number of live fetuses and

fetal body weight were lower, and skeletal development was slowed (reduction in ossified hindlimb phalanges). The latter was considered secondary to a general reduction in fetal growth rate.

The NOAEL for effects on fetal development was 30 mg/kg/day, which produced average maternal C_{max} and AUC_{0-24h} values of 6.28 µg/mL and 104.2 h*µg/mL, respectively, on DG 18 (day 12 of dosing), indicating an exposure margin of 2.0 and 1.8, respectively.

In the rabbit EFD the maternal NOAEL for pretomanid was 10 mg/kg/day, based on reduced maternal body weight and/or body weight gain and feed consumption during the entire dosage period at ≥ 30 mg/kg/day. At ≥ 60 mg/kg/day, dams also had reduced fecal output. Pretomanid did not affect fetal survival, growth, or development at doses up to 60 mg/kg/day. In the DRF study fetal body weights were reduced at 100 mg/kg/day. Based on these results, pretomanid was not teratogenic and did not affect embryofetal development in rabbits.

The NOAEL for effects on fetal development was 60 mg/kg/day, which produced average maternal C_{max} and AUC_{0-24} values of 8.24 µg/mL and 87.8 h*µg/mL, respectively, on gestation day 19, corresponding to an exposure margin of 2.7 and 1.5, respectively. Mean fetal plasma concentrations were approximately 59% to 110% of those observed in maternal plasma, demonstrating the pretomanid crossed the placenta.

In the PPND study in the rat the NOAEL for toxicity in the F0 dams 20 mg/kg/day, based on reduced maternal body weight and/or body weight gain and feed consumption during late gestation and lactation at 60 mg/kg/day. The NOEL for effects on F1 pups also was 20 mg/kg/day, based on lower pup weights and a slight delay in the age at which the air-drop righting reflex developed in pups born to and nursed by dams given pretomanid at 60 mg/kg/day. These effects were probably secondary to effects on dams, rather than direct effects of pretomanid itself. Pretomanid did not affect pup survival or post-weaning growth or otherwise clearly affect pup development at any dose level. There were no effects on the development of the neuromuscular or reproductive systems. Administration of pretomanid to nursing dams resulted in the presence of pretomanid in milk at all dose levels. Although pretomanid concentration was higher in milk than plasma at each dose level, it was much lower in plasma of pups than plasma of dams at each dose level.

The findings in the reproductive toxicity studies are adequately concluded in the SPC section 4.6 and 5.3.

Other toxicity studies

No melanin binding was observed in pigmented rats. A positive signal for phototoxicity was observed in an *in vitro* 3T3 NRU-PT assay and a subsequent *in vivo* assay was performed in pigmented rats. Oral administration of pretomanid at a dose of 100 mg/kg/day elicited cutaneous reactions indicative of phototoxicity (erythema, grade 1 barely perceptible light redness).

2.3.5. Ecotoxicity/environmental risk assessment

An Environmental Risk Assessment concerning pretomanid conducted according to EMEA/CHMP/SWP/4447/00 corr 2 was provided.

The LogD was measured experimentally at various pH values using the shake flask method. The LogD ranged from 2.3 to 2.4 at pH 3.0-11.0. The study was not performed in accordance with GLP, however it is considered sufficient for the purpose of determining if further PBTscreening is needed. It is concluded that pretomanid is not bioaccumulative.

The Applicant provided crude prevalence data in the EU in 2017 as published by the WHO. The crude prevalence of TB within Europe was 1.11 per 10.000. The proportion of patients with MDR-TB was 3.3% giving a prevalence of MDR-TB of 0.037 per 10.000. This corresponds to an Fpen of 0.0000037. Based on this the PECsurfacewater for pretomanid was calculated to 0.00018 µg/l, thus a Phase II assessment is not required.

It is noted that the sentence "Any unused medicinal product or waste material should be disposed of in accordance with local requirements" is present in the SmPC section 6.6 as required.

Table 1. Environmental risk assessment

Substance (INN/Invented Name): Pretomanid			
CAS-number (if available): 187235-37-6			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107 or …	2.38	Potential PBT (N)
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log K_{ow}	2.38	not B
	BCF	--	B/not B
Persistence	DT50 or ready biodegradability	--	P/not P
Toxicity	NOEC or CMR	--	T/not T
PBT-statement :	The compound is not considered as PBT nor vPvB		
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$, default or refined (e.g. prevalence, literature)	0.0005	µg/L	> 0.01 threshold (N)
Other concerns (e.g. chemical class)	None		(N)
Phase II Physical-chemical properties and fate			
Study type	**Test protocol**	**Results**	**Remarks**
Adsorption-Desorption	OECD 106 or …	K_{oc} =	List all values
Ready Biodegradability Test	OECD 301		
Aerobic and Anaerobic Transformation in Aquatic Sediment systems	OECD 308	$DT_{50, water}$ = $DT_{50, sediment}$ = $DT_{50, whole\ system}$ = % shifting to sediment =	Not required if readily biodegradable

Phase IIa Effect studies					
Study type	**Test protocol**	**Endpoint**	**valu e**	**Unit**	**Remarks**
Algae, Growth Inhibition Test/*Species*	OECD 201	NOEC		µg/L	species
Daphnia sp. Reproduction Test	OECD 211	NOEC		µg/L	
Fish, Early Life Stage Toxicity Test/*Species*	OECD 210	NOEC		µg/L	species
Activated Sludge, Respiration Inhibition Test	OECD 209	EC		µg/L	
Phase IIb Studies					
Bioaccumulation	OECD 305	BCF		L/kg	%lipids:
Aerobic and anaerobic transformation in soil	OECD 307	DT50 $\%CO_2$			for all 4 soils
Soil Microorganisms: Nitrogen Transformation Test	OECD 216	%effect		mg/ kg	
Terrestrial Plants, Growth Test/*Species*	OECD 208	NOEC		mg/ kg	
Earthworm, Acute Toxicity Tests	OECD 207	NOEC		mg/ kg	
Collembola, Reproduction Test	ISO 11267	NOEC		mg/ kg	
Sediment dwelling organism		NOEC		mg/ kg	species

2.3.6. Discussion on non-clinical aspects

Pharmacology

The non-clinical pharmacology program, which has in general been performed in accordance with current guidelines and the data package appears to support the rationale to use pretomanid in the intended disease indication. The selection of the rat as the primary rodent species and the monkey as the primary non-rodent species in the pivotal toxicity studies is acceptable, considering the low systemic exposure of pretomanid in dogs.

Secondary pharmacology

The potential off-target effects of pretomanid on common targets, such as different receptors, enzymes and ion channels, was not provided initially. In response to this issue the applicant performed a receptor off-target screen against common pharmacological targets in vitro. Pretomanid (10 µM = 3.6 µg/mL; Clinical C_{max}= 3.1 µg/mL at 200 mg dose) showed no significant interaction, defined as inhibition or stimulation >50%, for any of the 86 human targets tested.

Safety pharmacology

Non-clinical safety concerns identified for pretomanid include CNS-related clinical signs, including convulsions, as observed in mice, rats, and monkeys following administration of pretomanid after

single and repeat oral dosing. The exposure margins for CNS effects in animals were low (~ 2-3-fold above clinical C_{max}). Albeit there only has been described a few cases of convulsions in the clinical trials, a potential relation to pretomanid could not be excluded. The applicant was initially asked to discuss the potential mechanism for pretomanid-induced convulsions in animals, and how this mechanism is translated into the clinical setting and if the pretomanid-induced convulsions observed in the non-clinical studies is of clinical relevance. In the response, the applicant did not propose any mechanism for the convulsions. Since the mechanism is still not determined, it is unclear if the four cases of seizures observed in clinical trials are related to pretomanid or not. However, the clinical seizure cases all had alternative aetiologies (prior seizure history, CNS tuberculoma, moxifloxacin, heavy alcohol use) that may explain the seizure activity. In sum, even though the mechanism for non-clinical convulsion is still unknown, the clinical relevance of this non-clinical finding seems low. Due to the lack of mechanistic knowledge, the findings are described in section 5.3 of the product information.

A potential for pretomanid to cause QT prolongation is indicated by the in vitro hERG studies, showing a hERG signal (ratio of hERG IC_{50} to clinical free C_{max} ratio < 3x), and the evidence of QT prolongation as observed at all dose levels in monkeys given single oral doses of pretomanid (50, 150, 450 mg/kg; C_{max} = 5.8 µg/mL at 50 mg/kg). In order to further investigate the torsadogenic potential of pretomanid, the applicant was asked to considered performing further in-vitro electrophysiological studies with special emphasis on the effects of pretomanid on other cardiac ion channels. in response to this question, the applicant performed novel in vitro electrophysiology studies (patch clamp) to characterise the potential interaction of pretomanid with cardiac ion channels including hNav1.5, hKCNQ1/mink, and hCav1.2. The provided data indicate that pretomanid is a mixed ion channel blocker. Whereas pretomanid did not inhibit hNav1.5 late and hCav1.2 ion channels (IC50-values > 30 µM), pretomanid showed inhibition of hNav1.5 peak and hKCNQ1/minK ion channels with IC50-values of 28.6 µM and 23.24 µM, respectively, which is approximately 19-23 fold higher compared to clinically free plasma concentration of pretomanid. The rather unspecific ion channel blocking effect of pretomanid on e.g. Nav1.5, KCNQ1 and hERG is in line with the effects on QTc prolongation observed in human studies (mean ΔΔQTcN estimated as 4.9 ms at Cmax of 200 mg daily dose) as well as the QT interval prolongation induced by pretomanid in monkeys. It is agreed that further non-clinical studies are not warranted since such animal data is not likely to contribute to the overall human benefit/risk assessment of cardiac safety. In addition, non-clinical safety results are superseded by available data from clinical ECG studies.

Pharmacokinetics

The non-clinical pharmacokinetic profile of pretomanid is in general considered to have been adequately characterized. Rats and monkeys were chosen as target species for the toxicology studies. These species showed extensive metabolism and it has been shown that all human circulating extractable metabolites detected following a single oral dose of pretomanid were formed in the relevant toxicological animal species and that they are adequately qualified in rats and monkeys following repeated administration.

The $AUC_{0\text{-}24h}$ of un-extractable radioactivity in plasma of humans following a single dose was however estimated to represent more than 10% of the $AUC_{0\text{-}24h}$ of total radioactivity which, due to the long half-life of 18 days, is expected to increase at steady state. Thus, qualification of the un-extractable metabolites in non-clinical species is warranted. As analyses of the bound radioactivity could only identify 3 of several peaks in the chromatograms, the identities and relative amounts of each metabolite bound to human plasma proteins are unknown, as well as the half-lives of all individual plasma bound metabolites. It was therefore considered adequate to use the AUC and half-life for the un-extractable radioactivity for evaluation of non-clinical qualification of the metabolites bound to plasma proteins. i.e. whether the AUC of un-extractable radioactivity during a dosing interval at steady state in a toxicological species represent at least 50% of the exposure in humans.

The human exposure to pretomanid metabolites at steady state following the proposed dosing regimen of 200 mg pretomanid once daily (AUCtau,ss) was predicted by using the accumulation ratios determined for pretomanid and estimated for total radioactivity based on the $t_{1/2}$, respectively (see Section Pharmacokinetics, *Metabolism Plasma*). Corresponding steady state exposures were calculated for rats and monkeys in the repeated toxicity studies by using an accumulation ratio of 1 for pretomanid (no significant accumulation was observed for pretomanid) and of 2.9 in rats and 9.1 in monkeys for total radioactivity (based on the shortest half-life of 40 and 149 h, respectively, obtained for total radioactivity). From 100 mg/kg in rats and 25 mg/kg in monkeys, the predicted AUCtau,ss values for un-extractable radioactivity (1005 and 747 µgxh/mL, respectively) represented at least 50% of the exposure in humans (when using the corrected human AUCtau,ss). Metabolites bound to plasma proteins are thus considered sufficiently qualified in relevant toxicological species and studies (including studies of general toxicity, reproductive toxicity and carcinogenicity [ongoing study in rats will be provided when available]).

Toxicology

Pretomanid shows clear signs of toxicological effects on male rodent reproductive organs without any safety margin. The findings were only partially reversible. In rodents, the usual pattern of hormone alteration after a toxicant exposure that targets the seminiferous epithelium and produces germ cell loss leading to testicular atrophy is a progressive increase in FSH and LH, a decrease in InhB, and little or no change in testosterone. The data in the preclinical studies are consistent with this pattern of serum levels of male reproductive hormones. Generally, serum levels of male reproductive hormones are biomarkers of testicular injury that are altered as a compensatory response after the onset of the injury; therefore, the Applicant do not expect them to be changed early during treatment and to precede testicular injury. This is agreed. No clear mechanism behind the pretomanid-induced rodent testicular toxicity was provided, although oxidative stress was hypothetised. Testicular findings (i.e. sperm abnormalities) were also found in mature monkeys, but at a significantly lower degree than in rodents. The Applicant considers the findings of decreased sperm motility and total sperm count, and increased abnormal sperm ratio in the cynomolgus to be secondary to decreased food consumption and significant weight loss rather than direct effects of pretomanid on testes because the majority of these changes disappeared after recovery of physical condition, and there were no histopathological correlates in the male genital organs. Direct effects of pretomanid is not considered to be clearly ruled out. Sufficient clinical information regarding the effect of pretomanid on human male reproduction organs is not available. Due to the observed testicular toxicity, male reproductive hormones were studied in clinical Studies NC 002, NC-005, and NC 006, which showed no evidence of treatment-associated testicular toxicity. It was concluded that the available clinical data does not raise concern that pretomanid may cause testicular toxicity in the proposed regimen. Applicant is planning a Male Reproductive Safety Study that will evaluate sperm parameters. This is supported.

Lens fibre swelling was seen in several studies in rats and monkeys with and without cataracts. In some of the study reports the swelling of lens fibres were interpreted as artefacts caused by the fixation fluid (Davidson's fluid), since the fixative, according to the applicant, is known to sometimes cause cell swelling during tissue preparation. However, in the 3-month monkey study, the lens fibre changes correlated with clinical observations of cataract and were not typical of artefacts caused by Davidson's fixative. Similar, in the 26-week rat study, the lens fibre swelling generally correlated with the in-life ophthalmic examination identification of cataracts. In some pathologic conditions, such as diabetes, osmotic stress may develop due to accumulation of certain molecules in the eye. This may again lead to cataract formation and extensive swelling of cortical lens fibres (Kelkar et al. 2018; Pollreisz et al. 2010). There is a clear species difference in the cataract induction with the rat being

more susceptible than the monkey and the mouse (where no cataracts were observed). It also appears to be individual differences in susceptibility as shown in the difficulty to study the onset and recovery of cataracts in the performed repeat-dose studies. In a CHMP scientific advice it was recommend that the Applicant should further study the mechanism for cataract development in the rat. Such study has not been performed. The applicant is of the opinion that lens swelling is not related to cataract development, since only four out of nine animals in the 26-week rat study had microscopic lens fiber swelling, and two females with lens swelling did not have cataracts. It is not agreed that these data show a lack of relation between cataract and lens swelling. The results are considered as equivocal. According to the applicant, few of the over 1100 subjects in clinical studies administered pretomanid for over 6 months, had findings related to cataract formation and lens abnormalities. In conclusion, even though the mechanisms for cataract development in rats and monkeys is still unclear, considerable clinical experience suggest that the risk of cataract development in patients following treatment with pretomanid is low.

Signs of immunotoxicity were seen in most of the repeat-dose toxicity studies and in all three species, such as lymphoid depletion in the spleen, thymus, Peyer's patches and bronchial-associated lymphoid tissue in the lungs, and alterations in lymphoid organ weights. The applicant refers to publications showing that stress can have an impact on the immune system at dose levels producing overt toxicity. With pretomanid, adverse immunological changes were only observed in animals at toxic dose levels. Thus, the applicant is of the opinion that the immunological changes were caused by stress associated with administration of high doses of pretomanid. This assumption seems plausible. However, stress induced immunological responses do not exclude that other mechanisms also may cause such adverse events.

The applicant states that a 2-year oral carcinogenicity study in the rat is ongoing. A genotoxic concern based on the positive mutagenicity results of the M50 metabolite cannot at this point be excluded. However, based on the absence of pre-neoplastic or neoplastic findings in the chronic toxicity studies in the rat, or in the 26-week carcinogenicity study in transgenic mice combined with the severity of the indication (extensively drug resistant or treatment-intolerant/nonresponsive multidrug-resistant tuberculosis) and the observed positive therapeutic effect it is accepted that this study is submitted as a post marketing commitment. The applicant has committed to submit this study once available.

Oral administration of pretomanid at a dose of 100 mg/kg/day elicited cutaneous reactions indicative of phototoxicity (erythema, grade 1 barely perceptible light redness). The no observed adverse effect level (NOAEL) for phototoxicity in this study was 30 mg/kg/day indicating an exposure margin of 1.5- to 3-fold. The findings of cutaneous erythema was of low incidence, low severity, rapidly reversible and observed only at doses that exceed human doses and anticipated human exposures. The event of erythema was searched throughout the entire clinical safety datasets of the pretomanid clinical studies. Erythema occurred only in four subjects in phase 1 studies and out of 1153 subjects exposed to pretomanid. In the Nix-TB study, where pretomanid was used for as long as 6-9 months, there was no single event of erythema, no drug discontinuation due to skin event, nor any of the severe forms of skin reactions (e.g. Stevens Johnson Syndrome) were reported. It is concluded that the clinical relevance is minimal. There were no AEs in the clinical safety data set that were considered to represent allergic and/or indicative of immunotoxicity.

2.3.7. Conclusion on the non-clinical aspects

Pretomanid FGK could be approvable from a non-clinical point of view.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Table 2 Overview of studies included in the clinical pharmacology package of pretomanid

Description	Phase	Subject	N	Dose (mg)	Reference
SAD	1	HV	53	50, 250, 500, 750, 1000, 1250, 1500	CL-001, 2005
MAD	1	HV	24	200, 600, (1000)	CL-002, 2005
Food effect	1	HV	16	1000	CL-003, 2007
Food effect	1	HV	32	50, 200	CL-009, 2010
ADME	1	HV	6	873 mg [benzyl-14C]-pretomanid	CL-004, 2008
ADME	1	HV	6	1100 mg[imidazooxazinele-14C]-pretomanid	CL-008, 2009
DDI efavirenz lopinavir/ritonavir rifampicin	1	HV	52	200 mg od	A5306, 2017
DDI - midazolam	1	HV	14	400	CL-006, 2011

HV= healthy volunteer; pat=patient

Table 3 Overview of modelling and simulation reports with pretomanid

Description	**Reference**
PopPK	Population Pharmacokinetics of pretomanid, 2018
Conc-QTc modelling	Concentration-QTc modeling of pretomanid, 2018
Pkpd	Pharmacokinetics/Efficacy modeling of pretomanid, 2018

2.4.2. Pharmacokinetics

Bioanalysis

Plasma concentrations of pretomanid were initially determined by a HPLC-MS/MS following liquid-liquid extraction and using triazolam as internal standard. A sample volume of 50 µL K_2EDTA plasma was used. The calibration range was 10-10000 ng/ml. The assay was validated and cross-validated in heparin plasma. Further partial validation was performed following transfer of the assay.

The initial assay was further developed and validated by using an UPLC/with MS/MS detection following liquid-liquid extraction. The calibration range was 10-10000 ng/ml and deuterium-labelled-pretomanid was used as internal standard.

An assay for simultaneous determination of pretomanid, pyrazinamide and moxifloxacin in plasma by using LC-MS/MS detection was developed and validated. Plasma samples with heparin as anticoagulant was used and sample preparation by protein precipitation. d5-pretomanid, ^{15}N,d3-pyrazinamide and d4-moxifloxacin was used as internal standard for pretomanid, pyrazinamide and moxifloxacin, respectively.

During analysis of study samples from the phase-2 study NC-005, determining plasma levels of pretomanid, pyrazinamide and moxifloxacin simultaneously by a validated UPLC-MS/MS method, ISR (incurred sample reanalysis) criteria failed for all three analytes. The assay working procedures was changed/redeveloped. The method was then re-validated.

An analytical assay for simultaneous determination pretomanid, bedaquiline/N-monodesmethyl bedaquiline and linezolid in heparin plasma by LC-MS/MS was validated. The analytes and the internal standards were isolated from the biological matrix by protein precipitation. d5-pretomanid, d6-bedaquiline and d3-linezolid were used as internal standards.

A bioanalytical method for determination of pretomanid in the urine was developed and validated. Urine concentration was determined by HPLC with MS/MS detection using a sample volume of 100 µL and triazolam as internal standard.

Absorption

The absorption was rather slow with a t_{max} of *ca* 5h following single oral doses of pretomanid in fasted condition.

Total exposure and C_{max} increased in a linear manner with dose but less than dose proportionally up to 1000 mg and then levelled off.

Steady state was reached after *ca* 5-6 days with a $R_{AC}\approx 2$ following repeated dosing once daily. A less than dose-proportional increase in plasma levels was seen.

Co-administration with food resulted in about a 1.9- and 1.5-fold higher exposure of pretomanid after 200 mg and 50 mg, respectively, compared to when administered in fasted state. The increase in exposure was *ca* 4-fold following co-administered pretomanid 1000 mg with food compared to fasted state. The clinically recommended dose for pretomanid is 200 mg od.

Distribution

The f_u (unbound fraction) was determined to 13.6% and independent of plasma concentration in the studied concentration range 0.5-12 µg/ml (1.4-33 µM).

The of f_u of [14C]pretomanid in HAS (human serum albumin) was calculated to 17.3% indicating that binding to albumin is responsible for the plasma protein binding of pretomanid.

The blood-to-plasma ratio of pretomanid in human blood was determined to about 0.75.

There is no knowledge about pretomanid distribution to tissues of therapeutic interest (lung, epithelial lining fluid, sputum).

Elimination

The $t_{1/2}$ ranged between 15-20h.

The total recovery of radioactivity was 90 and 91% following an oral dose of [benzyl-14C]-pretomanid 873 mg and [imidazooxazinele-14C]-pretomanid 1100 mg, respectively. *Ca* 70-75% of the dose was excreted within the first four days. The $t_{1/2}$ of plasma radioactivity was calculated to 17 days.

About 55-65% of the radioactive dose was excreted in the urine and about 25-40% in faeces.

Metabolism

In vitro
The metabolic turnover of pretomanid was low in human liver microsomes (HLM) and hepatocytes. Four radioactive components were characterized, M10, M20, M50 and M13, following 2-h incubations in HLM. Eleven metabolites were detected after 4-h incubations in hepatocyte. Six metabolites (M37, M42, M10, M11, M20 and M43) each represented ≥1% of total radioactivity in the incubates. M11 was the major metabolite produced.

Pretomanid was metabolically stable with almost no detectable loss of parent compound in incubations with recombinant CYP 2C9, 2C19 and 2D6. CYP3A4 seemed to be involved, with 80% of parent compound remaining after a 60-min incubation.

The reactions involved in the elimination pathways of pretomanid and its metabolites are nitro-reduction, oxidations; including oxidative dealkylation and oxidative deamination, and conjugations; including glycine, glucuronide, and glutathione.

There is no evidence of chiral interconversion based on *in vitro* data from human hepatocytes and preclinical data.

Bacterial contribution to pretomanid metabolism

Nitro-reduction within gastrointestinal microflora and MTB are involved in the metabolism of pretomanid.

- Pretomanid enters MTB and is there converted to desnitro pretomanid. Bioreduction of pretomanid's aromatic nitro group is part of the activation of pretomanid within the target MTB.
- Six other metabolic products has been detected within the bacteria, named 2, 3, and M1 – M4. It is not clear whether these are released from the MTB; indeed, M3 is putatively a precursor to desnitro pretomanid as well as M1 and M4.
- Because of their well-known capacity for such nitro-reduction, gut microflora may thereby be implicated in pretomanid metabolism. It is likely that pretomanid enters gut bacteria where it is converted into one or more nitro-reduced species that are then released from the bacteria. However, more detailed characterization is not available.
- It is unknown whether any pretomanid molecules that enter bacteria are released unchanged from the bacteria.
- Although intestinal flora are likely involved in pre-systemic metabolism of pretomanid, quantitative effects on systemic exposure have not been definitively characterized.
    - Nitro-reduction products of pretomanid were detected in feces in human mass balance studies.
    - The detection in the urine of M29, the initial nitro-reduction product, as well as some of its downstream products, suggests that metabolites of gut bacteria may be absorbed systemically, although there is also evidence of hepatic nitro-reduction.

Urine and faces
The metabolic profiling following a single oral dose of either [benzyl-14C]-pretomanid or

[imidazooxazine-14C]-pretomanid) has identified more than 20 minor metabolites in urine and faces excreta. *Ca* 60% of the dose was characterized after [benzyl-14C]-pretomanid and 40% after [imidazooxazine-14C]-pretomanid).

Pretomanid was extensively metabolized as only a very low percentage of the dose was excreted as unchanged compound, <0.5% in the urine and <2% in faeces.

Several radioactive peaks were detected in urine and faeces. Following administration of [benzyl-14C]-pretomanid, M11 was the largest metabolite in the urine *ca* 13% of the radioactive dose and M26, M8/M10 and M13 each represented *ca* 5% of the dose. A number of radioactive peaks were identified in faeces but all represented <3% of the radioactive dose each.

After [imidazooxazine-14C]-pretomanid) 15 minor metabolites were characterized in the urine each accounting for <5% of the radioactive dose. In faeces were 31 radioactive peaks detected and 12 minor metabolites, each accounting for <2% of the dose.

Plasma

The concentration of total radioactivity in plasma and blood was much higher than the plasma concentration of pretomanid. The $t_{1/2}$ of total plasma radioactivity was calculated to *ca* 18 days compared to 16h for pretomanid. Pretomanid represented 29% of total plasma radioactivity at C_{max} and c*a* 80, 55 and 2.5% of radioactivity $AUC_{0\text{-}24h}$, $AUC_{0\text{-}48h}$ and AUC_{total}, respectively.

The extraction efficiency of 14C-related material from plasma samples decreased with time (see **Figure 2**). Unextractable radioactivity associated with plasma proteins might be the reason to the long $t_{1/2}$ of 18 days for total plasma radioactivity.

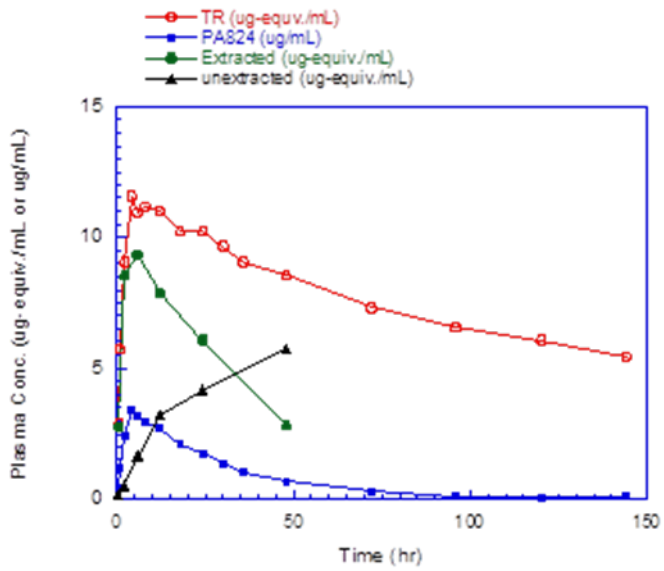


***Figure 2*:** PK profiles of Pretomanid, total plasma radioactivity, extractable plasma radioactivity and unextractable plasma radioactivity in CL-008 Study. Note: Source: CL-008, Table 11-4; extracted (acetonitrile) and unextracted radioactivity profiles are derived from extraction efficiency (CL-008, Section 16.2.5.3).

The exposures listed in Table 8 were used for the qualification of the different components of radioactivity, including an accumulation ratio of 2 for pretomanid (according to the dose-proportionality study) and 26 for total radioactivity (R_{Ac} based on $t_{1/2}$ of 18 days).

Table 4: Plasma exposures (AUCtau,ss) of pretomanid and components of radioactivity calculated from study CL-008 data

Analyte	AUCtau,ss [µg.hr/mL]
A) Pretomanid at 200 mg	50.9[a]
B) Pretomanid at 1100 mg	216
C) Total Radioactivity at 1100 mg	6373[b]
D) Extractable Radioactivity at 1100 mg	382
E) Total Radioactivity at 200 mg = (A/B)×C	*2796* [c] / 1502[d]
F) Extractable Radioactivity at 200 mg = (A/B)×D	*168* [c] / 90[d]
G) Unextractable Radioactivity at 200 mg = E – F	*2629* [c] / 1411[d]
H) Extractable Metabolites at 200 mg = F – A	*117* [c] / 39[d]

[a] Nix-TB update; [b] Calculated based on t1/2 424 hrs, study CL-008; [c] as calculated by the applicant, probably including a mistake; [d] recalculated using the formulas listed by the applicant.

The three most abundant metabolites detected, during the first 24h *post* dose, were M27, M50, and M52 representing 8, 6 and 5% compared to the exposure of pretomanid.

Dose proportionality and time dependencies

A less than dose-proportional increase in systemic exposure of pretomanid was seen following single doses of 50-1500 mg as well as after repeated dosing of 200 and 600 mg od.

Time dependency

No signs of time dependency were seen following 200 and 600 mg od in the MAD-study (CL-002).

PK in target population

The reference subject is: 55 kg, male, HIV-, DS-TB subject with baseline TBIL of 5 µmol/L and ALB of 35 g/L administered 200 mg qd of pretomanid alone in a fed condition for 8 weeks. The geometric mean (CV%) Cavg,ss for this subject is 2.37 µg/mL (32.8%).

Special populations

Renal impairment

No data have been provided. A study in subjects with decreased renal function is ongoing.

Hepatic impairment
No data have been provided. A study in subjects with hepatic impairment is ongoing.

Gender, race, weight, elderly
Clearance and volume of distribution scaled allometrically with weight. Apparent clearance in females was 18% less than in males according to the popPK model.

Age did not influence pretomanid PK over the age range investigated (18-77 years, five subjects ≥65 years in the popPK dataset). There is limited data on pretomanid PK in the elderly.

According to NCA analyses, Black, Mixed and White races had similar systemic exposure. There was a tendency towards higher exposure in Asian subjects, however, the data are limited (five Asian subjects with rich PK sampling across the development program).

None of the differences identified between subpopulations appears to warrant any differentiation of the dose.

Pharmacokinetic interaction studies

In vitro

- Pretomanid is a CYP3A4 substrate *in vitro*
- CYP inhibition/induction by pretomanid *in vitro*

Enzyme	**In vitro IC50 (µM)**	**Clinical relevance**	**Induction Clinical relevance**
CYP 1A2			
CYP2B6			
CYP 2C8	Study ongoing	TDI*	
CYP 2C9	Study ongoing		*
CYP 2C19	Study ongoing	TDI*	*
CYP 2D6		TDI*	
CYP 3A4	50	GI / TDI*	*

* In vitro studies to evaluate inhibition and induction of CYP2C8/9/19 by pretomanid are ongoing, clinical relevance will be assessed based on study results.

- *In vitro* – pretomanid was not a substrate of Pgp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2-K mediated transport

- *In vitro* inhibition of transporters

Transporter	IC50 (µM)	Clinical relevance
Efflux transporters		
Pgp	Study ongoing*	
BCRP	Study ongoing*	
Uptake transporters		
OATP1B1		
OATP1B3	Study ongoing*	
OAT1		
OAT3	Ki=2	Yes
OCT1		
OCT2	Study ongoing*	
MATE1		
MATE-K		

* An in vitro study to evaluate inhibition of PgP, BCRP, OATP1B3 and OCT2 by pretomanid is ongoing.

In vivo

- The exposure of midazolam (CYP3A4 substrate) was 0.85-fold when co-administered with pretomanid (CYP3A4 - inhibitor GI-tract, TDI signal, induction potential)
- The exposure of pretomanid was
  - 0.45-fold when co-administered with rifampicin (enzyme and transporter inducer, OATP1B1, inhibitor)
  - 0.75-fold following co-treatment with efavirenz (inducer of CYP3A4 and a CYP3A4 inhibitor)
  - 0.85-fold when dosed together with lopinavir (inducer of CYP450)/ritonavir (CYP3A4 inhibitor and an inducer of CYP 1A2, 2C8, 2C9, 2C19)
- *Conclusion*
  A clinically relevant decrease in systemic exposure of pretomanid was seen when co-administered with rifampicin and efavirenz thus pretomanid should not be co-treated with strong and moderate CYP inducers (SmPC 4.5).

  No clinically relevant effect on midazolam (CYP3A4 substrate) was seen when co-administered with pretomanid (CYP3A4 - inhibitor GI-tract, TDI signal, induction potential), however, potential effect on other PXR mediated enzymes, such as CYP2C9 and CYP2C19, *in vivo* is unclear.

Population PK modelling

A population pharmacokinetic (popPK) analysis was performed using nonlinear mixed effects modelling. Data were combined across 14 of the studies in the pretomanid development programme, constituting a total of 17725 observations from 1054 subjects. The purposes of the analysis were generally descriptive, with the main aim to identify important covariates and serve as basis for estimation of exposure metrics for exposure-response investigations. Absorption was described using three transit compartments and dose-dependent bioavailability, while disposition was described using a one-compartment model. A total of 33 covariate-parameter relationships were considered necessary to describe the combined data adequately.

Exposure-response relationships

No reliable exposure-response relationships have been characterized.

Concentration-QTc modelling was performed based on pooled data from across studies. For pretomanid monotherapy, a rich dataset was available, and a linear increase in QTc was estimated as a function of pretomanid concentration. For the BPaL regimen, limited data were available, with no data at pretomanid concentrations >~5 ug/mL. The final model for the BPaL regimen described a linear increase in QTcN (using a data-specific correction) as a function of both pretomanid concentration and bedaquline M2 concentration. With pretomanid at a concentration of 3.2 µg/mL (mean estimated C_{max} at 200 mg od) and with bedaquiline M2 at a concentration of 0.25 µg/mL (mean observed M2 concentration in Nix-TB), the mean ΔΔQTcN (upper 90% CI) was estimated as 9.5 ms (10.8 ms). Including a "secular trend" estimated from all the trials, ΔQTcN for the BPaL regimen would be 13.6 (14.9 ms).

2.4.3. Pharmacodynamics

Mechanism of action

Pretomanid is a new chemical entity and a member of the nitroimidazooxazines class, whereas delamanid is a member of the nitroimidazooxaole class). The mechanism of action seems complex and has not been fully elucidated. Under aerobic conditions, pretomanid appears to inhibit MTB cell wall biosynthesis by inhibiting the synthesis of mycolic acid, while under anaerobic conditions, it generates reactive nitrogen species.

In some further detail, for bactericidal action, activation of an aromatic nitro group is required, accomplished through an oxygen-sensitive bioreduction in the milieu found in microbes, but not in mammalian cells. The reaction, involving the transfer of two electrons, is dependent on three enzymes: a co-factor F420 (8-hydroxy-5-deazaflavin), an F420-dependent nitroreductase encoded ddn, and an F420-dependent glucose-6-phosphate dehydrogenase encoded fgd1.

Mutations in genes encoding for either Ddn, Fgd1, or the F420 biosynthetic pathway (fbiA, fbiB, or fbiC) result in high-level resistance (discussed in a coming section).

Following activation, pretomanid inhibits cell wall production by inhibiting the synthesis of mycolic acid, a process mainly affecting replicating bacteria. However, studies in a variety of in vitro models also provide evidence for a prominent effect on non-replicating MTB. Activated pretomanid yields NO-production, which may be the key to this action, where NO targets ATP synthase (energy production) as well as DNA and multiple enzymes within the MTB.

In vitro, in vivo activity, resistance and secondary pharmacology

In vitro activity

In vitro activity – MTB, aerobic conditions

The effect in vitro has been studied by a number of groups over the years, with the use of different assays (Stover 2000, Feuerriegel 2011, Upton 2015, the TBA-354-NLCN-072A study). The MIC-range was fully similar in these studies, ranging 000.5 to 0.48 µg/ml regardless of resistance classification (i.e DS-, MDR- and XDR-TB isolates). Of note, isolates with mutations known to yield resistance to pretomanid have MIC values of ≥16 µg/ml (below).

The Pretomanid-NCLN-MICRO-004 study evaluated the activity of pretomanid using different methods: MGIT, REMA and agar proportion. In this study, 35 patient isolates from the NC-006 trial (discussed in efficacy/safety section), 2 pretomanid-resistant mutants, 14A1 and T3; and MTB H37Rv (control) were

evaluated using independent inocula over three different dates in the same testing laboratory. The MGIT results in this comparison were from a previous study (Pretomanid-NCLN-MICRO-003); the REMA results were from the NC-006 trial. In this comparison, there was a method-dependent difference in MIC values, in the order agar, REMA and MGIT, where MICs by MGIT were typically 2 to 4-fold higher than the REMA-results.

In vitro activity– MTB, anaerobic conditions

The in vitro activity under anaerobic conditions, with and without acidification, and efficacy against intracellular MTB (in whole blood experiments) has also been studied and results published by a number of groups. Results are not presented in any detail. In summary, bactericidal activity was shown also against non-replicating MTB under conditions including hypoxia, microaerophila and acidified hypoxia. Intracellular activity in mouse macrophages was demonstrated in the 2018-Pretomanid In Vitro Anti-TB Activity Study 1. However, in two other studies investigating intracellular activity (Wallis et al. 2012 and Matsumoto et al. 2006), pretomanid displayed antagonistic effect to bedaquiline and sutezolid and was inferior to rifampicin and delamanid. The results of the three studies were discussed. There is firm documentation from study A and B that pretomanid alone is effective against intracellular MTB. The antagonistic effect observed for one of the conditions with pretomanid, bedaquline and sutezolid in study C, was deemed to be explained by a low concentration of pretomanid. Results obtained in the mouse and guinea pig models confirms a satisfactorily intracellular effect of the 3-drug regimen.

In vitro activity –other mycobacterial species and other bacteria

Among tested mycobacterial species besides MTB, M. bovis, M. africanum, and M. pinnipedi were susceptible, with MICs from ≤0.0312 to 0.125 µg/mL. MICs for M. ulcerans ranged ≤4 to >16 µg/mL (which would be considered resistant).

MICs were not measurable against M. avium, M. phlei, M. smegmatis, M. fortuitum or M. kansasii. M. leprae is naturally resistant as well (it lacks a functional ddn gene, needed for activation of pretomanid).

Pretomanid lacks activity against other aerobic bacteria and yeast including E. coli, S. aureus, and C. albicans; however, it does display some activity against a small number of Gram-positive and Gram-negative *anaerobic* bacteria, with MICs in the range of 1 to 32 µg/mL.

In vivo activity

In vivo activity in Murine Models of tuberculosis

Pretomanid has been evaluated in several murine models of TB, as monotherapy and in combination with a variety of first- and second-line TB drugs, including compounds in clinical development. For most, the MTB strain H37Rv was used. For all but one study, aerosol infection of mice was used; in that study, mice were infected via the intravenous route. Efficacy was generally assessed by CFU counts in lungs and/or spleens, either at the end of the treatment period (assessment of bactericidal activity), or 12 weeks after completion of treatment, to assess relapse (sterilizing capacity). A large number of studies by various investigators have been performed (Stover 2000, Tyagi 2005, Lenaerts 2005, Lannoix 2014, Li 2016). These were summarized by the applicant in an extensive review. In addition to studies in mice, a few studies were also performed in guinea pigs; results in that species was similar to that in mice and are not further commented.

In brief summary, Pa dosed 25 mg/kg yielded similar effect in an acute model as INH control (25 mg/kg).

The minimal effective dose (the lowest dose that prevents development of gross lung lesions and splenomegaly) and minimal bactericidal dose (the lowest dose that reduces lung CFU burdens by 99% compared to pretreatment) was found to be 12.5 and 100 mg/kg, respectively, in aerosol infected mice.

Also in a latent TB model (low aerosol inoculum, with treatment starting 6 weeks later) results with Pa were similar to that seen with INH.

A number of different Pa-containing regimens have been evaluated in mice. Three regimens have been studied in the clinical studies, the BPaMZ, BPaZ and BPaL regimens (efficacy section). The agents included have been evaluated in monotherapy and in different combinations by Li et al (publication 2016) and the TB Alliance, tables below.

In the study by Li et al, table below, the challenge dose was 3.5-4.0 log10 CFU, and treatment (5 days per week) started 14 days post challenge. Bactericidal activity was assessed by studying CFU counts in lung tissue up to 3 months of treatment, whereas relapse was assessed 3 months after completion of 1, 1.5, 2, 3, or 4 months of treatment (sterilizing capacity).

Table 5. Lung CFU Counts during treatment and proportions of mice relapsing after treatment

	Log10 CFU count (mean ±SD)			**Number relapsing/total number (%)**				
	M 1	M 2	M 3	M 1	M 1.5	M 2	M 3	M 4
RHZ	4.93±0.22	2.97±0.07	1.05±0.28					5/15
BPa	5.33±0.26	2.78±0.10	1.39±0.45					1/15
BPaMZ	1.96±0.25			15/15	4/17	0/15		
BPaZ	2.52±0.19				12/15	2/15		
BPaM	4.62±0.27	1.17±0.20	0.16±0.22			15/15	1/15	0/15

B= bedaquiline; CFU = colony-forming unit; H = isoniazid; M = moxifloxacin; Pa = pretomanid; R = rifampicin; SD = standard deviation; Z = pyrazinamide; Untreated mean lung log10 CFU counts at day 0 = 8.09±0.24. Source: adapted from (Li et al., 2017)

The B-Pa-M-Z regimen is presently evaluated in the NC-008 study (discussed in efficacy section), where both DS-TB (here as a means to shorten therapy) and MDR-TB patients are included. The added activity of pyrazinamide is clear in the mice model. This may question to what extent pyrazinamide can be justified as part of a standardized MDR-TB regimen: at least in the high epidemic setting more than half of MDR-TB patients were reported to have baseline isolates harbouring pyrazinamide resistance.

Two studies performed for the TB Alliance (Johns Hopkins University) evaluated the bactericidal activity of Pa, B and L in all types of combinations (of relevance for the pivotal study, Nix-TB and ZeNix). The doses used in these studies were pretomanid (100 mg/kg), bedaquiline (25 mg/kg), and linezolid (100 mg/kg), rifampicin (10 mg/kg), isoniazid (10 mg/kg), and pyrazinamide (150 mg/kg). The exposures obtained with that dosing of bedaquiline and linezolid are relevant for the exposures achieved in the clinical trials.

Mice where challenged with aerosol (~4.2 log10 CFU), and treatment started 2 weeks thereafter. Results (similar between studies) are shown below for the larger of the two experiments. This study clearly indicates that all 3 drugs in the BPaL regimen (the regimen of main interest for this application) clearly contribute to the efficacy of the combination, next table.

Table 6. Mean lung CFU counts following 1- and 2- month treatment of murine TB with Pretomanid, Bedaquiline, and Linezolid (JHU study 2015, expt 2a)

	Mean Lung Log_{10} CFU Count (±SD)	
	4 weeks	8 weeks
RHZ	5.47±0.19	1.43±0.81
Pa	6.02±0.19	4.57±0.20
B	4.73±0.20	2.09±0.28
L	6.81±0.13	5.61±0.25
BL	4.79±0.17	2.85±0.25
BPa	4.57±0.21	1.80±0.82
PaL	5.33±0.24	3.34±0.52
BPaL	2.75±0.31	0.00±0.00

Day 0 untreated mean lung log10 CFU counts = 7.68±0.27.

In a third study from Johns Hopkins (2016, expt 3a) also relapse frequency was evaluated for the BPaL regimen versus that obtained with control (RIF, INH and pyrazinamide given for 4 months). Following 3 months of therapy, the relapse rate with control was 7/15 versus 1/15 with the BPaL regimen given for 3 months. Two months of BPaL treatment resulted in relapse in 4/15.

Exposure response in mice

Ahmad et al (2011) studied exposure response in an acute mouse model with various dosing schedules over 24 days, with log CFU reduction in lung tissue as the end point. In brief, these experiments showed that free drug time over MIC had the best correlation to effect (R2 = 0.87), followed by *f*AUC/MIC (R2 = 0.60) (time over MIC being the cumulative percentage of the dosing interval that the drug concentration exceeds the MIC under steady state conditions, and *f*AUC (the area under the concentration time curve for free, unbound drug).

Ahmad et al. also concluded that T>MIC greater than 48% is needed for a 1-log_{10} kill, and T>MIC above 77% is near-maximal bactericidal effect. The EBA_{CFU}s observed in the phase 2 clinical trials for doses of 100 mg and above, are comparable to the maximal kill rate demonstrated in mice treated with 96 mg/kg twice daily.

Resistance

Mechanism of resistance

In general resistance to TB drugs arises via chromosomal mutations, linked to a spontaneous error rate of 1 per 10.000-100.000 per round of replication, and the lack of a mismatch repair system.

The mechanism of pretomanid resistance has been studied by several groups, using different methodologies. The mutation rate has been studied in so called fluctuation analyses, spontaneous mutation resistance studies and in serial passage studies. When summing up the results, the in vitro rate of mutations causing pretomanid resistance was around 10^{-7}, and quite dependent on the size of the inoculum.

Resistance selection studies has also been done in vivo, looking at mice exposed to pretomanid where comparisons have been made on mutations seen here as compared to those reported from mentioned in vitro studies. In summary, in resistant strains a number of point mutations are found in 5 different genes coding for the three enzymes that have been postulated to activate pretomanid (see section

Mechanism of action). The 5 genes are the following, encoding for either Ddn, Fgd1, or the F420 biosynthetic pathway (fbiA, fbiB, or fbiC):

- ddn (also called Rv3547),
- fgd1 (also called Rv0407), and
- three genes encoding for co-factor F420: fbi A (Rv3261), fbiB (Rv3262) and fbiC (Rv1173).

Precise MICs were not determined for these mutations, but MICs >3.6 µg/mL (highest exposure tested) were in practice yielded by all mutations.

In these studies (large number of strains tested), mutations in these genes accounted for the resistance in around 80% of pretomanid resistant isolates; around 20% of isolates were wild type (in these 5 genes), indicating that other cellular functions may be involved in the resistance to pretomanid.

Two studies (mice model) performed at Johns Hopkins for the Alliance (JHU 2017 3d-1 and 3d-2) also evaluated potential remaining activity of pretomanid in the presence of pretomaind resistance (H37Rv ddn mutant with a MIC of ≥16 µg/mL). Two weeks post challenge, the mice were treated with pretomanid alone (doses 50 and 100 mg/kg) and in various combinations with bedaquiline, linezolid, moxifloxacin and pyrazinamide. On the basis of the results in this study, no remaining activity can be expected from pretomanid in the presence of such (high level) resistance, neither when pretomanid was given in monotherapy, nor when it was added to other combinations.

Cross resistance to other TB agents

As summarized previously, MICs were fully similar in DS-, MDR- and XDR-TB isolates, i.e. there is no signal for a reduced susceptibility to pretomanid in isolates resistant to other TB agents in these studies. Baseline MICs in the phase 2/3 studies of this application will be discussed further below.

The other way around, looking at pretomanid laboratory-derived resistant mutants, cross resistance was seen towards delamanid, but not towards other TB agents. Cross resistance to delamanid is generally expected (and was observed in all but one pretomanid-resistant mutant), since delamanid shares the same activation pathway as pretomanid.

Resistance in vivo (phase 2 and 3)

Pretomanid MIC values at baseline and in case of failure (phase 2 and 3)

For the phase 2 studies, the applicant notifies that adequate laboratory practices were not in place prior to the start of these studies (realized by the applicant post-hoc), and that baseline MIC values in these studies need to be interpreted with caution. That included the large NC-005 study, where baseline MICs cannot be reported.

However, pretomanid MIC data obtained at baseline and at post-baseline visits is available from NC-002 study (8 weeks study, n = 148). Samples were examined for presence of high MIC values at baseline, evidence of primary (or pre-existing resistance), and for shifts in MIC values between baseline and post-baseline visits. None of this was seen; the pretomanid MICs at baseline ranged from < 0.025 to 0.2 µg/mL, (MIC50 = 0.05 µg/mL and MIC90 = 0.1 µg/mL for all treatment arms), which is in the range of values obtained for presumed pretomanid susceptible strains tested using a similar

methodology (Study TBA-354-NCLN-072-A). No pretomanid MIC shift from baseline to post-baseline greater than 4-fold was observed.

Data are also available from NC-006 (STAND, phase 3, DS TB) and the Nix-TB study (pivotal, MDR/XDR-TB).

In NC-006, MIC determinations from isolates captured at baseline and at/after end of treatment were done by REMA method, and in addition whole genome sequencing (WGS) was performed (Central lab, London). Following a repeated analysis, excluding isolates that had been contaminated with non-MTB mycobacteria, baseline resistance to pretomanid was seen in 2/213 baseline isolates tested (MIC>4 µg/ml for both isolates). One of the isolates had a mutation in the ddn gene, analysis of the second isolate was ongoing at the time of submission. Both subjects (one treated with control regimen, one with Pa-containing test regimen) had a favourable treatment outcome. Elevated pretomanid MIC values were not observed in any of the post Week 17 isolates, indicating no pretomanid resistance development, table below. There was no significance for baseline pretomanid MICs and treatment outcomes. In summary, there was no association between pretomanid baseline MICs and outcome, and no case of pretomanid resistance development was captured.

Table 7. Pretomanid MIC Values at Baseline and Post-week 17 for NC-006 Trial (REMA)

IC (µg/mL)	Baseline		Post-week 17	
	per stratum	cumulative	per stratum	cumulative
≤0.03	64 (30.1)	64 (30.1)	2 (8.3)	2 (8.3)
0.06	64 (30.1)	128 (60.1)	9 (37.5)	11(45.8)
0.12	52 (24.4)	180 (84.5)	9 (37.5)	20 (83.3)
0.25	23 (10.8)	203 (95.3)	4 (16.7)	24 (100)
0.5	5 (2.4)	208 (97.7)	0	24 (100)
1	0	208 (97.7)	0	24 (100)
2	0	208 (97.7)	0	24 (100)
4	1 (0.5)	209 (98.1)	0	24 (100)
>4	4 (1.9)	213 (100)	0	24 (100)

Note: Three out of the 5 baseline isolates with a MIC ≥4 were found to be wrong (including contamination with non-MTB species) in a subsequent re-analysis.

In the Nix-TB study, pure MTB cultures obtained at baseline (Day 1, or Screening to Week 4 if Day 1 cultures were negative or contaminated) were analysed in the central London laboratory. Pretomanid, bedaquiline and linezolid MIC values were analysed using the MGIT method, and whole pairwise genome sequencing was performed.

Available baseline isolates (n=38 out of 109 subjects) were susceptible to pretomanid, as well as to bedaquiline and linezolid, according to the cut-offs used (those recommended for MGIT by WHO 2018 = 1 µg/mL for both agents).

In the few isolates (n=4) obtained from 16 weeks post baseline or onwards, there were none with a fold shift to pretomanid or linezolid. One isolate developed bedaquiline resistance (baseline MIC 0.12 µg/ml versus 4 µg/mL at relapse 3 months post EOT). Sequencing revealed the development of mutation in the Rv0678 gene, associated to bedaquiline resistance, not present in the baseline isolate. Details on this patient (and others with relapse) are provided in the clinical efficacy section.

Table 8. Baseline Distribution of Minimum Inhibitory Concentrations in the Nix-TB Trial - Based on all available cultures from 109 included patients (interim report, 29 June 2018)

MIC (µg/mL)	**Pretomanid**	
	actual n (%)	cumulative n (%)
≤0.063	7 (18%)	7 (18%)
0.12	11 (29%)	18 (47%)
0.25	16 (42%)	34 (89%)
0.5	3 (8%)	37 (97%)
1	1 (3%)	38 (100%)
2	0	38 (100%)
MIC (µg/mL)	**Bedaquiline**	
	actual n (%)	cumulative n (%)
≤0.063	0	0
0.12	1 (3%)	1 (3%)
0.25	13 (39%)	14 (42%)
0.5	18 (55%)	32 (97%)
1	1 (3%)	33 (100%)
2	0	33 (100%)
4	0	33 (100%)
MIC (µg/mL)	**Linezolid**	
	actual n (%)	cumulative n (%)
0.25	0	0
0.5	36 (95%)	36 (95%)
1	2 (5%)	38 (100%)
2	0	38 (100%)
4	0	38 (100%)
8	0	38 (100%)

Source; MIC tables - CSR addendum v1.2

Positive baseline cultures (or at screening up to 4 weeks prior to day 1) are available for 93/109 subjects (85%), and 16/109 had a negative culture (this data is presented in the Efficacy section). Baseline MIC determinations were achieved for 58/93 (62%) (updated data during the procedure). In practice all isolates that did not grow for MIC determinations (34/35) had been handled at one laboratory, i.e. a lab without proper quality of methods. This laboratory is no longer part of ongoing studies, procedures have been further quality controlled and MIC determinations in the ongoing ZeNix study is presently running as planned.

Secondary pharmacology

QTc prolongation

The effect of pretomanid as monotherapy on QTc prolongation was studied in a thorough QT (TQT)-study (DMID-0058). Pretomanid exerted a mild effect on QTc prolongation (estimated placebo-adjusted change from baseline 4.4 ms [upper 90% CI 6.1 ms] at a pretomanid concentration of 2.3 µg/mL). Important concerns with this study were that pretomanid was only tested at systemic exposure approximately similar to that expected for the proposed clinical dose, i.e. without margins (mean Cmax after the 1000 mg pretomanid single dose in the QT-study was 2.3 µg/mL, while it was 3.1 µg/mL in the Nix-TB study) and that the relevant pretomanid+bedaquiline combination was not tested. Therefore, the results from the TQT study cannot be considered fully relevant, neither for pretomanid monotherapy nor for the BPaL regimen, and is considered inconclusive. However, a multiple dose pretomanid thorough QT study would not provide very useful information. The sum effect

of the BPaL regimen is of main interest, where a thorough QT study (versus placebo in healthy volunteers) of bedaquiline plus pretomanid in combination is not feasible, having in mind the time to steady state and prolonged time to maximum QT prolongation seen for bedaquiline. ECGs were taken throughout the Nix-TB study, and although this study does not allow for a more precise estimate of mean QTc prolongation, very few patients did have QTc increases of a magnitude associated with a substantial risk of arrythmias. Strict and clear recommendations on how to handle QTc prolongation during therapy with the B-Pa-L regimen is provided in the pretomanid SmPC.

Serum creatinine

Pretomanid treatment leads to increased serum creatinine, but apparently not a change in GFR as measured by iohexol clearance (Study CL-005). The Applicant suggests this is attributable to inhibition of tubular secretion of creatinine.

2.4.4. Discussion on clinical pharmacology

Pharmacokinetics

Pretomanid is a small molecule with one chiral centre, the Pretomanid FGK product consist of the S-isomer. Pretomanid was practical insoluble at physiological pH (BCS2).

Plasma concentrations of pretomanid was determined by validated LC/MS/MS methods either as a separate analyte or simultaneously with bedaquiline/linezolid or pyrazinamide/moxifloxacin following co-treatment. Where available, within study validations of the bioanalytical assays seem appropriate for the intended purpose. One bioanalysis report (DDI study A5306) including within study validation was missing and could not be provided since it had never been written. The issue was not further pursued.

During analysis of study samples from the phase-2 study NC-005, determining plasma levels of pretomanid, pyrazinamide and moxifloxacin simultaneously by a validated UPLC-MS/MS method, ISR (incurred sample reanalysis) criteria failed for all three analytes. Investigations concluded that the best way to alleviate the ISR issue was to change the working procedures/redevelop the assay. The method was then re-validated, and all reported results were generated using the re-validated method.

The effect of food on the bioavailability of pretomanid is considerable, especially at higher doses. This is expected for an oral solid drug product for which the rate of absorption is limited by the solubility in the GI-tract (presence of food/bile acids increase the solubility). The systemic exposure was about doubled at the dose intended for marketing (200 mg) when co-administered with food. In the pivotal study Nix-TB, pretomanid was administered with food to ensure adequate bioavailability. Non-clinical data show no effect of a high fat/calorie breakfast intake compared to without in monkeys, while in another study, bioavailability was higher in fasted compared in fed animals (*cf* Non-clinical AR). Surprisingly the effect of food on pretomanid bioavailability seems to be opposite in humans and monkeys, which could be attributed to pH variations. The food effect needs to be considered when assessing the outcomes of the clinical studies. For instance, the QTc study (evaluating doses up to 1000 mg) was conducted in fasting subjects and due to the saturation of the uptake in fasting state, the achieved exposures were low and generally not higher than what was observed after administration of the 200 mg at fed conditions.

The Applicant has suggested that pretomanid could be taken without food without loss of efficacy. However, there is currently no reliable evidence that adequate efficacy would be achieved at lower exposure than that obtained in Nix-TB. Therefore, the clinical recommendation should be that

pretomanid is administered with food. Across the Phase II studies and the Nix-TB study, pretomanid was administered with varying instructions regarding time and meal content. Still, exposure in terms of mean AUC0-24, ss was relatively stable (range 54-80 µg*h/mL), indicating that the exact food content and timing is not crucial.

Pretomanid was labelled in two different positions [benzyl-14C]-pretomanid and [imidazooxazinele-14C]-pretomanid to follow/determine metabolites after oral single doses. The concentration of total radioactivity in plasma was much higher than the plasma concentration of pretomanid. The $t_{1/2}$ of total plasma radioactivity was calculated to *ca* 18 days compared to 16h for pretomanid. An implication of the large difference in $t_{1/2}$ between parent compound and radioactivity is that the ratio between metabolites and parent compound might change with time, the PK of the metabolites are unknown. Pretomanid represented 29% of total plasma radioactivity at C_{max} but only 2.5% of total AUC of radioactivity. One major metabolite was determined, M19 representing 35% of the systemic exposure compared to pretomanid. None of the other detected circulated metabolites, during the first 24h *post* dose, were determined to be as large as 10% of the exposure of parent compound. The three others most abundant detected were M27, M50, and M52 representing 8, 6 and 5% compared to the exposure of pretomanid. The extraction efficiency of 14C-related material from plasma samples decreased with time. Thus, unextractable radioactivity associated with plasma proteins might be the reason to the long $t_{1/2}$ of total plasma radioactivity. The consequences following repeated dosing once daily with respect to systemic accumulation of drug related material, considering the long $t_{1/2}$ (18 days) of total plasma radioactivity, are unknown. May there be metabolites that have not been qualified in the non-clinical setting? The applicant provided a calculation that assumed a fixed ratio of parent/metabolites over time, which is not agreed with. After recalculation with the appropriate accumulation ratios for pretomanid and total radioactivity, the qualification of metabolites in non-clinical species was demonstrated for pretomanid, its known metabolites, and the sum of extractable or non-extractable radioactivity in the monkey and in the rat. This is when using the data as calculated by the applicant, which seems to include an error that leads to an overestimation of the human exposure. The consequence is that the true qualification ratios may be higher, which has no impact on the fact that the diverse metabolite fractions are considered qualified.

Taking into account the linearity of plasma protein binding and the lack of active transport, it can be assumed that the total plasma concentration of pretomanid represents a reasonable surrogate for unbound plasma and lung concentration.

The chemical structure of pretomanid has one chiral centre but the applied product is the S-enantiomer of the racemate. The applicant provided convincing non-clinical data and in vitro data from human hepatocytes where the other enantiomer (PA-1147) was not detected. It is endorsed that there is no evidence of chiral inversion at this point.

The total recovery of radioactivity was 90% following oral single doses of 14C-labelled-pretomanid with *ca* 70-75% of the dose was excreted within the first four days after administration and in total about 90% during the 11-days sampling collection. About 60% of the dose was excreted in the urine and *ca* 30% in faeces.

About 60% of the elimination pathways have been characterized, according to the EMA guideline 80% of the excreted radioactivity should be characterized *ie ca* 70%. Less than 1% was excreted unchanged compound in the urine and <2% in faeces. Depending on the radioactive labelling, [benzyl-14C]- or [imidazooxazinele-14C]-pretomanid, some differences in the metabolic pathways were characterized. However, independently of labelling many radioactive peaks were detected. M11 (M19-glycine conjugate) was the largest metabolite, with 13% of dose excreted in the urine. The second largest metabolite in the urine was M26 (7% of the dose). Several metabolites were excreted in the urine and faces with each representing <5% and <3% of the dose, respectively.

The reactions involved in the elimination pathways of pretomanid and its metabolites are oxidations, including oxidative dealkylation and oxidative deamination; nitro-reduction; and conjugations, including glycine, glucuronide, and glutathione. *In vitro* metabolism data showed that pretomanid was relatively stable but CYP3A4 seemed to be involved. In the *in vivo* DDI study (A5306) where pretomanid was co-administered with lopinavir/ritonavir (ritonavir - known CYP3A4 inhibitor) was a small decrease in exposure of pretomanid seen, with plasma levels of *ca* 0.85-fold compared when dosed alone. Thus, it can be concluded that CYP3A4 is partly involved in the metabolism of pretomanid *in vivo.* The co-administration of pretomanid with efavirenz (known CYP3A4 inducer) or rifampicin (known CYP inducer) resulted in a slight decrease in exposure of pretomanid with levels of 0.65- and 0.35-fold compared when dosed alone. The impact of genetic polymorphisms has been evaluated on the premises:

- Genes relevant to elimination of pretomanid and its metabolites vary from having little polymorphism (e.g., glycine N-acyltransferase) to substantial polymorphism (e.g. CYP3A4).
- Nothing specific is known about the impact of any polymorphism on pretomanid or its metabolites.
- However, polymorphisms are not expected to be clinically relevant:

    - The multiplicity of metabolic pathways reduces sensitivity to changes in any particular pathway. If genetic polymorphisms were important for the elimination of pretomanid itself, one consequence might be a large variability in pretomanid exposure.

The literature review conducted by the applicant (D151 response) supports that major genetic sources of variability is not expected, but overall there is uncertainty left on sources of variability in pretomanid PK and their relative impact.

The risk of pretomanid to interact with other active compounds, both from victim and perpetrator horizon, and potentially result in changes clinical efficacy/safety needs to be further investigated, in particular the potential impact of TDI of CYP2C8 and CYP2C19. Depending on emerging *in vitro* results further *in vivo* studies may be needed. The applicant agreed to perform the *in vitro* studies and adapted the SmPC accordingly.

Pretomanid is an inhibitor of the OAT3 transporter in vitro, which could result in increased concentrations of OAT3 substrate drugs clinically and may increase the risk of adverse reactions of these drugs. This has been described in the SmPC (section 4.5). An in vivo study is not planned.

Pretomanid enters MTB and is there converted to desnitro pretomanid. As part of the D151 response, the applicant has provided a literature review and a discussion of the potential impact of bacteria on the PK of pretomanid. Bioreduction of pretomanid's aromatic nitro group is part of the activation of pretomanid within the target MTB. However, limited data has been presented to understand the bacterial contribution to the metabolism of pretomanid. While no significant difference is observed in healthy volunteers versus patients, indicating that the MTB burden does not impact the overall elimination of pretomanid, pre-systemic metabolism by intestinal microflora is likely to occur, but the extent has not been investigated. In principle, in vitro studies and/or a mechanistic model could be have been used to explore this. Parallel to the requirements for in vitro incubations with CYP enzymes and transporters to understand the potential need for further in vivo investigation of involved ADME pathways and consequent potential impact on DDIs or genetic polymorphisms, in vitro incubations to investigate bacterial metabolism and potential release of pretomanid and metabolites from the bacteria could have been performed. Such exploratory investigations are not requested at this stage and as it is considered that the metabolic pathways of pretomanid have not been thoroughly investigated, these uncertainties are suggested described in the SmPC, and EPAR.

As pretomanid seems to be mainly eliminated by metabolism, the clinical consequences of potential increase in systemic exposure of pretomanid due to decreased hepatic function needs to be understood. No PK of pretomanid in subjects diagnosed with different degrees of hepatic impairment have been presented. A study in hepatic impairment is ongoing and expected to be reported mid 2021.

Less than 1% of the dose was excreted unchanged in the urine. A lack of a PK study in RI is acceptable for a drug that is predominantly hepatically eliminated and for which safety data are available indicating that dose adjustments are not necessary even at a markedly increased exposure to the drug. Such safety data are currently not available for pretomanid. A study in subjects with decreased renal function with a reduced/staged design is ongoing. While awaiting these results and derived treatment recommendations, there should be a restriction in the use of pretomanid in patients with reduced renal function. In the provided link to the renal impairment study details at clinicaltrials.gov, it is noted that pretomanid will be administered as a single 200 mg dose in the fasting condition. In such situations with dose-dependent, food-dependent and potentially time-dependent pharmacokinetics, steady state pharmacokinetics of pretomanid may not be readily predicted by single dose fasting data. It is questioned whether it will be possible to draw conclusions regarding dosing recommendations in renally impaired subjects based on this study.

The presented popPK model has several shortcomings, including strong signals of over-parameterisation and several covariates without a biological basis, while the final model appears to describe the data set well. The current popPK model can be considered exploratory only and is not considered qualified for extrapolation purposes. The predictability into future data is not expected to be adequate, which was also demonstrated by the model without the Nix-TB data not being able to predict the Nix-TB data adequately. A credible popPK model is necessary to improve the current understanding of sources of variability and increase confidence regarding posology recommendations in sub-populations. Pretomanid will be further developed for a paediatric indication. The key binding elements of the EMA opinion (EMA/PDCO/777955/2018 Corr) on the Paediatric Investigation Plan (EMEA-002115-PIP01-17) specify that a popPK model should be developed based on the adult data, scaled to the paediatric population and used for planning the paediatric studies. The specified objective of the model is to inform the dose finding in paediatric patients as well as determining the PK sampling scheme. It is important to note that the current model is not qualified for this purpose. The Applicant has agreed to refine the current popPK model before paediatric development.

Models describing increasing $EBA_{CFU(0-14)}$, $EBA_{TTP(0-14)}$ and time to sputum culture conversion (TSCC) as a function of average concentration (C_{avg}) were presented in the MAA. However, these models were not considered credible due to very limited data in the lower exposure region to support the functional shape estimated, no pre-specification of data handling and model discrimination and no model evaluation. The current exposure-response models cannot be used to derive a lower target concentration threshold for pretomanid where adequate efficacy is expected to be achieved or as an evidence of the contribution of pretomanid to the overall treatment effect.

The QTc-concentration model for the combined effects of bedaquiline-M2 and pretomanid is currently not considered qualified to predict QTc effects at supra-therapeutic exposure due to lack of relevant data. Current uncertainties regarding QTc prolongation risk for the bedaquiline+pretomanid combination are unresolved and will remain as part of the benefit-risk evaluation.

Pharmacodynamics

Pretomanid has activity to MTB, with MIC values in the range of of ≤0.015 to 2 mg/L, where MICs are fully similar in DS-, MDR- and XDR TB strains. Values seem method dependent (MICs on solid agar < REMA < MGIT, with an up to 4-fold difference for MGIT vs agar. The evaluation of breakpoints (seemingly method dependent) is a future matter for the TB team of the EU-CAST committee.

In addition to MTB, some other mycobacterial species (M. bovis, M. africanum, and M. pinnipedi) were susceptible, while other strains are fully resistant (M. avium, M. phlei, M. smegmatis, M. fortuitum or M. kansasii as well as M. Leprae).

In resistant MTB strains, 80% of resistant cases were explained by point mutations in 5 genes encoding for 3 enzymes involved in the activation of pretomanid in 80% of cases; i.e. some unknown mechanisms of resistance still remain to be found. There seems to be no relevant cross resistance to other TB agents, with the exception for delamanid, which is activated by the same enzymes and where cross resistance was anticipated.

The activity of pretomanid in monotherapy as well as in various combinations with other TB agents has been studied in large numbers of pre-clinical studies, mainly in mice. The results in these studies support the dose of pretomanid as well as the contribution of each agent to the present regimen.

When looking at baseline pretomanid MIC values in the clinical program, pre-existing resistance has so far only been seen in a couple of cases (indicating a prevalence of <1%). To date, no cases of treatment-emergent pretomanid resistance has been seen, neither in the NC-006 study (mainly concerning DS-TB patients) nor in the Nix-TB study.

2.4.5. Conclusions on clinical pharmacology

Pharmacokinetics

A rather limited clinical pharmacology package has been provided in the pretomanid submission. Further basic data considering elimination and potential DDIs are needed to secure efficacious and safe use pretomanid in all patients independently of co-treatments, organ function and will be provided as a follow-up measure (REC):

1. Renal impairment study
2. Hepatic impairment study
3. In vitro data shows signs of both PXR-mediated induction and time-dependent inhibition of CYP2C8 and CYP2C19 caused by pretomanid. In vivo data on the effect of pretomanid on midazolam cannot be used to exclude the risk for CYP2C induction in vivo, as pretomanid is also a CYP3A4 inhibitor. Thus, a clinically relevant effect of pretomanid on CYP2C substrates (induction and/or inhibition) cannot be excluded based on available data. The Applicant is recommended to provide further data. The risk for induction may be further addressed in vitro, by providing data on the effect of pretomanid on the PXR co-regulated enzymes CYP 2C9, 2C19 and 2C8 in hepatocytes. In case of positive signal in vitro, a multiple-dose DDI study with a sensitive CYP2C substrate would be recommended
4. In vitro inhibition of Pgp, OATP1B3, OCT2 and BCRP by Pretomanid

In addition, Pretomanid will be further developed for a paediatric indication. The key binding elements of the EMA opinion (EMA/PDCO/777955/2018 Corr) on the Paediatric Investigation Plan (EMEA-002115-PIP01-17) specify that a popPK model will be developed based on the adult data, scaled to the paediatric population and used for planning the paediatric studies. The specified objective of the model is to inform the dose finding of pretomanid in paediatric patients as well as determining the PK sampling scheme of the two planned studies. A credible adult model is a necessary step for proceeding to a scaled paediatric model and to obtain reliable predictions of the doses in the paediatric population. The plan included involvement of dedicated expert following pharmacometricians and external validation/refinement of the popPK model as more data become available, including the key activities:

- The popPK model will be updated/refined with paediatric data from PIP Study 3 in July 2020 and used to inform the dose and PK sampling strategy for PIP Study 4 (PAEDIATRIC-1, final protocol August 2020).

- The popPK model will be refined with data from ZeNix, SimplicitiTB and interim data from PAEDIATRIC-1 prior between June 2021 and February 2022 and used to inform the dose and PK sampling strategy for PIP Study 5 (PAEDIATRIC-2, final protocol January 2023

Pharmacodynamics

Pretomanid shows potent effect against MTB in vitro, where MIC values are similar in the presence of resistance to other approved agents in other classes.

Pre-clinical in vivo efficacy has been shown in a large number of murine models, both in monotherapy and in various combinations with other agents. For the present application, experiments performed in the mouse model at the Johns Hopkins University (2015) convincingly show that each of the agents in the B-Pa-L regimen (the regimen used in the pivotal study, Nix-TB) add to the activity of this triple regimen.

On basis of available data of limited size, baseline resistance to pretomanid is presently very uncommon (around 1%). In the clinical studies no cases of pretomanid resistance development has been documented so far.

2.5. Clinical efficacy

Introduction

The pretomanid clinical program (kinetic studies excluded) is outlined in the next table. All studies concern pulmonary TB.

EBA studies included Pa monotherapy in doses 50 –1200 mg qd, and Pa dosed 100 (1 study) or 200 mg per day in various combinations with bedaquiline, moxifloxacin, pyrazinamide and clofazimine.

In efficacy studies *in DS TB patients*, Pa (dosed 100 or 200 mg) has been explored as regimens Pa/B/Z and Pa/M/Z in phase 2. The Pa/M/Z regimen (Pa dosed 100 or 200 mg) was also evaluated in a phase 3 study, a study that failed; study NC- 006 ("STAND") was halted by the FDA for safety reasons and when this halt was lifted recruitment was not re—started. The sample size was therefore much lower than planned, and non-inferiority versus standard of care was not achieved (point estimate favouring the control regimen). Instead another study was started (NC-008, "SimpliciTB ", ongoing) evaluating regimen BPaMZ for 4 months versus SOC (HRZE/HR for 6 months).

In MDR-TB patients the regimen Pa/B/M/Z has been explored in phase 2 (8 weeks efficacy) and is being explored in one arm of the NC-008 study (treatment duration 26 weeks for the MDR-TB patients). A very limited number of MDR-TB patients were also part of the NC006 study (Pa/M/Z for 26 weeks, too few patients to evaluate).

In XDR-TB patients or MDR-TB where patients do not tolerate or respond to their regimen, the NIX-TB study (pivotal study of the present application) and the ZeNix study (ongoing) both evaluate Pa in combination with bedaquiline and Linezolid (B/Pa/L). Linezolid has an unfavourable toxicity profile, and the Zenix study explores outcomes for 4 different linezolid dosing schedules. In the NIX-TB study Linezolid is given at high dose throughout 26 weeks of therapy.

To be noted, the bedaquiline dosing differs in these studies. According to the present EU label (Sirturo), bedaquiline is dosed 400 mg qd during the first 2 weeks, followed by 200 mg thrice weekly. In addition to that dosing, 200 mg daily (26 weeks) and 200 mg daily for 8 weeks followed by 100 mg for the remaining 16 weeks, is evaluated. The latter dose has been chosen for the two larger ongoing studies ZeNix and SimpliciTB.

Table 9. The pretomanid clinical development program

Pivotal phase 3 study in subjects with XDR-TB or TI/NR MDR-TB		
Nix-TB / Ongoing N=109	BPaL	XDR-TB, or MDR-TB where patient do not tolerate/respond to regimen Single-arm. Treatment duration 26 weeks Pa200 + B according to labeling + L1200
Supportive phase 3 study in subjects with XDR-TB, TI/NR MDR-TB, or pre-XDR-TB		
ZeNix / NC-007 / Ongoing N=180	BPaL	XDR-TB, or MDR-TB where patient do not tolerate/respond to regimen 4-armed, blinded for linezolid dosing. Treatment duration 26 weeks. Pa200+ B 200 mg for 8 weeks, then 100 mg + L: 600 mg or 1200 mg for 9 or 24 weeks (1:1:1:1)
Phase 3 studies in subjects with MDR-TB or DS-TB		
STAND / NC-006 / Complete N=284	PaMZ	DS-TB (randomized, N=271) or MDR-TB (assigned, N=13). DS TB: 4-armed, open-label, randomized (1:1:1:1) Pa100 + M + Z for 17 weeks Pa200 + M + Z for 17 weeks Pa200 + M + Z for 26 weeks HRZE/HR for 26 weeks (control regimen) MDR-TB: Pa200 + M + Z for 26 weeks
Phase 2 studies in subjects with DS-TB or MDR-TB		
SimpliciTB / NC-008 / Ongoing Planned : 450	BPaMZ	DS-TB (randomized) or MDR-TB (assigned) DS-TB Pa200 + B 200 mg for 8 weeks, then 100 mg + M + Z for 17 weeks HRZE/HR for 26 weeks (control regimen) MDR-TB Pa200 + B 200 mg for 8 weeks, then 100 mg qd + M + Z for 26 weeks
NC-005 / Complete N=240	BPaZ and BPaMZ	DS-TB (randomized, N=180) or MDR-TB (assigned, N=60) DS-TB Pa200 + B according to label + Z Pa200 + B 200 mg qd + Z HRZE (control) } 8 weeks, followed by SOC MDR-TB Pa200 + B 200 mg qd + M + Z 8 weeks, followed by SOC
NC-002 / Complete N=207	PaMZ	DS-TB (randomized, N=181, 1:1:1) or MDR-TB (assigned, N=26) DS-TB Pa100 + M + Z Pa200 + M + Z HRZE (control) } 8 weeks, followed by SOC MDR-TB Pa200 + M + Z 8 weeks, followed by SOC - In addition to 8 weeks efficacy, also a 14-day EBA sub-study included.
Phase 2 studies in subjects with DS-TB (14-Day EBA Studies)		
NC-003 / Complete N=102	BPaZC, BPaZ, BPaC, BZC, Z alone, C alone	Pa dosed 200 mg days 1-14 B dosed 400 mg day 1, 300 mg day 2, 200 mg days 3-14 C dosed 300 mg days 1-3, 100 mg days 4-14 Z dosed 1500 mg days 1-14 Control HRZE (15 in all groups, including control)
NC-001 / Complete N=85	PaMZ, BPa, PaZ,	Pa dosed 200 mg days 1-14 B dosed 700 mg day 1, 500 mg day 2, 400 mg days 3-14 M dosed 400 mg days 1-14 Z dosed 1500 mg days 1-14

	BZ, B alone	Control: HRZE	(15 per group, 10 in control)
CL-010 / Complete N=69	Pa alone	Pa dosed 50, 100, 150 or 200 mg qd. Control: HRZE	(around 15 per group, 8 in control)
CL-007 / Complete N=67	Pa alone	Pa dosed 200, 600, 1000 or 1200 mg qd. Control: HRZE	(around 15 per group, 8 in control)

Microbiological methods – general outline

Microbiological methods – entry criteria

Phase 2

The main inclusion criterion for the phase 2 studies was a new diagnosis of pulmonary TB, confirmed by a positive test for acid-fast bacilli (AFB) in a sputum smear direct microscopy test. In studies involving DS-TB patients, the key exclusion criterion was the presence of rifampicin- and/or isoniazid-resistant bacteria in the pre-treatment sputum sample; conversely, in studies involving MDR-TB patients, rifampicin-resistance was a key inclusion criterion, and fluoroquinolone-resistance a key exclusion criterion. Thus, at Screening, spot sputum samples were subjected to the following tests:

- Direct AFB microscopy
- Rapid test for rifampicin and isoniazid (and for fluoroquinolones if the trial required it)
- Identification/speciation of the infecting organism by rapid test

Rapid tests for detection of resistance to rifampicin, isoniazid or fluoroquinolones (and for MTBC bacteria) included Cepheid GeneXpert MTB/RIF, and line probe assays Hain GenoType MTBDR*plus* and GenoType MTBDR*sl*. Cultures of screening samples were also subjected to rapid tests for identification/speciation. These included a non-commercial PCR-based test (Warren et al., 2006) or a commercial immunochromatographic assay based on detection of antigen MPT64 (e.g., Becton Dickinson TBc ID).

In EBA studies and in 8 week-studies, primary outcomes were based on sputum sample cultures on solid media and/or in liquid media. Pooled overnight (over approximately 16 hours) sputum samples collected at various time points during the trial, were digested and divided into 2 fractions; one was serially diluted and inoculated onto Middlebrook 7H11S medium for quantitative sputum culture (CFU counts per mL of sputum, mean of 4 counts), and the other was decontaminated and cultured in the MGIT system to determine time-to-positivity (TTP; in hours).

Phase 3 - Nix-TB (pivotal for this application)

Subjects were included in the trial as either having XDR-TB or TI/NR MDR-TB based on documented resistance to a panel of anti-TB drugs at any time prior to enrollment. In addition, a culture positive for MTB within 3 months prior to screening or MTB confirmed in sputum based on a molecular test within 3 months prior to or at screening could be used to confirm a pulmonary TB diagnosis. These "historical data", obtained at non-study laboratories, were collected for all subjects. Historical drug susceptibility information took precedence for inclusion over any results from the Screening molecular tests.

At Screening, a coached spot sputum sample was subjected to:

- AFB microscopy

- A molecular test to identify MTB and detect resistance to rifampicin (line probe assay Hain GenoType MTBDR*plus* or Cepheid GeneXpert MTB/RIF)
- Culture in the MGIT system followed by a rapid identification or speciation test to detect MTB and determine TTP.

Metrics of Efficacy

CFU Counts and Time to Positivity (TTP)

Two measurements of the mycobactericidal activity of a drug or a drug regimen have been used commonly to characterize efficacy in phase 2 studies, using cultures derived from sampled sputum: 1) the number of CFUs counted on solid media and 2) the time to culture positivity (TTP) in liquid media (MGIT system). These two effects are measured longitudinally for each patient.

CFU counts are related to the number of live bacteria in the sputum sample and hence in the infected lungs. Killing of bacteria by antibiotics commonly results in exponential or biphasic exponential decline in bacteria populations over the first several weeks of treatment. Thus, a plot of the serial measurements of a patient's CFUs, transformed to log10 (CFU) versus time, may appear to decrease in a linear or piecewise linear manner.

TTP represents the time until the cultured population of TB organisms from the sputum sample grows to a sufficient size to trigger a positive signal from the MGIT device. TTP is inversely related to the number of live bacteria in the sputum sample and hence in the infected lung. Profiles of TTP or log10(TTP) versus time have also been described by linear or piecewise linear relationships, although for TTP, a positive slope is associated with a declining bacteria population.

CFU counts and TTP were then used to calculate the following parameters:

- EBA_{CFU}: the rate of change of log_{10}CFU in sputum over time, characterized by linear or non-linear regression of log10CFU over time
- EBA_{TTP}: the rate of change in TTP (or log_{10}TTP) in the MGIT system during treatment over time
- BA_{CFU}: the rate of change of log10CFU in sputum over 56 days of treatment, determined from the model-fitted log_{10}CFU results as calculated by the regression of the observed log10CFU over time
- BA_{TTP}: the rate of change in TTP in the MGIT system over 56 days of treatment, determined from the model-fitted log_{10}TTP as calculated by the regression of the observed log_{10}TTP over time

Sputum Culture Conversion

Sputum is said to have been converted to negative if culturing in solid media yields no colony growth, and similarly if culturing in liquid media (MGIT) yields no signal of positivity, by 42 days after initiation of the culture.

Table 10 Sputum Collection and Conversion Assessment by Study

Study	Comment
NC-002	Overnight sputum collection. Time to conversion = study day of first conversion from positive to negative.
NC-005	Overnight and Coached Spot sputum collections. Overnight considered reference. Culture negative defined as first of two consecutive negative cultures, except for the week-8 timepoint, where a singular negative was acceptable.
NC-006	Early Morning and Coached Spot sputum collections; both samples analysed. A positive culture takes precedence over a negative culture at the same visit. Culture conversion required negative results at 2 consecutive visits at least 7 days apart. Subjects who were unable to produce sputum but who were clinically responding well were considered culture negative.
Nix-TB	Early Morning and Coached Spot sputum collections, both samples analysed. A positive culture takes precedence over a negative culture at the same visit. Culture conversion required negative results at 2 consecutive visits at least 7 days apart. Subjects who were unable to produce sputum but who were clinically responding well were considered culture negative.

In the phase 3 studies, sputum culture conversion at 6 months post end of treatment is primary endpoint of success. The time point is justified by an analysis of 15 TB treatment trials showing that 78% of all relapses occurred within 6 months of stopping treatment and 91% occurred within 12 months (Nunn 2010).

Drug susceptibility testing

Phase 2 studies

Susceptibility testing was performed on MTB isolated from participants prior to treatment start (baseline) and, in some studies, also at end of treatment, as follows:

- Drug susceptibility testing (DST) for streptomycin, isoniazid, rifampicin, and ethambutol in the MGIT system, using the standard critical concentrations recommended by the manufacturer
- MGIT DST and/or DNA sequencing analysis of pncA gene (Whitfield et al., 2015) for detection of pyrazinamide resistance in trials involving the drug.
- MIC testing for the study drugs.

MIC testing for pretomanid was performed using a modified agar proportion method in all studies, except for NC-003 where REMA was used.

Phase 3 studies

<u>MIC testing (study regimens)</u>

In the phase 3 trials, the REMA (NC-006) and MGIT (Nix-TB) methods were used for determining MIC values for pretomanid and the other drugs studied. The decision to use broth-based methods in these studies was made based on the potential large numbers of MTB clinical isolates to be tested, requiring simpler, high-throughput methods. In addition, in the case of Nix-TB, as all isolates were expected to be extensively drug-resistant *M. tuberculosis* (XDR-MTB or MDR-TB). The issue of biosafety was therefore deemed important, where the MGIT system is considered to be the safest assay for staff to handle.

Recently, a direct comparison of the MGIT, REMA and agar proportion methods was performed using pretomanid-susceptible and pretomanid-resistant laboratory strains, as well as clinical isolates. This comparison revealed a trend towards slightly higher MIC values by MGIT, followed by the REMA and agar proportion methods.

Details on baseline MIC determinations are discussed in the pharmacodynamics section.

Baseline Susceptibility to Other Anti-TB Drugs

Data on susceptibility to anti-TB drugs other than the study drugs included information obtained prior to subject enrollment (historical data) and information gathered at screening using molecular tests, or at baseline during the trial using the MGIT. Phenotypic DST in liquid media or on solid media were acceptable for historical data. Because these two data sets were generated at different laboratories using different methodology and at different timepoints in subjects' TB history, discrepancies were expected. When they occurred, historical data took precedence.

2.5.1. Dose response studies

EBA studies (14 days duration)

Study CL-007 [August-Dec 2007, South Africa]
The study explored Pa dosed 200, 600, 1000, and 1200 mg (all qd, fasting). EBA outcomes (log_{10} CFU-reduction and mean TTP) were fully similar for the 4 doses of Pa (around 1.5 log_{10} reduction in CFU counts), and the effect of control similar in magnitude to that reported previously for the HRZE (around 2.0 log10-reduction).

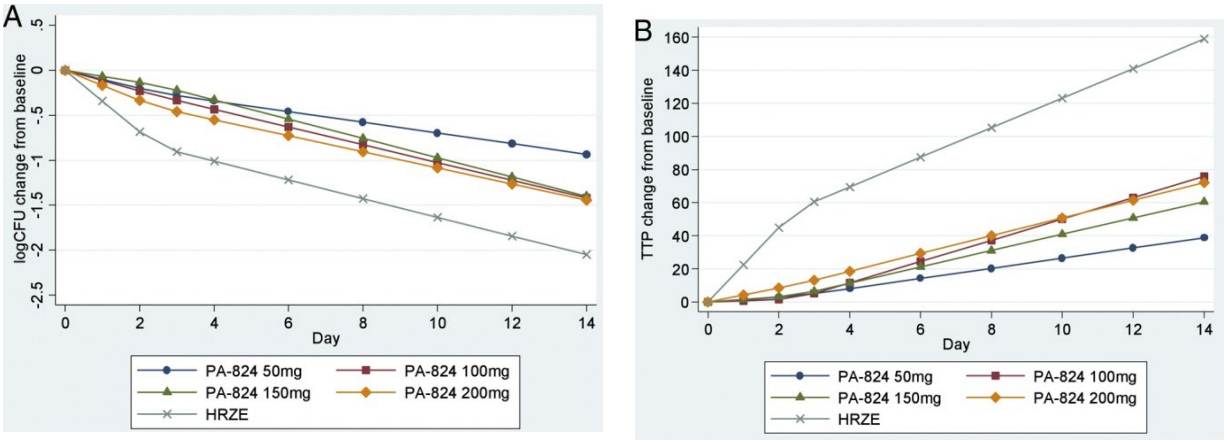
Hence, this study tried doses that were above the efficacy maximum of Pa. A second EBA study was therefore initiated, see below.

Study CL-010 [August 2009-May 2010]
The study tested Pa in doses 50,100,150 and 200 mg, all qd and fasting. Again, EBA outcomes did not differ significantly from each other, but there was a trend toward significance, indicating that the 50 mg dose may have lower EBA than the higher doses taken together, see table below and figure next page. The EBAs yielded with the 200 mg dose in studies C-007 and C-010 were very similar.

Table 11. EBA_{CFU} and EBA_{TTP} by Study Arm and Time Period, study C010

Method	days	Pretomanid							
		n	50 mg	*n*	100 mg	*n*	150 mg	*n*	200 mg
EBA_{CFU}	0-14	12	0.063 (0.06)	15	0.091 (0.07)	14	0.078 (0.07)	14	0.112 (0.07)
	0-2	14	0.093 (0.21)	15	0.111 (0.32)	15	-0.009 (0.29)	14	0.160 (0.25)
	2-14	12	0.059 (0.06)	15	0.088 (0.08)	14	0.096 (0.10)	14	0.104 (0.08)
EBA_{TTP}	0-14	13	2.621 (2.53)	14	4.969 (3.64)	15	4.633 (3.69)	16	4.640 (3.45)
	0-2	15	1.483 (8.15)	14	-1.345 (8.58)	15	4.867 (12.75)	13	3.096 (8.20)
	2-14	13	2.958 (2.65)	15	5.744 (3.97)	15	4.594 (5.03)	13	5.391 (3.61)

Figure 3 Bilinear Regression of Change from Baseline of log_{10}(CFU) (A) and TTP (B)



EBA - PA in combination with other agents (DS TB, HRZE as control)

Study NC-001 [October 2010-August 2011, South Africa)

Dosing and regimens are shown below, HRZE served as control.

Pa: 200 mg days 1-14; B: 700 mg day 1, 500 mg day 2, 400 mg days 3-14

M: 400 mg days 1-14; Z: 1500 mg days 1-14

The study was partially blinded and the microbiological assessments were fully blinded. Outcomes are shown below. The study was not powered to test for statistical differences between the treatment groups with respect to the primary efficacy variable, and results are exploratory.

In summary, Pa-M-Z showed a higher activity than HRZE control. The Pa-M-Z regimen was subsequently tested for 8 weeks in study NC-002, and further on for 16 or 24 weeks in the NC-006 study.

Table 12. EBA_{CFU}(0-14) and EBA_{TTP}(0-14), NC-001

Study Arm	n	Mean (SD) EBA_{CFU}	Mean (SD) EBA_{TTP}
Pa-M-Z	13	0.23 (0.128)	18.5 (22.58)
Pa-Z	14	0.15 (0.040)	8.8 (3.47)
Pa-B	15[a]	0.11 (0.050)	5.9 (2.79)
B	14	0.07 (0.068)	5.4 (3.52)
B-Z	15	0.13 (0.102)	10.0 (6.99)
HRZE	10	0.14 (0.094)	11.8 (3.93)

[a] n=14 for EBA_{CFU}(0-14).

EBA_{CFU} = absolute value of the average daily rate of change of log_{10}(CFU) over the indicated time period; EBA_{TTP} = average daily rate of change in TTP over the indicated time period.

Study NC-002 [March 2012-July 2013 in South Africa, Tanzania and Brazil]

This was an 8-week study; it also contained a 14-day EBA substudy, not summarized by the applicant (data below taken from the CSR). A small group of MDR TB patients was also included, allocated to the

higher dose of Pa. The EBA outcomes in this study are similar to those seen in NC-001, although in this case there is no clear advantage with the 200 mg dose over the 100 mg dose. However, the 8 week outcomes is in favour of the higher dose (see section on 8 weeks efficacy).

Table 13. Mean EBA_{CFU} (0-14), EBA Analysis Population, NC-002

Study Arm	n	Mean (95% CI) EBA_{CFU}
Pa100-M-Z (N=16)	14	0.24 (0.145; 0.328)
Pa200-M-Z (N=13)	10	0.16 (0.047; 0.260)
HRZE (N=15)	15	0.14 (0.022; 0.249)
Pa200MZ – MDR TB (N=18)	6	0.19 (-0.018; 0.403)

Source : CSR Table 14.2/5.3.

Study NC-003 [October 2012-April 2013, South Africa]
This was a 6-armed study, evaluating several regimens, table below. Only DS-TB patients were included.

Pa, B and Z was dosed as in study NC-001 ; clofazimine (C) was dosed 300 mg days 1-3, 100 mg days 4-14.
The change in CFU counts over time was the highest for the B-Pa-Z regimen. If allowing for a cross study comparison, the 14 day bactericidal activity of B-Pa-Z is somewhat lower than with Pa-M-Z (NC-001) or quite similar to this regimen (NC-002, EBA substudy).
- These two regimens were evaluated for 8 weeks (Pa-M-Z in NC-002, and B-Pa-Z in NC-005).

Table 14. EBA_{CFU}(0-14) by Study Arm, NC-003

Study Arm	Mean log(CFU) Reduction at Day 14 [CI]
B-Pa-Z-C (N=14)	0.111 [0.037-0.184]
B-Pa-Z (N=12)	0.167 [0.078-0.256]
B-Pa-C (N=15)	0.076 [0.007-0.147]
B-Z-C (N=14)	0.119 [0.031-0.211]
Z (N=15)	0.037 [-0.025-0.100]
C (N=15)	-0.017 [-0.086-0.055]
HRZE (N=15)	0.151 [0.070-0.231]

Pa:200 mg qd, **B:** 700 mg day 1, 500 mg day 2, 400 mg days 3-14, **M:** 400 mg qd, **Z:** 1500 mg qd

In this study it is noted that pyrazinamide (Z) in practice lack activity in short term therapy. The use of this agent has been a means to shorten relapse free standard treatment in DS-TB patients from 9 months to 6 months, when used as part of this regimen (HRZE) for the first 2 months.

It is also noted that clofazimine in monotherapy did not show any significant EBA, and did not seem to add to the activity of other regimens tested. This issue was evaluated in pre-clinical studies by Ammerman et al (JAC 2016). They explored the activity of clofazimine in a number of studies (activity in vitro and pre-clinical models mimicking EBA-studies), where it was shown that the response to clofazimine was quite delayed, and only seen with higher doses. On the basis of clinical studies in

MDR-TB patients, there is some evidence that the addition of clofazimine is a means of shortening TB therapy, and the agent is presently listed as a preferred agent for the treatment of MDR-TB by the WHO. Clofazimine is not part of regimens further evaluated by the applicant.

No EBA comparison has been made to delamanid, an agent of the same class, and no data on delamanid was summarized in the application. However, EBA studies of delamanid and (now) pretomanid (CL-010) have been assessed by the Agency, and results have also been published (both by Diacon et al, in 2011). Both studies were done in South Africa, had the same principal investigator and samples were analysed at the department of Biomedical Sciences at Stellenbosch University, using identical procedures. In CL-010 (Pa EBA) the 3 higher doses (100-150-200 mg qd) yielded around 1.5 log_{10} reductions in CFU counts/ml sputum (vs -2log_{10} with HRZE control). Delamanid in doses 100-400 mg qd, yielded around 0.5 log_{10} reductions, without a statistical difference between doses, and the activity with HRZE control was similar to that seen in CL-010, tables next page. Hence, the EBA activity of pretomanid is likely substantially higher than for delamanid, even though this was not compared within one study.

Tables 15-16. Delamanid (upper) and pretomanid (lower) EBA studies. Values are mean and (SD)

	Delamanid					
	100 mg (*n* = 11) mean (SD)	200 mg (*n* = 10) mean (SD)	300 mg (*n* = 10) mean (SD)	400 mg (*n* = 11) mean (SD)	All (*n* = 42) mean (SD)	HRZE (*n* = 6) mean (SD)
BL counts (log_{10} cfu/ml)	7.06 (0.40)	6.75 (0.71)	6.72 (0.49)	6.82 (0.65)	6.84 (0.57)	6.36 (0.61)
EBA: mean fall log_{10} cfu/ml per day						
Day 0–2	0.066 (0.165)	0.138 (0.271)	0.023 (0.193)	0.049 (0.205)	0.069 (0.208)	0.553 (0.379)
Day 2–14	0.026 (0.044)	0.038 (0.060)	0.063 (0.096)	0.018 (0.033)	0.035 (0.062)	0.100 (0.156)
Day 0–14	0.026 (0.042)	0.052 (0.045)	0.065 (0.089)	0.020 (0.027)	0.040 (0.056)	0.147 (0.164)

	Pretomanid				HRZE (8)
	50 mg (15)	100 mg (15)	150 mg (15)	200 mg (15)	
BL counts (log_{10} cfu/ml)	6.1 ± 0.6	5.8 ± 0.9	6.0 ± 0.7	6.1 ± 1.2	6.1 ± 1.0
EBA: mean fall log_{10} cfu/ml per day					
Day 0–2	0.093 (0.211)	0.111 (0.332)	0.009 (0.290)	0.160 (0.255)	0.470 (0.316)
Day 2–14	0.059 (0.060)	0.088 (0.085)	0.096 (0.098)	0.104 (0.083)	0.128 (0.070)
Day 0–14	0.063 (0.058)	0.091 (0.073)	0.078 (0.074)	0.112 (0.070)	0.177 (0.042)

Note: Figures for the tables were taken from the publication by Diacon et al.

8 weeks efficacy of PA in combination with other agents

In these studies change in CFU counts and TTP (days 0-56) are still the primary endpoints. Sputum culture conversion at week 8 is a secondary endpoint. Following the 8 weeks test period, patients received treatment according to local standard of care.

Study NC-002 [March 2012-July 2013 in South Africa, Tanzania and Brazil] evaluated the regimens below. In addition to DS TB patients (randomized), a small group of MDR TB patients were included, allocated to therapy with Pa 200 mg + M + Z.

In this study Pa dosed 200 mg + M + Z had a significantly higher bactericidal activity (over 8 weeks) than the HRZE control regimen (not significance for the 100 mg dose regimen).

Table 17. BA_{CFU} (0-56) and BA_{TTP} (0-56), study NC-002

Study Arm	BA_{CFU}(0-56) Posterior Estimate [BCI]	BA_{TTP}(0-56)[a] Posterior Estimate [BCI]
Pa200-M-Z	0.155 [0.133; 0.178] * (N=54)	0.020 [0.016; 0.024] (N=57)
Pa100-M-Z	0.133 [0.109; 0.155] (N=56)	0.020 [0.015; 0.025] (N=55)
Pa200-M-Z (MDR)	0.117 [0.070; 0.174] (N=9)	0.015 [-0.001; 0.031] (N=9)
HRZE	0.112 [0.093; 0.131] (N=54)	0.017 [0.013; 0.021] (N=58)

* p<0.05 vs. HRZE. BCI = 95% Bayesian credible interval

Note: BA_{CFU} and $_{TTP}$(0-56) = absolute value of the average daily rate of change of $\log_{10}$(CFU)/$\log_{10}$ TTP over Days 0 to 56.

In liquid media (MGIT) the proportion of patients who were sputum culture negative at week 8 (secondary endpoint) was significantly higher with the Pa + M + Z regimens (both doses) than with control (not significance with the use of solid media). Similar results were seen in MDR-TB patients (low numbers, descriptive data).

Table 18. Sputum culture conversion at day 57 (Efficacy Analysis Population), NC-002

Study Arm	solid media	liquid media
Pa100-M-Z	29/35 (83)	23/35 (66)
Pa200-M-Z	34/35 (94)	25/35 (71)
Pa200-M-Z (MDR)	5/8 (62)	4/8 (50)
HRZE	28/32 (87.5)	14/37 (38)

Source: Table 14.2/10.1 and /10.2 of the CSR.

The fact that liquid media is more sensitive to grow still remaining mycobacteria (i.e. from lower numbers, and perhaps less active forms) is very clear from the table above.

Study NC-005 [October 2014-January 2018, in South Africa, Tanzania, and Uganda] evaluated Pa (dosed 200 mg) in combination with bedaquiline, where bedaquiline was dosed in two different dosing schedules, plus pyrazinamide, versus HRZE control. A 4th arm included MDR TB patients, allocated to Pa + bedaquiline (dosed 200 mg daily) + moxifloxacin + pyrazinamide. Around 15% of DS TB patients were HIV-positive; in the MDR TB arm this figure was 42%.

DS-TB

Pa200 + **B** according to label + **Z** (N=59)
Pa200 + **B** 200 mg qd + **Z** (N=60)
HRZE (control) (N=61)

MDR-TB

Pa200 + **B** 200 mg qd **+ M** + **Z** (N=60)

The differences between test regimens versus HRZE (control regimen) for the primary end point, TTP in liquid media in overnight samples, were statistically significant, see table below. Differences for this end point between test regimens were not significant. This analysis is based on the mITT population (211/240 randomized patients), where cases of pyrazinamide resistance was excluded; 29 patients were excluded due to resistance to pyrazinamide at/prior to baseline, 7/180 (4%), of randomized DS-TB patients and 22/60 (37%) of randomized MDR-TB patients.

Table 19. Bactericidal activity (BA) days 0-56, mITT population, study NC-005

	Study Arm	**Posterior Estimate [95% BCI]**	
		BA_{TTP}(0-56) [a]	**BA_{CFU}(0-56)**
DS	B(label)-Pa200-Z (n=57)	4.865* [4.308; 5.467]	0.123 [0.109; 0.139]
	B200-Pa200-Z (n=56)	5.168* [4.613; 5.774]	0.109 [0.097; 0.121]
	HRZE (n=59)	4.038 [3.669; 4.423]	0.116 [0.106; 0.127]
MDR	B200-M-Pa+Z-MDR	5.180* [4.598; 5.830] (n=37)	0.156* [0.125; 0.198 (n=38)

The median time to conversion (MGIT) for the B-Pa-M-Z regimen in MDR-TB patients in study NC005 was 41 days (5.8 weeks) in the absence of resistance to pyrazinamide (n=38) and 49 days (7 weeks) in the presence of such resistance (n=22).

The Kaplan-Meier estimate of the *cumulative probability of sputum culture conversion* at Day 56 (liquid media), was presented, and highest for the B200-Pa-Z regimen. The table below shows the frequencies (not Kaplan-Meier approach); the table only includes data for patients without baseline resistance to Z (mITT).

Table 20. Proportion with sputum culture conversion day 57 (based on overnight sputum samples), mITT population, study NC-005

Regimen	**Solid media**	**Liquid media**
B(label)-Pa200-Z (n=57)	39/48 (81)	24/38 (63)
B200-Pa200-Z (n=56)	35/45 (78)	33/45 (73)
HRZE (n=59)	37/45 (82)	18/42 (43)
B200-Pa-M-Z (MDR) (n=38)	25/26 (96)	25/26 (96)

Source: Tables 14.2/1.1.7 and 14.2/4.1.1.7 in the CSR

In summary, in cross study comparison, 8 weeks results in NC-002 and NC-005 seemed rather similar with the Pa-B-Z regimen, and the Pa-M-Z regimen. In the 2 weeks EBA studies the Pa-M-Z regimen perhaps yielded a somewhat more rapid response (NC 001 vs NC 003). The Pa-M-Z regimen was chosen for a phase 3 study of full treatment duration in DS TB patients (NC-006, STAND), where the main objective was to evaluate whether that regimen given for 4 months is non-inferior to standard of care. This study, which failed, is discussed in the section Supportive studies.

Conclusions on EBA results:

- A noticeable EBA was measured during monotherapy with pretomanid at all doses.
- Dose range-finding studies supported a clinical dose of 200 mg of pretomanid per day
- The combinations containing pretomanid that showed the greatest early (over 14 days) bactericidal activity were PaMZ in NC-001 and BPaZ in NC-003
- The PaMZ and BPaZ combinations appeared more efficacious than the standard HRZE therapy for the treatment of DS-TB in 8-week trials
- The BPaMZ combination used to treat MDR-TB patients showed the highest BACFU(0-56) and BATTP(0-56) observed in 8-week trials
- No evidence was found of pretomanid resistance among baseline isolates and no emergence of pretomanid resistance was observed during treatment

Linezolid dose choice for Main study

Linezolid is an oxazolidinone antibacterial drug approved for the treatment of drug-resistant, gram-positive bacterial infections, including Staphylococcus aureus, streptococci, and enterococci. Linezolid is not formally approved for the treatment of TB infection but has been part of treatment for patients with advanced resistance (pre-XDR/XDR-TB) for quite some time, and a recommended agent by the WHO as part of such therapy.

Linezolid is active against MTB in vitro, including against MDR strains, with MICs ranging from 0.125-1 µg/mL (Tato et al, 2006). In the lack of data to indicate what dose of linezolid that is optimal to effectively treat TB infection, the TB Alliance has completed an EBA study, LIN-CL-001. This study (performed Nov 2014 – Nov 2016) explored linezolid dosed 300 mg qd/bid, 600 mg qd/bid and 1200 mg qd and thrice weekly in patients with newly diagnosed DS-TB. HRZE served as control. Change in CFU counts and TTP (both as model-fitted log CFU counts/TTP) over time were primary end points, in line with the other EBA studies presented.

A dose response was seen up to 1200 mg daily dosing, without a significant difference when comparing bid and qd dosing, see table below.

Table 21. Mean linezolod EBA_{TTP} and EBA_{CFU}, LIN-CL-001

	log(TTP)		log(CFU)	
Lin 300 QD [N=14]	2.269	[1.071; 3.535]	0.024	[-0.020; 0.071]
Lin 300 BID [N=15]	3.303	[1.949; 4.663]	0.060	[0.008; 0.114]
Lin 600 QD [N=30]	4.128	[2.943; 5.342]	0.094	[0.060; 0.126]
Lin 600 BID [N=15]	4.071	[2.521; 5.666]	0.072	[0.014; 0.127]
Lin 1200 QD [N=15]	4.458	[3.301; 5.630]	0.104	[0.052; 0.158]
Lin 1200 TIW [N=15]	2.178	[1.101; 3.253]	0.069	[0.034; 0.105]
HRZE [N=8]	6.918	[4.825; 9.141]	0.167	[0.088; 0.245]

Expressed as the daily rate of change in log(CFU) count/log TTP days 0-14. Source: LIN_CL_001 CSR

When looking at pharmacokinetics, Linezolid administered 1200 mg QD as well as 300 and 600 mg BID yielded values above MIC throughout the dosing interval in all participants. This was not achieved with the 300 mg qd and 600 mg qd dosing. It was concluded that linezolid has a concentration-dependent

bactericidal activity against Mycobacterium tuberculosis. The dose chosen for the main study was 600 mg bid (changed to 1200 mg qd following an amendment).

Main study

Nix-TB: A Phase 3 Open-label Trial Assessing the Safety and Efficacy of Bedaquiline Plus Pretomanid Plus Linezolid **(B-Pa-L)** in Subjects with Pulmonary Infection of Either Extensively Drug-resistant Tuberculosis (XDR-TB) or Treatment Intolerant/Non-responsive Multi-Drug Resistant Tuberculosis (MDR-TB).

First Patient Enrolled:	16 April 2015
Date of data cut-off for efficacy presented in this report:	29 March 2019
Expected Last Patient's Last Visit:	13 July 2020

Centres: 3 sites in South Africa (Cape Town, Durban, Johannesburg)

Methods

During the procedure efficacy for all 109 patients up to the time for the primary endpoint (6 months of follow-up post EOT) was provided.

Study Participants

Main entry criteria

Patients with min weight of at least 35 kg, and age ≥ 14 years with pulmonary XDR-TB, or with MDR-TB and not responding to (6 months record)/tolerating the MDR-TB regimen.

A positive culture (fulfilling XDR-TB, or MDR-TB criteria) within 3 months prior to screening was requested, as well as an X-ray picture (within a year prior to Screening) consistent with pulmonary TB.

Patients may have previously been treated for DS/MDR-TB. No prior treatment with pretomanid was allowed, and a maximum of 14 days prior therapy with bedaquiline and/or linezolid, provided that this treatment was discontinued at least 3 days prior to the first trial treatment administration.

Patients co-infected with HIV needed to have a CD4 count of at least 50 cells/uL; for those on HIV therapy, efavirenz (strong inducer) was not allowed. Any NRTIs could be used.

Note: a weight of at least 35 kg and having strong inducer/inhibitors as part of exclusion criteria was implemented as part of amendment 2, when 44 patients already had been included.

Treatments

Patients received orally administered trial treatment as follows:

Bedaquiline according to label + Pretomanid 200 mg QD + Linezolid 1200 mg daily for 24 weeks, if culture positive or reverted to being culture positive Months 4-6 and clinical condition suggestive of ongoing TB infection, treatment could be extended to 9 months. (Note: For the present interim efficacy analysis (N=81) only 1 patient was given prolonged treatment of 39 weeks).

The initial 44 patients were dosed linezolid 600 mg BID. After this Amendment 2 was in place, and the dosing was changed to 1200 mg QD.

Treatment was to be taken with food (within 30 min before or after meal)

Toxicity and dose adjustments

Reduction in linezolid dose, including temporary cessation up to 35 days, was allowed in case of linezolid-specific toxicity. A step down in dose was to proceed from 1200 mg QD to 600 mg QD and then to 300 mg QD. While linezolid was withheld, patients could remain on bedaquiline and pretomanid, provided that they had received linezolid 1200 mg for at least the first 4 weeks and were smear-negative/had trace results.

If drug-related toxicity caused by bedaquiline and/or pretomanid was suspected, the full treatment regimen may have been halted for up to 35 consecutive days. It was not allowed to halt just bedaquiline and pretomanid and continue linezolid as monotherapy.

Objectives

The objective of the NIX-TB study is to evaluate the efficacy and safety and pharmacokinetics of pretomanid + bedaquiline + linezolid given for 6(-9) months for the treatment of XDR-TB (or MDR-TB in those intolerant to the recommended MDR-TB regimen), and to show a cure rate of at least 50%. The reasons for not including a control regimen, and the 50% cure rate as a minimum target was discussed by the applicant, and this is reflected in the end of the efficacy section.

Outcomes/endpoints

Primary end-point: incidence of clinical failure, bacteriologic relapse, or bacteriologic failure (re-infection) through 6 months after the end of treatment.

Secondary endpoints:

- Incidence of bacteriologic failure, or relapse, or clinical failure through follow-up until 24 months after the end of treatment
- Time to sputum culture conversion
- Proportion with sputum culture conversion at 4, 6, 8, 12, 16, and 26 or 39 weeks;
- Exploring Linezolid dosing (actual) and efficacy
- Change from baseline TB symptoms on the basis of a 10 symptom questionnaire
- Change from baseline in the patient-reported health status.
- Change from baseline weight.

Sample size

The study was originally planned as an exploratory study without formal statistical testing and a sample size calculation was therefore not performed.

The study was originally planned to enrol a total of up to 200 patients. However, 109 patients were enrolled in the trial (recruitment completion on 15 November 2017), and another study, the ZeNix study, exploring the BPaL regimen with 4 different dosing regimens of linezolid, was initiated rather than increasing the size of the Nix-TB study.

Statistical methods

Analysis populations

- The **Intent-to-treat (ITT)** analysis population was defined as all patients, excluding late Screening failures. For the ITT population patients who were not proven to have a favourable outcome were classified as having an unfavourable outcome.
- The **Modified intent-to-treat (MITT)** analysis population is defined as the ITT analysis population with extra exclusions:
    - Patients who completed treatment, were lost to follow-up or withdrawn from the trial, their last status being culture negative and their last positive culture result followed by at least 2 negative culture results
    - Women who became pregnant during treatment and stopped their trial treatment
    - Patients who died during treatment from violent or accidental cause (not including suicide)
    - Patients who died during follow-up with no evidence of failure or relapse of their TB
    - Patients re-infected with a new strain after being classified as having culture negative status
    - Patients with only contaminated or missing sputum samples for primary endpoint visit, provided patient had not already been classified as unfavourable, and provided their last positive culture was followed by at least 2 negative cultures.
- The **Per-protocol (PP)** analysis population is defined as the MITT population with extra exclusions.

Primary efficacy analysis
The primary efficacy endpoint was treatment failure, defined as bacteriologic failure or relapse or clinical failure through follow-up until 6 months after the End of Treatment. The probability of treatment failure through follow-up until 6 months after the End of Treatment, as a function of time after assignment of treatment, was analysed using Kaplan-Meier analysis. The binomial proportion for patients with bacteriologic failure was presented.

The proportion of assessable patients with a favourable and unfavourable outcome, with 95% confidence intervals (CIs), was presented. For success, the lower bound of the 95% CI for a favourable outcome had to be >50%.

Adjustment for covariates was not performed. Due to small numbers, no subgroup analyses were conducted and no multiplicity adjustments for alpha were done. The latter was motivated by the study being (initially) exploratory.

Sensitivity analyses
The following sensitivity analyses of the primary endpoint were performed:

- Analyses of the ITT analysis population, MITT analysis population, and PP analysis population including only the XDR-TB patients.
- Analyses of the ITT analysis population, MITT analysis population, and PP analysis population excluding patients who were never culture positive during the baseline period.

Missing data

Patients who dropped out of the trial were included in the analyses. For the ITT population patients who were not proven to have a favourable outcome were classified as having an unfavourable outcome.

Interim analyses

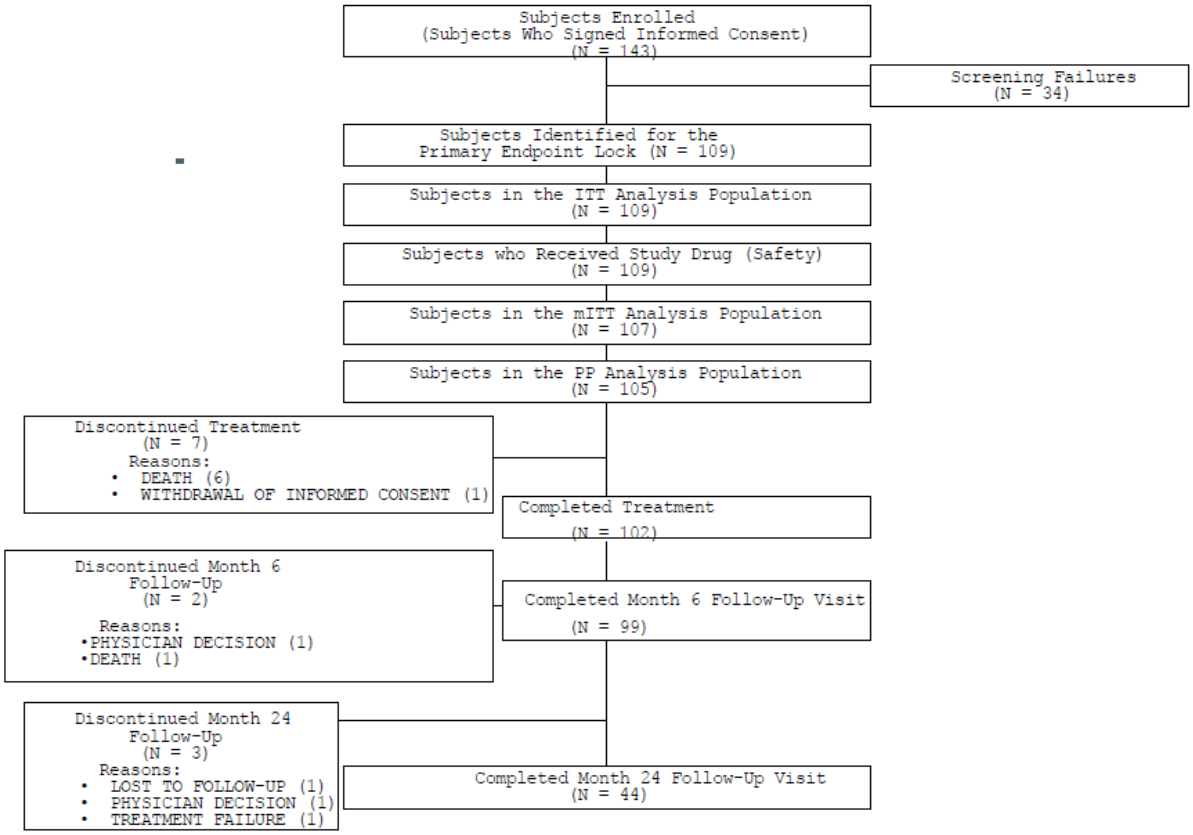
No formal interim analysis for efficacy was planned. However, interim analyses were performed once the first 15, 30, and 45 patients completed the 6-month follow-up after completion of the Treatment Period, withdrew from the trial, were lost to follow-up, or died.

Results

Participant flow

Figure 4

Figure 10-1 Overall Disposition of Patients in the Trial



Recruitment

Conduct of the study

There were 4 amendments with a number of details. The first was in place prior to the inclusion of the first patient. Amendment 2 (22 January 2016, and already mentioned) is considered of highest interest, where linezolid dosing was changed from 600 mg BID to 1200 mg QD based on preliminary data from a linezolid clinical trial that noted similar bactericidal effect on TB when either dosing scheme

was given for 14 days (discussed previously). This amendment also added exclusion of patients who had received more than 2 weeks of bedaquiline or linezolid and added cytochrome P450 3A4 inhibitors and inducers to specific treatment exclusion.

Baseline data

Age ranged from 17 years to 60 years (mean 35.6 years) and the weight from 29 to 112 kg (mean 57). The gender mix was even (48% females). Most patients were black (83/109) or of mixed race (n=25).

Just over half were HIV-positive (56/109). The mean CD4 count (available for 51/56) was around 400 cells µL (range 55-1023). Viral loads were not provided.

TB disease characteristics

Abnormal chest X-ray results compatible with TB were reported for all 109 patients. Around 50% had unilateral cavities, followed by bilateral cavities (41, 38%). Seventeen (16%) patients had no cavities. The chest X-ray and cavity type results were similar by linezolid dosing group (background mentioned previously). The majority of patients had a previous MDR diagnosis as the original type of TB and entered this study with XDR TB.

Table 22. TB disease characteristics, NIX-TB study (N=109)

	600 mg BID (44)	1200 mg QD (65)	All (109)
Original TB classification			
DS	6 (14)	5 (8)	11 (10)
MDR	33 (75)	43 (67)	76 (70)
XDR	5 (11)	16 (25)	21 (19)
MISSING	0	1	1
Months since original TB diagnosis, median (range)	19 (0.5-99)	8 (0.8-141)	12 (0.5-141)
Current TB classification			
XDR-TB	37 (84.1)	34 (52.3)	71 (65.1)
MDR-TB NON-RESPONSIVE	3 (6.8)	16 (24.6)	19 (17.4)
MDR-TB INTOLERANT	4 (9.1)	15 (23.1)	19 (17.4)
Months since original TB diagnosis, median (range)	3 (0.4-90)	3 (0.4-69)	3 (0.4-90)
Days since most recent positive culture, median (range)	66 (16-106)	38 (17-93)	49 (16-106)

The proportion of HIV co-infected was very high, around 50% At baseline, the majority of these patients were treated with lopinavir/ritonavir-based therapy (25/56) or nevirapine-based therapy (17/56); efavirenz based therapy was not allowed (interaction). Eight were untreated fir the HIV-infection. Lopinavir/ritonavir causes an around 2-fold increase of bedaquiline exposure (M2-metabolite not affected) (Brill et al 2017), and also pretomanid exposure is likely increased (CYP inhibition).

Outcomes and estimation

Primary endpoint (MITT)

A favourable outcome was seen in 98/107 patients (92%), and similar for those with XDR and MDR-TB, next table. Hence, the favourable outcome rate is well in line with the outcomes achieved in DS-TB patients treated with standard of care.

Table 23. Outcome ,mITT population, and reasons for unfavorabe outcome (23 March 2019)

Status	Outcome		Total	XDR	TI/NR MDR
	Total Enrolled		109	71	38
	Total Assessable (%)		**107 (98%)**	**70 (99%)**	**37 (97%)**
Favorable	Culture negative status at 6 month post treatment		98	63	35
	Sputum not produced at 6m post treat, but culture neg. earlier		0	0	0
	Total Favorable (% of assessable)		**98 (92%)**	**63 (90%)**	**35 (95%)**
Unfavorable	During treatment	Death (not violent or accidental)	6	5	1
		Lost to follow-up	0	0	0
		Withdrawn (not for failure)	1 [a]	1	0
		Retreated for TB	0	0	0
		Treatment failure (culture confirmed)	0	0	0
	Post treatment	Withdrawn; never culture negative status	0	0	0
		Never culture negative status by 6m post treat	0	0	0
		Withdrawn; Relapse	1 [b]	1	0
		Withdrawn; Relapse (not WGS confirmed)	1 [c]	0	1
		Relapse at 6m post treat	0	0	0
		Death (TB related)	0	0	0
	Total Unfavorable (% of assessable)		**9 (8%)**	**7 (10%)**	**2 (5%)**

Abbreviations: MDR, multidrug-resistant; MITT, modified intent-to-treat; N, number of patients; NR, nonresponsive; tb, tuberculosis; TI, treatment-intolerant; WGS, Whole Genome Sequencing; XDR, extensively drug-resistant.

Eleven patients had unfavourable outcomes in the ITT analysis, summarized in the table below. Seven patients had unfavourable outcomes due to death, with 6 of the deaths occurring during the treatment period and one approximately 6 months after the end of treatment. BL MIC values are not available for all patients, due to negative baseline culture or problems with quality of methods at one lab, discussed previously. However, it is clear that there is no association between baseline MIC values (all 3 drugs evaluated) and outcomes in the NIX-TB study. One patient had an increased baseline MIC for bedaquiline, however, this patient died of other reasons than TB at day 75. Four out of 5 patients without available BL MICs died for reasons that would not be linked to efficacy problems with the regimen. In the 5th case, relapse day 187, the isolate at failure was fully susceptibly to all 3 drugs (data not shown).

Table 24. Unfavourable patients at the primary endpoint (ITT population)

Patient ID	TB type	Baseline MIC (µg/mL)	Reason	Treatment or follow-up
	XDR	B=0.5 L=0.5 Pa=0.12	relapse 167 days post EOT, confirmed by WGS	follow-up
	XDR	B=1.0 L=0.5 Pa=0.25	withdrawal day 133, not for treatment failure.	treatment
	XDR	B=1.0 L=0.5 Pa=0.25	death day34, severe (disseminated) tuberculosis	treatment
	XDR	B=0.12 L=0.5 Pa=0.5	death day50, Upper GI bleeding	treatment
	XDR	N/A	death day54, worsening of tuberculosis	treatment
	XDR	N/A	death day52, acute haemorrhagic pancreatitis and multiorgan failure	treatment
	TI/NR MDR	N/A	Relapse, 187 days post EOT, not confirmed by WGS	follow-up
	XDR	N/A	death185 days post EOT, natural causes	follow-up
	TI/NR MDR	N/A	death day 92, pneumonia	treatment
	XDR	B=2.0 L=0.5 Pa=0.12	death day 75, septic shock secondary to pneumonia	treatment
	TI/NR MDR	B=0.5 L=0.5 Pa=0.12	lost to follow-up, 730 days post EOT	follow-up

Sensitivity Analyses

Patients could enter the study on the basis of a positive culture within 3 months prior to the study; as expected a proportion would have a negative baseline culture (turned out to be around 15%).

When excluding patients who were never culture positive during the baseline period, the outcome is fairly identical (90% favourable outcome), see table below.

Table 25. Outcomes in the mITT population, excluding patients without a positive BL culture

	Total	XDR	TI/NR MDR
Total enrolled	109	71	38
Not positive at baseline	16	9	7
N in analysis	93	62	31
Unassessable	2	1	1
Total Assessable	91	61	30
Favorable	82(90%)	54 (89%)	28 (93%)
Unfavorable	9 (10%)	7 (11%)	2 (7%)
95% CI for Favorable	82-95%	78-95%	78-99%

Selected secondary endpoints

- Time to Sputum Culture Conversion

For the 91 patients of the mITT population with a positive culture at baseline (requested for this analysis) 87 converted to negative with a median time to negative culture (MGIT) was of 6.0 weeks (IQR 4.1 to 8.1), see table below. All but 1 were negative by week 12.

Table 26. Median time (weeks) to negative culture, NIX-TB study

	Total	**XDR**	**TI/NR MDR**
Total in analysis	91	61	30
Time (weeks)	6.0	6.0	5.9
IQR	4.0-8.1	4.1-8.3	3.9-8.1

- Culture Conversion Status at 4, 6, 8, 12, and 16 Weeks

For the assessable patients (n=93) the conversion rate over time was fully similar in patients with XDR vs MDR TB.

- **Relapse (29 March 2019 cut off)**

In total, three cases of relapse (2 cases as part of the present mITT population, n=80) have been recorded at this cut-off date, where all patients had the chance to pass 6 months EOT, and 44 had passed the 24 months follow-up.

- Subject 01-9026-018 3 months post EOT (patient died after relapse, non-TB related)
- Subject 02-9038-031 2 months post EOT
- Subject 02-9006-005 15 months post EOT

The first patient, with advanced HIV co-infection (low CD4 count), fulfilled therapy with reported high compliance, with linezolid dose reductions in second part. For the second patient a low adherence to therapy was suspected. The third case is a very late relapse. WGS is lacking to verify that this is the same strain and not a re-infection, although resistance data suggests a relapse.

Ancillary analyses

The success rate was similar for patients with and without HIV-infection, and the linezolid dosing (600 mg bid vs 1200 mg qd) also yielded similar outcomes, numerically higher for the 1200 mg dose, see tables below.

Table 27. Planned Primary Endpoint Analysis by HIV Status (MITT Population)

	Total	HIV positive	HIV negative
Total enrolled	109	56	53
Total Assessable	107	55	52
Favorable	98 (92%)	50 (91%)	48 (92%)

Table 28. Ad-hoc Primary Endpoint Analysis by Linezolid Dosing at Enrolment (MITT Population)

	Total	1200 mg once daily	600 mg bi-daily
Total Assessable	107	63	44
Favorable	98 (92%)	59 (94%)	39 (89%)
95% CI for Favorable	85% to 96%	85% to 98%	75% to 96%

- **Comparison of Nix-TB outcomes to results obtained in prior XDR/MDR TB studies**

The results obtained in the Nix-TB study may be considered outstanding, and a positive B/R could be inferred in the absence of a control arm; results obtained in prior studies are far lower than those seen here.

In order to provide a background of reference data for comparative assessment of the outcomes observed in the Nix-TB trial, TB Alliance conducted two additional assessments of XDR-TB treatment.

-First, the applicant undertook a comprehensive review of the published outcome data in XDR-TB patients treated with drugs not including any of the Nix-TB study regimen drugs or delamanid. The search (criteria presented in the application) identified 18 studies that met search criteria and that described outcomes that could be mapped to the standardized WHO outcome of treatment success. These studies reported outcomes in 1731 patients. The majority of the patients in these studies came from South Africa (1300 patients from 8 studies), where the Nix-TB study was conducted. Rates of treatment success across the South Africa studies were consistent, averaging 14%, with a range of 2% to 22%. Outside of South Africa, reported rates of treatment success were more varied, ranging from 15% to 60%; two studies reported rates above 50%.

- Second, to evaluate the outcomes in historical comparator groups more closely matched to the Nix-TB study population, analyses were undertaken of two comprehensive datasets of XDR-TB patients treated with a) drug regimens not including bedaquiline, linezolid, or delamanid (N=204) and b) with a more contemporary regimen of bedaquiline + linezolid + other agents not including Pretomanid were done (N=102, of whom 82% had linezolid as part of the regimen). These data sets were provided by the Head of the Division of Pulmonology at the University of Cape Town, (South Africa), and concerns patients admitted between 2008-2014, and 2013—2016, respectively. The proportion with a favourable outcome in the first cohort was 13.4%. and in the second around 65% (here counted achieving cure or treatment completion), similar in those with and without linezolid. In the latter cohort patients were treated with a median of 6 TB drugs in addition to bedaquiline and linezolid; death rate was 19/102.

Details as follows:

The Nix-TB population included in this comparative analysis consisted of all 109 patients enrolled into the trial; these patients were enrolled between April 2015 and November 2017, thus substantially overlapping in time with the control cohort. Both the Nix-TB and the control population were all from South Africa, treated in a hospital setting, and 19 died. There was one potentially significant difference in the baseline characteristics. All the patients in the cohort were diagnosed with XDR-TB, while patients in Nix-TB had XDR or TI/NR MDR-TB.

All 102 control patients received a bedaquiline-based regimen which contained a median of 8 drugs. Eighty-two percent also received linezolid. The control patients received a mean of 16.5 months of

treatment. All Nix-TB trial patients received 6 months of treatment with the investigational regimen except for the patients who died during treatment, one who withdrew during treatment, and 2 who extended treatment to 9 months of therapy.

Table 29 Key differences in the treatment regimen, Nix-TB Study vs. Control

Treatment Regimen	Total treatment duration	Regimen
Control (N = 102)	mean 16.5 months	B + L* + median of 6 other drugs
Nix-TB (N = 109)	6 months	B + L + Pa

* L was part of the regimen in 82% of the control population
B = bedaquiline; L = linezolid; Pa = pretomanid

The criteria for the timing of assessment of the primary outcome and the criteria for being categorized as favourable or unfavourable for the two cohorts is detailed below in table 30.

Table 30 Outcome Assessments, Contemporaneous control and Nix-TB cohorts

	Control Cohort	Nix-TB Cohort
Timing of primary outcome assessment	24 months after start of treatment	6 months after completion of treatment
Criteria for favourable outcome	Patients who achieved cure or treatment completion	Patients with a negative culture status at 6 months from end of therapy who had not already been classified as having an unfavorable outcome (such as death), and whose last positive culture result was followed by at least two negative culture results
Criteria for unfavourable outcome	Patients who died, who had treatment failure (Treatment terminated or need for permanent regimen change of at least 2 anti-TB drugs), defaulted (did not comply with therapy), or were lost to follow-up	Patients who died, who had a relapse during the primary follow-up within 6 months after treatment completion (defined for this analysis as "failed treatment"), or who were lost to follow up

Results

The two populations were similar in baseline characteristics (Table 31).

Table 31 Demographics and baseline characteristics, Comparison between Nix-TB and contemporaneous controls

		Nix-TB	-Control	p-value	Standardized differences
N		109	102		
Sex				0.123	0.233
Female	n (%)	52 (47.7%)	37 (36.3%)		
Male	n (%)	57 (52.3%)	65 (63.7%)		
Age at treatment	Mean (SD)	35.6 (10.1)	36.8 (11.2)	0.406	0.115
Weight (kg)	Mean (SD)	56.9 (15.0)	53.1 (10.0)	0.031	0.302
HIV status:				1.000	0.008
Negative	n (%)	53 (48.6%)	50 (49.0%)		
Positive	n (%)	56 (51.4%)	52 (51.0%)		
Linezolid				N/A	N/A
No	n (%)	0 (0%)	18 (17.6%)		
Yes	n (%)	109 (100%)	84 (82.4%)		

Data are presented as means (standard deviations) for continuous variables and numbers and percents for categorical variables. P-values are derived from chi-square tests and Student T-test with equal variance assumption for categorical and continuous variables respectively. Standardized differences are the differences in means for continuous variables (or difference in proportions for dichotomous variables) between the two groups divided by the pooled standard deviation.

Patients outcomes were as follows:

Controls:

- Favourable outcome - (n=66)
- Unfavourable Outcomes - Death (n=19), Default (n=1), Treatment Failure (n=5) or Lost to Follow-Up (n=11)

Nix-TB:

- Favourable outcome – (n=98)
- Unfavourable Outcomes –Death (n=7), Relapse (n=2), Withdrawn (n=1), or Lost to follow-up (n=1)

The proportion of favourable outcomes in the two populations was different; 89.9% of the Nix-TB population had favourable outcomes compared to 64.7% of the control population (Table 36), which yielded a significant estimated risk ratio of 1.39 (1.19 – 1.62, 95% CI) of having a favourable outcome in the Nix-TB population compared with the control population (Table 32).

Table 32 Frequency and Proportions for the 2 by 2 Table of Nix-TB and Control Populations by Outcome

	Outcome (Favorable)		
	No	**Yes**	**Total**
Control	36 (35.3%)	66 (64.7%)	102
Nix-TB	11 (10.1%)	98 (89.9%)	109

No = unfavorable, Yes = favorable

Table 33 Comparison between Outcomes in the Nix-TB and Control Populations

Risk ratio (Nix-TB/Control)	**Estimate (95% CI)**	**p-value**
Unadjusted	1.39 (1.19 – 1.62)	<0.0001
Adjusted	1.36 (1.16 – 1.59)	0.0002

Abbreviations: CI = confidence interval, p-value derived from the likelihood ratio chi-square test

Controls: Subjects receiving both bedaquiline and linezolid

Similar results were obtained in the sensitivity analysis comparing the outcomes in the same populations for all participants with linezolid in addition to bedaquiline and a background regimen.

The proportion of favourable outcomes in these subsamples was also different; in the Nix-TB population subsample, 89.9% had favourable outcomes compared to 66.7% in the control population subsample. This yielded a significant (P = 0.0003) estimated risk ratio of 1.35 (1.14 – 1.59, 95% CI) of having a favourable outcome in the Nix-TB compared with the control population.

Matched Controls

A final analysis compared the two population subsamples individually matched by sex, age, body weight, and HIV status at baseline. The outcomes in Nix-TB were very similar between the patients with XDR-TB and TI/NR-MDR-TB and thus matching was not done by type of TB infection. To perform the matching, a propensity score methodology was applied (Austin 2011). The propensity score matching method was selected because different types of covariates (dichotomous and continuous) were used to match subjects. The results of the matching procedure were effective as there was little difference between the two populations.

The proportion of favourable outcomes in this matched sample was also different; in the Nix-TB population subsample, 90.0% had favourable outcomes compared to 63.3% in the control population subsample. This yielded a significant estimated risk ratio of 1.42 (1.20 – 1.69, 95% CI, P<0.0001) and significant odds ratio of 4.00 (1.84 – 8.68, 95% CI, P=0.0005) of having a favourable outcome in the Nix-TB compared with the control population.

In addition to the analyses provided by the applicant, the Nix-TB results could also be compared to those generated in the C209 study, part of the MAA of bedaquiline (Sirturo). That study included MDR- and XDR-TB patients (single arm), and patients received bedaquiline for 6 months on top of an optimized background regimen given for a minimum of 18 months. Participants were recruited in 11

countries in Asia, Eastern Europe plus in Peru and South Africa. The proportion of patients with no lung cavities were higher in the C209 study (34% vs 16%; bilateral cavities at baseline were seen in 12% of patients in C209 vs 38% in the Nix-TB study). Further, HIV co-infection was much less common in C209 (4% vs 50% in the Nix-TB study), (Pym et al, 2016). At study completion, 125/205 mITT patients (61.0%) were "cured", 63 (31%) had failed treatment or defaulted/transferred out and 14 (7%) had died. In summary, the B-Pa-L, given for 6 months to patients that likley had a more severe disease status (including a 50% HIV prevalence, many with a low baseline CD4 count), in a poor setting, yielded a markedly higher proportion of favourable outcome than did Bedaquline-based prior therapy given for a minimum of 18 months, in many cases in combination with linezolid as part of the background regimen.

Supportive study

The Pa phase 2 dose ranging studies of 8 weeks duration were summarized at the start of the efficacy section. This section concerns the **NC-006 study** [or **STAND**, Feb 2015-Nov 2017] which was conducted in Georgia, Kenya, Malaysia, Philippines, South Africa, Thailand, Uganda, Ukraine, and Tanzania (vast majority recruited in Africa, mainly South Africa)

The study, non-blinded, evaluated 3 Pa-containing regimens vs control in patients with pulmonary tuberculosis; MDR-TB patients were allocated to a specified regimen of 6 months (no control).

The plan was to include 1500 patients (1200 with DS TB and 300 with MDR-TB). However, the study was put on partial hold by the FDA in 2015, due to a potential signal for liver toxicity. When this safety hold was lifted in August 2017, the TB Alliance chose not to re-open the study. Already enrolled patients (n=284) were followed to the trial end points; the study is therefore significantly underpowered.

DS-TB patients were randomized to receive:

- Pa 100 mg + M 400 + Z 1500 for 4 months (N=65), or
- Pa 200 mg + M 400 + Z 1500 for 4 months (N=71), or
- Pa 200 mg + M 400 + Z 1500 for 6 months (N=67), or
- HRZE/HR, standard-of-care regimen for 6 months (N=68).

MDR-TB patients: $MPa_{200}Z$ regimen for 6 months (N=13).

The primary objectives were to evaluate the efficacy, safety, and tolerability of the 3 test regimens to that seen with the control regimen. Pre-specified hierarchy for ordering of analyses was as follows: the high dose regimen for 6 months vs control, followed by high dose 4 months and low dose 4 months vs control.

The secondary objective was to evaluate the outcomes of the 6 months MPa200Z regimen in patients with MDR-TB versus in patients with DS-TB.

In this study around 70% of subjects were black, around 15% of mixed race (around 7% Asian and 5% white). Median BMI was around 19. Overall, around 25% were HIV-positive (even proportions between randomized arms).

The primary efficacy endpoint was the incidence of bacteriologic failure or relapse or clinical failure within 12 months from start of therapy in the DS TB group (non-inferiority to control, with an NI margin of 12%).

For the MITT population, 13% of patients were unfavorable in the control group vs 23% in the $6MPa_{200}Z$ group, showing a difference of 9.88% (upper bound of the 95% CI for this difference =23.89%). Hence, non-inferiority of the test regimen (first in hierarchy) versus control was not achieved. Further analyses were not performed. Results in the PP population as well as in sensitivity analyses including analysis of all randomized and ITT populations were consistent with the overall findings.

Although the NC-006 study was underpowered as a result of the safety hold, the outcomes obtained with the 4 months regimens indicate a very high risk of relapse (9 + 2=11 out of 57 and 6 +1=7 out of 46 assessable patients, respectively), see table below. The failed outcome with the test regimen given for 6 months was mainly driven by withdrawals during treatment, and the relapse rate in this arm was around 3%.

Table 34. Outcomes for randomized regimens (DS-TB) in NC-006 (mITT population)

Outcomes		2HRZE/4HR	4MPa100Z	4MPa200Z	6MPa200Z	Total
Total Randomized		68	65	71	67	271
Total Assessable (% of N randomized)		60 (88%)	57 (88%)	61 (86%)	56 (84%)	234 (86%)
Culture negative at 12 months		52	38	46	43	179
Total Favourable (% of assessable)		52 (87%)	38 (67%)	46 (75%)	43 (77%)	179 (77%)
During treatment	Death (not violent/accidental)	0	1	1	2	4
	LTFU or withdrawn	0	1	0	0	1
	Withdrawal, not treatment failure	7	4	4	9	24
	Retreated for TB	0	0	1	0	1
	Treatment failure	1	0	1	0	2
Post treatment	Withdrawal (culture positive last visit)	0	9	6	1	16
	Withdrawal, clinical deterioration	0	1	0	0	1
	Confirmed relapse	0	2	1	1	4
	Death (TB related)	0	1	1	0	2
Total unfavourable.		8 (13%)	19 (33%)	15 (25%)	13 (23%)	55 (24%)

The median time to culture negative status was fairly similar for the test regimens and control in this study (somewhat longer with control), next table

Table 35. Median Time to Culture Negative Status (MITT Population)

	2HRZE/4HR	4MPa100Z	4MPa200Z	6MPa200Z	Total	MDR*
Total in analysis	60	57	61	56	234	11
Time (weeks)	8.0	7.9	7.0	7.4	7.9	4.9
IQR	6.9 to 16.9	5.9 to 11.9	4.9 to 11.9	5.9 to 11.9	5.9 to 12.0	4.9 to 12.0

NC-006 is a failed study, and, despite being under-powered, clearly indicate that the Pa/M/Z regimen is not an option in the attempt to shorten treatment duration in DS TB, despite the more rapid early bacterial clearance with this regimen as compared to standard HRZE therapy.

High (unacceptable) relapse rates when shortening therapy to 4 months were seen in two fairly recent large scale studies (Gillespie et al, and Merle et al; both studies published in NEJM Oct 2014). Moxifloxacin and gatifloxacin was added to the common DS TB regimen, and despite the fact that early bacterial clearance was more rapid, non-inferiority to HRZE control given for 6 months was not achieved. Relapse rates of 10-15% were seen with the shorter regimens. Clearly, it is hard to predict final outcomes by early response.

Despite the safety issues in study NC-006, the same regimen, with the addition of bedaquiline (B-Pa-M-Z) is being evaluated in a more recent, ongoing study (Simplici-TB or NC-008), where 4 months treatment is again tested for patients with DS-TB, and 6 months treatment is tested in patients with MDR-TB.

Ongoing trial

ZeNix Trial

The ongoing Phase 3 linezolid optimization study of the BPaL regimen, ZeNix (also referred to as NC-007) was designed to optimize linezolid dosing with the aim of potentially limiting toxicity while preserving efficacy. The ZeNix trial is a Phase 3 partially-blinded, randomized trial assessing the safety and efficacy of various doses and treatment durations of linezolid plus bedaquiline and pretomanid in participants with pulmonary infection of either extensively drug resistant tuberculosis (XDR-TB), pre-XDR-TB or treatment intolerant or nonresponsive multi-drug resistant tuberculosis (MDR-TB). This 4-arm study randomizes subjects to varying linezolid doses (1200 or 600 mg once daily [QD]) and varying lengths of linezolid treatment (2 or 6 months). A total enrolment of 180 XDR-TB, pre-XDR-TB, or TI/NR MDR-TB subjects is planned; 45 of those patients will receive BPaL with the linezolid dose and duration the same as in Nix-TB.

The preliminary blinded data shared with the CHMP on-treatment efficacy (N=137) with the B-Pa-L regimen for the same treatment population in the ZeNix study (XDR-TB/intolerant or unresponsive MDR-TB) provides data supporting Nix-TB results.

Summary of main study

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 36. Summary of Efficacy for trial Nix-TB

Title: A Phase 3, Open-label Trial Assessing the Safety and Efficacy of Bedaquiline Plus Pretomanid 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)			
Study identifier	Nix-TB		
Design	Ongoing Phase 3, open-label, single treatment group, with interim analysis and report on all 109 enrolled patients followed to the primary endpoint		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		26 weeks treatment (option for 9 months if culture positive at 4 months), with 6 months follow up after treatment completion to primary endpoint Screening period up to 9 days Patients followed to secondary endpoint 24 months after treatment completion
Hypothesis	The primary objective is to evaluate the efficacy, safety, tolerability and pharmacokinetics of bedaquiline plus pretomanid plus linezolid after 6 months of treatment (option for 9 months for subjects who remain culture positive or revert to being culture positive between month 4 and month 6 visits) in Subjects with either pulmonary XDR tuberculosis, treatment intolerant or non-responsive multi-drug resistant tuberculosis (MDR-TB). For success, the lower bound of the 95% confidence interval of a favourable outcome should be above 50%.		
Treatments groups	Bedaquiline / Pretomanid / Linezolid		Bedaquiline 400 mg orally once daily D1-14, then 200 mg 3 times/week for 26 weeks (option for 9 months if culture positive at 4 months), plus Pretomanid 200 mg orally once daily for 26 weeks (option for 9 months if culture positive at 4 months), plus Linezolid at a starting dose of 1200 mg orally daily for 26 weeks (option for 9 months if culture positive at 4 months) 109 patients enrolled
	Primary endpoint	Unfavourable rate	Incidence of bacteriologic failure or relapse or clinical failure through follow up until 6 months after the end of treatment.
	Key Secondary endpoints		Incidence of bacteriologic failure or relapse or clinical failure through follow up until 24 months after the end of treatment as a confirmatory analysis. Time to sputum culture conversion to negative status through the treatment period.
Database lock	Interim data cutoff of 29 March 2019		
Results and Analysis			

Analysis description	**Primary Analysis**			
Analysis population and time point description	The primary analysis was done with the intention-to-Treat (ITT) analysis population that comprises all subjects who were assigned study treatment.			
Descriptive statistics and estimate variability	**Population**	**Total**	**XDR-TB**	**TI/NR-MDR TB**
	Number of subjects	109	71	38
	Favourable rate (6 months after end of treatment) 95% Confidence Interval]	90% 83%-95%	89% 79%-95%	92% 79%-98%
	Favourable rate (24 months after end of treatment 95% Confidence Interval	N=41 / 47 87% (74% - 95%)	N=34 / 40 (85%) (70%-94%)	N=0 / 7 (100%) (59%-100%)
	Time to sputum culture conversion (N=91) Interquartile range	6.0 weeks 4.0–8.1 weeks	6.0 weeks 4.1-8.3 weeks	5.9 weeks 3.9-8.1 weeks
Notes	The study met the success criterion requiring the lower bound of the 95% confidence interval of a favourable outcome to be above 50%.			

2.5.2. Discussion on clinical efficacy

Pretomanid has been evaluated in monotherapy (14 days EBA studies) in doses ranging from 50 mg qd to 1200 mg qd, where maximum effect was reached with a dose of 200 mg qd. The effect with that dose seemed higher than that obtained with 50 mg, while the difference versus results achieved with 100 and 150 mg qd was minor. All pretomanid doses in these studies were administered in the fasting condition, whereas in most of the Phase II-III studies, including Nix-TB, pretomanid was administered under fed conditions. Because fed status is the major determinant for the dose-exposure relationship (2-4 times higher exposure with food), the interpretation of dose-response results across studies is complicated, and it is therefore essential to consider actual plasma exposure as part of the evaluation.

During 8 weeks of therapy, combining pretomanid and moxifloxacin + pyrazinamide (Pa-M-Z), the effect with the 200 mg dose was again somewhat higher (NC-002 study) than that yielded with 100 mg in DS TB patients, without apparent differences in safety, and this dose of pretomanid has been chosen for the further studies in MDR/XDR TB patients. It should be noted that the systemic exposure for both the 100 mg (mean AUCss 39.5 µg*hr/mL) and 200 mg (mean AUCss 80.0 µg*hr/mL) cohort in NC-002 was higher than the systemic exposure at 200 mg (30.9-37.9 µg*h/mL) in the 14-day EBA studies.

The clinically proposed dose of 200 mg qd is not strongly supported by the data. Post-hoc exposure-response analyses were performed, but no reliable relationship could be characterised. A lower target exposure threshold for efficacy could therefore not be derived. However, from the available data, it does appear that 200 mg QD (with food) would allow plateau effect to be achieved for the majority of patients.

Several regimens have been evaluated. In the NC-005 study, bedaquiline was added to the Pa-M-Z regimen (B-Pa-M-Z) and evaluated for 8 weeks in MDR-TB patients. The early bactericidal activity of this regimen was very promising, which is presently studied in an ongoing study (SimpliciTB); for 4 months vs 6 months of standard therapy in DS TB patients, and for 6 months in MDR-TB patients (results not presented as part of this application). Of note, the Pa-M-Z regimen (without bedaquiline) has been evaluated in a phase 3 study in DS TB patients, where 4 months of such therapy was associated with a high relapse rate; this regimen is not further studied.

The regimen for which licensure is sought concerns pretomanid in combination with bedaquiline and linezolid (B-Pa-L), aimed for XDR-TB patients or MDR-TB patients not responding/being intolerant to standard MDR therapy. While this regimen was never studied and compared to the other regimens in EBA studies or 8 weeks studies, pre-clinical studies are supportive of the combination. With regards to the contribution of the individual agents, the following may be summarized:

All three agents have potent in vitro activity to MTB (DS-TB as well as XDR-TB strains). In the murine model, the B-Pa-L regimen showed substantially higher effects than did any 2-drug combination constructed of the 3 agents (B-L, B-Pa, Pa-L). Clinically, the effect size per se in the Nix-TB study is considered to verify a contribution of all 3 agents. The highest cure rates so far presented in XDR-TB patients (70-80% favourable outcomes in smaller cohorts) were generated with regimens consisting of bedaquiline plus linezolid in combination with a large number of other agents (regimens typically consisting of 10 agents), and with treatment durations of 2 years; to be compared to the 90% success rate with the B-Pa-L regimen given for 6 months. Efficacy (and safety) wise, the issue is not the contribution of pretomanid to the efficacy, but rather the time and dose of linezolid needed, which is being studied in the ongoing ZeNix study (below).

The pivotal study (Nix-TB) is a standalone single arm study of 6 months duration. The study included 109 patients (78 XDR-TB patients, 31 MDR TB patients), and results covering 6 months of follow-up post end of treatment (primary end point), with a favourable outcome of 92% in the mITT population (90% in ITT) must be considered as outstanding. Results are in line with the cure rate seen in trials of DS-TB patients treated with standard of care, and considerably higher than previously reported in studies of XDR/MDR-TB patients. The relapse rate at this time point is low (3/107), where all patients had a chance to pass 6 months of follow-up EOT, and 44 have passed 24 months of follow-up.

Additional efficacy data needed in the context of a conditional MA

As discussed, the dose of pretomanid (200 mg qd) used in the Nix-TB, and other ongoing studies, is supported on the basis of EBA/8 weeks studies. The dose of bedaquiline used in the Nix-TB study is the one approved according to Sirturo labelling. The dose chosen for linezolid (1200 mg qd) is supported by outcomes in a specific EBA-study, exploring doses from 300 to 1200 mg per day, as well as pharmacokinetics where this dose results in Cmin values that are well above MIC values in MTB. There are indications that linezolid, which has an unfavourable safety profile, may be given at a lower dose and/or for shorter duration, without a negative impact on efficacy; this is presently studied in a large ongoing trial in a similar patient population (the ZeNix study). The BPaL regimens in the ZeNix study differ from that of the pivotal Nix-TB study in 3 of 4 study arms, and the ZeNix trial reflects the sponsor's commitment to attempt to improve the benefit-risk ratio for pretomanid in combination with bedaquiline and linezolid in the treatment of TI/NR MDR and XDR through further optimization of linezolid dosing in the BPaL regimen. The sponsor has furthermore committed to provide the final results of the NixTB trial as well as the results of the ZeNix trial to confirm the outcome of the NixTB trial reported so far.

2.5.3. Conclusions on the clinical efficacy

The efficacy outcomes in the pivotal study (Nix-TB), generated with pretomanid 200 mg qd, in combination with bedaquiline dosed according to label and linezolid dosed 1200 mg qd, all for 6 months, are considered outstanding, where 98/109 (90%) of the ITT population (98/107 (92%) of mITT population) have a negative sputum culture at 6 months past end of treatment. The risk for relapse is obviously low. It is acknowledged that the data are based on a single arm study. However, results are consistent for all relevant subgroups of patients within the study, with fully similar results for the patients with XDR-TB and MDR-TB, for patients with and without HIV-co-infection. Further, preliminary blinded data shared with the CHMP on-treatment efficacy (N=137) with the B-Pa-L regimen for the same treatment population in the ZeNix study (XDR-TB/intolerant or unresponsive MDR-TB) provides data supporting Nix-TB results. In summary, the efficacy data with the B-Pa-L regimen are considered outstanding.

The CHMP considers the following measures necessary to address the missing efficacy data in the context of a conditional MA:

To confirm the outcome of the NixTB trial and to evaluate efficacy (and safety /tolerability) of various doses and durations of linezolid plus bedaquiline and Pretomanid after 26 weeks of treatment in participants with either pulmonary XDR-TB, pre-XDR-TB, or treatment intolerant or non-responsive MDR-TB the results of the ZeNix trial will be provided. Final reporting by Q42022.

In addition, in order to confirm the efficacy of pretomanid the marketing authorisation holder will complete and submit results from the ongoing Nix-TB.

2.6. Clinical safety

Organization of Safety Database

An integrated safety database was constructed from the individual study databases (19 studies, for details see intro to Efficacy section: three phase 3 studies, 6 phase 2 studies and 10 phase 1 studies. The TB Alliance conducted 17/19 studies while two phase 1 studies were conducted by the National Institutes of Health.

The integrated data for the 2 ongoing phase 3 studies, Nix-TB and ZeNix are currently for 109 and 15 subjects, respectively (26 March 2018). This data cut-off date is the basis for all integrated safety results. Analysis groups of this data base are based on treatment/regimens given, as shown, followed by data on pretomanid exposure within clinical program.

Table 37. Analysis Groups for Pretomanid Integrated Summary of Safety

Analysis Group	Included Studies	Included Populations	Included Pa-Containing Regimens
Pivotal Study for Safety Analysis			
Nix-TB	Pivotal phase 3 study Nix-TB (not pooled; interim [1] data)	XDR-TB or TI/NR MDR-TB	BPaL
Supporting Study for Safety Analysis			
ZeNix	Supporting phase 3 study ZeNix (ZeNix; not pooled; partially blinded interim[1] data)	XDR-TB, pre-XDR-TB, or TI/NR MDR-TB	BPaL
Supporting Analysis Pooling Groups			
MDR-TB pooling group	Phase 3 study NC-006 Phase 2 studies: NC-002 NC-005	MDR-TB	PaMZ, BPaMZ No HRZE control arm
DS-TB pooling group for combination studies	Phase 3 study NC-006 arm Phase 2 studies or arms: NC-001, NC-002 NC-003, NC-005 CL-007, CL-010	DS-TB	BPa, BPaC, BPaZ, BPaZC, PaMZ, PaZ, pretomanid alone (different doses) HRZE control arm
Phase 2 pretomanid-alone pooling group	Phase 2A studies: CL-010 CL-007	DS-TB	Pretomanid alone (different doses) HRZE control arm
Phase 1 pooling group	Phase 1 studies: CL-001, CL-002 CL-003, CL-004 CL-005, CL-006 CL-008, CL-009 A5306, DMID-10-0058	Healthy subjects	Pretomanid (different doses and conditions)

[1] 26 March 2018

Note: Main baseline demographics by pooling group are provided below.

Patient exposure

The table is not showing exposure by dose of pretomanid, which can be summarized as follows: the number of subjects who received pretomanid for 6 months (the minimum duration for the sought indication) is limited to around 150 subjects (all treated with the 200 mg dose). When adding those who were treated for at least 3 months the number adds up to around 300 subjects. The group who received 3-6 months treatment are mainly those treated with Pa – M – Z for 4 or 6 months as part of the NC-006 study (DS TB). Of those treated for < 3 months (n=874), around 250 subjects were treated for 8 weeks as part of the NC-002 and NC-005 studies (the majority with the 200 mg dose).

Table 38. Overall Exposure by Exposure Category (Population: Safety - All Studies)

Tuberculosis Diagnosis Duration of Exposure	Pretomanid Alone (N = 411)	Pretomanid Combination Therapy [1] (N = 633)	BPaL [2] (N = 124)	Comparator/ Control [3] (N = 339)	Total Pretomanid (N = 1168)
All Subjects					
< 3 months	411 (100.0)	443 (70.0)	20 (16.1)	277 (81.7)	874 (74.8)
3 – <6 months	0 (0.0)	131 (20.7)	7 (5.6)	5 (1.5)	138 (11.8)
>= 6 months	0 (0.0)	59 (9.3)	97 (78.2)	57 (16.8)	156 (13.4)
MDR/XDR-TB					
< 3 months	0 (0.0)	88 (13.9)	20 (16.1)	0 (0.0)	108 (9.2)
3 – <6 months	0 (0.0)	2 (0.3)	7 (5.6)	0 (0.0)	9 (0.8)
>= 6 months	0 (0.0)	9 (1.4)	97 (78.2)	0 (0.0)	106 (9.1)
DS-TB					
< 3 months	122 (29.7)	355 (56.1)	0 (0.0)	242 (71.4)	477 (40.8)
3 – <6 months	0 (0.0)	129 (20.4)	0 (0.0)	5 (1.5)	129 (11.0)
>= 6 months	0 (0.0)	50 (7.9)	0 (0.0)	57 (16.8)	50 (4.3)
Healthy Subjects					
< 3 months	289 (70.3)	0 (0.0)	0 (0.0)	35 (10.3)	289 (24.7)
3 – <6 months	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
>= 6 months	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

DS-TB = drug-susceptible tuberculosis; HRZE = isoniazid, rifampicin, pyrazinamide, and ethambutol regimen
[1] Includes pretomanid with any combination of drugs except bedaquiline and linezolid (BPaL), which is summarized separately.
[2] BPaL = bedaquiline + pretomanid + linezolid.
[3] The main comparator numberwise is standard of care - HRZE (for details, please refer to the efficacy section) Note: Pretomanid dose is not reflected in this table.

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Although limited, the safety data base is considered sufficient for the evaluation of safety in the context of the MAA, having in mind the patient population for the sought indication, and what other alternatives they have. Of note, two fairly large studies are ongoing (NC007 and NC-008) where some 500 patients will be treated with a pretomanid-containing regimen (pretomanid dosed 200 mg) for 4-6 months

Table 39. Overview of Subject Demographics (Population: Safety - All Studies)

Characteristic	Pretomanid Alone (N = 411)	Pretomanid Combination Therapy (N = 633)	BPaL (N = 124)	Control (N = 339)	Total Pretomanid (N = 1168)	Total (N = 1507)
Mean age	30.1	33.8	36.9	31.6	32.8	32.5
Male sex	248 (60.3)	444 (70.1)	72 (58.1)	234 (69.0)	764 (65.4)	998 (66.2)
Race						
White	197 (47.9)	13 (2.1)	9 (7.3)	31 (9.1)	219 (18.8)	250 (16.6)
Black	134 (32.6)	436 (68.9)	90 (72.6)	198 (58.4)	660 (56.5)	858 (56.9)
Asian	3 (0.7)	15 (2.4)	0 (0.0)	5 (1.5)	18 (1.5)	23 (1.5)
Other	77 (18.7)	169 (26.7)	25 (20.2)	105 (31.0)	271 (23.2)	376 (25.0)
Region						
Africa	122 (29.7)	595 (94.0)	116 (93.5)	294 (86.7)	833 (71.3)	1127 (74.8)
Asia	0 (0.0)	29 (4.6)	8 (6.5)	7 (2.1)	37 (3.2)	44 (2.9)
Europe (Ukraine)	0 (0.0)	9 (1.4)	0 (0.0)	3 (0.9)	9 (0.8)	12 (0.8)
US	289 (70.3)	0 (0.0)	0 (0.0)	35 (10.3)	289 (24.7)	324 (21.5)
Mean weight	69.15	54.81	56.95	56.24	60.12 (	59.24

Characteristic	Pretomanid Alone (N = 411)	Pretomanid Combination Therapy (N = 633)	BPaL (N = 124)	Control (N = 339)	Total Pretomanid (N = 1168)	Total (N = 1507)
Mean BMI	23.49	19.68	20.60	19.87	21.12	20.84
HIV Status						
Positive	14 (3.4)	135 (21.3)	58 (46.8)	50 (14.7)	207 (17.7)	257 (17.1)
Negative	107 (26.0)	496 (78.4)	66 (53.2)	253 (74.6)	669 (57.3)	922 (61.2)
Unknown	290 (70.6)	2 (0.3)	0 (0.0)	36 (10.6)	292 (25.0)	328 (21.8)

Nix-TB study – the B-Pa-L regimen

The combination of B-Pa-L was used in the pivotal study and the application concerns an approval of pretomanid as part of this specific regimen. The safety results in the Nix-TB should be viewed, firstly with the known toxicities of B and L in mind, and secondly with the safety (and efficacy) of present alternative (non-Pa containing) regimens for XDR TB patients (or MDR TB patients who cannot tolerate or do not respond to recommended MDR TB regimens) in mind.

The main established ADRs of bedaquiline is liver reactions (mainly asymptomatic increases of transaminases typically seen after a number of weeks of therapy) and QT-prolongation (again with a slow onset in study C208, reaching a maximum of around 15 msec after 12-16 weeks of treatment). Pancreatitis is a potential safety issue on the basis of pre-clinical findings, but clinical cases were not seen in the C208 study and were not reported in bedaquiline PSURs so far.

Linezolid has a mitochondrial toxicity which has been studied in some detail (reference e.g. Milosevic 2018). The mechanism of action is impairment of mitochondrial protein synthesis; the effect is reversible, and the mitochondrial function is normalizing much faster than what is seen with the mitochondrial toxicity caused by toxic nukes of HIV therapy, such as stavudine (effects on mitochondrial DNA and mitochondrial numbers). The toxicity is dose and duration dependent and typical associated ADRs are neuropathy, myelosuppression and lactic acidosis.

Target organs of pretomanid toxicity in pre-clinical studies concern central nervous (ocular, convulsions), respiratory, and cardiovascular systems. In mouse and rat studies with pretomanid, several hepatotoxic effects were observed, while for monkeys there were fewer findings. Testicular toxicity was observed in male mice and rats in all repeat-dose studies, but not in monkeys. Modest, but likely acceptable margins of safety relative to the expected exposure for the 200 mg/day dose in humans were shown (2-4 times). Cardiovascular effects (QT) were not seen in a thorough single dose QT study (doses 400 and 1000 mg). The QT study was however, inconclusive as this study was flawed with several insufficiencies (see further below).

Adverse events

Common AEs reported from the Nix-TB study are only briefly summarized. Having in mind the patient population, the focus is on serious adverse events, AEs of special interest, numbers needing to stop therapy due to AEs (and in particular ADRs), and deaths.

Table 40. Overview of Treatment-Emergent Adverse Events, Nix-TB

	BPaL (N = 109) n (%) / Events
Any TEAE	109 (100.0) /1254
Serious TEAEs	19 (17.4) /36
Study drug-related TEAEs	108 (99.1) /688
TEAEs by worst severity Grade 4/Potentially Life-Threatening	17 (15.6) /31
Grade 3/Severe	41 (37.6) /90
Grade 2/Moderate	43 (39.4) /261
Grade 1/Mild	8 (7.3) /872
TEAEs leading to discontinuation of any study drug	33 (30.3) /44
TEAEs leading to discontinuation of Linezolid	29 (26.6) /31
TEAEs leading to discontinuation of Bedaquiline/Pretomanid*	6 (5.5) /12
TEAEs leading to death	6 (5.5) /10

* All discontinuations due to death

Discontinuation/interruption and dose reduction of linezolid occurred frequently, this is discussed in a subsection below. Those who permanently stopped the entire regimen were those who died (not treatment related according to investigators).

As outlined in the efficacy section, linezolid dosing was changed from 600 mg bid to 1200 mg qd when 44/109 patients had entered the study (i.e. not randomized).

Treatment-emergent AEs occurred in all patients. The most frequently (≥10% of subjects) reported TEAEs were the following (AEs indicated with * are known adverse effects of linezolid):

Term	**1200 mg qd** (n=65)	**600 mg bid** (n=44)	**all** (n=109)
peripheral sensory neuropathy/ neuropathy peripheral*	53 (82)	32 (73)	67 (78)
skin disorders	39 (60)	28 (64)	67 (61)
anemia *	20 (31)	20 (45)	40 (37)
GI events	41 (63)	32 (73)	73 (67)
nausea*	22 (34)	18 (41)	
vomiting*	21 (32)	16 (37)	
dyspepsia	19 (29)	7 (16)	
abdominal pain	5 (8)	6 (14)	
diarrhea*	4 (8)	6 (14)	
headache*	13 (20)	15 (34)	28 (26)
decreased appetite	15 (23)	9 (21)	24 (22)
liver enzyme increased			
GT	11 (17)	7 (16)	18 (16)
transaminases	3 (5)	9 (21)	12 (11)
hypoglycemia (10%)	8 (12)	4 (9)	12 (11)
abnormal loss of weight (10%)	7 (11)	4 (9)	11 (10)

Note: terms clearly not related to therapy not shown (upper respiratory tract infection, pleuritic pain, hemoptysis, back pain)

There were some differences in frequency of AEs by linezolid dosing, however numbers are low, and patients are not randomized to either dosing schedule. For the main linezolid toxicity, neuropathy, there is a numerical difference (not significant) favoring the BID regimen. In contrast, anemia (second most common problem) was more frequently reported with the BID regimen. Increased transaminases were numerically less common with the QD dosing schedule. However, overall the safety profile did not differ markedly by qd or bid dosing.

Maximum TEAE grades of 3 and 4 were reported in 37.6% and 15.6% of subjects, respectively. The most frequently (≥2 subjects [1.8%]) reported TEAEs with graded 3 and 4 were as follows:

Grade 3

peripheral sensory neuropathy /neuropathy peripheral (20.2%)
transaminases increased (5.5%)
amylase increased/hyperamylasemia (8.3%)
GT increased (4.6%)
lipase increased (3.7%)
anemia (3.7%)
neutropenia (2.8%)
bone marrow failure (1.8%)
abdominal pain upper (1.8%).

Grade 4

hypoglycemia (2.8%), and for following 1.8% (n=2) for each term
upper gastrointestinal haemorrhage
pneumonia
pulmonary tuberculosis
sepsis
anemia
gamma-glutamyltransferase increased

Neuropathy linked to linezolid was graded 1-2 in the majority of cases (total frequency 78%), and resulted in a large proportion of linezolid dose reductions/interruptions/discontinuations (discussed later). Increased transaminases are a known ADR of bedaquiline, with an ALT grade 3 increase in 5.1% in the C208 study (vs 1.3% in the placebo arm). The frequency of increased amylase and lipase (where lipase is considered more specific for pancreatitis) is fairly high. This is a potential ADR of bedaquiline (according to the Situro RMP), with a 2.6% incidence of amylase increase grade 3 in the C208 study (low numbers). Two cases of pancreatitis were reported in the Nix-TB study.

Serious adverse event/deaths/other significant events

An overall summary of serious TEAEs reported in Nix-TB is shown below. In a subsequent table more details are provided. To a great extent the events are those expected in patients with severe TB (50% co-infected with HIV, including those with low CD4 counts), and few events are considered related to therapy. The majority of the latter events are likely linked to linezolid (anemia, neutropenia). The one case of "lactic acidosis" was reported as part of a sepsis (i.e. the term is in a way misleading).

Table 41. Serious TEAEs by System Organ Class and Preferred Term, NIX-TB (N=109)

SUBJECTS WITH AT LEAST ONE TEAE	19 (17.4)	Fatal cases
INFECTIONS AND INFESTATIONS	7 (6.4)	
PNEUMONIA	3 (2.8)	(2, both also sepsis)
TUBERCULOSIS (pulmonary/disseminated)	4 (3.7)	2
SEPSIS/ SEPTIC SHOCK	3 (2.8)	2
DISSEMINATED TUBERCULOSIS	1 (0.9)	
TUBERCULOMA OF CENTRAL NERVOUS SYSTEM	1 (0.9)	
GASTROINTESTINAL DISORDERS	5 (4.6)	
ABDOMINAL PAIN UPPER	1 (0.9)	
HAEMATEMESIS	1 (0.9)	
PANCREATITIS	1 (0.9)	
PANCREATITIS HAEMORRHAGIC	1 (0.9)]	(1, also multiorgan failure)
UPPER GASTROINTESTINAL HAEMORRHAGE	1 (0.9)	1
METABOLISM AND NUTRITION DISORDERS	4 (3.7)	
HYPOGLYCAEMIA	2 (1.8)	
ABNORMAL LOSS OF WEIGHT	1 (0.9)	

LACTIC ACIDOSIS	1 (0.9)	
NERVOUS SYSTEM DISORDERS	4 (3.7)	
GENERALISED TONIC-CLONIC SEIZURE	1 (0.9)	
OPTIC NEURITIS	1 (0.9)	
SEIZURE	1 (0.9)	
SYNCOPE	1 (0.9)	
BLOOD AND LYMPHATIC SYSTEM DISORDERS	3 (2.8)	
ANAEMIA	2 (1.8)	
NEUTROPENIA	1 (0.9)	
RESPIRATORY, THORACIC, MEDIASTINAL DISORDERS	3 (2.8)	
ASTHMA	1 (0.9)]	
DYSPNOEA	1 (0.9)	
HAEMOPTYSIS	1 (0.9)	
PNEUMOTHORAX SPONTANEOUS	1 (0.9)	
PSYCHIATRIC DISORDERS	2 (1.8)	
DEPRESSION SUICIDAL	1 (0.9)	
GENERALISED ANXIETY DISORDER	1 (0.9)	
EYE DISORDERS	1 (0.9)	
OPTIC NEUROPATHY	1 (0.9)	
GENERAL DISORDERS	1 (0.9)	
MULTIPLE ORGAN FAILURE	1 (0.9)	1
INVESTIGATIONS	1 (0.9)	
TRANSAMINASES INCREASED	1 (0.9)	

Table 42. Details on serious TEAEs by SOC and Preferred Term Nix-TB (N=109) (Fatal cases indicated)

Subject ID	Preferred Term/ Verbatim Term	Study Day	Severity/ Relationship	Action Taken/ Outcome
1	Abnormal loss of weigh	160	Severe/ Not related	Dose not changed/ Recovered or resolved
	Pneumothorax spontaneous	184	Life threatening/ Not related	Dose not changed/ Recovered or resolved
2	Asthma	197	Severe/ Unlikely related	Not applicable/ Recovering or resolving
	Depression suicidal	126	Life threatening/ Not related	Dose not changed/ Recovered or resolved
	Dyspnoea	197	Severe/ Unlikely related	Not applicable/ Recovering or resolving
3	Generalised tonic-clonic seizure/	30	Severe/ Unlikely related	Drug interrupted/ Recovered or resolved
	Haematemesis	30	Severe/ Unlikely related	Drug interrupted/ Recovered or resolved
	Neutropenia	50	Life threatening/ Probably related	Drug interrupted/ Recovering or resolving
4	Hypoglycaemia	42	Life threatening/ Not related	Dose not changed/ Recovered or resolved
5	Intermittent seizures, CNS tuberculoma	209	Severe/ Not related	Not applicable/ Recovered or resolved
	Pancreatitis	65		

Subject ID	Preferred Term/ Verbatim Term	Study Day	Severity/ Relationship	Action Taken/ Outcome
			Severe/ Possibly related	Drug interrupted/ Recovered or resolved
6	Syncope	155	Severe/ Not related	Dose not changed/ Recovered or resolved
7	Optic neuritis	117	Life threatening/ Probably related	Drug withdrawn/ Recovered or resolved
8	Pneumonia		Severe/ Not related	Dose not changed/ Recovering or resolving
	Post-TB bronchiectasis	187	Moderate/ Not related	Not applicable/
9	Disseminated tuberculosis	34	Life threatening/ Not related	Not applicable/ Fatal
	Severe pulmonary tuberculosis	34	Life threatening/ Not related	Not applicable/ Fatal
10	Upper gastrointestinal bleeding	50	Life threatening/ Possibly related	Dose not changed/ Fatal
11	Acute severe worsening of pulmonary tuberculosis	47	Life threatening/ Not related	Drug withdrawn/ Fatal
	Symptomatic anaemia	19	Life threatening/ Probably related	Drug interrupted/ Recovered or resolved
12	Acute haemorrhagic pancreatitis	51	Life threatening/ Possibly related	Drug withdrawn/ Fatal
	Hypoglycaemia	51	Life threatening/ Possibly related	Drug withdrawn/ Recovered or resolved
	Multi-organ failure	51	Life threatening/ Possibly related	Drug withdrawn/ Fatal
13	Generalised anxiety disorder	34	Moderate/ Unlikely related	Dose not changed/ Recovering or resolving
14	Worsening epigastric pain	29	Severe/ Possibly related	Drug interrupted/ Recovered or resolved
15	Severe symptomatic anemia	64	Life threatening/ Related	Drug interrupted/ Recovered or resolved
16	Lactic acidosis	47	Life threatening/ Possibly related	Drug interrupted/ Recovered or resolved
	Sepsis	47	Life threatening/ Not related	Drug interrupted/ Recovered or resolved
17	Sepsis	85	Life threatening/ Not related	Not applicable/ Fatal
	Pneumonia	85	Life threatening/ Not related	Not applicable/ Fatal
	Transaminitis	85	Life threatening/ Unlikely related	Not applicable/ Not recovered or resolved
18	Septic shock	76	Life threatening/ Unlikely related	Not applicable/ Fatal
	Pneumonia	76	Life threatening/ Unlikely related	Not applicable/ Fatal
19	Optic neuropathy	143	Mild/ Probably related	Drug withdrawn/ Recovered resolved
	Haemoptysis	17	Life threatening/ Not related	Dose not changed/ Recovered or resolved

Deaths (Nix-TB, 26 March 2018 cut-off)

There were 8 deaths in the study, so far, where two occurred a long time after therapy was stopped (both subjects fulfilled treatment of 26 weeks, table 43 below).

Table 43 Deaths in Nix-TB

Subject ID	Age/Sex/ Race	Study Day of Death	Reason
During treatment period			
a	34/M/B	35	Severe (disseminated) tuberculosis
b	20/F/O	51	Upper gastrointestinal bleeding
c	31/F/B	55	Worsening of tuberculosis
d	35/M/B	53	Acute haemorrhagic pancreatitis and multiorgan
e	29/F/O	93	Pneumonia
f	26/F/O	76	Septic shock secondary to pneumonia
During Follow-up			
g	55/M/B	486	Sepsis secondary to gangrene (vascular disease)
h	38/M/B	369	Natural causes

In one case (acute hemorrhagic pancreatitis) causality with respect to treatment cannot be ruled out. The other 5 deaths that occurred during the treatment period are not indicative of causality to the regimen. Another two deaths occurred: one during the 6-month follow-up after EOT and the second more than 1 year after EOT.

AEs leading to dose reduction/interruption/discontinuation

Refer to Efficacy section around rules for interruptions of linezolid/full regimen, and dose reductions (linezolid).

Discontinuation of study treatment: No patients, except those 6 who died during therapy, permanently stopped the entire B-Pa-L regimen.

In addition to those 6 cases, *linezolid* was permanently discontinued in 28 patients (28 of remaining 103 patients =being 27%), and in the vast majority of cases due to peripheral neuropathy (compare to grade 3 neuropathy reported in 20% of the patients, section on AEs above).

Interruption of study treatment: The entire regimen was interrupted at least once in 20 patients (18%); 41 events. Reasons for interruptions were spread on a large number events (similar to the AE frequency), with maximum 2 events per term.

Linezolid was interrupted at least once in 50 subjects (46%), 70 events, dominated by peripheral neuropathy (two terms combined), 23%, and anaemia, 16%. The total mean duration of linezolid dose interruption was 44 days (min 2 days, maximum 145 days).

Reduction of linezolid dose: The linezolid dose was reduced at least once in 69 patients (63%) and the reasons were mainly due to peripheral neuropathy, 30 patients (28%) and anaemia, 13 patients (12%). The incidence of onset of events for peripheral neuropathy increased steadily over time:

≤2 weeks, 5%, >2 weeks to 8 weeks, 21%, >8 weeks to 26 weeks, 65%

The rate of dose interruption/reduction due to myelosuppression (in practice anaemia) was greatest from week 4 to week 12.

As a consequence of (mainly) these two major toxicities, neuropathy and anaemia, the rate of linezolid dose reductions/interruptions increased steadily from week 4 to week 20, see the figure below.

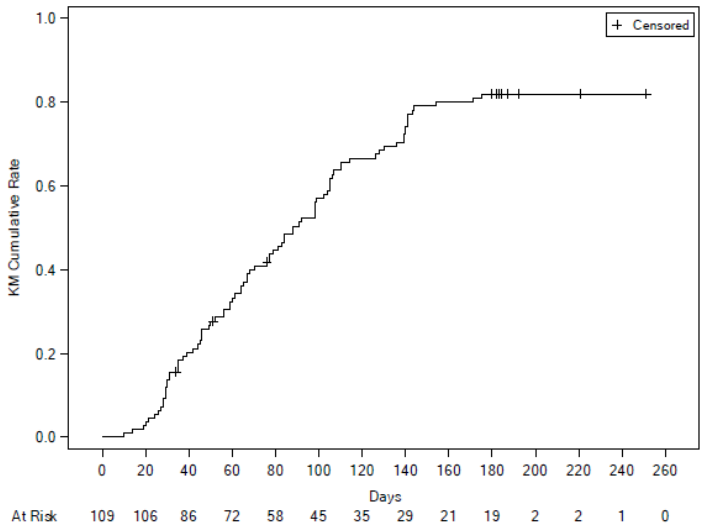

Figure 5. Time to First Dose Interruption and/or Dose Reduction of Linezolid due to an AE (Population Safety)

Patients were assessed for peripheral neuropathy at each visit by the use of a questionnaire and examinations were done monthly through treatment, and at the follow-up time points. A key question in the questionnaire was "During the past 14 days have you had pain, aching or burning in your feet or legs? The response to this question is visualized in the graph below.

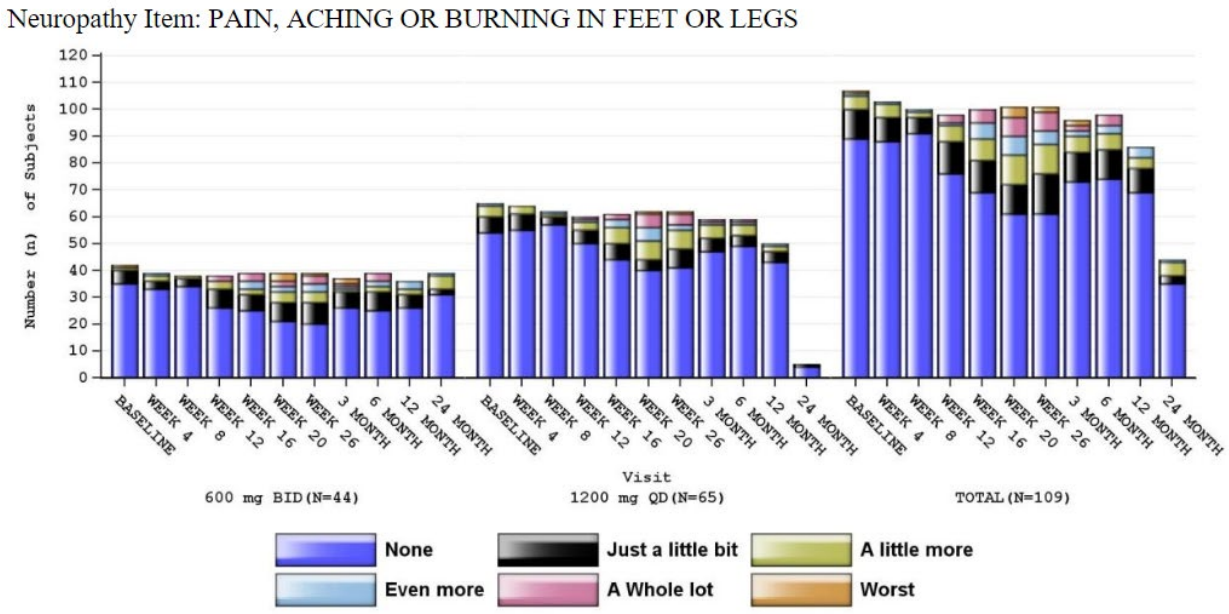

Figure 6

In summary, peripheral neuropathy was very frequent with the present dosing schedule, mild in most cases, and for the most reversible, however a proportion of patients reported symptoms (higher than at baseline) still 2 years post EOT. Of those 44 patients with a follow-up of 24 months, 14 had a higher total neuropathy score at this time point than at baseline. In 9 of these 14 cases the score was low at the 24 months follow-up (5 out of total 30 points) and lower than their scores at EOT; in 2 cases with a maximum score at EOT the score was reduced to half. The remaining 3 patients reported a considerably higher score at the 24 months follow-up timepoint than at the previous time points (however data are hard to interpret).

Upon request, a guide on how to manage linezolid dosing in the case of typical linezolid toxicities has been introduced in the Pretomanid SmPC, since relevant information is not provided in the Linezolid SmPC (where a maximum of 28 days of Linezolid therapy is recommended).

The ZeNix study is ongoing, with the target to find a more optimized linezolid dose (B-Pa-L, with 4 different dosing schedules for linezolid).

AEs of special interest (B-Pa-L)

- *Pretomanid*

Cataracts – Lens Disorders

Cataract development was observed in rats in long term toxicity studies. No cataract development was seen in monkeys. Due to the rat findings, since July 2009, clinical studies of pretomanid given for >14 days have included slit-lamp examinations with AREDS2 scoring of lens opacities.

In the Nix-TB study, slit lamp examinations were done at screening, Week 26, and 3 months after completion of treatment to assess potential development of cataracts. The applicant concluded that the findings of small percentages of both increases and decreases in AREDS2 scores suggest normal variation in the rates and/or age-related changes, i.e. no signal was seen for cataract formation.

Per 26 March 2018, data were available for 175 eyes in 88 subjects in Nix-TB. Score increases (worsening) of ≥1 from baseline for cortical, nuclear, and posterior subcapsular opacity were observed in 3 (1.7%), 2 (1.1%), and 6 (3.4%) eyes, respectively. Conversely, score decreases (improvement) of ≥1 from baseline were observed in 4 (2.3%), 13 (7.4%), and 0 (0%) eyes for cortical, nuclear, and posterior subcapsular opacity, respectively. One subject had an AREDS2 score increase of ≥2 from baseline. It was concluded that this patient might have already developed lens opacities when initially screened, which then progressed over the following months.

In NC-006**,** a score increase (worsening) of ≥1 from baseline in cortical opacity was observed in 2 eyes (1.6%) in the 100 mg PaMZ group and 1 eye (0.8%) in the 4-month 200 mg PaMZ group. Three eyes (2.3%) in the 4-month 200 mg PaMZ group and 4 eyes (2.7%) in the 6-month 200 mg PaMZ showed increases of ≥1 from baseline in nuclear opacity scores. Increases of ≥1 from baseline in posterior subcapsular scores were observed in all treatment groups: 3 eyes (2.4%), 2 eyes (1.5%), 1 eye (0.7%), and 1 eye (0.8%) for 100 mg PaMZ, 4-month 200 mg PaMZ, 6-month 200 mg PaMZ, and HRZE control, respectively. Three subjects in Study NC-006 had an increase of ≥2 in AREDS2 score.

It is acknowledged that based on the currently available data (and the doses and durations studied), it is difficult to see any clear clinically meaningful effect of pretomanid on the potential for cataract formation. However, ocular monitoring should be continued in the pretomanid clinical development

program in order to collect more data, and according to the study protocol for ZeNix, this is attended to. In study NC-006, there was one subject reporting an event of cataract (treated with the 6MPa200Z regimen) in the DS-TB pooling group. This subject seems to be the only subject among the included patients in the pretomanid safety database that reported an AE with the term "cataract". There was also one subject treated with the same regimen reporting "lenticular opacities". It is stated that all of the reported events in the SMQ of Lens disorder in the all-pretomanid group resolved.

Convulsions

Convulsions and other CNS-related effects were seen with pretomanid administration in rats and mice, at plasma exposures at least 2-fold higher than those yielded with the 200 mg dose. In summary, no signal for an increased risk of seizures was seen in the Nix-TB study. Two patients experienced serious convulsions (grade 3), however, both cases were considered heavily confounded (prior seizures, advanced tuberculoma in the brain).

Convulsions have been reported to occur in patients when treated with linezolid. In most of these cases, a history of seizures or risk factors for seizures were reported. However, whether pretomanid might add to that risk currently not known.

Testicular Degeneration

Testicular toxicity, reflected in seminiferous tubule degeneration, germ cell depletion, and infertility, was observed in mice and rats treated with pretomanid for 3 months. The effects were reversible in rats given lower doses (at edge of having toxic effects) and with shorter duration, but irreversible at high doses for longer durations. Effects were also seen on sexually mature male monkeys given daily oral doses of ≥150 mg/kg/day for 13 weeks (decreased sperm count and motility and an increased ratio of abnormal to normal sperm), in this case without histopathological testicular findings.

Reproductive hormone levels (testosterone, LH and FSH) were evaluated in male subjects in Studies NC-002, NC-005 (both 8 weeks duration), and NC-006 (16 or 24 weeks duration). The findings from the 3 studies showed no evidence that pretomanid is a testicular toxicant in humans at the doses and exposure times evaluated (up to 26 weeks). However, the results are insufficient in order to exclude that pretomanid may exhibit testicular toxicity.

A male reproductive study is planned to evaluate whether 6 months of pretomanid 200 mg daily dosing has an effect on sperm parameters and associated male reproductive hormones. A final report submission is predicted for 1Q 2024.

Liver toxicity

Liver safety was pre-defined as an adverse event of special interest of pretomanid on basis of pre-clinical findings. This is the main safety issues in parts of the program (mainly the Pa-M-Z regimen), in particular in the NC-006 study, which was halted for reasons of liver safety. A thorough assessment of liver safety in the entire program, including that seen in the Nix-TB study, is placed in a separate section below.

- *Bedaquiline*

(Pancreatitis) Amylase and lipase elevations were frequent in the Nix-TB study (grade 3-4 amylase in 17/109 and grade 3-4 lipase in 6/109). Of note, enzyme elevations (in particular amylase) were quite

frequent already at baseline. Further, the frequency of graded enzyme toxicity was fully similar with the Pa-B-Z regimen as with HRZE control in the NC-005 study.

There were 3 cases reported as clinical pancreatitis. One fatal case of multiorgan failure + haemorrhagic pancreatitis, where a causality to the regimen cannot be ruled out. The other 2 reported cases are questionable; one concerned a patient without enzyme elevations and other evidence of pancreatitis (resolved) and the other reported case was asymptomatic and enzyme elevations were present already at baseline. No clinical cases of pancreatitis were reported from the other studies in the Pa-program.

There is no indication for an association of pancreatitis (or such enzyme elevations) to pretomanid therapy per se when looking at the entire program. The non-clinical studies did not indicate that pretomanid affected the pancreas.

(Rhabdomyolysis/myopathy) In the NIX-TB study these events (reported by around 10% of patients) were grade 1 or 2, except one case (grade 3). None were considered serious.

(Liver safety and QT-prolongation) These issues are discussed in separate sections later.

- *Linezolid*

Linezolid is toxic to mitochondria, with a consequent risk for peripheral neuropathy and hematopoietic cytopenias (dose reductions/interruptions discussed above), optic nerve disorders and lactic acidosis.

In the Nix-TB study monthly ophthalmic examination (visual acuity and colour assessment) was part of the study procedures, in line with the recommendations provided in the linezolid SmPC. Two subjects were diagnosed with optic neuritis (one grade 4, one grade 1). Both events started after week 16, and both resolved when linezolid treatment was stopped.

Lactic acidosis: Eight (7.3%) patients reported TEAEs associated with lactic acidosis. All TEAEs resulted in study drugs being interrupted (7 patients) or withdrawn (1 patient). Linezolid was interrupted for four TEAEs, BPaL regimen for two TEAEs, and study drug(s) was not recorded for one TEAE. All except one TEAE had resolved by the data cut-off date. The unresolved event was hyperlactacidemia which occurred in one of the patients who died (cause of death was stated to be pneumonia and septic shock). Lactic acidosis is described as an AE of linezolid, however, it occurs rather rarely (unknown frequency in the SmPC of linezolid). Trial centres did not have the capability to test arterial blood gas parameters to confirm the diagnosis of acidosis and the incidence may have been underestimated. The risk of lactic acidosis with the regimen (i.e. linezolid) is reflected in the SmPC.

DS-TB pooling group

When comparing frequencies of AEs (and graded lab toxicities) between pretomanid monotherapy in phase 2 (CL-007 and CL-010, total N=122) and HRZE control, differences in treatment durations between these groups must be taken into account, table below.

Table 44. Exposure to Treatment DS-TB Subjects (Pa and control)

	Pretomanid Alone (N = 122)	Pretomanid Combination Therapy (N = 534)	HRZE Control (N = 229)
Time on treatment (days) [1]			
N	122	534	229
Mean (SD)	13.7 (1.60)	73.6 (51.72)	80.0 (63.57)
Median	14.0	56.0	56.0
Minimum, Maximum	2, 14	1, 202	4, 198
Treatment time <= 2 weeks	122 (100.0)	103 (19.3)	46 (20.1)
Treatment time >2-8 weeks	0 (0.0)	222 (41.6)	107 (46.7)
Treatment time >8-26 weeks	0 (0.0)	205 (38.4)	74 (32.3)
Treatment time > 26 weeks	0 (0.0)	4 (0.7)	2 (0.9)

Pretomanid monotherapy

For the phase 2 pretomanid-alone pooling group (i.e., monotherapy with pretomanid): The highest proportion of patients reporting AEs occurred in the category skin and subcutaneous tissue disorders (10/122, 8.2%) followed by gastrointestinal symptoms (8/122, 6.6%) and headache (3/122, 2.5%). It is noted that skin-related AEs seems to be rather frequently reported with pretomanid alone and when pretomanid is combined with other anti-TB drugs. In the non-clinical documentation, it was shown that oral administration of pretomanid at a dose of 100 mg/kg/day to rats, followed by exposure to UVR, elicited cutaneous reactions indicative of phototoxicity (erythema, grade 1 barely perceptible light redness) in the non-pigmented and/or pigmented skin sites. There was no signal of photo toxicity in the clinical studies. Of note, in the monotherapy studies, pretomanid was administered without food. As the bioavailability of pretomanid 200 mg seems to be approximately halved when administered in the fasting condition compared with in the fed condition, the representativeness of the incidences of AEs reported from the additional studies examining pretomanid alone at the proposed clinical dose is questioned. Furthermore, absorption became saturated in the fasting condition, and increasing doses led to significantly less than dose proportional increases in exposure. The studied exposure margin may therefore be considerably smaller than implied by the studied dose range.

Pretomanid combination therapy

Two different regimens have been tested for 8 weeks or longer in DS-TB patients, Pa-M-Z in studies NC002 (8 weeks) and NC006 (16 or 24 weeks), and B-Pa-Z in study NC005 (8 weeks), with HRZE/HR control in all 3 studies. The latter 3 studies cover 444/534 (83%) of the patients treated with Pa combination therapy in the DS pooling group. The remaining 90 were those treated in 14-day EBA-studies (NC-001 [Pa-M-Z, Pa-Z, Pa-B] and NC-003 [Pa in several combinations], also here with HRZE as control).

Grade 3/4 treatment emergent, drug-related, AEs were considerably more common with pretomanid combo therapy than with control, see table below.

Table 45. Overview of Treatment-Emergent Adverse Events (DS-TB Subjects in Phase 2/3 Studies)

		Pa combo(N = 534) n (%) / Events	HRZE Control (N = 229) n (%) / Events
Any TEAE		440 (82.4) /1938	176 (76.9) /725
Serious TEAEs		32 (6.0) /48	8 (3.5) /12
Study drug-related TEAEs		348 (65.2) /1057	133 (58.1) /365
TEAEs by worst severity	Grade 4	56 (10.5) /86	14 (6.1) /27
	Grade 3	112 (21.0) /242	38 (16.6) /73
	Grade 2	116 (21.7) /396	58 (25.3) /165
	Grade 1	156 (29.2) /1213	66 (28.8) /460
	Missing Grade	0 (0.0) /1	0 (0.0) /0
TEAEs leading to discontinuation of regimen		63 (11.8) /134	13 (5.7) /36
TEAEs leading to death		7 (1.3) /9	1 (0.4) /2

The frequencies of grade 4 events reported in the studies of longer duration (8 weeks in NC-002 and NC-005; 16-24 weeks in study NC-006) are shown below. Numbers who discontinued study drugs were indeed high with the test regimens, around twice as frequent as with control. Liver reactions/chemistry are the driver of the difference.

Table 46. Overview of treatment emergent AEs in NC-002 and NC-005 (8 weeks), DS-TB patients

NC-002 (South Africa, Tanzania and Brazil)	**Pa-M-Z (N=122)**	**HRZE (N=59)**
Grade 4 AEs	12 (9.8)	6 (10.2)
Liver disorder (numbers w/ at least one event)	28 (23.0)	7 (11.9)
Discontinuation of drug due to AE	20 (16.4)	7 (11.9)
NC-005 (South Africa, Tanzania, and Uganda)	**B-Pa-Z (N= 119)**	**(N=61)**
Grade 4 AEs	15 (12.6)	2 (3.3)
Liver disorder (numbers w/ at least one event)	13 (10.9)	4 (6.6)
Discontinuation of drug due to AE	11 (9.2)	2 (3.3)

Source: CSRs of NC002 and NC005

Of note, study NC-006 was halted due to severe liver events (3 fatal events with the test regimen, onset at around week 5 in all 3 cases). A large number of serious liver related events were reported in the test arms, none with control, see the table below. The frequency of grade 4 events with the test regimen given for 6 months was very high (20.9%), and 11/67 (16.4%) discontinued study drug due to an AE during the 6 months course (vs 5.9% in the control arm, same treatment duration).

Table 47. Overview of treatment emergent AEs in NC-006, DS-TB patients

	Pa100-M-Z	Pa200-MZ	Pa200-MZ	Pa-MZ	HRZE/HR
	4M	4M	6M	total	6M
	65	71	67	203	68
SERIOUS	3 (4.6)	8 (11.3)	8 (11.9)	19 (9.4)	3 (4.4)
LIVER-RELATED	19 (29.2)	17 (23.9)	24 (35.8)	60 (29.6)	21 (30.9)
SERIOUS LIVER-RELATED	1 (1.5)	4 (5.6)	4 (6.0)	9 (4.4)	0
=> WITHDRAWAL FROM STUDY	6 (9.2)	6 (8.5)	10 (14.9)	22 (10.8)	4 (5.9)
=> EARLY DISCONTINATION OF DRUG	6 (9.2)	6 (8.5)	11 (16.4)	23 (11.3)	4 (5.9)
=> DEATH	1 (1.5)	2 (2.8)	2 (3.0)	5 (2.5)	0
GRADE III	25 (38.5)	21 (29.6)	22 (32.8)	68 (33.5)	19 (27.9)
GRADE IV	9 (13.8)	9 (12.7)	14 (20.9)	32 (15.8)	6 (8.8)

Source CSR, table 12.2. => TEAE leading to

Deaths (DS-TB pooling group)

Deaths occurring in this group are shown in the table below; the deaths only occurred in the studies of longer duration. It is noted that many of the death occurred a long time after treatment was stopped (not considered related to study drug).

The main reasons for treatment emergent deaths were TB complications. Three patients died in NC-006, of liver failure, possibly related to the Pa-M-Z regimen.

Table 48. Deaths in the DS-TB pooling group

Subject ID/Treatment	Age/Sex/Race	Day	Reason
NC-002			
PaMZ	37/F/B	-	Only received 1 dose of study medication, withdrawn from study due to baseline grade 3 AST/ALT. Died when out of study.
NC-005			
BPaZ	34/M/B	23	Spontaneous pneumothorax
BPaZ	38/M/B	5	Pneumothorax
HRZE	46/M/B	44	Acute liver and renal failure
BPaZ	29/M/B	731	Fatal shooting
BPaZ	35/M/B	508	GI bleeding
BPaZ	64/M/B	403	Unknown (possibly HIV disease)
HRZE	39/M/B	437	Unknown
NC-006			
PaMZ	39/F/B	163	Natural causes (unknown)
PaMZ	45/M/M	114	Pneumothorax

Subject ID/Treatment	Age/Sex/Race	Day	Reason
PaMZ	44/F/B	28	Hepatotoxicity
PaMZ	21/M/B	39	Fulminant liver failure
PaMZ	23/F/B	34	Liver failure with hepatic encephalopathy
PaMZ	60/M/O	482	Accident
PaMZ	45/M/B	343	Sepsis
PaMZ	31/M/B	305	Haematemesis
PaMZ	47/M/B	469	Anal canal
PaMZ	37/M/A	436	Massive hemoptysis
HRZE	51/M/M	576	Left lobar pneumonia
HRZE	54/F/B	707	Lower respiratory tract infection

MDR-TB pooling group

This concerns MDR-TB patients in NC002, NC005 and NC-006:

NC-002: Pa200-M-Z (N=26, 8 weeks)
NC-005: B(200 daily)-Pa200-M-Z (N=60, 8 weeks)
NC-006: Pa200-M-Z (N=13, 6 months)

98% of the patients were recruited in Africa (75% in South Africa and 23% in Uganda); 38% where co-infected with HIV.

The majority (part of NC-005) were treated with the B-Pa-M-Z (for 8 weeks), the regimen presently evaluated in Simplici-TB (NC-008). There is no randomized control (HRZE control concerned DS TB subjects). Hence, the safety evaluation of the MDR-TB pooling group is less informative than that of the DS-TB pooling group.

Despite the fact that the vast majority were treated for a limited duration of 8 weeks, grade 4 events were reported in 6/99 patients (as compared to 1/109 with the B-Pa-L regimen given for 6 months in the Nix-TB study), and the same number discontinued the study regimen (as compared to none of the 103 who survived in the Nix-TB study).

Table 49. Overall AE summary, MDR-TB pooling group

	Pa combo therapy (N = 99) n (%) / Events
Any TEAE	92 (92.9) /372
Serious TEAEs	6 (6.1) /13
Study drug-related TEAEs	75 (75.8) /195
TEAEs by worst severity Grade 4	6 (6.1) /11
Grade 3	17 (17.2) /38
Grade 2	35 (35.4) /83
Grade 1	34 (34.3) /239
Missing Grade	0 (0.0) /1
TEAEs leading to discontinuation of regimen	6 (6.1) /14
TEAEs leading to death	1 (1.0) /1

The most frequent grade 3 or 4 TEAEs concerned ALT and AST increases, and this was, again, the main driver of treatment discontinuations.

Five deaths were reported in the MDR-TB pooling group, all were considered non-related to study drug and 4 out of the 5 deaths occurred a long time after test therapy was completed.

Evaluation of liver safety in the pretomanid program

No major liver safety findings were seen in the pre-clinical program; hepatocellular hypertrophy in repeat dose toxicity studies in mice, rats, and monkeys with pretomanid at high dose levels were considered an adaptive response. In addition, a few instances of minimally increased serum transaminase activity in rats and one instance of single-cell hepatocellular necrosis in mice at dose levels that exceeded the maximum tolerated dose and were not the major cause of animal morbidity.

No relevant ALT toxicity was seen with pretomanid monotherapy in studies CL-007 (doses 200-1200 mg) and CL-010 (doses 50-200 mg), where around 15 patients per dose arm (total 121 patients) were treated for 14 days.

B-Pa-L regimen (Nix-TB)

In Nix-TB, consideration of stopping study drug administration, at least temporarily, in subjects with liver function abnormalities or signs and symptoms of hepatitis was to be discussed with medical monitor in the following situations.

- ALT or AST >8 x ULN
- ALT or AST >5 x ULN for more than 2 weeks
- ALT or AST >3 x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%).
- IF ALT / AST ≥ 3 X ULN and Total Bilirubin >2x ULN, the IMP should be interrupted and the subject's clinical course discussed with the Sponsor Medical Monitor.

More or less identical stopping rules applied in the other studies (discussed below).

In 8/109 patients (7%) treatment was interrupted due to liver enzyme elevations; all were able to re-start without further clinically relevant elevations. No liver-related deaths were seen in the Nix-TB study.

With regards to ALT/AST toxicity, data from the Nix-TB study are shown below.

Table 50 Graded toxicity- transaminases, bilirubin elevated (Nix-TB)

	Nix-TB (BPaL) (N=109) n/N (%)
Grade 3 ALT	11/ 109 (10.1)
Grade 4 ALT	1/109 (0.9)
Grade 3/4 ALT elevation	12/109 (11.0)
Grade 3 AST	9/ 109 (8.3)
Grade 4 AST	1/ 109 (0.9)
Grade 3/4 AST elevation	10/109 (9.2)
Total bilirubin >2 ULN	2/109 (1.8)

ALT/AST grade 4 toxicity = >8 X ULN

The figures for the BPaL regimen are considered reassuring; the frequency of grade 4 toxicity is low (with a toxicity scale defining grade 4 as > 8 x ULN, a modest increase in clinical practice).

Pa-M-Z and B-Pa-M-Z regimens (NC-006 and NC-008)

NC-006 explored 3 different PaMZ regimens vs control in DS-TB patients. An additional arm included MDR-TB patients (not randomized, data not shown here).

Three fatal liver events were seen with the test regimen, all 3 had a similar pattern with an onset of ALT/AST elevations after 2-3 weeks of therapy, rapidly increasing to extreme values and fulminant liver failure shortly thereafter. In all 3 cases the liver tests were normal at study entry and prior to the time patients fell ill (with GI symptoms of vomiting and diarrhoea). The cases are indicative of severe drug toxicity. These cases resulted in a safety hold, initiated by DSMC and followed by the FDA, which was lifted after an analysis of overall data provided by the applicant. A fatal case of live failure had also occurred with the HRZE control regimen, and the ratio of test treatment vs control was 3:1.

Liver related treatment emergent AEs were more frequent with the test regimens than with control; around twice as frequent in the study arm of 6 months duration (i.e. same duration as HRZE/HR control). Numbers withdrawn from the study, as well as discontinuing therapy due to hepatic events were substantially higher with the test regimen given for 6 months as compared to HRZE/HR control (around 12% vs 3%), less marked for those treated with the 4 months test regimens. Graded ALT/AST toxicity, considerably more frequent with the test regimen, see table below.

Table 51. Graded toxicities for liver enzymes and BIL for the NC-006 study, DS TB subset

	Pa(100 mg)MZ 4 months (N=65) n/N1 (%)	Pa(200 mg)MZ 4 months (N=71) n/N1 (%)	Pa(200 mg)MZ DS-6 months (N=67) n/N1 (%)	HRZE Control (N=68) n/N1 (%)
Parameter Grade				
ALT (U/L)				
Grade 1	16/ 65 (25)	15/ 71 (21)	10/ 67 (15)	12/ 68 (18)
Grade 2	5/ 65 (8)	3/ 71 (4)	3/ 67 (4)	1/ 68 (1)
Grade 3	5/ 65 (8)	7/ 71 (10)	8/ 67 (12)	1/ 68 (1)
Grade 4	4/ 65 (6)	5/ 71 (7)	7/ 67 (10)	4/ 68 (6)
AST (U/L)				
Grade 1	14/ 65 (22)	10/ 71 (14)	13/ 67 (19)	22/ 68 (32)
Grade 2	5/ 65 (8)	2/ 71 (3)	9/ 67 (13)	1/ 68 (1)
Grade 3	7/ 65 (11)	4/ 71 (6)	6/ 67 (9)	2/ 68 (3)
Grade 4	5/ 65 (8)	8/ 71 (11)	6/ 67 (9)	4/ 68 (6)
BIL (umol/L				
Grade 1	2/ 65 (3)	1/ 71 (1)	1/ 67 (1)	0/ 68 (0)
Grade 2	1/ 65 (2)	1/ 71 (1)	1/ 67 (1)	0/ 68 (0)
Grade 3	0/ 65 (0)	0/ 71 (0)	1/ 67 (1)	0/ 68 (0)
Grade 4	2/ 65 (3)	1/ 71 (1)	1/ 67 (1)	1/ 68 (1)

AST /ALT: Grade 1: 1.1 - <2.0 x ULN Grade 2: 2.0 – <3.0 x ULN Grade 3: 3.0 – 8.0 x ULN Grade 4: > 8 x ULN

Hyperbilirubinemia (when accompanied by any increase in other liver function test):

Grade 1:1.1 - < 1.25 xULN Grade 2:1.25 - <1.5 x ULN Grade 3:1.5 – 1.75 xULN Grade 4:> 1.75 x ULN

Hyperbilirubinemia (when other liver function tests are in the normal range):

Grade 1:1.1 - < 1.5 x ULN Grade 2:1.5 - <2.0 x ULN Grade 3:2.0 – 3.0 x ULN Grade 4:> 3.0 x U

The reasons for the frequent and seemingly severe toxicity in NC-006 are not clear, and this raised concerns about the liver safety of pretomanid, at least as part of this particular regimen.

It was questioned whether the severe liver toxicity seen in some cases in the studies above may be due to an PD interaction between Pa and pyrazinamide.

However, from what is known around transporters and the metabolism of the agents no reasons were found to suspect pretomanid as a victim of pyrazinamide. The other way around, potential effects of Pa on the metabolism of pyrazinamide cannot be ruled out.

In the NC-005 study pyrazinamide and its metabolites were indeed analysed. Regimens and results are shown in the table below. The exposure of pyrazinamide and its metabolites is very similar with B-Pa-Z as with HRZE control.

In contrast it is noted that the level of 5-hydroxypyrazinoic acid (5-OH-PA) is around 2-fold higher with the addition of *moxifloxacin* (arm 4*)*. The effect on 5-OH-PA is of interest since it has been cited as the major mediator of toxicity (Shih, et al, 2013; Rawat, et al, 2018), although there is disagreement on this matter (Tostmann, et al, 2010). However, there are in fact no relevant difference in frequencies of liver related AEs, severe liver related AEs and graded ALT/AST toxicities with the 3 different test regimens in NC-005 t (Table 52).

Table 52. NC-005 pharmacokinetic assessment of pyrazinamide and its metabolites

Analyte	**Pyrazinamide (Z)**							
Treatment (Z dose) mg)	**HRZE† (1350)**		**B_{200}PaZ (DS-TB) (1500)**		**B_{label}PaZ (DS-TB) (1500)**		**B_{200}PaMZ (MDR-TB) (1500)**	
Dosing Days	Day 14	Day 56	Day 14	Day 56	Day 14	Day 56	Day 14	Day 56
N	15	15	15	11	12	13	12	12
PZA	404379	400754	407534	413182	391882	399664	408485#	439086
PA	148449#	132100	148156	113636	124997	108017	175430##	196121
5-OH-PA	10946	10538†	11290	8643	10938	9232	21778##**	20860*
T1/2 (hr)	7.02 (n=12)	7.38 (n=14)	9.54 (n=14)	9.49	8.28 (n=11)	7.98 (n=10)	6.86 (n=8)	7.54 (n=10)

† values normalized to 1500 mg (HRZE arm was dosed based on body weight. # N = 14; ## N = 11. *p<0.05, ** p<0.01 compared to HRZE control. NC-005 Pyrazinamide Metabolites Report

Table 53. Selected outcomes related to hepatotoxicity and QT in pretomanid arms, in NC-005

Outcome	Drug Sensitive		Multi-Drug Resistant
	$B_{label}PaZ$	$B_{200}PaZ$	$B_{200}PaMZ$
Number of subjects	59	60	60
Hepatotoxicity: n (%)			
≥ 1 liver related TEAE[a]	6 (10.2)	7 (11.7)	9 (15.0)
≥ 1 serious liver related TEAE[b]	2 (3.4)	0 (0)	2 (3.3)
ALT > 3×ULN[c]	6 (10.3)	4 (6.7)	5 (8.3)
AST > 3×ULN[c]	6 (10.3)	3 (5.0)	5 (8.3)
ALT or AST > 3×ULN[c]	7 (12.1)	4 (6.7)	6 (10.0)
BILI ≥ 1.1×ULN[c]	0 (0)	0 (0)	2 (3.3)

The applicant concluded that there are no clear indications for a PD interaction between Pa and pyrazinamide. This is endorsed, with the caveat that Pa metabolites have not been thoroughly studied and fully understood. Further, that the finding of high 5-OH-PA exposures (potentially toxic driver of pyrazinamide) in the regimen that included moxifloxacin not necessarily explains the toxicity findings in NC-006. This is agreed. Of note, in a very large study exploring 3 different regimens for DS TB (1:1:1, N=1930); 6 months of HRZE vs 2 experimental arms where moxifloxacin replaced isoniazid (H) and ethambutol respectively (i.e. was given with pyrazinamide for the first 8 weeks), no difference in hepatic safety was seen between arms (Gillespie et al 2014).

Figures on hepatic toxicity (grade ¾ ALT toxicity or potential HY´s law cases) in the Safety analysis pooling groups do not point to a relevant difference of risk by gender.

QT changes – review of the pretomanid program

On the basis of pre-clinical data, pretomanid has a potential for QT-prolongation (see pre-clinical section). In a thorough QT-study in healthy volunteers (Study DMID-10-0058, single centre in the U.S.), pretomanid in single doses of 400 mg and 1000 mg yielded minor QTc effects of 2.7 and 4.5 msec, versus an expected +10.9 msec with 400 mg moxifloxacin. When combining Pa 400 mg and moxifloxacin, the effect was 12.5 msec (additive). Hence, no relevant QTc increase would be expected with the 200 mg dose, on the basis of single dose data. However, the thorough QT study may be considered inconclusive (see further above under "Secondary pharmacology").

Having those results in mind, mean QTc changes from baseline were higher than expected in the clinical studies with the Pa-M-Z regimen (around + 20 msec), since pyrazinamide is not known to cause an increase. The B-Pa-M-Z regimen yielded, over time, an increase of around 25 msec (NC-005, 8 weeks duration). In contrast, the B-Pa-L regimen in Nix-TB yielded a modest increase of 10 msec, somewhat lower than expected.

Table 54. Mean QTc(F) changes from baseline by various regimens in the Pretomanid program

	Week 4	Week 8	Week 16/17	Week 24
NC-002 (DS TB) (n~60 per arm)				
Pa100-M-Z	8.6	12.9	-	-
Pa200-M-Z	15.0	20.5	-	-
HRZE	4.3	6.8	-	-
NC-006 (DS-TB) (n~60 per arm)				
Pa100-M-Z (4 months)	-	~12	~15	-
Pa200-M-Z (4 months)	-	~18	~20	-
HRZE/HR (6 months)	-	~12	-	
NC-005 (n~60 per arm)				
DS-TB				
Pa200 –B(load)-Z	20.1	23.6	-	-
Pa200-B200-Z	18.8	21.7	-	-
HRZE	10.0	11.6	-	-
MDR-TB				
Pa200-B200-M-Z	24.4	25.0	-	-
Nix-TB (XDR/MDR) (n=109)				
Pa200-B(load)-L1200	6.1	7.9	9.9	9.1

When scrutinizing data, a main difference noted between subjects in the Nix-TB study and the other studies is a substantial difference in *baseline* QTc values. Among all subjects, the median baseline QTcF was 414.5 ms in Nix-TB (and similar in the ongoing ZeNix, 408 ms), but around 390 ms in NC-002, NC-005, and NC-006. A reasonable, yet speculative, explanation to that difference in baseline QTc values would be a residual QT-prolongating effect of the previous failing regimens for patients included in Nix-TB and ZeNix, while the other studies included newly diagnosed patients without TB therapy.

When looking at treatment emergent, potentially clinically relevant ECG results in the Nix-TB study it is noted that only 5/109 had a QTc of >480 ms, and 1/109 >500 msec on treatment. However, it has to be kept in mind that the Nix-TB study was not designed in order to fully detect the adverse effects on the QT interval of the pretomanid + bedaquiline combination. Few patients were examined, there was no comparator group and ECG was recorded before the trial drugs were administered (i.e. not at C_{max}) and with low frequency.

Further QTc data was presented for all subjects in Nix-TB who died from "natural causes", "cause unknown" or "worsening of TB". These data do not indicate that unclear deaths were attributed to treatment related QTc increases. Reported cases of cardiac-related AEs in the Nix-TB study (e.g., dizziness, palpitations, hypotension and syncope grade 3) were also scrutinized. There were 14 patients with reported AEs that possibly could be cardiac-related. For four of these patients there were no information as the events occurred outside the treatment window. For the majority of remaining patients (6), there were no ECG recordings at the time the AEs occurred (the Nix-TB study did not employ continuous ECG recordings). Notwithstanding this limitation, for the ECGs that were recorded, no reading showed QTcF over 440 ms before or after the events in any of the patients.

In summary, the totality of data indicates that pretomanid has a more QT-prolongating effect than what is seen in the thorough QTc study. However, the results obtained in the Nix-TB study are still compatible with an acceptable profile that can be handled with appropriate patient selection, monitoring and stopping criteria, which will be implemented in the SmPC.

Laboratory findings

Liver chemistry, discussed in the previous section, is not repeated below.

With pretomanid monotherapy (2 weeks, studies CL-007, CL-010, doses of 50 mg to 1200 mg daily) there were no relevant changes in haematology parameters; mean decreases in platelets was seen in some arms, including with HRZE control. For other lab chemistry, a moderate increase in creatinine was noted with doses 200 mg qd and higher. A maximum of around +15 umol/L were seen at doses 600 mg qd and higher. However, no findings were seen in urine analyses (including, among other parameters, protein, glucose, microalbumin, blood, leukocytes). This indicates inhibition of tubular secretion of creatinine, however which transporter that is inhibited has not been clarified. Increases of amylase were seen in all treatment arms in these studies, including with HRZE control, without any pattern indicating a specific problem for pretomanid.

Nix-TB (B-Pa-L)

Graded toxicity of interest, outside liver chemistry, is shown below. Decreases in haemoglobin, and less marked in neutrophils, is linked to linezolid therapy, where the frequency of linezolid dose interruptions/reductions and discontinuations due to anaemia were discussed previously.

Change from baseline in creatinine has been summarized for the first 45 patients at the data cut off. The pattern was very similar to that seen in the study CL-007 with an increase of around 15 umol/L from week 2 and onwards. This is not considered a cause of concern.

Table 55. Graded toxicity for haematology (left) and selected lab chemistry (right) in NIX-TB (N=109)

HEMOGLOBIN	n	%
Grade 1	27	(24.8)
Grade 2	26	(23.9)
Grade 3	6	(5.5)
Grade 4	0	(0.0)
NEUTROPHILS, ABSOLUTE COUNT		
Grade 1	19	(17.4)
Grade 2	9	(8.3)
Grade 3	4	(3.7)
Grade 4	1	(0.9)
PLATELETS		
Grade 1	3	(2.8)
Grade 2	1	(0.9)
Grade 3	2	(1.8)
Grade 4	0	(0.0)
WHITE BLOOD CELL COUNT		
Grade 1	22	(20.2)
Grade 2	6	(5.5)
Grade 3	6	(5.5)
Grade 4	0	(0.0)

CREATININE	
Grade 1	24 (22.0)
Grade 2	4 (3.7)
Grade 3	0
Grade 4	0
LIPASE	
Grade 1	12 (11.0)
Grade 2	4 (3.7)
Grade 3	4 (3.7)
Grade 4	1 (0.9)
AMYLASE	
Grade 1	33 (30.3)
Grade 2	26 (23.9)
Grade 3	16 (14.7)
Grade 4	1 (0.9)
URIC ACID	
Grade 1	17 (15.6)
Grade 2	2 (1.8)
Grade 3	1 (0.9)
Grade 4	0 (0.0)

In the DS-TB pooling group, no signals for specific lab toxicity, outside liver chemistry, were seen when comparing the test regimens vs control. In these studies, there were no indications for myelo-

suppressive effect of pretomanid (linked to linezolid in the B-Pa-L regimen). In this pooling group increases in amylase and lipase were similar with Pa combo as with control (similar exposures/durations for test and control). Still, having in mind the very high frequency in the Nix-TB study (B-Pa-L regimen), for which bedaquiline has a potential for an impact on these enzymes, it could be interesting to compare the frequencies of graded toxicities in the randomized DS-TB patients in studies NC-002 vs NC-005, for which baseline characteristics seem quite similar, including around 20% HIV co-infected patients in both studies.

Safety in special populations

No data, neither efficacy/safety nor PK data, has been generated in special populations, such as patients with renal or liver impairment, children etc. Only a handful of patients in the clinical studies (all combined) were aged 65 years or older, and a safety assessment by age is therefore not relevant.

With regards to sex, the gender mix is fairly even in the larger studies. The main safety issue is liver safety, and figures on hepatic toxicity (grade 3-4 ALT toxicity or potential HY´s law cases) in the Safety analysis pooling groups did not point to a relevant difference of risk by gender.

The lack of PK data in patients with severe renal impairment is considered acceptable, having metabolism/elimination in mind.

Immunological events

There seems to be very few TEAEs reported as "hypersensitivity" in the pretomanid database. There was one subject reporting a serious TEAE of hypersensitivity in the all-pretomanid group in the DS-TB pooling group. This subject received the PaMZ regimen (100 mg pretomanid daily), the event started on study Day 59 (12 days after the final dose of trial medication), lasted 9 days and were considered moderate in severity. The event was considered related to treatment of gouty arthritis with allopurinol, this drug was stopped, and the event resolved. One other subject in the DS-TB pooling group, receiving PaMZ (100 mg pretomanid daily) discontinued the study due to a TEAE described by the investigator as "drug induced hypersensitivity skin reaction moxifloxacin". The event was resolved and considered "moderate" in severity. There were a few other TEAEs (some cases of e.g. dermatitis allergic, rash, pruritus, eczema and other PTs connected to the SOC skin and subcutaneous tissue disorders in addition to PT like conjunctivitis allergic) that could potentially represent allergic reactions to treatment. It is possibly only the data from the phase 2 pretomanid-alone pooling group that could assist in elucidating this potential risk for pretomanid. In total 10/122 subjects reported different types of rash from this pooling group, but there has been no targeted discussion by the applicant of this issue. In the non-clinical part, there are indications of pretomanid being the cause of immunotoxicity in some species. There were no AEs in the safety data set indicative of immunotoxicity.

Safety related to drug-drug interactions and other interactions

The issue of a potential PD interaction was raised and is discussed in the Liver safety and QTc sections. In summary, there is no evidence for such interactions at present.

2.6.1. Discussion on clinical safety

Pretomanid in monotherapy in doses 50-1200 mg per day was well tolerated over 14 days treatment (EBA studies). Reported AEs were mainly grade 1, with no high frequencies for any particular SOC, and

graded lab toxicity was unremarkable, including liver enzymes (none had a grade 2 shift from baseline). In the monotherapy studies, pretomanid was administered without food. As the bioavailability of pretomanid 200 mg seems to be approximately halved when administered in the fasting condition compared with in the fed condition, the representativeness of the incidences of AEs reported in these studies of limited duration is limited. Furthermore, absorption became saturated in the fasting condition, and increasing doses led to significantly less than dose proportional increases in exposure. The studied exposure margin is therefore considerably smaller than implied by the studied dose range.

Liver safety – main safety issue in the program as a whole, but not with the B-Pa-L regimen

The applicant is seeking approval for pretomanid as part the specific B-Pa-L regimen on the basis of outcomes in the Nix-TB study. Liver safety in this study, of limited size (N=109), is considered re-assuring. Graded ALT toxicity was rather common, but grade 4 toxicity (here 8 x ULN) was only seen in 1/109 patients in the Nix-TB study, a low figure for a treatment regimen of XDR-TB patients. While 8/109 interrupted the regimen due to ALT increases, in accordance with the protocol and by rules that are being reflected in the SmPC, all of these patients could resume and finish treatment. No deaths in the study were related to liver toxicity.

The liver safety in study NC-006 raised serious concerns around the liver safety of pretomanid as part of the Pa-M-Z regimen with 3 fatal liver events/203 treated and a high frequency of ALT 3/4 toxicity. With regards to deaths, 1 fatal liver event was seen also in the HRZE control group, and patients were allocated to test vs HRZE in a ratio of 3:1.

In summary, liver safety with the B-Pa-L regimen is considered acceptable, and manageable. There is presently no clear understanding of whether the pretomanid component could explain a potentially worse safety profile of the other regimens, where both moxifloxacin and pyrazinamide (per se) have an acknowledged DILI potential. Adequate wordings around frequent liver monitoring during treatment and stopping rules are included in section 4.4 of the SmPC.

Linezolid toxicity – the main identified safety issue for the B-Pa-L regimen

Linezolid has a mitochondrial toxicity, which is dose and duration dependent, and typical associated ADRs are neuropathy, myelosuppression and lactic acidosis. With the 1200 mg daily dose throughout 6 months of treatment that was used in the Nix-TB study, incidences of neuropathy and anaemia are very high, with a high need for dose interruptions/reductions and discontinuations. While anaemia typically occurred within the first 12 weeks, neuropathy typically started after 8 weeks of therapy. Linezolid was permanently discontinued in around one third of the patients and interrupted in around 50%; dose reductions were common (> 60% had to reduce the linezolid dose at least once). Neuropathy was mild in most cases, and for the most reversible. A proportion of patients reported symptoms (higher score than at baseline) still 2 years post EOT, for the most at a low score, or with a substantial reduction as compared to a very high score at EOT. A guide on how to manage linezolid dosing in the case of typical linezolid toxicities has been introduced in the Pretomanid SmPC, since such information is not provided in the Linezolid SmPc (where a maximum of 28 days of Linezolid therapy is recommended). The ZeNix study, presently evaluating optimized dosing schedules for linezolid, is predicted to be fully recruited within 1Q2020, and with top line results for efficacy (6 months of follow-up) in July 2021.

Another potential issue of the B-Pa-L regimen is bedaquiline and pancreatitis. One of the deaths in the Nix-TB study was a fatal event of haemorrhagic pancreatitis with multi-organ failure, where causality

to the regimen could not be ruled out. However, when viewing enzyme elevations and pancreatic events (very few) in the pretomanid program as a whole, there are no indications for an association to pretomanid. Furthermore, in the non-clinical documentation, there were no signs of pretomanid affecting the pancreas.

QT-prolongation with Pa-containing therapy

QT-prolongation is an issue of MDR/XDR-TB therapy, since several agents used in recommended treatment regimens have such a potential (bedaquiline, delamanid, some of the quinolones, clofazamine). While pre-clinical studies indicate a potential for QT prolongation for pretomanid, no relevant QTc prolongation was seen with a single dose of 400 mg in a thorough QT study (twice the dose used in the present regimen). Furthermore, when combined with moxifloxacin the modest effect was simply additive reflecting mainly the known QTc prolonging effect of the latter. However, the thorough QT study is considered inconclusive due to several insufficiencies, including the single dose design.

When looking at the entire program, a higher impact of pretomanid on QT prolongation than that seen in the thorough QT study is predicted. However, the number of patients with maximum QTc values of levels associated with a substantial risk of arrythmia with the B-Pa-L regimen in the Nix-TB study were low; 5/109 had a maximum QTc of >480 msec, and 1/109 >500 msec. However, it has to be noted that the Nix-TB study had some limitations in that respect; few patients were examined, there was no comparator group and ECG was recorded before the trial drugs were administered (i.e. not at C_{max}) and with low frequency.

In conclusion, although the QTc effect of pretomanid has not been fully elucidated, the issue can be managed by appropriate patient selection, monitoring and stopping rules, which will be part of the SmPC. Further dedicated studies are not considered likely to yield further information that would impact these recommendations.

Deaths in Nix-TB and the other studies

In the Nix-TB study six patients died prior to completion of therapy, and another two quite some time thereafter. Apart from the patient who potentially died due to pancreatitis, other treatment emergent deaths concern deaths in other infections (sepsis) or worsening of TB, where an association to therapy is not suspected.

Deaths occurring during treatment/follow-up in the other clinical studies were not considered associated to the study treatment, with the potential exception of the 3 cases of deaths in liver failure in study NC-006. Other causes of death were related to tuberculosis, other infections and accidents.

Each death in Nix-TB is a complex combination of many factors such as the underlying disease of XDR-TB, other concomitant medical conditions and use of a variety of co-medications (including the 3 study drugs). This makes it challenging to pinpoint specific drugs and/or medical reasons as definite causes for the deaths.

Other uncertainties

From the non-clinical studies, it was observed a toxic effect of pretomanid on testes and male fertility. The duration of the follow-up periods for examining pretomanid's possible detrimental effect on male fertility in humans was limited to 8 weeks in the phase 2 studies NC-002 and NC-005 and 26 weeks in

the prematurely terminated phase 3 study NC-006. Analyses of hormones did not point to negative effects, but semen analyses have not been performed. A dedicated study is planned where both semen and hormones will be studied for 6 months therapy of 200 mg pretomand daily therapy.

Convulsion and other CNS-related effects were seen with pretomanid administration in rats and mice. Two subjects in Nix-TB experienced serious convulsions (grade 3); whether the combination of linezolid and pretomanid might increase the risk of provoking convulsions is not known.

Adverse drug reactions (ADRs) reported from the Nix-TB trial have been included in the Summary of Product Characteristics.

Additional safety data needed in the context of a conditional MA

An optimized dosing of linezolid is being studied in the ZeNix study, concerning the same treatment population (Category 2 study).

2.6.2. Conclusions on the clinical safety

Pretomanid as part of the specific regimen B-Pa-L shows an acceptable safety profile in the Nix-TB study. One main obstacle of this regimen is linezolid toxicity, in particular peripheral neuropathy, and it is clear that the dose/duration is not optimized yet. Linezolid is however considered manageable, and instructions around dose adjustments in cases of toxicity are provided in the SmPC. Of note, linezolid toxicity is an issue also for alternative (non-pretomanid) regimens, as linezolid is a recommended part of XDR-TB therapy for efficacy reasons.

Liver safety is an important safety concern but considered acceptable and manageable for the B-Pa-L regimen. Frequent liver monitoring is crucial, and instructions on when to interrupt the regimen is presented in the SmPC.

The impact of Pretomanid, and of the B-Pa-L regimen, on QTc has not been fully characterized. However, this is a recognized safety issue of pretomanid as well as of bedaquiline. Results obtained in the Nix-TB study are compatible with an acceptable profile that can be handled. Appropriate patient selection, monitoring and stopping criteria are therefore mandated, and are implemented in the SmPC. Further dedicated studies are not considered meaningful.

Testicular toxicity is a potential safety concern on the basis of pre-clinical studies in rats. Human toxicity cannot be ruled out on the basis of the data studied in the clinical studies so far (hormonal levels). A dedicated study, which will study the also any impact on sperm quality, has been initiated.

In summary, the safety profile of pretomanid as part of the Pa-B-L regimen for treatment of patients with XDR-TB, and/or MDR-TB and not responding to/tolerating the MDR-TB regimen, is considered acceptable. However, due to the limited number of patients treated for 6 months or more, and the single arm nature of the data providing information on the safety of the B-Pa-L, available data are considered non-comprehensive.

The CHMP considers the following measures necessary to address the missing safety data in the context of a conditional MA:

Safety and Efficacy of Various Doses and Treatment Durations of Linezolid Plus Bedaquiline and Pretomanid in Participants With Pulmonary TB, XDR-TB, Pre- XDR-TB or Non-responsive/Intolerant MDR-TB (ZeNix).

Study performed in order to evaluate the safety, efficacy and tolerability of various doses and durations of linezolid plus bedaquiline and Pretomanid after 26 weeks of treatment in participants with either pulmonary XDR-TB, pre-XDR TB, or treatment intolerant or non-responsive MDR-TB.

This study was fully recruited in December 2019, with topline results available 2Q/3Q 2021.

In addition, in order to confirm the safety and efficacy of pretomanid the marketing authorisation holder should complete and submit results from the ongoing Phase 3 Open-label Trial (Nix-TB).

2.7. Risk Management Plan

Safety concerns

Summary of safety concerns	
Important identified risks	QT prolongation
Important potential risks	Hepatotoxicity Testicular toxicity
Missing information	Use in pregnancy

Pharmacovigilance plan

Study	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
A Phase 3 partially-blinded, randomized trial assessing the safety and efficacy of various doses and treatment durations of linezolid plus bedaquiline and pretomanid in participants with pulmonary infection of either extensively drug-resistant tuberculosis (XDRTB), pre-XDR-TB or treatment intolerant or non-responsive multidrug resistant tuberculosis (MDR-TB) NC-007-(B-Pa-L) (ZeNix) Category 3 Ongoing	To evaluate the efficacy, safety and tolerability of various doses and durations of linezolid plus bedaquiline and pretomanid after 26 weeks of treatment in participants with either pulmonary XDR-TB, pre-XDR-TB, or treatment intolerant or non-responsive MDR-TB.	Hepatotoxicity	Interim report Final report	Sep 2021 Sep 2022

Risk minimisation measures

Safety concern	**Risk minimisation measures**	**Pharmacovigilance activities**
Important identified risk: QT prolongation	Routine risk communication: SmPC sections 4.4, 4.8, 5.3 PL sections 2, 4	Additional pharmacovigilance activities: None

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	Routine risk minimisation activities recommending specific clinical measures to address the risk: An ECG should be obtained before initiation of treatment, and at least monthly during treatment with the combination regimen of pretomanid, bedaquiline, and linezolid (SmPC section 4.4). Serum potassium, calcium, and magnesium should be obtained at baseline and corrected if abnormal. Follow-up monitoring of electrolytes should be performed if QT prolongation is detected (SmPC section 4.4). Other routine risk minimisation measures beyond the Product Information: Legal status: prescription only Additional risk minimisation measures: None	
Important potential risks: Hepatotoxicity	Routine risk minimisation measures: SmPC sections 4.4 and 4.8 PL sections 2 and 4 Routine risk minimisation activities recommending specific clinical measures to address the risk: recommendation to avoid alcohol and other hepatotoxic medicinal products, other than those specified in the indication statement, is included in the SmPC section 4.4 recommendation for monitoring liver function, including for symptoms and signs of liver injury and laboratory tests, are included in SmPC section 4.4 Other routine risk minimisation measures beyond the Product Information: Legal status: prescription only Additional risk minimisation measures: None	Additional pharmacovigilance activities: Study ZeNix
Important potential risks: Testicular toxicity	Routine risk communication: SmPC sections 4.6, 5.3 Routine risk minimisation activities recommending specific clinical measures to address the risk: None Other routine risk minimisation measures beyond the Product Information: Legal status: prescription only Additional risk minimization measures: None	Additional pharmacovigilance activities: None

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Missing information: Use in pregnancy	Routine risk minimisation measures: SmPC section 4.6 PL section 2 Routine risk minimisation activities recommending specific clinical measures to address the risk: None Other routine risk minimisation measures beyond the Product Information: Legal status: prescription only Additional risk minimisation measures: None	Additional pharmacovigilance activities: None

Conclusion

The CHMP and PRAC considered that the risk management plan version 0.3 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date IBD. The IBD is 14.08.2019. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. New Active Substance

The applicant compared the structure of pretomanid with active substances contained in authorised medicinal products in the European Union and declared that it is not a salt, ester, ether, isomer, mixture of isomers, complex or derivative of any of them.

The CHMP, based on the available data, considers pretomanid to be a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Labelling exemptions

A request of translation exemption of the labelling as per Art.63.1 of Directive 2001/83/EC has been submitted by the applicant and has been found acceptable by the QRD Group for the following reasons:

The group has agreed to grant an English only label for the following components: Outer carton for the blister, outer carton for the bottle, blister label and bottle label.

The labelling subject to translation exemption as per the QRD Group decision above will however be translated in all languages in the Annexes published with the EPAR on EMA website, but the printed materials will only be translated in the language(s) as agreed by the QRD Group.

2.10.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Pretomanid FGK (pretomanid) is included in the additional monitoring list as it pertains a conditional marketing authorisation for a product containing a new active substance.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Tuberculosis remains one of the world's major causes of illness and death – prompting the WHO in 1993 to declare tuberculosis to be a global health emergency. While tuberculosis is found in every country in the world, it disproportionately affects people in resource-poor settings, particularly those in Asia and Africa, but outbreaks still occur in industrialized nations. Approximately one quarter of the world's population are infected with *M. tuberculosis* (WHO 2018), and where the risk to fall ill in active disease is many times higher in case of immunosuppression, e.g. in case of (untreated) HIV-infection. In 2017 there were an estimated 10 million incident cases of tuberculosis (around 10% co-infected with HIV), and 1.6 million people died from tuberculosis. Emerging resistance is a great threat to TB therapy – in 2017 the number of cases of MDR-B (rifampicin resistance) was estimated to 330,000, and where the proportion of these cases fulfilling criteria for XDR-TB (resistance to isoniazid, rifampicin, quinolones and at least one injectable agent) is increasing.

3.1.2. Available therapies and unmet medical need

Prior to the approval of bedaquiline in 2013/2014 typical cure rates of XDR-TB patients in the high epidemic setting were very low, around 15%, despite two years of therapy with multiple agents. Following that approval and with the use of off-label linezolid, treatment success have increased, where some groups more recently have reported cure rates of 70-80%. However, the latter regimens were complex, typically including >10 drugs, and with treatment durations of around 2 years. For MDR-TB (less advanced resistance) regimens are also still complex, with a large number of agents and with treatment durations of at least 9 months. Hence, new shorter and standardized regimens are highly warranted for the treatment of XDR-TB, as well as for MDR-TB patients who have tolerability problems with present therapies.

3.1.3. Main clinical studies

The TB Alliance has systematically tested various pretomanid-containing regimens aimed to simplify MDR-TB therapy, as well as to shorten standard therapy for patients with Drug Sensitive TB (DS-TB). As part of this program, the Nix-TB study, pivotal for this application, was initiated in April 2015. This single arm study (N=109), which is running at 3 MDR-TB centres in South Africa, concerns the hardest to treat, i.e. patients with XDR-TB and patients with MDR-TB who are intolerant/non-responsive to the MDR-TB regimen. Around 50% of the patients are co-infected with HIV, and the baseline disease characteristics very severe. The regimen is standardized and includes only three agents: bedaquiline (dosed according to label), pretomanid (dosed 200 mg daily) and linezolid (dosed 1200 mg daily) (the "B-Pa-L" regimen). The treatment duration is 6 months, which could be extended to 9 months if the patient was still culture positive after 4 months of therapy (occurring in only 1/109, who had a favourable outcome with 6 months of therapy). The Nix-TB study was the first clinical trial to test the B-Pa-L regimen, which had shown promising results in a murine TB model. Baseline resistance to any of the three agents is still very low also in the XDR-TB setting.

3.2. Favourable effects

The primary endpoint (negative sputum culture 6 months after EOT) was achieved by 98/109 patients (ITT 90%, mITT 98/107 (92%))

Furthermore, secondary endpoint data at 24 months follow-up from EOT is provided in 47 patients who were evaluable for efficacy at this timepoint, demonstrating a relapse-free cure rate is largely sustained at 87%.

The success rate was similar in XDR-TB (89%) and MDR-TB (92%) patients and identical in those with or without HIV co-infection. The outcome was unfavourable in 8/80 for the primary endpoint, and this concerns 2 relapses and 6 deaths (4 cases directly/indirectly associated to severe TB disease, 1 case of upper GI bleeding and 1 case of haemorrhagic pancreatitis).

A comparison of the BPaL regimen of the Nix-TB study with a contemporaneous and geographically matched control group where all patients received bedaquiline and 82% of patients received linezolid, showed superior efficacy of the BPaL regimen, with cure rates of 89.9% and 64.7%, respectively ($P<0.0001$). The contribution of pretomanid to the treatment effect is supported by animal model data indicating additive effects.

3.3. Uncertainties and limitations about favourable effects

Nix-TB is a single-armed study of limited size; thus, there is no calibration with regards to the cure rate in the selected population with standard of care.

The primary endpoint data concern the outcome 6 months post end of treatment. In prior analyses, based on other treatment regimens, around of 80% of all relapses occurred within that time period (90% within 12 months). Data in 47 patients that have reached 24 months are supportive; however, the small dataset of available data constitute a limitation on the long-term outcomes.

3.4. Unfavourable effects

The most common TEAEs in the pivotal study, occurring in ≥10% of patients, were peripheral neuropathy, nausea, anemia, vomiting, headache, dyspepsia, dermatitis acneiform, decreased appetite, GGT increased, rash, pruritus, transaminases increased, abdominal pain, musculoskeletal pain and hyperamylasaemia. With regards to more common AEs (i.e. potential ADRs) causality to the regimen and individual agents are hard to assess, considering the patients population studied.

The present B-Pa-L regimen includes linezolid dosed 1200 mg daily throughout the treatment duration, where, notably, linezolid is a preferred XDR-TB agent also with other regimens. Linezolid has a well-established mitochondrial toxicity and is outside the XDR-TB setting therefore seldom used for prolonged times (more than 4 weeks of therapy is off-label use). This toxicity resulted in a high frequency of peripheral neuropathy (total frequency around 70%, mainly grade 1-2) and anaemia (around 35%), resulting in a high frequency of linezolid dose reductions/interruptions and discontinuations: linezolid was interrupted at least once in around 50% of the patients (mean total duration of interruptions around 6 weeks), and discontinued in around 25% of the patients. Over 60% of the patients had to reduce the dose of linezolid at least once.

The entire regimen was halted (but not discontinued) in a modest number of patients (7%), a low number in the setting of XDR TB therapy. In these cases, the interruptions were mainly caused by increases in liver enzymes, where it is unclear what agent that caused the reactions, since also bedaquiline (and possibly other co-medications) has the potential for such reactions. In all cases

therapy could be re-started and therapy completed. Of note, the SmPC includes a detailed guidance around monitoring of liver enzymes, and how to handle cases of enzyme elevations.

Pretomanid appears to contribute to the QTc prolonging effects of the BPaL regimen, which also includes bedaquiline, a known QT-prolongator. The SmPC includes instructions on appropriate monitoring.

3.5. Uncertainties and limitations about unfavourable effects

Pretomanid was administered without food in the monotherapy studies (dosed 100 to 1200 mg), and in fed state in longer term efficacy and safety studies (dosed 100 or 200 mg). The food effect is around +50%, and further, absorption became saturated in the fasting condition. The studied exposure margin is therefore smaller than implied by the studied dose range.

While pretomanid has a half-life of 16h, the t1/2 of total plasma radioactivity in the mass balance study is significantly longer. Despite extraction attempts, a proportion of the radioactivity remains unextractable (39% unextracted based on AUC0-48). Unextractable radioactivity associated with plasma proteins might be the reason to the long t1/2 of 18 days for total plasma radioactivity. The composition of the unextractable radioactivity is unknown, as is its reactive potential. The sum of unextractable radioactivity is however qualified in non-clinical species.

Pretomanid liver safety in this clinical setting of XDR-TB is deemed acceptable on basis of Nix-TB data (the B-Pa-L regimen, tested without a control group). Only 1/109 patients had an ALT/AST grade 4 increase, and those who stopped the regimen due to liver enzyme elevations (8/109) could all resume therapy and complete the planned treatment duration. No grade 3-4 ALT increases were seen during short term monotherapy up to 1200 mg qd. In contrast, seemingly worrisome data were seen in a planned phase 3 study, NC-006, which was halted by the FDA for reasons of liver safety (safety hold later lifted). In this study Pa-M-Z was given for 4 or 6 months and was compared to HRZE-control in DS-TB patients. Of those randomized to Pa-M-Z, 12% had ALT/AST elevations > 10 x ULN (grade 4) and liver chemistry/toxicity lead to treatment discontinuations in many cases. Three patients (still Pa-M-Z-arms) had fulminant liver failures with a fatal outcome, all 3 with an onset around week 4-5. Of note, one liver related death was seen also in the control arm, and patients were randomized 3:1 (test regimen vs control). The reasons for the high frequency of severe liver reactions in NC-006 is unknown. Although the liver safety of pretomanid is not fully understood, present data are deemed acceptable, and detailed stopping criteria are provided in the SmPC.

On basis of pre-clinical data, pretomanid has a potential for QT-prolongation, but the results in a single dose thorough QT study would predict no relevant effect for doses used in the clinical studies (100 or 200 mg qd, with 200 mg in the Nix-TB study). However, this QT study is considered to have deficiencies, including the single dose design. Across other studies a varying degree of QTc increase is seen. Based on the totality of data a QTc increase higher than that seen in thorough QT study is expected. When looking at treatment emergent, potentially clinically relevant ECG results in the Nix-TB study it is noted that 5/109 were reported to have a QTc reading of >480 msec, and 1/109 >500 msec. The Nix-TB study was not designed in order to fully detect the adverse effects on the QT interval of the pretomanid + bedaquiline combination. Notwithstanding this, the uncertainty is considered manageable, with proper selection of patients, and monitoring in accordance with relevant wordings in section 4.4. of the SmPC.

In non-clinical studies a toxic effect of pretomanid on testes and male fertility was observed. The duration of the follow-up periods for examining pretomanid's possible detrimental effect on male fertility in humans was limited to 8 weeks in the phase 2 studies NC-002 and NC-005 and 26 weeks in the prematurely terminated phase 3 study NC-006. A dedicated study on semen is planned.

3.6. Benefit-risk assessment and discussion

3.6.1. Importance of favourable and unfavourable effects

A favourable treatment outcome of 90% in XDR/TB patients, and MDR-TB patients intolerant/non-responsive to their MDR-TB regimen, with the use of standardized 6 months treatment course consisting of 3 agents is an outstanding result. The applicant has provided further contextualisation, comparing outcomes with 102 concurrent control patients treated in a single large centre in Cape Town, South Africa (which also participated in Nix-TB) who received a bedaquiline-based regimen which contained a median of 8 drugs, including linezolid in 82% of cases. This cohort consisted of XDR-TB only. The median duration of treatment was 16.5 months, to be compared with the 6 months with BPaL regimen. The proportion with a favourable outcome was around 65% (here counted as all patients with a sputum conversion 6 months EOT, or having fulfilled treatment), compared to 90% with the considerably simpler B-Pa-L regimen. Given the considerable value of treatment simplification, also in case the relative increment in efficacy of BPaL would be overestimated, the regimen would still constitute an important improvement in TB treatment. In addition, preliminary data from the ZeNix study are supportive of the effects seen with the B-Pa-L regimen in the Nix-TB study.

The single-arm design of the study makes it difficult to discern any AEs caused specifically by pretomanid from the AEs instigated by bedaquiline and linezolid. Few patients have been treated with the BPaL regimen and important patient groups have not been studied (e.g., patients with renal and hepatic impairment, elderly and patients that might be prone to QT prolongations or increases in liver transaminases). Possible additive and/or synergistic effects of pretomanid on potentially serious AEs caused by one of the other drugs in the triplet is not known, especially with regards to fragile patient groups.

One important obstacle with the present regimen is the high frequency of linezolid-associated side effects (neuropathy and anaemia) as a result of the 1200 mg daily dose given throughout the treatment course. However, linezolid is a recommended agent also in alternative regimens in XDR-TB therapy (85% in the mentioned concurrent cohort), and these side effects were manageable with the use of dosing rules that are provided in the SmPC. An optimized dosing schedule of linezolid is presently evaluated in the ZeNix study (N=180).

Apart from linezolid-related adverse events, liver toxicity and QTc prolongation have been identified as important risks when using the BPaL regimen. The liver safety in the Nix-TB study was acceptable, and those few patients who halted the regimen due to ALT increases could resume and complete therapy without further problems. Supportive preliminary data have been presented for the B-Pa-L regimen from the ZeNix-study. Although the impact of Pretomanid on QTc has not been fully characterized and is likely larger than predicted in the thorough QT study, this uncertainty is considered manageable, with proper selection of patients, and monitoring of patients. Of note, substantial QT prolongation is a general issue for available XDR TB regimens, where several agents have such properties.

In summary, the safety profile of the BPaL regimen is considered acceptable and the AEs manageable provided that close monitoring and surveillance of the patients during treatment and follow-up is in place. However, due to the limited number of patients treated for 6 months or more, and the single arm nature of the data providing information on the safety of the B-Pa-L, available data are considered non-comprehensive.

3.6.2. Balance of benefits and risks

The efficacy with pretomanid as part of the B-Pa-L regimen is considered to outweigh the risks for treatment of patients with XDR-TB or patients with MDR-TB intolerant/unresponsive to the MDR TB regimen, on the basis of efficacy and safety based on the available data.

3.6.3. Additional considerations on the benefit-risk balance

Since safety data are deemed non-comprehensive, it needs to be justified that the prerequisites for a Conditional Marketing Authorisation are fulfilled. The balance of benefits and risks is however considered positive.

It is recognised that a randomised controlled trial versus the presently recommended XDR-TB regimens is not feasible. The ongoing ZeNix study, however, is anticipated to provide a further understanding of the safety profile of the B-Pa-L regimen, including the frequency of hepatic reactions and issues related to QT prolongation, and will, as an additional benefit, provide further data on the optimal linezolid dose. Given the progress of this study, it is likely that the applicant will be able to provide comprehensive data.

Given the efficacy seen in the pivotal Nix-TB study, as well as the shortening of XDR therapy to six months, the product addresses an unmet need and provides a major therapeutic advantage over all available therapies for the treatment of XDR-TB/ unresponsive MDR-TB infection.

Therefore, notwithstanding the non-comprehensiveness of safety data, benefits to public health of the immediate availability of the medicinal product outweigh the risks inherent in the fact that additional data are still required.

Conditional marketing authorisation

As comprehensive data on the product are not available, a conditional marketing authorisation was proposed by the CHMP during the assessment, after having consulted the applicant.

The product falls within the scope of Article 14-a of Regulation (EC) No 726/2004 concerning conditional marketing authorisations, as it aims at the treatment of a life-threatening disease. In addition, the product is designated as an orphan medicinal product.

Furthermore, the CHMP considers that the product fulfils the requirements for a conditional marketing authorisation:

- The benefit-risk balance is positive, as discussed.
- It is likely that the applicant will be able to provide comprehensive data. The ongoing ZeNix study (examining linezolid dose and duration within this treatment regimen) will give more data on efficacy of the regimen as well as gather information on an optimized dose of linezolid, limiting the side effects of that agent. In addition, 24 months follow-up data of the Nix-TB study will also become available. Overall, this is expected to provide comprehensive data about the efficacy and safety of pretomanid in the proposed indication. Both studies are ongoing and the applicant is expected to be able to provide the results.
- Unmet medical needs will be addressed, as in adult patients with pulmonary extensively drug resistant (XDR), or treatment-intolerant or nonresponsive multidrug-resistant (MDR) tuberculosis, the improved efficacy and considerably shorter and simpler treatment regimen are marked improvements compared to currently authorised treatments for these patients.

- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required. Given the considerable value of treatment simplification, also in case the relative increment in efficacy of BPaL would be overestimated, the regimen would still constitute an important improvement in TB treatment. In addition, preliminary data from the ZeNix study are supportive of the effects seen with the B-Pa-L regimen in the Nix-TB study, decreasing the risks inherent in the fact that additional data are still required.

3.7. Conclusions

The overall B/R of Pretomanid in the presently sought indication is positive.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Pretomanid FGK is not similar to Sirturo (bedaquiline fumarate), Granupas (para-aminosalicylic acid) or Deltyba (delamanid) within the meaning of Article 3 of Commission Regulation (EC) No. 847/200. See Appendix 1.

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Pretomanid FGK is favourable in the following indication:

Pretomanid FGK is indicated in combination with bedaquiline and linezolid, in adults, for the treatment of pulmonary extensively drug resistant (XDR), or treatment-intolerant or nonresponsive multidrug-resistant (MDR) tuberculosis (TB), see sections 4.2, 4.4 and 5.1.

Consideration should be given to official guidance on the appropriate use of antibacterial agents.

The CHMP therefore recommends the granting of the conditional marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see section 4.2 of the Summary of Product Characteristics).

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Specific Obligation to complete post-authorisation measures for the conditional marketing authorisation

This being a conditional marketing authorisation and pursuant to Article 14-a of Regulation (EC) No 726/2004, the MAH shall complete, within the stated timeframe, the following measures:

Description	**Due date**
In order to further evaluate the safety, efficacy and tolerability of linezolid plus bedaquiline and pretomanid after 26 weeks of treatment in participants with either pulmonary XDR-TB, pre-XDR TB, or treatment intolerant or non-responsive MDR-TB, the marketing authorisation holder should complete and submit results from the ongoing study ZeNix – A Phase 3 Partially-blinded, Randomized Trial Assessing the Safety and Efficacy of Various Doses and Treatment Durations of Linezolid Plus Bedaquiline and Pretomanid in Participants With Pulmonary Infection of Either Extensively Drug-resistant Tuberculosis (XDR-TB), Pre-XDR-TB or Treatment Intolerant or Non-responsive Multi-drug Resistant Tuberculosis (MDR-TB)	Annual reports to be submitted Final report by Q4 2022
In order to confirm the safety and efficacy of pretomanid the marketing authorisation holder should complete and submit results from the ongoing Phase 3 Open-label Trial Assessing the Safety and Efficacy of Bedaquiline Plus Pretomanid Plus Linezolid (B-Pa-L) in Subjects with Pulmonary Infection of Either Extensively Drug-resistant Tuberculosis (XDR-TB) or Treatment Intolerant/Non-responsive Multi-Drug Resistant Tuberculosis (MDR-TB) (Nix-TB)	Final report by Q2 2021

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that pretomanid is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.