A Phase 2 Randomized, Open-Label Trial of PA-824-Containing Regimens versus Standard Treatment for Drug-Sensitive Sputum Smear-Positive Pulmonary Tuberculosis

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Study Protocol containing Statistical Analysis Plan

7 February 2018

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U.S. Investigators:
Kelly E. Dooley – Johns Hopkins University – Co-Principal Investigator
Nikhil Gupte, Johns Hopkins University – Statistician and Co-Investigator
Grace Barnes, Johns Hopkins University – Study Coordinator

South Africa Investigators:
Rodney Dawson – University of Cape Town – Site Principal Investigator
Helen McIlleron – University of Cape Town – Co-Investigator and Pharmacologist
Bronwyn Hendricks – University of Cape Town – Co-Investigator

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STUDY TEAM

Principal Investigator, United States
Kelly E. Dooley, MD, PhD
Associate Professor of Medicine, Pharmacology & Molecular Sciences
Divisions of Clinical Pharmacology and Infectious Diseases
Johns Hopkins University Center for Tuberculosis Research
600 N. Wolfe Street, Osler 527
Baltimore, MD 21287
Phone 443-287-0517
Fax 410-614-9978
Email kdooley1@jhmi.edu

Co- Investigator, United States
Susan E. Dorman, MD
Associate Professor of Medicine and International Health
Johns Hopkins University Center for Tuberculosis Research
1550 Orleans Street, Room 1M-06
Baltimore, MD 21231
Phone 410-502-2717
Fax 410-955-0740
Email dsusan1@jhmi.edu

Co-Investigator, Study Coordinator, United States
Grace Barnes, BSN, MPH
Johns Hopkins University Center for Tuberculosis Research
1550 Orleans Street
CRB-2, 1M
Baltimore, MD 21287-0014
Phone 410-614-3812
Email glbarnes@jhmi.edu

Co-Investigator, Biostatistician, United States/India
Nikhil Gupte, MSc, MPhil, PhD
Center for Global Health and Education, Johns Hopkins University School of Medicine &
BJ Medical College and Sassoon General Hospitals
Jai Prakash Narayan Road
Pune, India 411001
Phone +91 98220-70853
Email ngupte1@jhmi.edu

Site Principal Investigator, Cape Town
Rodney Dawson, MB ChB,FCP (SA), Cert. Pulm.(SA)
Centre for TB Research Innovation, University of Cape Town Lung Institute
P.O.Box 34560
Groote Schuur 7937, South Africa
Phone +27214066864
Fax +27214067770
Email rodney.dawson@uct.ac.za

Co-Investigator, Cape Town
Helen M. McIlleron, MB ChB, PhD
Head, Pharmacokinetic Unit, Division of Clinical Pharmacology
Department of Medicine
University of Cape Town
Email helen.mcIlleron@uct.ac.za

Co-Investigator, Cape Town
Bronwyn Hendricks, MB,ChB
Centre for TB Research Innovation, University of Cape Town Lung Institute
P.O.Box 34560
Groote Schuur 7937, South Africa
Phone +27214047747
Fax +27214047770
Email bronwyn.hendricks@uct.ac.za

Co-Investigator, Cape Town
Kim Narunsky
Centre for TB Research Innovation, University of Cape Town Lung Institute
P.O.Box 34560
Groote Schuur 7937, South Africa
Phone +27 21 406 6605 (Office)
Fax +27214047770
Email Kim.Narunsky@uct.ac.za

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1 PROTOCOL SYNOPSIS

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<tr><td>Protocol Title:</td><td>A Phase 2 Randomized, Open-Label Trial of PA-824-Containing Regimens versus the Standard Regimen for the Treatment of Drug Sensitive Sputum Smear-Positive Pulmonary Tuberculosis</td></tr>
<tr><td>Treatment Indication:</td><td>Pulmonary Tuberculosis (TB)</td></tr>
<tr><td>Trial Objective:</td><td>Assess the mycobactericidal activity of PA-824 (given at 200 mg daily for 12 weeks) when added to first-line TB treatment (isoniazid, pyrazinamide, and a rifamycin antibiotic) after 12 weeks of treatment</td></tr>
<tr><td>Trial Design:</td><td>Open-label, randomized clinical trial in three treatment groups. Patients with drug-sensitive TB will all receive once daily isoniazid and pyrazinamide for 8 weeks followed by 4 weeks of daily isoniazid. In addition, Arm 1 participants will receive PA-824 200 mg daily and rifampin 600 mg daily for 12 weeks. Arm 2 participants will receive PA-824 200 mg daily and rifabutin 300 mg daily for 12 weeks. Arm 3 participants (control group) will receive rifampin for 12 weeks and ethambutol for 8 weeks. Patients will be screened following TB diagnosis, will receive 12 weeks of study treatment and will return for follow-up visits at 4, 12, and 36 weeks after study treatment completion. All patients will be referred to the local TB treatment program to complete their 24-week TB treatment course.</td></tr>
<tr><td>Patient Population:</td><td>A total of approximately 183 male and female patients, newly diagnosed with sputum smear positive pulmonary tuberculosis aged 18 years or older.</td></tr>
<tr><td>Treatment arms:</td><td>Study participants will be randomized 1:1:1 to receive one of the following:

<table>
<tr><th>Arm</th><th>Weeks 0-8</th><th>Weeks 9-12</th><th>Weeks 13-24*</th></tr>
<tr><td>1</td><td>PaHRZ</td><td>PaHR</td><td>HR</td></tr>
<tr><td>2</td><td>PaHRbZ</td><td>PaHRb</td><td>HR</td></tr>
<tr><td>3 (standard Rx)</td><td>EHRZ</td><td>HR</td><td>HR</td></tr>
</table>
*Note: During Weeks 13-24, patients will receive standard TB treatment through local TB programs.

PA-824 (Pa): 200 mg once daily (7/7)
Rifampin (R): 600 mg once daily (7/7)
Rifabutin (Rb): 300 mg once daily (7/7)
Isoniazid (H): 300 mg once daily (7/7)
Ethambutol (E) and Pyrazinamide (Z) will be given at standard doses, according to weight bands (see Section 5.1)</td></tr>
</table>

Criteria for evaluation:

Primary Endpoints:

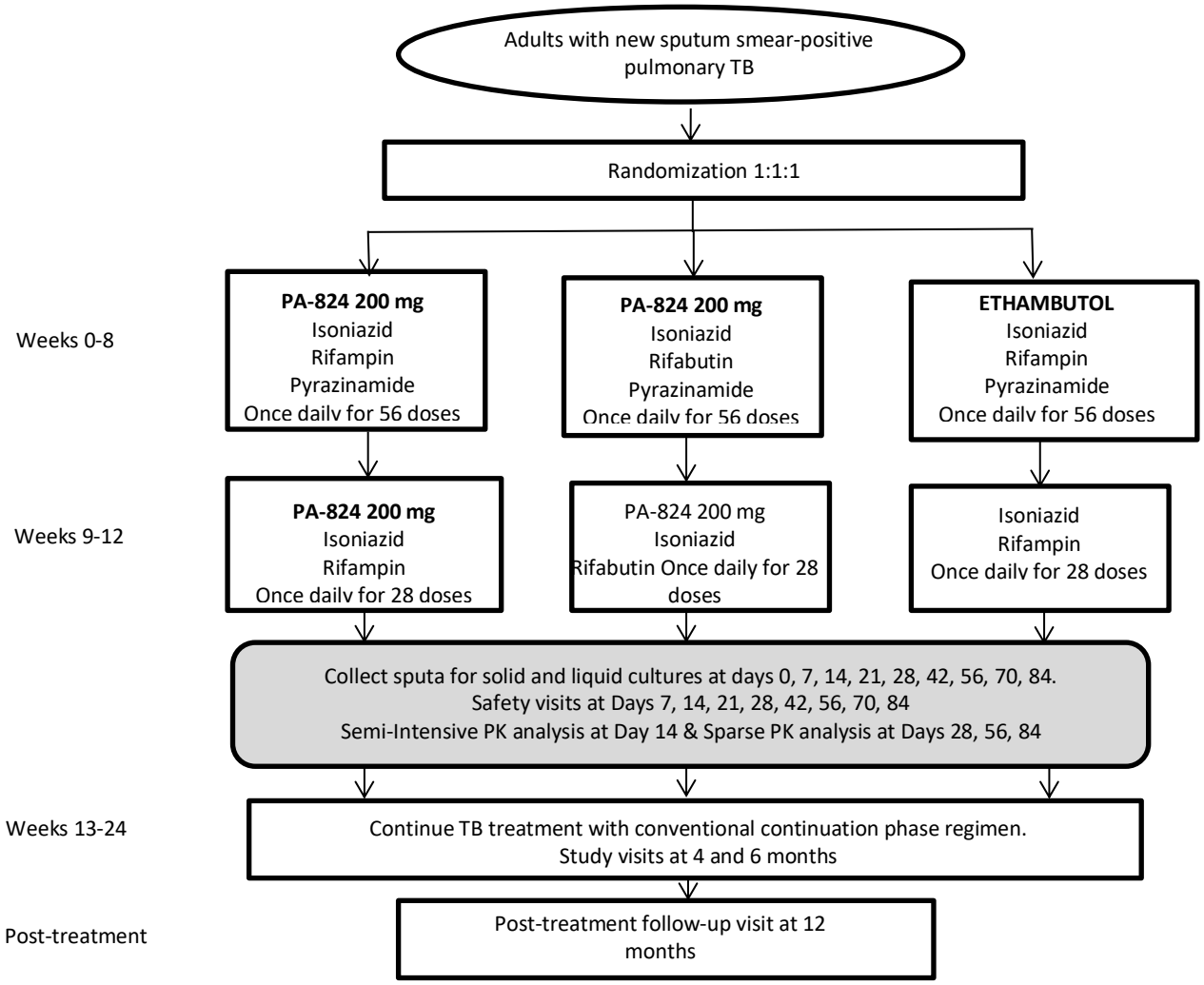
(1) Time to sputum culture conversion in liquid media, defined as the time from the start of treatment until the first negative sputum culture, over 12 weeks
(2) Proportion of subjects with a severe adverse event (defined as grade 3, 4, or 5 adverse event)

Secondary Endpoints:

(1) Discontinuation of study treatment for any reason
(2) Time to sputum culture conversion in solid media, defined as the time from the start of treatment until the first negative sputum culture, over 12 weeks
(3) Proportion of participants with sputum culture conversion at 8 weeks or at 12 weeks (separately, on solid and liquid media).
(4) The rate of change in time to sputum culture positivity (TTP) through 12 weeks in the Mycobacterial Growth Indicator Tube (Bactec MGIT960) system in sputum over 12 weeks in participants, which may be described with linear, bi-linear or non-linear regression of TTP on time
(5) Pharmacokinetics (PK) of PA-824 delivered at a dose of 200 mg once daily in the context of concomitant rifampin- or rifabutin-containing treatment
(6) Pharmacokinetic/pharmacodynamic (PK/PD) relationship between PA-824 exposures (area under the concentration-time curve (AUC) or maximum concentration (Cmax)) and outcomes (time to culture negativity or rate of change in TTP over 12 weeks) using non-linear mixed effects modeling

Study site: Study subjects will be recruited from the University of Cape Town inpatient wards and outpatient clinics.
Study duration: Duration of study: It is estimated that 18 months will be required for recruitment and enrollment of study subjects. The estimated duration of participation for each study subject is 12 months, including 3 months (12 weeks) of experimental phase TB treatment, 3 months (12 weeks) of non-experimental conventional continuation phase TB treatment, and an additional 6 months for follow-up for TB relapse. For those in Arms 1 and 2, a post-treatment ophthalmology follow-up 12 weeks after stopping PA-824 will be performed.

2 STUDY SCHEMA



3 OBJECTIVES

3.1 Primary objectives

(1) To compare the efficacy of the test regimens, using the intermediate endpoint of time to culture conversion on liquid medium, to the efficacy of standard treatment
(2) To compare the safety and tolerability of each test regimen to the safety and tolerability of standard treatment

3.2 Secondary objectives

(1) To compare the discontinuation rate of study treatment, by arm
(2) To compare the efficacy of each regimen, using the intermediate endpoint of time to culture conversion on solid medium, to the efficacy of standard treatment
(3) To compare the proportion of participants in each arm who convert sputum cultures to negative with the control arm after 8 and 12 weeks of treatment (on liquid medium and on solid medium)
(4) To compare the rate of change in TTP through 12 weeks, comparing the test regimens to standard treatment
(5) To describe the steady-state pharmacokinetics of PA-824 among patients with pulmonary TB receiving co-administered isoniazid, rifampin or rifabutin, and pyrazinamide
(6) To evaluate the relationship between PA-824 pharmacokinetics (AUC and Cmax) and treatment outcomes, as measured by time-to-culture conversion or rate of change in TTP over time among participants in the experimental treatment arms

4 INTRODUCTION

4.1 Scientific background

Tuberculosis (TB) as a Global Health Problem: The Need for Novel Treatment Regimens

Tuberculosis (TB) continues to be a global public health threat. In 2011, there were an estimated 8.7 million incident cases of TB and 1.4 million deaths.(1) TB control efforts are hampered by the lengthy, complex treatment regimens necessary for cure without relapse. Although the current regimens and drugs have been very successful in controlled clinical trials resulting in the permanent cure of more than 95% of trial participants, treatment of drug-sensitive TB takes 6 months to complete. In practice, treatment completion and cure rates vary by country, with poor adherence most likely to occur after the second month of treatment. The full application of the directly observed therapy short course (DOTS) strategy is becoming more and more difficult in the developing countries of the world where TB incidence is high, countries that are also battling to control the HIV epidemic. As a result of poor treatment adherence, drug resistance is becoming more common, and multidrug-resistant (MDR) TB has emerged as a worldwide epidemic with approximately 440,000 cases and 150,000 deaths per year (2). Shorter treatment duration can decrease the logistical burden and expense of extended therapy, improve adherence, and help prevent the emergence of acquired drug resistance. An urgent research priority is, thus, to evaluate new drugs and new combination regimens that can shorten treatment duration for drug-sensitive TB, both for the benefit of individual patients and to improve TB control from a public health standpoint. PA-824, a nitroimidazole with impressive activity against *Mycobacterium tuberculosis* (MTB) in in vitro and mouse models and in early phase clinical trials, holds promise as a novel anti-tuberculosis agent.

Nitroimidazoles for the treatment of TB

Tuberculosis in humans is an extremely complex disease that primarily affects the lung in discrete consolidated foci known as granulomas. In contrast to murine models of TB disease, human disease is characterized by discrete types of lesions including both aerobic (cavities) and anaerobic (caseous necrotic nodules) areas. The bacteria found in these different areas represent metabolically distinct populations against which individual TB drugs have varying activity(3). In TB-infected rabbits and non-human primates that have necrotic lesions similar to those seen in human TB disease, nitroimidazoles have been demonstrated to be highly efficacious against TB(4), presumably because of their activity against "persister" organisms, those *M. tuberculosis* subpopulations that are phenotypically resistant to first-line drugs like isoniazid and rifampin but can be killed by TB drugs that have activity against the anaerobic sub-populations thought to cause relapse if TB treatment is stopped too early. Metronidazole, for example, is active *in vitro* against *M. tuberculosis* maintained under anaerobic conditions, but demonstrated little to no activity in mouse

models of TB treatment (5). However, in the rabbit and primate models, the drug was highly efficacious (4,6). More importantly, in a recent clinical trial involving 31 patients, metronidazole shortened the time to sputum culture conversion among patients with MDR-TB receiving a standard MDR-TB regimen compared to placebo, but two-month culture conversion proportions among those who did and did not receive metronidazole were not statistically different (7). High resolution CT and positron emission tomography (PET) testing suggested that resolution of TB lesions over 2-6 months was associated with successful treatment outcome, but these results require confirmation in a larger population of patients with TB. Metronidazole causes unacceptably high rates of peripheral neuropathy (7), and it is dosed three times a day, so is not a good candidate drug for treatment of drug-sensitive TB; also, it has activity against TB that is limited to TB in anaerobic conditions. Delamanid (previously OPC-67683) , another nitroimidazole under development for the treatment of TB, increased 2-month culture conversion from 30% to 45% when given at a dose of 100 mg twice daily or 200 mg twice daily and added to standard treatment among patients with MDR-TB (8). However, long-term favorable outcomes assessed over 24 months were only improved among those patients who received extended treatment with delamanid for 6 months (versus those that received the drug for $\leq$2 months)(9). Delamanid, a twice-a-day drug, has never been tested among patients with drug-sensitive TB who are receiving first-line drugs with good sterilizing activity (like rifamycins and pyrazinamide).

PA-824 is a nitroimidazole with potent in vitro activity against MTB with minimum inhibitory concentrations (MIC) for antibiotic-susceptible and drug-resistant strains ranging from 0.015 to 0.25 (10,11), a narrow spectrum of activity limited to the *M. tuberculosis* complex, and no demonstrable cross-resistance with marketed TB drugs. In vitro, PA-824 has activity against metabolically active as well as non-replicating *M. tuberculosis* isolates (11). Thus, PA-824 may be expected to have both bactericidal and sterilizing activity, the latter being the key to treatment shortening. The mechanism of action of PA-824 has not been fully elucidated, but it appears to inhibit oxidation of hydroxymycolate to ketomycolate, thus interfering with cell wall biosynthesis (10). However, it has other, less specific, activity. PA-824 is a prodrug, like its cousin, metronidazole. It is activated by *M. tuberculosis* via reduction to a des-nitroimidazole metabolite by a deazaflavin (F420)-dependent nitroreductase (10). Once the compound is enzymatically reduced to its des-nitroimidazole metabolite, reactive nitrogen species including nitric oxide are generated which poison *M. tuberculosis'* respiratory apparatus and likely contribute to the drug's lethal effects, particularly in nonreplicating bacilli (12). The preclinical and clinical data suggesting that PA-824 may play an important role in TB treatment shortening are provided in detail below.

PA-824 has been studied in three 14-day early bactericidal activity (EBA) trials to date. In EBA studies, quantitative sputum cultures are collected daily over the course of 7-14 days among patients with pulmonary TB receiving monotherapy or combination therapy to detect differences in microbiologic activity among doses, drugs, or regimens (13,14). Typically, these studies are used as dose-finding trials to determine which drugs or combinations should be tested in Phase 2B trials (longer-duration studies, in which regimens are tested over 2-3 months). In two EBA studies, PA-824 monotherapy has demonstrated substantial mycobactericidal activity (15,16). The efficacy data from the TB Alliance-sponsored study PA-824-CL-007 indicated that all doses of PA-824 (200, 600, 1000 and 1200 mg) produced an equivalent decrease in sputum CFU counts over the 14-day treatment period; no difference could be discerned among the PA-824 treatment groups (17). In study PA-824-CL-010, an EBA study with a similar design to study PA-824-CL-007 except for the use of lower doses of PA-824 (50, 100, 150 and 200 mg/day), results indicated that PA-824 treatment resulted in a measurable dose-dependent mycobactericidal activity over the dose range studied, and supported a clinical dose of 100-200 mg per day (16). In study NC-001-(J-M-Pa-Z), an EBA study with multiple treatment combinations, preliminary results suggested that the three drug combination of PA-824 (200 mg per day), moxifloxacin and pyrazinamide (PaMZ) had similar or better activity to standard first-line TB treatment. This combination was just tested in a Phase 2B trial (NC-002) for drug-sensitive and MDR-TB with PA-824 given at doses of 100 mg or 200 mg (18). NC-002 was recently completed; 207 subjects were enrolled, including 60 randomized to M-PA100-Z, 62 randomized to M-PA200-Z, and 59 to HRZE. In addition, 26 subjects with MDR-TB were treated with M-PA200-Z. . Preliminary results are as follows: In the M-PA200-Z arm, 8-week culture conversion was 94% on solid media and 71% on liquid media; in the M-PA-100-Z arm, 8-week culture conversion was 85% on solid media and 67% on liquid media. This is compared to 87% (solid) and 38% (liquid) culture conversion at 8 weeks in the control arm (unpublished data courtesy of TB Alliance). Phase 2A and 2B trials of PA-824, thus, support testing of this drug at doses of 100 mg or higher once daily. Preclinical studies suggest that adding PA-824 to standard first-line TB drugs may shorten the time needed to cure TB to less than 6 months(19), and this must be studied clinically in a Phase 2B trial prior to evaluation of a regimen of PA-824 and standard first-line TB drugs (isoniazid, a rifamycin antibiotic, and pyrazinamide) in a Phase 3 treatment-shortening study. Middle-duration (2-3 months) treatment studies have historically served as stepping stones to pivotal Phase 3 trials in development of new TB treatment regimens.

PA-824

Preclinical Studies

Please see the Investigator's Brochure (Appendix C) for full details of preclinical and clinical studies to date.

Microbiology

In vitro studies have demonstrated that the minimum inhibitory concentration (MIC) of PA-824 against a variety of drug-sensitive *MTB* isolates is similar to the MIC of isoniazid (MIC of PA 824, ≤0.015 to 0.25 µg/mL; MIC of isoniazid, 0.03 to 0.06 µg/mL) (10,11) . PA-824 was efficacious in vitro against drug-resistant clinical isolates of *M. tuberculosis* with MIC values ranging from 0.03 to 0.53 µg/mL. The minimum effective dose (MED) of PA-824 was 12.5 mg/kg/day in a mouse model of TB (20). The MED is defined as the lowest dose able to prevent the development of macroscopic lung lesions and splenomegaly. The minimum bactericidal dose (MBD) was 100 mg/kg/day in the same mouse model. The MBD is defined as the lowest dose able to reduce the lung TB colony forming unit (CFU) counts by 99%. The magnitude of CFU reduction by PA-824 at this dose is similar to that seen with the highest dose of isoniazid tested in the same study (25 mg/kg/day).

Nonclinical Safety Studies

The non-clinical safety evaluation of PA-824 includes pharmacology, pharmacokinetics, toxicology and metabolism studies that were conducted in accordance with current ICH guidelines.

Metabolite analyses in rats, monkeys, and humans indicate an overall similar metabolic profile in these species with some differences among minor metabolites. These studies have confirmed that rats and monkeys are appropriate species for the toxicology program.

PA-824 was negative in all genotoxicology studies performed. One of its metabolites (M50) that is found in rat, monkey, and human plasma was positive in a screening Ames assay. M50 is not a major metabolite in humans, and the exposure relative to parent drug is higher in the rat (24%) and monkey (18%) than in humans (6%).

PA-824-induced effects in respiratory, CNS, and cardiovascular safety pharmacology studies were generally mild and reversible; effects were most prominent at 450 mg/kg/day. PA-824 is a weak inhibitor (IC50=20uM) of the hERG channel. In a telemetry monkey study, in the dose range 50-450 mg/kg, there was no or minor prolongation of the QT interval, depending on the method of correction. The weight of evidence suggests that there should be no effect on QT in the concentration range being explored in the clinical studies.

Repeat-dose toxicology studies with PA-824 have been conducted in male and female rats (14 days to 6 months) and in male and female monkeys (7 days to 3 months). In all studies, dose-dependent reduced food consumption and reduced weight gain or weight loss were noted. In addition, testicular atrophy was observed in rats while cataracts were observed by indirect ophthalmoscopy in both rats and monkeys. In general, toxicity in both rat and monkey was significantly greater when exposures exceeded approximately 300 µg•hr/mL in the 14-day studies and approximately 200 µg•hr/mL in the 3-month studies.
Reproductive toxicology studies show that PA-824 is not teratogenic in rats or rabbits. In the rat fertility study, dose-dependent reduced fertility rates, due to decreased sperm numbers and decreased motility, were observed at doses of 30 mg/kg and greater. This effect was partially reversible. As in the 3-month rat toxicology study, irreversible testicular lesions were not observed at 30 mg/kg, only at 100 and 300 mg/kg.
To more fully characterize the cataract and male reproductive system findings, a 3-month monkey study in sexually mature males (0, 50, 150, 300 mg/kg/day) and a 6-month rat study (0, 30, 100, 300 mg/kg) in males and females were conducted. Ocular assessments were conducted in a much more careful and systematic manner than in the initial 3-month toxicology studies described above. In each of the later studies, all ophthalmologic examinations were conducted by a single ophthalmologist, using both indirect ophthalmoscopy and slit-lamp biomicroscopy. Animals were screened before dosing to ensure no animal had cataracts at baseline, and then monthly during dosing and recovery. In this monkey study, although similar drug exposures were attained as in the original 3 month monkey

study, no cataracts or testes effects (semenology, organ weights, histopathology, or hormones [testosterone, follicle-stimulating hormone, Inhibin B]) were observed at any point during dosing or during a 20-week recovery period. PA-824 does not appear to cause cataracts or testicular toxicity in monkeys. In the 6-month rat study, PA-824 caused irreversible cataracts at 100 mg/kg from Day 118 of the study in males and females. In contrast to the original 3-month rat report, rats in this more carefully conducted study developed cataracts while on drug but not during recovery. The NOAEL was 30 mg/kg for cataracts and 10 mg/kg for testicular toxicity. Rats that developed cataract and testicular toxicity also experienced marked decreases in body weight gain and food consumption. The AUC safety multiples (relative to the exposures obtained at the anticipated clinical dose of 200 mg/day) for cataract are ~1.5x in the rat; in the monkey at the highest dose tolerated, where there were no cataracts in the second well conducted study, the multiple is at least 3.7x.

To summarize, cataracts have been detected in multiple animals from two similar rat studies at mid-to-high doses. In contrast, the finding of cataracts in one monkey study could not be confirmed in a follow-up study. Thus, both cataracts and the testicular effects appear to be species-limited.

Preclinical efficacy

In an experimental murine model of TB, PA-824 was shown to have bactericidal activity during the early phase of TB treatment and sterilizing activity during the continuation phase of TB therapy when given as monotherapy (20). Further, when given with isoniazid, it prevented the development of isoniazid mutants. Subsequent studies demonstrated that substitution of PA-824 for pyrazinamide or rifampin (the two key sterilizing drugs in first-line TB treatment) was detrimental, and addition of PA-824 to standard treatment with isoniazid, rifampin, and pyrazinamide did not appear to provide additional benefit (21). In a subsequent study, substitution of PA-824 for isoniazid in a regimen containing rifampin and pyrazinamide reduced the treatment time required for cure from 6 months to 4 months, and the two-drug combination of PA-824-PZA was particularly potent (22). These mouse models, though, do not develop necrotic lesions, so the full anaerobic activity of PA-824 may not be realized, as was the case with metronidazole. In the "Kramnik" mouse model, in which C3H3B/FeJ mice develop hypoxic, necrotic lesions with their TB disease, Pa-824 added significantly to moxifloxacin plus pyrazinamide (MZ), but the combination of Pa-M-Z was not better than standard treatment with rifampin, isoniazid, and pyrazinamide (RHZ) (23). If findings in the mouse model are indicative of the human experience, retaining a rifamycin in the regimen and capitalizing on the synergistic activity of pyrazinamide and PA-824 is a promising treatment-shortening strategy.

Clinical Studies

Nine Phase 1 and four Phase 2 clinical trials with PA-824 have been completed. A Phase 2A EBA trial evaluating the combination of PA-824 plus moxifloxacin plus pyrazinamide showed that this combination had excellent early bactericidal activity (18), and the follow-on two-month Phase 2B trial of PA-824 at doses of 100 or 200 mg plus moxifloxacin plus pyrazinamide is complete, and preliminary efficacy results are described above. Table 1 provides a high-level summary of the completed and ongoing studies. Full details of the completed clinical studies are provided in the current Investigator's Brochure (Appendix C).

Table 1 PA-824 Clinical Studies to Date

Study	Design	PA-824 Dose Levels (mg)	Enrolled (active and control)	Key Safety/Efficacy Findings
Phase I				
CL-001	Double-blind, placebo-controlled, single-dose, dose-escalating, PK and safety study	0, 50, 250, 500, 750, 1000, 1250, 1500	53	• Well tolerated; no dose-limiting AEs or abnormal laboratory results; no effects on ECG, vital signs, or PE.
CL-002	Double-blind, placebo-controlled 7-day multidose, escalating, PK and safety study	0, 200, 600, 1000	24	• Well tolerated; no effects on ECG, vital signs, or PE. • After 5 days' dosing at 1000 mg/d, progressive moderate creatinine elevation: reversed during 7-day washout period. • No consistent effect on BUN. • A planned 1400-mg cohort not enrolled.
CL-003	Open-label, single-dose, food effects	1000	16	• Well tolerated; no dose-limiting AEs or abnormal laboratory results; no effects on ECG, vital signs, or PE. • Treatment-emergent AEs affecting more than one subject occurred more frequently after dosing in the fed condition than the fasted condition, and more frequently among women than men. • Bioavailability is 3.5-to-4.5-fold higher when PA-824 is administered within 30 minutes of a high-fat, high-calorie meal than when it is administered after an overnight fast.
CL-004	Open-label, single-dose, ADME	~860, oral suspension [benzyl-^{14}C]PA-824	6	• Well tolerated; no dose-limiting AEs or abnormal laboratory results; no effects on ECG, vital signs, or PE. • No significant radioactivity captured as [benzyl-^{14}C]CO_2. • ~91% of dose recovered (~65% in urine; ~26% in feces) Plasma: parent drug and one major metabolite. Urine: little or no parent drug; multiple major metabolites. • Feces: minimal unchanged parent drug; numerous low-abundance metabolites.
CL-005	Double-blind, 8-day multidose, renal effects	0, 800, 1000	47	• Well tolerated; no dose-limiting AEs or abnormal laboratory results; no effects on ECG, vital signs, or PE • As anticipated, serum/plasma creatinine levels increased significantly (up to ~ 40%) during treatment; reversed during 7-day washout period. • No effect during treatment on GFR, ERPF, FF, BUN or UA

Study	Design	PA-824 Dose Levels (mg)	Enrolled (active and control)	Key Safety/Efficacy Findings
CL-006	Open-label, multidose, DDI	400	14	• Well tolerated; no dose-limiting AEs • For midazolam, the geometric mean ratio of Day 17 (midazolam+Pa-824) vs. Day 1 (midazolam alone) for Cmax was 0.84 and AUC(0-infinity) was 0.85 • For the 1-hydroxy midazolam metabolite, the corresponding geometric mean ratio for Cmax was 1.05 and AUC(0-infinity) was 1.11
CL-008	Open-label, single-dose, ADME	~1100, oral suspension [imidazooxazine-^{14}C]PA-824	6	• Well tolerated; no dose-limiting AEs or abnormal laboratory results; no effects on ECG, vital signs, or PE. • No significant radioactivity captured as [imidazooxazine-^{14}C]CO_2. • ~91% of dose recovered (~53% in urine; ~38% in feces) Plasma: parent drug. Urine: little or no parent drug; multiple major metabolites. Feces: unchanged parent drug and numerous low-abundance metabolites.
CL-009	Open-label, single-dose, food effects	50 and 200	32	• Well tolerated; no dose-limiting AEs • In the presence of high fat, high calorie diet, Cmax and AUC of the 50-mg dose increased 1.40-fold and 1.45-fold respectively, whereas for the 200-mg dose, Cmax increased 1.76-fold and AUC increased 1.88-fold.
ACTG A5306	Open-label, multidose DDI with efavirenz (EFV), ritonavir-boosted lopinavir (LPV/r), or rifampin	200 mg	52	• Well tolerated; no dose-limiting AEs • In the presence of efavirenz, on average, AUC is reduced 35%; with LPV/r, AUC is reduced 15%; with rifampin, AUC is reduced 60%
Phase II				
CL-007	Partially double-blinded (blinded as to PA-824 dose), 14-day multidose, extended early bactericidal activity	200, 600, 1000, 1200	• 69	• Overall well tolerated with relatively few AEs and no dose-limiting AEs or laboratory findings. No clinically significant effects on ECG, vitals, or PE noted. • Two SAEs occurred during study, both were considered possibly related to TB disease (hemoptysis). • PA-824 treatment produced a measurable decrease in log CFU, with the magnitude of effect equivalent for all doses.

CL-010	Partially double-blinded (blinded as to PA-824 dose), 14-day multidose, extended early bactericidal activity	50, 100, 150, 200	• 69	• Well tolerated. • PA-824 treatment produced a measurable decrease in log CFU with some evidence of dose dependence.
NC-001-(J-M-Pa-Z)	Partially double-blinded (blinded as to combination within Pa or J containing arms), 14-day multidose, extended early bactericidal activity	200	• 85	• Well tolerated. • PA-824 plus moxifloxacin plus pyrazinamide combination treatment produced a decrease in log CFU at least comparable to that of the Rifafour e-275® control group.
NC-002-(Pa-M-Z)	Open-label, partially randomized (patients with drug-sensitive TB are randomized; patients with MDR-TB are assigned), 2-month multidose	100, 200	• 230	Based on preliminary results: • Well tolerated • Among patients with drug-sensitive disease, eight-week culture results on liquid media favored the experimental arms over the standard treatment arm (isoniazid, rifampin, pyrazinamide, ethambutol)

- **Phase 1**

In these trials, PA-824 has been administered in doses ranging from 50 to 1500 mg as 50 or 200 mg tablets or as an oral suspension. PK parameters have largely been consistent in each study and can be summarized as follows:

- PA-824 is moderately rapidly absorbed, with median T_{max} values across subjects and dose levels ranging from 4 to 7 hours.
- The mean half-life for elimination ($t_{½}$) across subjects and dose levels was approximately 16 - 25 hours.
- Exposure increased approximately linearly but less than dose-proportionally, with increasing doses up to approximately 600 – 1000 mg, while higher doses achieved minimal additional increases in either C_{max} or AUC.

Two studies using radiolabeled PA-824 in an oral-suspension formulation have been conducted to investigate the metabolism and excretion patterns of PA-824 in humans: Study PA-824-CL-004, which used [benzyl-^{14}C] PA-824 and Study PA-824-CL-008, which used [imidazooxazine-^{14}C] PA-824. The mass balance results from the two studies were very similar. In each study, the majority (53-65%) of radioactivity was excreted in the urine; an additional 26-38% was collected in the feces such that approximately 91% of the administered dose was ultimately recovered in the excreta.

Radioprofiling and metabolite identification work have been completed on samples from the two human studies as well as from analogous work in rat and monkey using both radiolabeled PA-824 preparations. The metabolism of PA-824 proceeds via a combination of reductive metabolism (~20 – 25% of the dose) and oxidative metabolism (remainder of the characterized metabolites). The metabolic profile of PA-824 *in vivo* was qualitatively similar in the three species, with quantitative differences being minor. No human unique metabolites were detected and there is no one single metabolic path that can be considered major. Furthermore, there are no major metabolites in human plasma.

Study PA-824-CL-006, a drug-drug interaction study with midazolam to assess the extent of CYP3A inhibition by PA-824, results indicate that dosing of PA-824 400 mg once daily for 14 days did not have a major effect on the exposure of midazolam or its major metabolite 1-hydroxy midazolam. For midazolam, the geometric mean ratio of Day 17 (midazolam+PA-824) vs. Day 1 (midazolam alone) for Cmax was 0.84 and AUC was 0.85. For the 1-hydroxy midazolam metabolite, the corresponding geometric mean ratio for Cmax was 1.05 and AUC was 1.11. The data suggests that PA-824 does not cause clinically significant drugs interactions with drugs metabolized by CYP3A.

Study PA-824-CL-009, a food effects study using lower doses of PA-824 (200 mg and 50 mg), results indicate that the food effect observed is dependent on the PA-824 dose administered. When a single dose of PA-824 was administered with a high fat, high calorie meal, Cmax and AUC of the 50 mg dose increased 1.40-fold and 1.45-fold respectively, whereas for the 200 mg dose, Cmax increased 1.76-fold and AUC increased 1.88-fold.

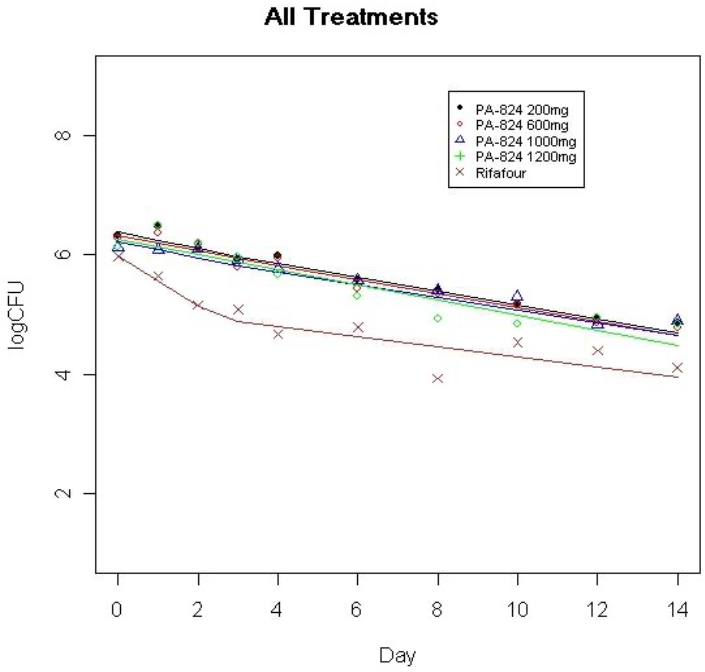
In the NIH-sponsored study ACTG A5306, a drug-drug interaction study with efavirenz, ritonavir-boosted lopinavir, or rifampin among healthy volunteers, results indicate that efavirenz reduces PA-824 AUC approximately 35%, lopinavir/ritonavir reduces AUC 15%, and rifampin reduces AUC 65%. Subjects were not co-administered isoniazid, an inhibitor of metabolizing enzymes, or other TB drugs.

- **Phase 2**

Study PA-824-CL-007 examined the effects of 14 days' treatment with PA-824 monotherapy at doses ranging from 200 to 1200 mg/day on TB participants' lung bacterial load measured as logCFU (log of Colony Forming Units) in the sputum. Four groups of approximately 15 men and women received 200, 600, 1000, or 1200 mg/day PA-824. An additional group of eight participants received the standard South African 4-drug combination TB treatment (Rifafour® e-275, comprised of a fixed-dose combination of isoniazid, rifampin, pyrazinamide and ethambutol). This group was included to provide

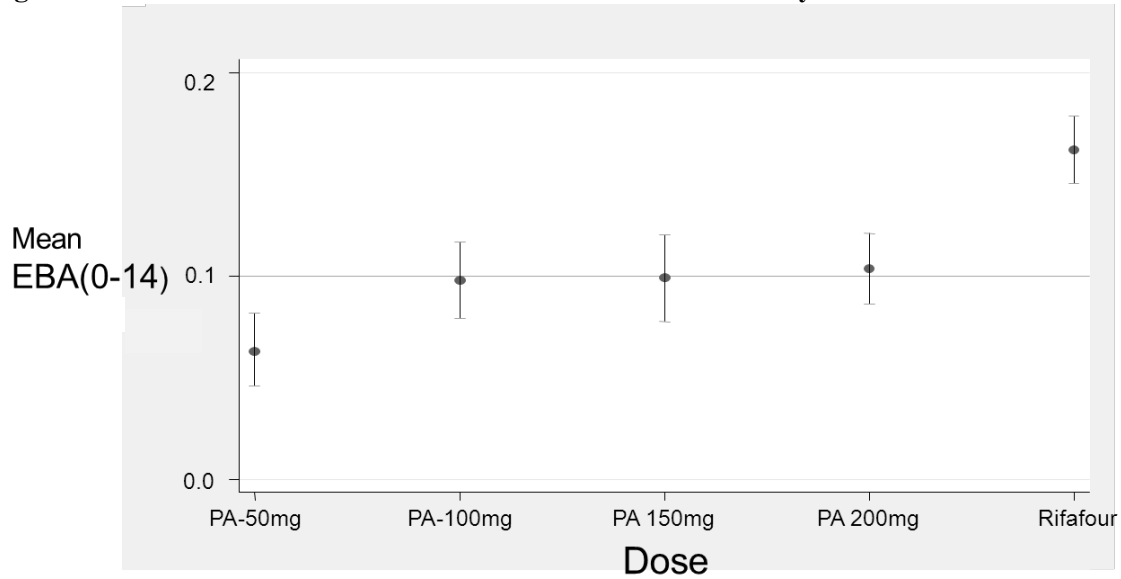
control for the microbiological techniques used to measure lung TB bacterial burden. The efficacy data from this study indicated that all doses of PA-824 including the lowest dose, 200 mg, produced a measurable and equivalent decrease in sputum CFU counts over the 14-day treatment period; no difference could be discerned among the PA-824 treatment groups (Figure 1) (17).

Figure 1 PA-824-CL-007 Mean Group logCFU Values Over Time



Study PA-824-CL-010 was an EBA study with a similar design to study PA-824-CL-007 except for the use of lower doses of PA-824 (50, 100, 150 or 200 mg/day). Results indicate that PA-824 treatment resulted in a measurable dose-dependent mycobactericidal activity, with the 50 mg dose demonstrating less activity than the 100, 150 and 200 mg doses, which were all equivalent (Figure 2) (16) .The results of the 200 mg and Rifafour arms were also consistent with the results for those same dose arms in study PA-824-CL-007, indicating these EBA studies are highly reproducible when conducted at these clinical and laboratory sites, and supported the choice of doses of 100 mg and 200 mg, PO, once per day, as the clinical doses of PA-824 tested in the eight-week Phase 2B NC002 trial.

Figure 2 PA-824-CL-010 Error Bar Plot over Treatment for Daily Mean EBA



Study NC-001-(J-M-Pa-Z) was a 2-week EBA study that assessed the two-week EBA of the following drug combinations: PA-824 plus pyrazinamide, PA-824 plus pyrazinamide plus moxifloxacin, along with two other non-PA-824 containing combinations. The two-week EBA activity of PA-824 plus pyrazinamide plus moxifloxacin was at least comparable to that of Rifafour (HRZE) (18).

Study NC-002 (Pa-M-Z) is a recently-completed 2-month study evaluating moxifloxacin plus PA-824 100mg plus pyrazinamide versus moxifloxacin plus PA-824 200mg plus pyrazinamide versus standard treatment (HRZE) among patients with drug-sensitive TB and PA-824 200mg plus moxifloxacin plus pyrazinamide among a small group of patients with MDR TB that had isolates sensitive to pyrazinamide. Preliminary efficacy results suggest that both experimental arms had higher 8-week culture conversion rates than the standard of care arm on liquid media; solid media results were similar in all arms. Final results are expected in mid-2014. The experimental regimens were safe and well-tolerated. These results suggest that PA-824 at doses of 100 mg and 200 mg daily is effective in combination regimens for TB.

Clinical Safety

Across the clinical studies with PA-824 completed to date, a total of 649 subjects have been exposed to PA-824, including 289 healthy subjects across the Phase 1 studies and 360 subjects with newly diagnosed smear positive pulmonary TB across the Phase 2 studies. Among the 289 healthy subjects, 174 received exposure to a single dose of PA-824 ranging from 50 to 1500 mg and 115 received exposures to repeated daily doses of PA-824 (50 to 1000 mg) for up to 14 days. The 360 subjects with newly diagnosed smear positive pulmonary TB were exposed to PA-824 either as a single agent at daily doses of 50 to 1200 mg for 14 days or in combination with other anti-TB agents (bedaquiline, moxifloxacin pyrazinamide and/or clofazimine) at a dose of 100 mg or 200 mg for up to 56 days.

Safety has been fully evaluated for the studies with final clinical study reports, and the safety evaluation is preliminary from the recently completed Phase 2B 8-week NC-002 study.

Safety in Completed Studies of Dosing up to 2 Weeks Duration

The overall safety profile from these completed clinical studies in healthy subjects and TB patients through 2 weeks of dosing indicated that orally-administered PA-824 was well tolerated when administered alone or a part of a multi-drug anti-TB regimen. In the Phase 2 studies, PA-824-CL-007, PA-824-CL-010 and NC-001-(J-M-Pa-Z), the overall frequency of treatment-emergent adverse events (TEAEs) during the 2-week treatment period was similar for the PA-824 treatment groups and for the standard first-line treatment for TB, Rifafour® e-275. In these Phase 2 studies, the overall frequency of TEAEs with PA-824 did not appear dose related when given at doses ranging from 50 to 200 mg/day in Study CL-010 or from 200 to 1200 mg/day in Study PA-824-CL-007. In Study NC-001-(J-M-Pa-Z), the three-drug regimen of moxifloxacin + PA-824 + pyrazinamide was associated with a slightly higher frequency of reported treatment-emergent adverse events (TEAEs) (60%) than were the two-drug regimens of PA-824 + pyrazinamide (53%) or PA-824 + TMC207 (47%).

Reported TEAEs across the 330 subjects exposed to PA-824 in completed clinical studies with final study reports thus far have generally been mild in intensity, with only about 3% of subjects (one healthy volunteer, nine subjects with pulmonary TB) having a TEAE that was assessed as severe. Almost all TEAEs resolved without sequelae. Five of the 330 subjects (all with pulmonary TB) exposed to PA-824 in the completed studies had a TEAE that met the criteria for being considered serious (pneumothorax, pneumonia, worsening pulmonary TB, hemoptysis, and neurocysticercosis). Each of the serious adverse events (SAEs) reported thus far in subjects exposed to PA-824 were assessed as unrelated to study treatment, and for two of these subjects, the SAE occurred more than 30 days after the last dose of PA-824.

TEAEs led to discontinuation from study treatment and/or from the study for 10 of the 330 subjects exposed to PA-824 (one healthy subject; nine subjects with pulmonary TB). Five of the nine subjects with pulmonary TB who were discontinued due to a TEAE were receiving PA-824 as part of a multi-drug regimen (three were on moxifloxacin + PA-824 + pyrazinamide). For five subjects, the TEAE leading to discontinuation was detected upon laboratory evaluations (alanine aminotransferase [ALT] increased in three subjects; ECG abnormalities [QTc prolonged; Wolff-Parkinson-White syndrome]) and the subjects were asymptomatic. Of the remaining five TEAEs leading to discontinuation, four were assessed as unrelated to study treatment (SAEs of hemoptysis, pneumothorax, neurocysticercosis; urinary tract infection [subject discontinued to take protocol-disallowed medication]). The remaining TEAE leading to discontinuation was a generalized rash in a healthy subject that presented approximately 32 hours after the final PA-824 dose (1000 mg/day).

In Phase 1 studies, headache was the most common TEAE. Among the 42 subjects across these studies who received a PA-824 dose of 200 mg (anticipated maximum clinical dose) or less, 10 (24%) reported headache. The incidence of headache in subjects treated with placebo in Phase 1 studies PA-824-CL-001 and PA-824-CL-002 was 31% and 22%, respectively. In the Phase 2 studies in subjects with pulmonary TB, in studies PA-824-CL-007 and PA-824-CL-010, headache was reported for three of the 122 subjects exposed to PA-824 as a single agent. In Study NC-001-(J-M-Pa-Z) seven of the 45 subjects exposed to PA-824 as part of a multi-drug regimen in reported headache.

Gastrointestinal disorder TEAEs (primarily nausea and vomiting) and skin and subcutaneous tissue disorder TEAEs (mainly rash) were the most frequently-reported TEAEs in the Phase 2 studies. Gastrointestinal disorder TEAEs were reported for 13 of the 122 subjects (11%) treated with PA-824 across Studies PA-824-CL-007 and PA-824-CL-010 and for four of the 45 subjects (16%) treated with a

PA-824-containing regimen in Study NC-001-(J-M-Pa-Z). None of these TEAEs were severe or serious, or resulted in discontinuation. Skin and subcutaneous tissue disorder TEAEs were reported for 18 of the 122 subjects (15%) across Studies PA-824-CL-007 and PA-824-CL-010, and for three of the 45 subjects (7%) treated with PA-824 in Study NC-001-(J-M-Pa-Z). Although treatment with PA-824 did appear to be associated with skin-related TEAEs, such as pruritus or rash, these events tended to be mild in intensity and generally resolved without sequelae. Across the 11 clinical studies with PA-824 completed to date (including 8 Phase I studies), only one skin-related TEAE (generalized rash) was severe and resulted in discontinuation from the study. None were serious.

No other clinically significant changes in laboratory parameters (hematology, clinical chemistry, and urinalysis) have been noted with PA-824 in the Phase 1 or 2 clinical studies. As indicated above, three of the 45 subjects (7%) exposed to a PA-824-containing regimen in Study NC-001-(J-M-Pa-Z) were withdrawn from treatment due to an elevation in ALT levels. The rate of discontinuation for this laboratory TEAE in this study was similar to that for bedaquiline-containing regimens (2/30, 7%). Moreover, none of the three subjects discontinued for increased ALT levels in Study NC-001-(J-M-Pa-Z) had associated symptoms, and in all cases, ALT levels resolved upon discontinuation of treatment. No other hepatic-related TEAEs have been reported in clinical studies with PA-824, and evaluation of hepatic laboratory parameters (ALT, aspartate aminotransferase [AST], total bilirubin) did not suggest any clinically significant changes in these parameters with PA-824.

The initial 3-month nonclinical toxicology studies in rats and monkeys reported the development of cataracts during both treatment and recovery in rats dosed with PA-824 300 mg/kg/day and during the 3-month recovery period in monkeys in the PA-824 450/300-mg/kg/day group. As a result, the Investigational New Drug (IND) application for PA-824 was placed on full clinical hold by the Food and Drug Administration (FDA) on 09 April 2008 until additional nonclinical and clinical data were provided. Following submission of nonclinical data from two repeat toxicology studies in rat and monkey, cross-species metabolism comparisons, clinical information on ophthalmologic evaluations in subjects who had participated in multi-dose clinical studies PA-824-CL-005 and PA-824-CL-007, and expert opinions from an independent Ophthalmology Review Board (ORB), the clinical hold was lifted in July 2009. In addition, the FDA requested planned ophthalmologic examinations in future clinical studies. Since the clinical hold was lifted, five additional clinical studies have been conducted, each of which included prospectively-planned ophthalmologic evaluations (slit-lamp examinations, visual acuity testing) predose and at 3- or 6-months after the final dose of study treatment (Studies PA-824-CL-010, PA-824-CL-006, PA-824-CL-009, NC-001-(J-M-Pa-Z) and NC-002-(M-Pa-Z). Mild lenticular opacities were noted upon ophthalmological slit lamp testing in 2 of the 152 subjects exposed to PA-824 across these five studies; both subjects were asymptomatic.

Rat toxicology studies demonstrated testicular toxicity although there was no evidence of testicular toxicity in mature male monkeys evaluated with PA-824 dosing over 3 months. To gather more information in male subjects regarding male reproductive hormones, serum testosterone, LH and FSH were measured in all male subjects in Study NC-002-(M-Pa-Z) at baseline and at the end of 8 weeks of exposure to PA-824 and the control H-R-Z-E therapy.

Preliminary Safety from Completed Phase 2b 8-Week Study NC-002

Preliminary data analyses of the study indicate that a total of 207 subjects were enrolled, with 60 randomized to M-PA100-Z, 62 randomized to M-PA200-Z, and 59 to HRZE. An additional 26 subjects were treated in the M-PA200-Z MDR arm. In this study 88% of all subjects had a treatment emergent adverse event (TEAE), including 87% in the M-PA100-Z group, 92% in the M-PA-Z group, 85% in the Rifafour group and 89% in the M-PA-Z MDR group. Adverse events were graded according to the NIH

Division of Microbiology and Infectious Diseases Adult Toxicity Table. TEAEs by grade were comparable by grade across the arms of the trial, as demonstrated in the table below.

NC-002 Adverse Events by DMID Grade

Severity	Statistic	M-PA100-Z (N=60)	M-PA200-Z (N=62)	Rifafour (N=59)	M-PA200-Z MDR (N=26)	Total (N=207)
Grade 1	N (%)	43 (71.7)%	48 (77.4%)	46 (78.0%)	18 (69.2%)	155 (74.9%)
Grade 2	N (%)	25 (41.7%)	31 (50.0%)	27 (45.8%)	13 (50.0%)	96 (46.4%)
Grade 3	N (%)	18 (30.0%)	20 (32.3%)	15 (25.4%)	6 (23.1%)	59 (28.5%)
Grade 4	N (%)	3 (5.0%)	9 (14.5%)	6 (10.2%)	2 (7.7%)	20 (9.7%)

Eleven serious adverse events (SAEs) were reported in 9 subjects, with one subject in each of the M-PA100-Z and the Rifafour groups, and 7 subjects in the M-PA200-Z group. The subject in the M-PA100-Z group died of an unknown cause 39 days after a single dose of study drug regimen, and the death was not considered related to study drug by the investigator or the sponsor. Four other SAEs were considered not related to study drug, including a pneumothorax, a bone fracture, dyspnea requiring hospitalization, and second degree heart block considered on evaluation to be existing prior to entry into the trial. SAEs considered possibly related or related to the study drug regimen included hyperuricemia likely secondary from pyrazinamide, drug-induced hepatitis, and elevated liver enzymes. One subject had an episode of agranulocytosis that resolved after the study drug regimen was stopped, and one subject had a seizure witnessed by the family and was discontinued from the study.

The protocol required that subjects with hepatic enzyme ALT or AST elevations greater than 3X the Upper limit of Normal (ULN) have study drug discontinued. Consequently, 25 subjects were withdrawn from the study across the study arms for elevations in hepatic enzymes. Among patients in the M-PA100-Z, M-PA200-Z, ,and HRZE arms, 4/60, 5/62, and 4/59 had ALT values higher than 5 times the upper limit of normal; among patients with MDR-TB receiving a regimen of M-PA200-Z, 2 of 26 did.

Additional preliminary safety findings of special focus from NC-002 are noted below.

Ophthalmologic Evaluations – All subjects received ophthalmologic evaluations including visual acuity testing and slit lamp examinations at baseline and 3 months after completion of study drug dosing. The ophthalmologists were trained in grading 3 regions of the lens using the AREDS2 grading system across a range of 0-4. All subjects enrolled with the required zero score grade for all regions of the lens except for 1 subject who was blind in one eye. Among all subjects in the trial, 4 subjects had lens evaluations with a grade of greater than zero. One subject in the M-PA100-Z group and 3 subjects in the M-PA200-Z group had grades of 0.5 or 1.0 in a single eye in one of the 3 zones of the lens. It is unlikely these findings represent a drug-induced lens opacity given the low incidence, the unilateral nature of all findings and the differing zone locations of the findings. It is common in persons with no clinical abnormalities to have grades of 0.5 – 1.0+ in the AREDS2 rating on a slit lamp evaluation.

Reproductive Hormone Evaluations – Preclinical toxicology studies had noted that rats dosed with PA-824 developed testicular toxicity, although a 3 month study in mature monkeys did not identify any drug related testicular toxicity. In Study NC-002 men were evaluated with plasma samples for the reproductive hormones LH, FSH and Testosterone at baseline and at the end of the dosing period. If the study drug regimen caused testicular toxicity, the most sensitive measure from these hormones would be an increase in levels of FSH. Among subjects in the M-PA100-Z group the mean baseline FSH was 9.027 U/L which

decreased to 8.338 U/L at the end of therapy. Among subjects in the M-PA200-Z group the mean baseline FSH was 6.531 U/L at baseline and this decreased to a mean of 6.061 at the end of therapy. Men in the Rifafour group had a mean baseline of 7.394 U/L which decreased to 6.714 at the end of therapy. This gives relative reassurance that the M-PA-Z regimen is not likely to cause testicular toxicity in men.

Electrocardiographic Conduction Interval Changes – Subjects in NC-002 had supine resting ECGs taken at baseline, Day 4 and weekly through the 8 week dosing period and 2 weeks after the end of dosing. All ECGs were read by a central cardiology service. No subjects had a corrected QT interval (QTcF) greater than 500 msec during the study. A small number of subjects had asymptomatic increases in QTcF from baseline over 60 msec: Two in the M-PA100-Z group, 4 in the M-PA200-Z group, none in the Rifafour group and 2 in the M-PA200-Z MDR group. An evaluation of the mean change from baseline across all post-baseline ECGs notes increases of 11.1 msec in the Rifafour group, 11.1 in the M-PA100-Z group, 17.8 msec in the M-PA200-Z group and 6.7 in the M-PA200-Z MDR group. Of note, many subjects were tachycardic at baseline with their active pulmonary MTB and had heart rates decrease over the first week of therapy, which complicates interpretation of heart-rate corrected calculated QT.. In addition, PA-824 was always given with moxifloxacin in NC-002, and moxifloxacin is known to prolong QTc modestly.

4.2 Study rationale

PA-824 is an investigational drug with bactericidal and sterilizing activity against *M. tuberculosis* that has significant potential as a treatment-shortening drug when used as part of combination TB therapy. The nitroimidazoles are of particular interest because of their activity against "persisters," those bacteria that are hard to kill and lead to relapse when TB is not treated for a long enough duration. Data from preclinical studies suggest that PA-824 is synergistic with pyrazinamide and that regimens including PA-824, rifampin, and pyrazinamide shorten the treatment duration required for cure from 6 months to 4 months. Although these results are promising, it is important to note that the pathophysiology of TB disease in humans and the mouse model differ substantially, and new regimens must be tested rigorously in humans to discover their true treatment-shortening potential. PA-824 is a once-daily, well-tolerated nitroimidazole that is in Phase 2 development. The proposed study would answer the following question: In a combination regimen that includes isoniazid, pyrazinamide, and a rifamycin, drugs with proven activity against TB, will addition of PA-824 improve the activity of the regimen?. While trials involving combinations of multiple investigational drugs for TB are under consideration and are likely to be informative, trials substituting one drug for another may also help us learn about the individual contributions of investigational drugs to regimens so that prioritizing drugs and drug combinations for Phase 3 trials can be done efficiently and effectively. Historically, adding rifampin to TB treatment resulted in a 20% difference in 8-week culture conversion (on solid media) and allowed for shortening of treatment duration from 18 months to 9 months; the addition of pyrazinamide increased the 8-week sputum culture conversion rate by 13% and allowed for treatment shortening from 9 to 6 months (24). In modern-day Phase 2B TB treatment trials, about 80-85% of patients with pulmonary TB convert their sputum cultures (on solid media) to negative by the end of 2 months of treatment, and culture conversion on liquid media at 2 months is about 65-70%. Trial endpoints with more discriminatory power than the binary 2-month culture conversion proportion, such as time to culture conversion or quantitative culture results over time, will be needed to inform Phase 3 trials(25). In addition, modalities that allow us to expand our evaluations of treatment response beyond the microbiology of sputum samples, such as CT scans, may be important as biomarkers of TB treatment response. Last, though many TB treatment trials have ended at 2 months, the time between 2 and 3 months of treatment is likely to provide important additional data about sputum culture clearance that can help us figure out which drugs and regimens among the many moving forward in clinical studies have the most promise of being an effective 3-4 month regimen.

4.3 Rationale for selection of doses and regimens

In this study, PA-824 will be substituted for ethambutol, a drug that generally does not provide additional activity when given together with isoniazid, rifampin, and pyrazinamide to treat drug-sensitive TB. The role of ethambutol, given at its minimal effective dose because of optic toxicity that is related to dose and cumulative exposure, in TB treatment is to protect companion drugs against the development of resistance (26). PA-824 also protects against isoniazid resistance, as noted in the Background section above. Isoniazid is the drug with the highest bactericidal activity (1-2 log reductions in sputum colony counts are generally seen after 2 days of dosing) (27), driving down bacterial burden quickly, and rifamycin antibiotics (rifampin or rifabutin) and pyrazinamide are the drugs with the highest sterilizing activity of all TB drugs evaluated to date. This study is designed to see if the addition of PA-824 to this combination can increase its potency further given its activity against "persisters", with an eye towards treatment shortening.

The dose of PA-824 selected for this study is based on data from the dose-ranging EBA studies described above in which increases in dose above 200 mg did not increase the activity of the drug and doses between 100 mg and 1000 mg produced similar results (17). In addition, preclinical studies suggest that PA-824's activity against *M. tuberculosis* is time-dependent, and human pharmacodynamic simulations based on Phase 1 data predict that 200 mg per day produces free drug time above mean inhibitory concentration (T>MIC) values near the target for maximal observed bactericidal effect, as determined in the mouse model (28). The PK/PD parameter that correlates best with treatment effect and the target value for that parameter in humans have not been determined. At a dose of 200 mg, the median %T>MIC is 99%. Further, preliminary 8-week NC-002 study results demonstrated that PA-824 at doses of 100 mg and 200 mg given together with moxifloxacin and pyrazinamide had higher two-month culture conversion rates on liquid media than standard treatment with isoniazid, rifampin, pyrazinamide, and ethambutol. This supports further testing of doses of 100 mg and 200 mg. However, preliminary results from a recent Phase I drug-drug interaction (DDI) study suggested that PA-824 at a dose of 200 mg given on an empty stomach with rifampin resulted in PA-824 exposures that are similar to a dose of about 100 mg given without rifampin. However, the combined effects of isoniazid, an inhibitor of metabolizing enzymes, and rifampin on PA-824 have not been tested. In Arm 1, PA-824 will be given at a dose of 200 mg together with rifampin, pyrazinamide, and isoniazid. In Arm 2, PA-824 will be given at a dose of 200 mg together with rifabutin, a drug with comparable efficacy to rifampin that is a significantly less potent inducer of metabolizing enzymes (29,30), plus pyrazinamide, and isoniazid (for example, among patients with TB and HIV requiring antiretroviral therapy, rifabutin is commonly substituted for rifampin to avoid drug interactions). Exposures to PA-824 are expected to be higher in Arm 2 than in Arm 1, and this will allow us to evaluate concentration-effect relationships when PA-824 is added to an effective first-line regimen. PA-824 will be given with food, which is expected to boost exposures, so exposures should be higher than in the DDI study of rifampin plus PA-824 because drugs were taken on an empty stomach in that trial.

5 DESIGN

5.1 Study treatment (First 12 Weeks)

"Study treatment "is defined as the TB treatment given for the first 12 weeks, during the experimental phase of the trial. These 12 weeks of study treatment includes the Intensive Phase (first 8 weeks) of TB treatment and four weeks of the Continuation Phase. This study will be a prospective, open-label Phase 2B clinical trial. Adult pulmonary TB suspects who meet eligibility criteria will be randomized to receive one of the following three regimens

Arm	**Weeks 0-8 (Intensive phase)**	**Weeks 9-12 (First 4 weeks of the continuation phase)**
1	**Pa**HRZ	**Pa**HR
2	**Pa**HRbZ	PaHRb
3 (standard Rx)	**E**HRZ	HR

Vitamin B6 (pyridoxine) 50 mg will be administered with each dose of isoniazid

All treatment will be administered once daily, 7 days per week. Study medication doses will be directly observed by study personnel, a designated healthcare worker, or a lay treatment supervisor who is aware of the study protocol and has received training in study procedures.

PA-824 will be given at a dose of 200 mg once daily, recommended to be taken with food. Exact doses of the other study medications are given in Table 2 below. Pyrazinamide and ethambutol doses should be based on weight at the time of study entry, and should not be increased if weight increases during the intensive phase.

The duration of the intensive phase of therapy will be determined by the number of doses ingested, not by calendar time. Patients will complete the intensive phase when they have had 56 doses. The total duration of study treatment will be determined by the number of doses ingested, not by calendar time. Patients will complete study treatment when they have had 84 doses.

Table 2. Doses of isoniazid, rifampin, pyrazinamide, and ethambutol during the intensive phase

Drug	**Dose**
Isoniazid	300 mg
Rifampin	600 mg
Rifabutin	300 mg
Pyrazinamide	
< 55 kg	1000 mg
≥ 55-75 kg	1500 mg
> 75 kg	2000 mg
Ethambutol	
< 55 kg	800 mg
≥ 55-75 kg	1200 mg
> 75 kg	1600 mg

5.2 Non-experimental continuation phase treatment

Study follow-up will continue during the non-experimental component of the continuation phase of TB treatment (Weeks 13-24). Since study endpoints will already have been reached by Week 12 and data collected, the composition and duration of non-experimental continuation phase treatment will not affect study results.

After completing the experimental phase of therapy, patients in all study arms will be treated with standard, non-experimental continuation phase treatment in accordance with South African care standards.

5.3 Endpoints

Primary Endpoints:
(1) Time to sputum culture conversion in liquid media, defined as the time from the start of treatment until the first negative sputum culture, over 12 weeks
(2) Proportion of subjects with a severe adverse event (defined as grade 3, 4, or 5 adverse event)

Secondary Endpoints:
(1) Discontinuation of study treatment for any reason.
(2) Time to sputum culture conversion in solid media, defined as the time from the start of treatment until the first negative sputum culture, over 12 weeks
(3) Proportion of participants with sputum culture conversion at 8 weeks or at 12 weeks (separately, on solid and liquid media).
(4) The rate of change in time to sputum culture positivity (TTP) through 12 weeks in the Mycobacterial Growth Indicator Tube (Bactec MGIT960) system in sputum over 12 weeks in participants, which may be described with linear, bi-linear or non-linear regression of TTP on time
(5) Pharmacokinetics (PK) of PA-824 delivered at a dose of 200 mg once daily in the context of multidrug treatment for TB
(6) Pharmacokinetic/pharmacodynamic (PK/PD) relationship between PA-824 exposures (area under the concentration-time curve (AUC) or maximum concentration (Cmax)) and outcomes (time to culture negativity or rate of change in TTP over 12 weeks) using non-linear mixed effects modeling

5.4 Study drug descriptions

5.4.1 PA-824

PA-824 is an investigational nitroimidazole being developed for the treatment of TB. It interferes with cell wall biosynthesis and may have other mechanisms of action as well in nonreplicating bacteria.

PA-824 Pharmacokinetics: At a dose of 200 mg, steady state PK parameters are as follows: C_{max} 1.7 mcg/mL, time to maximum concentration (T_{max}) 4.5 hours, half-life ($t_{1/2}$) 16.0 hours, $AUC_{0\text{-}tau}$ 30.2 mcg*h/mL, and CL/F 6.7 L/h. Food increases bioavailability, particularly at higher doses, with a high-fat, high-calorie meal increasing C_{max} of a 200 mg dose by 75% and AUC by 88% when compared to the fasting state (31). After being absorbed, PA-824 is extensively metabolized (<5% is recovered as parent drug in urine and feces) to multiple metabolites. In the human, the metabolism of [14C]-PA-824 proceeds via a combination of reductive metabolism (~20–25% of the dose) and oxidative metabolism (remainder of the dose). No single metabolite is predominant, and the most prominent component in the plasma is radiolabelled parent drug. The drug and its metabolites are largely eliminated in the urine (53-65%) and feces (26-38%). Clearance is not time- or dose-dependent.

Drug-Drug Interactions: Liver microsome studies were performed to assess the potential of PA-824 to inhibit or induce human cytochrome P450 (CYP) isoenzymes (31). PA-824 did not induce isoforms 3A4, 2C9, or 2E1 in vitro but is a weak competitive inhibitor of CYP3A in other experiments. PA-824 also inhibited 2C8, 2C9, and 2C19. PA-824 is not a substrate for 2C9, 2C19, or 2D6. It is 20% metabolized by 3A4. In a clinical drug interaction study using midazolam as a CYP3A probe, PA-824 dosed at 400 mg daily decreased midazolam C_{max} and $AUC_{0\text{-}inf}$ by 16% and 15%, respectively, a difference not likely to be clinically significant. In a drug interaction study with efavirenz, a CYP2B6 substrate, preliminary results suggest that PA-824 did not change efavirenz concentrations; efavirenz reduced PA-824 AUC by about

35% (32). Rifampin reduced PA-824 concentrations by about 65%. Ritonavir-boosted lopinavir reduced PA-824 concentrations modestly (about 15%), and PA-824 did not alter lopinavir concentrations appreciably. Participants in the drug interaction study did not take isoniazid, a known inhibitor of metabolizing enzymes, with rifampin, so the combined effects of the two drugs on companion drug concentrations are unknown.

Dosage: PA-824 is being studied in subjects with TB at doses of 100 mg and 200 mg daily to be administered orally for the treatment of human TB. PA-824 is available in tablet form containing 50 mg, 100 mg, or 200 mg of PA-824.

Tolerability: In the clinical trials of PA-824 to date, multiple dosing of PA-824 has extended for as long as 56 days. As described in detail above, the most common side effects or AEs associated with PA-824 exposure include:

Headache
Benign, isolated and reversible elevations of serum creatinine
Stomach discomfort (nausea, vomiting, flatulence, and/or diarrhea)
Skin and subcutaneous tissue disorders

Refer to section 4.1 for detailed safety information related to PA-824.

5.4.2 Rifampin

Rifampin is a semi-synthetic rifamycin derivative that is highly active against mycobacteria, most gram-positive bacteria, and some gram-negative bacteria. It is bactericidal for both intracellular and extracellular microorganisms. By inhibiting prokaryotic DNA-dependent RNA polymerase, it suppresses the early elongation of the nucleotide chain in RNA synthesis.

Pharmacokinetics: Rifampin is normally absorbed completely when taken orally, but food delays absorption. After 1.5 to 2 hours, a 600 mg dose yields a peak blood level of 8-20 mcg/mL. The half-life of rifampin varies from 2 to 5 hours, and it is shortened by approximately 20-40% after the first week of daily treatment because of the induction of hepatic microsomal enzymes. The half-life is unaffected by renal impairment but is increased by liver disease or biliary obstruction. Rifampin is deacetylated to an enterohepatically recirculated active metabolite, and 50% to 60% is excreted in the feces. Up to 30% of a dose is excreted in the urine. Approximately 85% of circulating rifampin is bound to plasma proteins, and is widely distributed throughout the body.

Drug-drug interactions: Rifampin is an inducer of a number of hepatic enzymes involved in the metabolism of drugs and some hormones. This enzyme induction causes more rapid elimination (and potential loss of efficacy) of many drugs. Medications for which concomitant rifampin is contraindicated include: HIV-1 protease inhibitors (other than ritonavir), delavirdine, cyclosporine, tacrolimus, itraconazole, and ketoconazole.For many other medications, the dose can be increased to compensate for the effect of rifampin.

Dosage: The recommended dose of rifampin for the treatment of TB during the intensive phase is 600 mg. Rifampin is available in 150 mg or 300 mg capsules. In this study, rifampin will be provided at a dose of 600 mg daily.

Tolerability: In the usual daily dose of 600 mg, rifampin is well tolerated. It often causes harmless but disconcerting red-orange discoloration of tears, sweat, saliva, feces, and urine. Less than 4% of TB patients experience significant adverse reactions to rifampin. Gastrointestinal AEs are the most common, and they include epigastric distress, anorexia, nausea, vomiting, cramps, and diarrhea. Hepatitis rarely

occurs in persons who have normal baseline hepatic function. The incidence of hepatitis may be increased for older persons and those who have chronic liver disease or alcoholism, but remains substantially lower than that for pyrazinamide or isoniazid. Rifampin can cause a flu-like syndrome of fever, chills, and myalgia, although this is uncommon using the 600 mg dose given daily or thrice-weekly. In a very small proportion of patients the flu-like syndrome is associated with interstitial nephritis, acute tubular necrosis, thrombocytopenia, hemolytic anemia, and shock. There may be changes in menstruation.

5.4.3 *Rifabutin*

Rifabutin is a semisynthetic ansamycin antibiotic derived from rifamycin S. It inhibits DNA-dependent RNA polymerase and is active against *E. coli, Bacillus subtilis, Mycobacerium avium, M. intracellulare, and M. tuberculosis.*

Pharmacokinetics: Following a single oral dose, rifabutin is rapidly absorbed reaching peak concentrations in 2 to 3 hours. Peak concentrations increase proportionally with dose over the range of 300 mg, 600 mg, 900 mg, and 1200 mg. The terminal half-life is approximately 38 hours. Rifabutin has high lipophilicity with an apparent steady-state distribution volume of 9.3L/kg. It is 85% protein-bound, and protein binding is concentration-independent. Following 28 days of oral dosing of rifabutin, the steady-state AUC is lower than the first dose AUC at each of the four dose levels. At the 300 mg daily dose, the AUC was approximately 30% lower on day 28 than on day 1. The modest decrease in AUC following 4 weeks of administration of rifabutin is presumed to be due to auto-induction of its metabolism. Bioavailability of rifabutin is about 20% following the first dose and 12% after multiple doses. High-fat meals slow the rate of absorption but do not alter the extent of absorption. Following the oral administration of ^{14}C-rifabutin (solution), 52.9% of the administered dose was excreted in the urine, predominantly as metabolites and 29.4% of the administered dose was recovered in feces. Rifabutin was the primary circulating compound with 31-OH rifabutin and 25-O-desacetyl-rifabutin produced by 4 hours. The 25-O-desacetyl metabolite has antimicrobial activity equal to the parent on a molar basis but the AUC ratio of parent to metabolite is approximately 10:1. Rifabutin undergoes extensive metabolism, along with 31-OH and 25-O-desacetyl-rifabutin, three other urinary metabolites have been identified. Based on the urinary metabolites, the primary routes of metabolism are deacetylation and oxidation; CYP3A plays a role in rifabutin metabolism.

Drug-drug interactions: Rifabutin is chemically related to rifampin, a potent inducer of P450 enzymes, including those of the CYP3A family. Rifabutin appears to induce P450 enzymes as well, although not as potently as RIF, as judged by effects on markers of P450 activity in humans. Thus, when given together with companion drugs that are metabolized by P450 enzymes, induction of metabolism is expected, though the magnitude of the drug interaction, if any, is generally not clinically significant. In addition, rifabutin is a CYP3A substrate. Drugs that inhibit CYP3A4 can increase rifabutin concentrations.

Dosage: The recommended dosage for rifabutin is 300 mg once daily. Dose adjustments are necessary for patients with severe renal impairment or for patients taking certain concomitant drugs. For this study, RBT will be given at a dose of 300 mg once daily, during the experimental phase, the standard treatment dose for TB.

Tolerability: In two pivotal trials (n=566 rifabutin and n=550 placebo), clinical adverse events occurring at >1% of patients include rash, gastrointestinal intolerance and neutropenia. Discoloration of urine

occurred in >30% of patients on RBT. In <1% of the patients, flu-like syndrome, hepatitis, chest pressure and pain with dyspnea and skin discoloration occurred. Increased alkaline phosphatase, SGOT, and SGPT occurred similarly in the rifabutin and placebo groups. There were no reported incidences of hyperbilirubinemia noted in the reports of these studies. Incidence of neutropenia is more common with rifabutin than with placebo. Uveitis is rare when given at 300 mg per day, but dose-limiting uveitis occurs at higher doses.

5.4.4 Isoniazid

Isoniazid is the hydrazide of isonicotinic acid and is one of the primary drugs for TB treatment. The activity of isoniazid is limited to the mycobacteria of the *M tuberculosis* complex; it is bactericidal for rapidly dividing organisms and bacteriostatic for "resting" bacilli. The probable mechanism of action is the inhibition of the biosynthesis of mycolic acids, a component of the mycobacterial cell wall.

Isoniazid Pharmacokinetics: Isoniazid is generally well absorbed; food and antacids decrease the rate, but not the extent of absorption. The peak blood levels of isoniazid, 3 to 5 mcg/ml, are obtained 30 minutes to 2 hours after ingestion of routine doses. It diffuses into all body fluids and cells and penetrates into the caseous material of a tuberculoma or pulmonary cavity. In the liver, it is acetylated to inactive metabolites, and 75% to 95% of the dose is excreted as inactive metabolites in the urine within 24 hours. Isoniazid clearance rates depend on 2 metabolic phenotypes, slow and fast acetylation. The isoniazid AUC among persons who have fast acetylation is 30% to 50% of that among persons who have slow acetylation. Because isoniazid is well tolerated over a wide range of therapeutic doses, a single dose per body mass is recommended. Persons who have rapid acetylation achieve effective concentrations, while persons who have slow acetylation do not experience increased toxicity. Half-life ($t_{1/2}$) may vary from 1 hour in fast acetylators ($t_{1/2}$ < 90min) to 3 hours in slow acetylators ($t_{1/2}$ >90min).

Isoniazid Drug-drug interactions: Isoniazid decreases the clearance of some medications that are metabolized in the liver (particularly carbamazepine, phenytoin, diazepam).

Isoniazid Toxicity: The total incidence of all adverse effects from isoniazid is approximately 5%, many of which do not require discontinuation of the drug. Peripheral neurotoxicity is dose dependent and it is uncommon (<0.2%) at conventional doses. The risk of peripheral neuritis increases for persons who are malnourished or predisposed to neuritis by other illnesses. Concomitant administration of pyridoxine (vitamin B_6) is recommended for these persons and will be given to all patients receiving isoniazid in this trial. Other nervous system reactions are rare at normal doses, and they include convulsions, encephalopathy, optic neuritis, memory impairment, and psychosis. Gastrointestinal adverse effects include nausea, vomiting, and epigastric distress. Asymptomatic elevation of aminotransferases is common and occurs in 10-20% of persons receiving isoniazid. However, idiosyncratic severe hepatic reactions are uncommon but are more likely in older persons (up to 2.3% hepatitis incidence in persons more than 50 years old), and may be life threatening. Daily consumption of alcohol increases the risk of isoniazid-associated hepatotoxicity by approximately 4-fold. The risk of isoniazid-induced hepatotoxicity may also be increased in the postpartum period. The prodromal symptoms of hepatotoxicity are anorexia, nausea, vomiting, fatigue, malaise, and weakness; persons who take isoniazid and have these symptoms should stop therapy and be evaluated immediately.

5.4.5 Pyrazinamide

Pyrazinamide is an analog of nicotinamide and has unique activity against *M tuberculosis*, allowing the duration of treatment to be decreased from 9 months to 6 months (assuming rifampin is used throughout). The mechanism of action of pyrazinamide remains unknown.

Pyrazinamide Pharmacokinetics. Pyrazinamide is well-absorbed from the gastrointestinal tract and widely distributed into all tissues. Usual doses are 15-30 mg/kg/d, up to 2 gm/d. Peak serum concentrations of about 45 mcg/ml are achieved approximately 2-3 hours after a dose. Food and antacids do not significantly affect the absorption of pyrazinamide. The half-life of pyrazinamide is approximately 9-10 hours, and is prolonged in the presence of hepatic insufficiency. Pyrazinamide is metabolized to pyrazinoic acid by the hepatic microsomal enzyme pyrazinamide deamidase. Approximately 40% of a dose is recovered in the urine as pyrazinoic acid and an additional 4% is excreted in the urine as the unchanged parent drug. The remaining drug is thought to be excreted in the bile.

Pyrazinamide Drug-drug interactions: There are no known clinically significant drug-drug interactions involving pyrazinamide.

Pyrazinamide Toxicity: The most frequent side effects are skin rash, gastrointestinal intolerance, hepatotoxicity (1.3%), arthralgias (1-7%), hyperuricemia due to blockade of urate excretion (up to 66%), and rarely acute gouty arthritis. These side effects are seldom dose-limiting. Asymptomatic elevations in serum uric acid are frequent, usually occur during the first or second month of treatment, and are self-limited and require no specific treatment. Minor arthralgias also may occur during pyrazinamide treatment and can usually be treated with salicylates or non-steroidal inflammatory agents such as indomethacin while continuing the drug. The most common serious side effect of pyrazinamide is hepatotoxicity.

5.4.6 Ethambutol

Ethambutol is an ethylene derivative of butane that interferes with cell wall synthesis in mycobacteria; other bacteria are uniformly resistant to ethambutol. In the treatment of human TB, ethambutol is effective in preventing the emergence of drug resistant strains, although it has no sterilizing activity at clinically-tolerated doses.

Ethambutol Pharmacokinetics: Ethambutol is well absorbed from the gastrointestinal tract, reaching peak serum concentrations of 3-5 mcg/ml in normal volunteers 2-4 hours after a dose. Food slows absorption and decreases the peak serum concentration by 10-20%, but has no effect on the total systemic exposure (AUC). Antacids decrease both the peak serum concentration and AUC, and so should not be administered at the same time. Ethambutol is primarily eliminated by the kidneys as unchanged drug; the serum half-life averages 4 hours. Patients with renal insufficiency are prone to accumulation of the drug and the resultant toxicity.

Ethambutol Drug-drug interactions: There are no known drug–drug interactions involving ethambutol.

Ethambutol Toxicity: Ethambutol is usually well-tolerated with low rates of skin rash, nausea, vomiting, or diarrhea. Fever, allergic reactions, abdominal pain, mental status changes, peripheral neuropathy, and increased liver function tests have rarely been associated with ethambutol. Adverse events occur in less than 2% of patients receiving ethambutol at the 15 mg/kg dose and include decreased visual acuity (0.8%), rash (0.5%) and asymptomatic hyperuricemia. The most common serious side effect of ethambutol is retinal toxicity, often first perceived as a decrease in color perception. Patients receiving ethambutol should be instructed about symptoms of ocular toxicity. If stopped promptly, permanent visual loss is rare among patients with ethambutol-related retinal toxicity. Rates of retinal toxicity are very low when the drug is given for relatively short periods, as is the case in this study.

5.5 Study drug supply and accountability

PA-824, formulated as 200 mg tablets, will be obtained from the Global Alliance for TB Drug Development ("TB Alliance"). Rifampin, rifabutin, isoniazid, pyrazinamide, and ethambutol will each be obtained from local suppliers compliant with Good Manufacturing Practice Regulations. The investigator will acknowledge receipt of and keep an inventory of all study drug supplied for this study. Study staff has the responsibility to assure that study drugs are dispensed to patients in compliance with the protocol. Procedures for drug handling and storage will be described in the study site SOPs.

5.6 Randomization

This will be a randomized trial. Trial randomization will be computer-generated. Eligible patients (who meet all of the inclusion criteria and none of the exclusion criteria) will be randomized in a 1:1:1 ratio to the study arms.

Randomization will be stratified by the presence of cavitation at baseline (time of diagnosis), since cavitation is associated with a decreased rate of 2-month culture conversion, and HIV status. Cavitation is defined as a gas-containing lucent space at least 1 cm in diameter within the lung parenchyma surrounded by an infiltrate or fibrotic wall greater than 1 mm thick seen on a standard chest radiograph (cavitation seen only on chest CT does not satisfy this definition).

This will be an open-label trial in which neither the subjects nor the study staff will be blinded as to treatment assignment after randomization. However, microbiologists performing mycobacterial cultures and statisticians will be blinded as to treatment assignment of individual patients.

6 POPULATION

6.1 General Considerations

Study entry is open to males and females age 18 years or older of any ethnic background who meet study eligibility criteria. Historically, approximately 60% of patients with TB at clinical sites are males. The gender, ethnicity, and socioeconomic background of study subjects are expected to mirror that of the population served by the study site, and that of the population most affected by TB. Study subjects will be recruited from the hospital and clinics of the University of Cape Town, South Africa.

6.2 Special Populations

6.2.1 Children, Pregnant Women, and Breast-Feeding Women

Children and pregnant or breast-feeding women will not be enrolled because of uncertainties about the safety of PA-824 in these groups of patients. Further, dose equivalence studies have not been performed in pediatric patients.

6.2.2 Prisoners

This study will not enroll prisoners. However, it is possible that a participant will be incarcerated after enrollment. If an enrolled individual is incarcerated, then study medications will be stopped and the participant will be treated for active TB according to the standards of the institution in which s/he is incarcerated. While incarcerated, individuals will not be followed in the study. When the individual is no longer incarcerated, study follow-up may continue, at the discretion of the investigator.

6.2.3 HIV-Positive Individuals

HIV-negative individuals and HIV-positive individuals with CD4 ≥ 350 cells/cu mm will be included in this study. HIV-positive individuals with CD4 <350 cells/mm^3 will not be included in this study, as these patients may be better served by individualized (non-study) TB and HIV therapy. If one or both of the proposed experimental regimens is demonstrated to be effective and safe in this study, then subsequent future studies including individuals with advanced AIDS may be warranted. Since drug-drug interactions between rifampin and protease inhibitors or nevirapine can compromise HIV treatment, patients with HIV co-infection who are receiving protease inhibitors or nevirapine will not be enrolled in this study. Further, patients taking efavirenz require a dose adjustment of rifabutin to 450 mg, and variability in dosing in the experimental regimen may compromise study results, so EFV use will not be permitted. Patients who, based on study testing, are newly identified to be HIV positive will receive individualized counseling about the risks and benefits of study therapy and delay of antiretroviral therapy. Individuals in whom prompt initiation of antiretroviral therapy is clinically indicated and appropriate will not be enrolled in the study. If a participant is already enrolled in the study, they will be taken off of study medicines and referred to the clinics for treatment with individualized (non-study) TB and HIV therapy.

6.3 Inclusion Criteria

Subjects must meet all inclusion criteria in order to participate in this study.

1. Suspected pulmonary tuberculosis with acid-fast bacilli in a stained smear of expectorated sputum at Screening. Patients having extra-pulmonary manifestations of tuberculosis, in addition to smear-positive pulmonary disease, are eligible for enrollment.

2. Age ≥ 18 years

3. Weight ≥ 40 kg and ≤ 80 kg

4. Karnofsky score of at least 60 (requires occasional assistance but is able to care for most of his/her needs; see Appendix B)

5. Signed informed consent

6. HIV negative, or positive with CD4 ≥350 cells/cu mm and not currently taking or planning to take combination antiretroviral therapy for HIV during the experimental phase of the treatment.

7. Ability to adhere with study follow-up

8. Contraceptive requirements

   Females may participate if they are: 1) of non-childbearing potential (have had a bilateral oophorectomy, tubal ligation and/or hysterectomy or have been postmenopausal for at least 12 consecutive months), 2) if they are using effective birth control methods and are willing to continue practicing birth control methods throughout treatment or 3) practice sexual abstinence or have a vasectomized male partner (confirmed sterile), or have a female partner. Therefore to be eligible for this study women of childbearing potential should either: 1) use a double barrier method to prevent pregnancy (i.e. use a condom with either diaphragm or cervical cap) or 2) use hormonal based contraceptives in combination with a barrier contraceptive, or 3) use an intrauterine device in combination with a barrier contraceptive. They must also be willing to continue these contraceptive measures until one week after the last dose of study medication or one week after

discontinuation from study medication in case of premature discontinuation. (Note: Hormone-based contraception alone may not be reliable when taking rifampin-containing TB regimens; therefore, hormone-based contraceptives alone cannot be used by female participants to prevent pregnancy).

Male participants who are having heterosexual intercourse with females of child-bearing potential are required to use one of the following birth control methods during their participation in the trial and for 12 weeks after their last dose of study medication to prevent pregnancy:

- a double barrier method which can include a male condom, diaphragm, cervical cap, or female condom; or
-a barrier method combined with hormone-based contraceptives or an intra-uterine device for the female partner.
The use of the above mentioned birth control method does not apply if the male participant has been vasectomised or has had a bilateral orchiectomy minimally one month prior to screening, or is not heterosexually active, or practices sexual abstinence or if the female sexual partner has had a bilateral oophorectomy, tubal ligation and/or hysterectomy or has been postmenopausal for at least 12 consecutive months.

6.4 Exclusion Criteria

<u>All subjects meeting any of the following exclusion criteria at baseline will be excluded from study participation:</u>

1. Pregnant or breast-feeding

2. Known intolerance or allergy to any of the study drugs

3. Concomitant disorders or conditions for which isoniazid, rifampin, rifabutin, pyrazinamide, or ethambutol is contraindicated. These include severe hepatic damage, acute liver disease of any cause, allergy to the drug, and acute uncontrolled gouty arthritis.

4. Current or planned therapy, during the intensive phase of TB therapy with cyclosporine or tacrolimus, which have unacceptable interactions with rifamycins.

5. Any medical or psychosocial condition, which, in the view of the study investigator, makes study participation inadvisable.

6. Pulmonary silicosis

7. Central nervous system TB

8. ECG at screening with QTc (Fridericia correction) interval >450 ms or any clinically-significant, in the opinion of the investigator, ECG abnormality

9. History and/or presence (or evidence) of neuropathy or epilepsy.

10. History of lens opacity or evidence of lens opacity on slit lamp ophthalmologic examination with a value of 1.0 or higher on AREDS2 Clinical Lens Opacity Classification and Grading System scale.

11. Infection with an isolate known to be resistant to a first-line TB drug (for example, patients with Gene Xpert screening through the local TB program with results suggesting resistance to rifampin)

12. Laboratory parameters done at, or ≤ 14 days prior to, screening (with results available for review by study personnel) demonstrating any of the following:
    - Serum alanine aminotransferase (ALT) activity > 3 times the upper limit of normal
    - Serum total bilirubin level > 2 times the upper limit of normal
    - Serum creatinine greater than the upper limit of normal
    - Hemoglobin level less than 7.0 g/dL
    - Platelet count less than 100,000/mm^3
    - Positive pregnancy test (women of childbearing potential)

13. More than five days of treatment directed against active tuberculosis in the past 6 months

7 STUDY SCHEDULE

7.1 Screening Visit

Subjects with a presumptive diagnosis of sputum smear positive pulmonary TB will be invited to participate. Because culture confirmation is rarely available when TB treatment is initiated, patients will be recruited based on having a sputum smear that is positive for acid fast bacilli or a sputum specimen that is positive for TB by Gene Xpert.

Informed written consent, using IRB-approved consent forms, will be obtained by trained study personnel prior to performing any study-specific procedures. Informed consent is a process that will be initiated prior to the individual's agreeing to participate in the study and will continue throughout the individual's study participation. The informed consent process is described in detail in the "Study Procedures" section.

The following will be performed after obtaining written informed consent:

- Eligibility criteria will be confirmed
- Participants will be asked questions related to demographics, current and past medical history, medications they are taking, alcohol, TB symptoms, and TB risk factors. They will be advised that they can decline to answer any question they consider too personal.
- An expectorated sputum sample will be obtained for smear, culture, and drug susceptibility testing at the study laboratory.
- Participants will have heart rate, blood pressure, respiratory rate, temperature, height and weight measured
- Blood will be drawn for a CBC and serum creatinine, ALT, total bilirubin, albumin and potassium, unless results from these tests within the previous 14 days or less are available.
- All participants will undergo HIV testing and counseling regardless of whether they have had a previous HIV test or not. HIV testing will be carried out with two rapid tests (the second test will be to confirm the result of the first); only one test need be carried out if the subject is known to have HIV, the single test will be confirmatory. Should there be a discrepancy between the two

rapid tests, blood will be sent to the laboratory for enzyme-linked immunosorbent assay Elisa and Western Blot test in order to confirm the diagnosis.

- For HIV-positive participants, a CD4 count should be performed unless written results are available from a test done within the preceding 3 months or less.
- Urine will be collected for pregnancy testing if a woman is of child-bearing potential.
- A posterior anterior chest radiograph will also be taken, unless a posterior anterior chest radiograph done within the previous 14 days or less is available for review. The chest radiograph must be read prior to randomization.
- Visual acuity testing and color vision perception testing will be performed. Slit lamp ophthalmologic examination will be performed to rule out opacities (cataracts).
- An ECG will be performed

Screening evaluations may be done in the context of one or more visits. For example, ophthalmologic examination may be performed after other screening tests indicate that the patient is likely to meet the criteria for study inclusion.

7.2 Baseline Visit

The baseline visit is defined as the visit at which study drug treatment is initiated. The baseline visit (and initiation of study drug treatment) should occur as soon as feasible after randomization. The Baseline visit may be done as soon as the Screening results are available, even if this occurs on the same day as a Screening test is performed.
Procedures to be performed at the Baseline Visit

- Review of eligibility criteria
- Review of concomitant medications
- Targeted symptom assessment
- Measurement of weight
- Interval medical history
- Pregnancy evaluation
- Vital signs (heart rate, blood pressure, respiratory rate, temperature)
- Sputum obtained for smear and culture
- Counseling about study procedures and drug toxicity

Participants who continue to meet eligibility criteria will be started on study drug treatment. Instructions for administering study drugs are provided in the "Administration of Study Drugs" section.
Any residual sputum sample may be stored for future testing.
Participants who no longer meet eligibility criteria may be referred to a local source of TB care. The study team will work in concert with the local source of TB care to provide timely, medically appropriate TB-related care.

7.3 Study Visits During Experimental Phase of TB Treatment (Weeks 0-12)

Visits listed below are protocol-specified study visits. Safety of study participants will be enhanced further by providing study medication doses by directly observed therapy (DOT). In this context, participants will be queried about signs/symptoms potentially related to drug toxicity at the time of medication doses per standard TB treatment DOT practices. Should signs/symptoms potentially related to drug toxicity be recognized at any time, then further evaluation including laboratory testing may be performed at the discretion of the treating clinician.

Study Visits at Days 7, 14, 21, 28, 42, 56, 70, (all +/- 2 days) and 84 (+5days)

The following will be performed:

- Targeted symptom assessment
- Interval medical history
- Adverse event assessment
- Pregnancy evaluation
- Concomitant medication assessment
- Vital signs (heart rate, blood pressure, respiratory rate, temperature)
- Measurement of weight
- Sputum obtained for smear and culture. Two sputum samples will be obtained at Day 84 visit. Drug susceptibility testing will performed on Day 56 and/or Day 84 positive cultures as relevant.
- Counseling about study procedures and drug toxicity

In addition,

- Complete blood count, serum creatinine, alanine aminotransferase (ALT), total bilirubin will be performed at days 7, 14, 28, 42, 56, and 84. ALT and bilirubin will be collected on Day 21
- Single 12-lead ECG will be performed on Days 7 and 28
- Testing of visual acuity and color perception should be performed at the Day 28 study visit
- Semi-intensive PK sampling will be performed on day 14 (+5) days (see Section 8.13) for patients in Arms 1 or 2
- Sparse PK samples will be collected on days 28, 56, and 84 for patients in Arms 1 or 2

7.4 Study Visits During Non-Experimental Continuation Phase TB Treatment (Weeks 13-24) and Following Completion of Treatment

Week 16 and Week 24 (+/- 7 days) Study Visits
The following will be performed:

- Targeted symptom assessment
- Interval medical history
- Review of interval TB treatment
- Concomitant medication assessment at Week 16 in case of Grade 1 or higher AE/SAE
- Measurement of weight
- Sputum will be obtained for smear and culture

Adverse event assessment and counseling about study procedures and drug toxicity will be performed at the Week 16 visit. In addition, ophthalmologic examination (including visual acuity and color perception) will be performed at the Week 24 visit. Arms 1 and 2 participants will also undergo slit-lamp ophthalmologic examination.

Week 48 (+/- 7 days) Study Visit
The following will be performed. This visit can also be conducted via the phone at the investigator's discretion and participant availability.

- Targeted symptom assessment
- Interval medical history

Additional investigations may be conducted at the investigator's discretion should active TB be suspected.

7.5 Early withdrawal

In case of early withdrawal during the study treatment period of the study (prior to Day 84), all efforts shall be made to complete the Early Withdrawal assessments as soon as possible or within 7 days of site notification of the participant withdrawal. At the early withdrawal visit the following information will be collected and procedures performed:

- Interval medical history
- Adverse event assessment
- Pregnancy evaluation
- Concomitant medication assessment
- Measurement of weight
- Targeted symptom assessment
- Complete blood count
- Serum creatinine, alanine aminotransferase (ALT), total bilirubin
- 12-lead ECG
- Single sample for PK evaluation (Arms 1 and 2), if study drug has been given within 24 hours of the visit

7.6 Unscheduled visit

In the case of an unscheduled visit, the following should be performed as relevant, based on the judgement of the site team.

- 12 lead ECG
- Targeted symptom assessment
- Vital signs (heart rate, blood pressure, respiratory rate, temperature)
- Measurement of weight
- Counseling about study procedures and drug toxicity
- Sputum obtained for smear and culture
- Pregnancy evaluation
- Poster anterior chest radiograph
- Complete blood count,
- Serum creatinine, alanine aminotransferase (ALT), total bilirubin

8 STUDY PROCEDURES

8.1 Informed Consent Process

Informed written consent, using IRB-approved consent forms, will be obtained by trained study personnel prior to performing study-specific procedures. Potential subjects will receive information about risks and possible benefits of study participation, study objectives and procedures, potential toxicities, and the informed consent process. Informed consent requires the signature or mark of the subject. A copy of the signed and dated informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to subjects that the quality of their medical care will not be adversely affected if they decline to participate in this study, and that they may withdraw consent at any time. Individuals who choose not to participate in the study will be referred to local sources of TB care.

8.2 Administration of Study Drugs

Patients should start on assigned study therapy as soon as possible after randomization. Doses of study therapy will be given as directly observed therapy (DOT) by study personnel, or by a health care worker or lay treatment supervisor who is aware of the study protocol and trained regarding the study protocol. Alternatively, doses of study therapy can be given via DOT by a family member or employer who has been trained by the study team. Adherence will be confirmed by review of the participant's treatment card, and retraining of the DOT provider will be provided if needed. DOT may be administered at the TB clinic or other health care facility, or, with the subject's permission, at the subject's residence, workplace, or other mutually agreed upon location convenient for the subject.

During the first three months of treatment (experimental phase), study treatment will be 7 days per week. During the experimental phase, non-study TB treatment, either in addition to or in place of study drugs, should not be given.

8.3 Obtaining Sputum Specimens

Sputa should be obtained by spontaneous expectoration whenever feasible. Sputa should be refrigerated at approximately 4°C after collection, and transported to the laboratory as soon as possible but within 2 working days of collection (i.e. received in the laboratory within 2 working days of collection).

8.4 Laboratory Aspects of Sputum Analysis

Laboratory evaluation of sputa will be performed at a qualified mycobacteriology reference laboratory in Cape Town and will be done according to detailed written lab SOPs. Briefly, sputa will be homogenized and decontaminated using the NALC-NaOH method. Fluorescence smear microscopy will be performed on an aliquot of the concentrated processed specimen. Aliquots will be added to Lowenstein Jensen (solid) media and MGIT (liquid) media. Nucleic acid and/or biochemical methods will be used for mycobacterial species determination. Quality monitoring procedures will be documented, and laboratory activities will be subject to regular study monitoring.

8.5 Drug Susceptibility Testing

Pre-treatment isolates of *Mycobacterium tuberculosis* collected at screening will undergo conventional drug susceptibility testing to isoniazid and rifampin. Susceptibility testing to, pyrazinamide, ethambutol and PA-824 may also be performed pending availability of drug susceptibility testing for these drugs. Susceptibility testing will performed on Day 56 and/or Day 84 positive cultures as relevant.

8.6 Electrocardiograms (ECG)

Single ECGs will be recorded for 10 seconds. Timing and registration technique for ECGs will be standardized for all participants. Participants should be lying down (recumbent) for at least 5 minutes prior to each 12-lead ECG evaluation.

8.7 Ophthalmologic examinations

Visual acuity will be tested using a Snellen chart. Color vision perception will be tested using the Ishihara charts to test for color-blindness. The slit lamp examination will be performed by an

ophthalmologist, and results will be documented using the AREDS2 Clinical Lens Opacity Classification and Grading System.

8.8 Review of Symptoms Since Last Study Visit

Patients will be asked whether they have experienced any of the following since the last study visit: fevers, sweats, cough, rash, itching, jaundice, nausea, vomiting, diarrhea, loss of appetite, vision problems, numbness/tingling of extremities, headache, joint pain, eye pain. In addition, patients will be asked whether they have had other symptoms (not listed above); if yes, then these symptoms will be recorded and graded. In addition, targeted diagnostic evaluations may be indicated to determine the underlying cause of symptoms and their association with study drug use.

8.9 Concomitant Medications

The use of all non-study drugs (including over-the-counter medications) from 14 days before starting study therapy through the end of experimental treatment will be monitored and recorded. In addition, if a grade 1 or higher AE or SAE occurs within 28 or fewer days after completion of experimental phase therapy, then the use of all non-study drugs (including over-the-counter medications) during the interval from study drug completion to AE/SAE onset will be recorded. The use of all non-study drugs (including over-the-counter medications) will be recorded at this study visit.

Concomitant antimicrobials with known antituberculous activity (amoxicillin/clavulanic acid, amikacin, kanamycin, streptomycin, moxifloxacin, ofloxacin, ciprofloxacin, levofloxacin, sparfloxacin, gatifloxacin, gemifloxacin, linezolid, capreomycin, cycloserine, ethionamide, para-aminosalicylate sodium) should not be used during the study phase therapy. Any patient who receives more than 2 doses of any of the medications listed above will be classified as being on a non-study regimen. Antimicrobials with no known antituberculous activity may be prescribed for intercurrent infections at the discretion of the investigator.

Ethanol can exacerbate the potential hepatotoxicity of isoniazid, rifampin, and pyrazinamide. Participants will be counseled to abstain from alcohol while on TB therapy.

8.10 Management of Subjects Deemed Protocol Ineligible Based on Culture Results

Because of the relatively slow growth of *M. tuberculosis* in culture, the results of sputum mycobacterial cultures and drug susceptibility testing are rarely available at the time of initiation of TB treatment. Therefore, for this study we plan to enroll patients on the basis of a positive sputum AFB smear microscopy result for TB. We anticipate that approximately 10% of enrollees subsequently will be deemed protocol ineligible based on culture results.

Management of individuals having either a) NO baseline sputum culture that is positive for *M. tuberculosis* OR b) a baseline *M. tuberculosis* isolate that is resistant to any one or more of the following: rifampin and/or isoniazid and/or pyrazinamide and/or ethambutol: these individuals will be taken off of their assigned study regimen, and treated with an individualized regimen at the discretion of the treating clinician. All patients receiving experimental phase therapy at the time of discontinuation of the study regimen will have a study visit at 2 weeks after discontinuation of the study regimen for assessment of delayed toxicity.

8.11 Management of Subjects that are not Protocol-Correct

Some subjects may have toxicity, adherence problems, or other problems that prevent completion of experimental phase therapy in the protocol-correct timeframe. These patients should continue to be followed in the study (unless consent is withdrawn), and continue to have protocol-specified evaluations.

8.12 Compensation of Study Subjects

Study subjects will be compensated for their time and travel to and from study visits and for time spent during PK sampling. The form and amount of compensation will be in accordance with local guidelines.

8.13 Steady State Pharmacokinetic Assessments

Semi-intensive PK sampling: At Day 14(+5) days each subject receiving PA-824 will have a steady state PK assessment for PA-824. An additional aliquot will be saved for possible future PK determination of companion drugs, should this become of scientific interest. Samples will be collected via an indwelling catheter or by direct venipuncture. Approximately 4 cc of blood will be obtained immediately prior to administration of study medications and then at 1, 2, 5, 8, and 24 hours post-dose. The 24 hour specimen should be drawn immediately before administration of the next medication dose. Specimens will be obtained, processed, and assayed according to procedures in a detailed procedures manual.

Sparse PK sampling: On Days 28, 56, and 84, patients receiving PA-824 will have a sparse sampling for PA-824 PK analysis. It is preferable that these samples be collected pre-dose (within 2 hours of scheduled dosing) and then 2-4 hours after an observed dose, if scheduling allows.

8.14 Management of HIV-Positive Subjects

All participants will undergo HIV testing and counseling regardless of whether they have had a previous HIV test or not. HIV testing will be carried out with two rapid tests (the second test will be to confirm the result of the first) for patients who are not known to have HIV. For patients with an existing HIV diagnosis, one rapid test will be performed to confirm the diagnosis. Should there be a discrepancy between the two rapid tests, blood will be sent to the laboratory for enzyme-linked immunosorbent assay Elisa and Western Blot test in order to confirm the diagnosis.

For HIV-positive participants, a CD4 count should be performed unless written results are available from a test done within the preceding 3 months or less.

Patients who, based on study testing, are newly identified to be HIV positive will receive further individualized counseling about the risks and benefits of study therapy. Individuals in whom prompt initiation of antiretroviral therapy is clinically indicated will not be enrolled in the study. If a participant is already enrolled in the study, he or she will be taken off of study medicines and referred to the clinics for treatment with individualized TB and HIV therapy. All HIV-positive individuals who screen for, or participate in, the trial will be referred to local sources of free HIV care by the study team. HIV-positive individuals who participate in the trial will be referred for HIV treatment following completion of experimental TB treatment.

8.15 Procedures to maximize compliance with study visits

Several methods will be used to maximize the proportion of participants who routinely keep their study visit appointments. First, only individuals with an expressed interest in participating and keeping

appointments will be enrolled. Second, at enrollment, information will be collected including the participant's name, address, phone number, and, with the permission of the participant, names and telephone numbers of friends/relatives who would normally know the participant's whereabouts. Third, participants will be given written appointment cards. Fourth, participants with telephones may be called the day before the scheduled visit to remind them. Fifth, participants who miss appointments will be contacted either by phone or by domiciliary visit or through friends/family whose names were provided by the participant. Finally, study subjects will be compensated for their time and travel to and from study visits and for time spent during PK sampling. The form and amount of compensation will be in accordance with local guidelines.

9 ASSESSMENT OF SAFETY AND TOLERABILITY

9.1 Safety and Tolerability Endpoints

The primary endpoint for analysis of safety and tolerability is Grade 3 or higher adverse events. Secondary endpoints include permanent discontinuation of assigned study regimen for any reason (other than new recognition of subject ineligibility based on absence of *M. tuberculosis* growth in baseline sputum cultures, or growth of *M. tuberculosis* resistant to isoniazid and/or rifampin and/or pyrazinamide and/or ethambutol). Other aspects of safety and tolerability that will be assessed include mortality and the rates and types of toxicity thought related to study drugs by the investigator.

9.2 Safety Evaluation

A Data and Safety Monitoring Board (DSMB) will review the study protocol and oversee progress of the trial. The DSMB will review safety data once 50% of the participants have completed study treatment. Since this is an open-label study, the DSMB will have access to treatment group assignment. The DSMB will be comprised of at least the following: an expert in statistics, an expert in clinical trials, and an expert in clinical TB. No early stopping rules will be formally adopted.

9.3 Interim Efficacy and PK Analysis

The study will randomize 183 individuals with smear positive TB to the 3 study arms. In alignment with the timing of the Safety Evaluation (Section 9.2), An interim efficacy and PK analysis is planned when 50% of participants have completed study treatment. Each experimental arm (Arm 1 and Arm 2) will be compared to the control arm (Arm 3). Interim analysis will be done for time to culture conversion on liquid media and to assess PK interactions.

Time to culture conversion

Unadjusted hazard ratio for time to culture conversion will be estimated using Cox-proportional hazards model. A hazard ratio of at least 1.20 will ensure enhanced efficacy of each experimental treatment arm. The interim analysis is powered at 95% to ensure the end of study power at 90%. An experimental arm that does not meet efficacy criteria at the interim analysis may be dropped, following a formal review of all data by the study team leadership and the DSMB.

PK analysis

PK parameters (e.g. area under the concentration-time curve over 24 hours (AUC_{0-24}), minimum concentration (Cmin), maximum concentration (Cmax), and oral clearance (Cl/F)), will be estimated for PA-824 from semi-intensive sampling data, using a noncompartmental approach using standard PK software, like Phoenix WinNonLin. Using all PK data (sparse and semi-intensive), a PK/PD model will be developed to evaluate the PK values for PA-824 when given with rifampicin vs. with rifabutin.

Results may be helpful as accessory information that will put interim efficacy results into pharmacologic context.

9.4 Early Stopping Rules

No early stopping rules will be formally adopted.

9.5 Study Treatment Discontinuation Criteria

Participants who prematurely discontinue study treatment after having received one or more doses of PA-824 will not be replaced. The exception to this is in cases of new recognition of subject ineligibility based on absence of *M. tuberculosis* growth in baseline sputum cultures, or growth of *M. tuberculosis* resistant to isoniazid and/or rifampin and/or pyrazinamide and/or ethambutol. Study treatment must be immediately discontinued as a result of the following:

- Withdrawal of informed consent.
- Investigator considers it for safety reasons in the best interest of the participant that he/she be withdrawn.
- If a participant develops a new onset stage 4 illness after enrollment, they would meet the criteria for withdrawal from the study and, if HIV positive, for fast track initiation of ART per the South African National Department of Health ART guidelines. The Interim WHO Clinical Staging of HIV/AIDS and HIV/AIDS Case Definitions for Surveillance, African Region (2005) will be used for guidance on definitions of stage 4 illnesses (34).
- Resistance to isoniazid, rifampin, pyrazinamide, or ethambutol determined during study.
- Participant experiences specific toxicities as outlined in Section 9.7.
- Participant becomes pregnant.
- Termination of the study by the sponsor.

All participants withdrawn from treatment will be referred to the local community TB clinics for standard antituberculosis chemotherapy according to National Guidelines. The participants will be provided with a referral letter to take with them to the TB Clinic. Participants will be transported by the study staff to the clinic, or a follow-up call will be made by the study site staff to the clinic to determine if the participant attended the clinic on the date as arranged. Every effort will be made to continue to follow participants who discontinue study medicine prematurely until the final study visit, if they are agreeable. Any participant who received at least one dose of PA-824 and is withdrawn or withdraws early from study treatment will undergo an early treatment discontinuation visit and will be requested to return for the applicable follow up visits. Evaluations at these visits will not include PK sampling.

9.6 Subject Study Withdrawal Criteria

Participants who withdraw from the trial after having received one or more doses of PA-824 will not be replaced. The exception to this is in cases of new recognition of subject ineligibility based on absence of *M. tuberculosis* growth in baseline sputum cultures, or growth of *M. tuberculosis* resistant to isoniazid and/or rifampin and/or pyrazinamide and/or ethambutol. A participant may be prematurely withdrawn from the trial as a result of the following:

- At their own request or at the request of their legally acceptable representative.
- If, in the investigator's opinion, continuation in the trial would be detrimental to the well-being of the participant.
- At the specific request of the sponsor.

Every effort will be made to reduce loss to follow-up, as described in 8.16. Subjects will be contacted by phone if they do not return for study visits. All participants withdrawn will be referred to the local community TB clinics for standard antituberculosis chemotherapy according to National Guidelines. The participants will be provided with a referral letter to take with them to the TB Clinic. The participants will either be transported to the clinic by the study staff, or else a follow-up call will be made by the study site staff to the clinic to determine if the participant attended the clinic on the date as arranged.

9.7 Adverse Events

The Investigators are responsible for eliciting adverse events by observing study participants and recording all adverse events observed by him/her or reported by study participants during the trial.

9.7.1 Definitions

Adverse event (AE): Any untoward medical occurrence in a study participant administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Serious adverse event (SAE): Any untoward medical occurrence that at any dose:

- results in death;
- is life threatening (any event in which the subject was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe);
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect; or
- is a medically important event.
  Note:
  Medical and scientific judgment should be exercised in deciding events are “medically important.” These events are those that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the study participant or may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse.

Unexpected Adverse Event (UAE): An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator’s Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product).

9.7.2 Association and Attribution/causality

An adverse event is considered associated with the use of the drug (Adverse Drug Reaction) if the attribution is possible, probable or definite.

The following definitions for rating attribution/causality will be:

Relatedness Rating	Definition
Not Related	An adverse event, which is not related to the use of the drug.
Doubtful	An adverse event for which an alternative explanation is more likely, e.g., concomitant drug(s) or concomitant disease(s), and/or the relationship in time suggests that a causal relationship is unlikely.
Possible	An adverse event, which might be due to the use of the drug. An alternative explanation, e.g., concomitant drug(s) or concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore the causal relationship cannot be excluded.
Probable	An adverse event, which might be due to the use of the drug. The relationship in time is suggestive, e.g., confirmed by dechallenge. An alternative explanation is less likely, e.g., concomitant drug(s) or concomitant disease(s).
Definite	An adverse event, which is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation, e.g., concomitant drug(s) or concomitant disease(s).

9.7.3 Severity

With the exception of QTc interval, all symptoms and laboratory findings will be graded according to severity using the Division of AIDS Table for Grading Severity of Adult Adverse Events (Appendix D), which, in our view is the most comprehensive grading scale for patients with infectious diseases, including TB or HIV.

The protocol-specific criteria for grading prolonged QTc are as follows:

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Prolonged QTc (using Fridericia QT correction formula)	Asymptomatic, QTc interval 0.45 – 0.47 sec	Asymptomatic, QTc interval >0.470 – <0.50 sec	Asymptomatic, QTc interval ≥0.50 sec OR a QTc >0.48 sec with an increase in interval ≥ 0.06 sec above baseline	Life threatening consequence, e.g., Torsade de pointes or other associated serious ventricular dysrhythmia

9.7.4 Reporting

***AE**:* Adverse events will be collected by the investigators from the time a study participant receives the first dose of study drug through the Week 16 visit. Any AE (serious or non-serious) observed by the investigator or reported by the study participant that meets the AE reporting requirements (see below) will be recorded on an Adverse Event Case Report Form. The Investigator will review each AE and assess its relationship to drug treatment based on all available information at the time of the completion of the case report form. The following information will be recorded for each Adverse Event reported:

- Diagnosis of the AE, if possible. In the case where an overall diagnosis cannot be made, each specific sign and/or symptom will be recorded as individual AEs;
- Date of onset;
- Stop Date (duration) if applicable;
- Severity;
- Action taken with study drugs;
- Other action taken;
- Outcome;
- Relationship to study drugs;
- Seriousness.

The following adverse events must be reported on an Adverse Event Report Form:

- new medical diagnosis (at the time of enrollment, if the patient already has a medical diagnosis whose signs or symptoms worsen during the study to a Grade 3 or 4, this is an adverse event that must be reported)
- any grade 1 or higher adverse event
- study drug discontinuation due to an adverse event
- pregnancy
- lab values that are considered clinically significant by the investigator

***SAE**:* Any SAE must be recorded and reported by the site investigator to the Johns Hopkins University study coordinator immediately (within 24 hours), to the relevant drug safety team at TB Alliance or Pfizer and to the local IRB and to regulatory authorities in accordance with local requirements and ICH guidelines for Good Clinical Practice. SAEs will be collected from the time a study participant receives the first dose of study drug through Week 16.

The study drugs are PA-824, ethambutol, isoniazid, rifampin, rifabutin, and pyrazinamide. The most common adverse effects associated with the study drugs are specified in the "Study Drugs" section. The investigator is responsible for monitoring all adverse events that are observed or reported during the study, regardless of whether they are related to study drugs.
In accordance with the FDA's Code of Federal Regulations, the study sponsor and the participating investigators are responsible for reviewing all information relevant to the safety of the study drugs. Reporting and monitoring of SAEs is required to alert the FDA, sponsor, institutional review boards, and the clinical investigators of real and potential safety issues. The investigators will carefully review the Adverse Event Reports and use this information to monitor the investigational drug's toxicity profile and patient safety. Any adverse experience associated with the use of the drug that is both serious and unexpected will be reported to the FDA in the form of a written Safety Report.
The responsible institutional review boards, FDA and South African Regulatory body (Medicines Control Council) will be notified of all SAEs and AEs according to their individual guidelines.

9.7.5 Pregnancy

The Investigator will immediately notify the sponsor of any pregnancy that is discovered during study drug administration or which started during study drug administration. The Investigator will also report pregnancies which occur within one month of the completion of PA-824. Pregnancy forms will be completed for all pregnancies reported during the clinical trial. In addition, with the permission of the study participant, or female partner of a male participant, the investigator will report follow-up information regarding the outcome of the pregnancy, including perinatal and neonatal outcome. Infants will be followed for 6 months.

All women of childbearing potential will be instructed to contact the study team immediately if they suspect they might have become pregnant (for example, missed or late menses) during study treatment or within one month of receiving their last dose of study medication.

If pregnancy is suspected for a participant in Arm 1 or Arm 2 while the subject is receiving PA-824, the PA-824 will be withheld immediately until the result of the pregnancy test is known. If pregnancy is confirmed, the PA-824 will be permanently discontinued in an appropriate manner and the subject withdrawn from the trial. Protocol-required procedures for trial discontinuation and follow-up will be performed unless contraindicated by the pregnancy. Patients will be referred to prenatal care and to the local TB program for continuation of their TB treatment. Women will be asked questions about pregnancy history as well as information about the current pregnancy and birth. Permission will also be requested to follow the infant for six months.

Should the female partner of a male Subject enrolled in Arm 1 or Arm 2 become pregnant during study treatment or in the 4 weeks after the completion of PA-824 and the investigator becomes aware that this situation has occurred, consent will be requested from the female partner for collection of information on her pregnancy history and for information on the current pregnancy and birth. Permission will also be requested to follow the infant for six months.

Pregnancy reporting will follow the same time lines and reporting structures as for a SAE (see above). SAE reporting will also occur if the pregnancy outcome is a congenital anomaly. This will follow the reporting procedures described above for SAE reporting plus an additional clinical report.

9.8 Clinical Management of Adverse Events

9.8.1 Liver toxicity

Study treatment will be stopped if any of the following criteria are met:

- ALT $\geq$ 5x the upper limit of normal (ULN)
- ALT $\geq$ 3x ULN with signs or symptoms suggestive of clinical hepatitis including one or more of the following: nausea, vomiting, abdominal pain, dark or clay colored stools, unexplained fever, jaundice, liver tenderness, or hepatomegaly
- ALT $\geq$ 3xULN and Total Bilirubin $\geq$ 2xULN

If a patient develops hepatic toxicity meeting the above stopping criteria, the following evaluation will be undertaken: assessment for history of injection or non-injection drug use, alcohol ingestion, use of other hepatotoxic drugs, and performance of serologic tests for viral hepatitis (IgM antibody for Hepatitis A, Hepatitis B surface antigen, IgM antibody to Hepatitis B core antigen, antibody to Hepatitis C). In addition, an AST and INR should also be checked, if not already done.
Participants will be followed until resolution (return to baseline) or stabilization of the ALT elevation.

For management of liver toxicities that do not meet these criteria, refer to section 9.7.3.

9.8.2 Cardiac rhythm disturbances

For Grade 1 (asymptomatic) or Grade 2 (asymptomatic, transient rhythm abnormality not requiring any treatment) cardiac rhythm disturbances**,** participants may continue study treatment and should be carefully evaluated and followed closely. For Grade 3 (recurrent, persistent, symptomatic arrhythmia requiring treatment) or Grade 4 (unstable dysrhythmia requiring treatment) cardiac rhythm disturbances**:** participants will permanently discontinue study drugs and be withdrawn from the trial. For QTc prolongation meeting Grade 3 or 4 criteria as described in Section 9.6.3, subjects should be permanently withdrawn from the trial.

9.8.3 Other toxicities

Grade 1 toxicities: In general, for grade 1 toxicities, the patient will be followed carefully, and the study drugs will be continued.
Grade 2 toxicities: For grade 2 toxicities, the patient will be followed more carefully, with additional laboratory and/or clinic visits as necessary, and the study drugs temporarily held at the investigator's discretion.
Grade 3 toxicities: For any grade 3 toxicity that, in the principal investigator's judgment is due to study drug(s), the causative study drug(s) should be held. The clinician should rule out other possible causes of the symptoms before discontinuing study medication. When possible, concomitant medications should be held first at the discretion of the principal investigator if he/she suspects they are contributing to the toxicity. Depending on the nature and severity of the toxicity, the degree to which it resolves, and/or the emergence of alternative explanations for the toxicity or the subject's deterioration, the study drugs(s) may be restarted at the discretion of the investigator.
Grade 4 toxicities: Any patient with grade 4 renal, hepatic, cardiac or hematological toxicity will be immediately discontinued from study therapy. The laboratory test or clinical finding in question will be reassessed as soon as possible. The repeat test will guide management of the event as follows:

- If the repeat assessment shows toxicity of grade 3 or lower, and if the patient has continued to receive study drugs between the two testing dates, then the patient will be managed according to the appropriate toxicity level of the repeat test.
- If the repeat test shows toxicity of grade 3 or lower, and if the patient has not received study drugs between the two testing dates, then the patient will be managed at the discretion of the investigator with regard to the re-administration of study drugs, and otherwise according to the toxicity level of the repeat test.
- If the repeat test shows grade 4 toxicity, then the patient will be permanently discontinued from study medications. Further treatment of TB will be directed by the investigator on an individualized basis. The patient will continue to be followed for study monitoring purposes (as are other patients who make a permanent departure).

For other grade 4 toxicities, the study drugs will be temporarily held and may be restarted or permanently stopped at the discretion of the investigator. Toxicities graded 3 or 4 and occurring during study therapy will be documented on the Adverse Event Form, according to the criteria described in the following sections. The maximum level of toxicity reached will be clearly indicated.
For all toxicities that are treatment-emergent and that require the study therapy to be temporarily or permanently discontinued, relevant clinical and laboratory tests will be obtained as clinically indicated and repeated as needed until final resolution or stabilization of the toxicity.

Temporary or permanent discontinuation of study drugs: Certain events or conditions may necessitate temporary or permanent discontinuation of the study medication. Patients who experience such events or conditions, however, will still be "on study" and will be followed until study completion. Any patient for whom the study medication is temporarily discontinued will be restarted on study medication as soon as possible. Study regimens will be discontinued and non-study regimens will be used with continued study follow-up for patients who fail to respond to study therapy either clinically and/or bacteriologically, for patients in whom treatment-emergent drug toxicity warrants discontinuation of study therapy, and for patients who become pregnant. If study drugs are permanently discontinued, further antituberculosis therapy may be administered at the investigator's discretion.

Criteria for temporary discontinuation of study therapy

- Development of a toxicity that, depending on its nature and severity, requires temporary discontinuation of the study medication until the toxicity resolves as indicated in the preceding toxicity management section.
- Development of another medical condition that makes the administration of the study drug inadvisable. The decision to discontinue temporarily the study medication in this situation will be at the investigator's discretion. The period during which the patient is off study medication will be as short as clinically possible.

10 DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

Details of the analyses specified in this section will be contained in a statistical analysis plan.

10.1 Hypotheses

- Among patients with pulmonary TB, the addition of PA-824 to first-line TB treatment that includes isoniazid, a rifamycin antibiotic, and pyrazinamide for 12 weeks will reduce the time to sputum culture conversion compared to standard treatment with isoniazid, rifampin, pyrazinamide and ethambutol, measured over 12 weeks

- PA-824 at a dose of 200 mg daily given for 12 weeks with first-line drugs will be safe and well-tolerated

10.2 Data Analysis

This is a three arm randomized controlled trial to estimate the rate of, and time to sputum culture conversion among pulmonary TB patients receiving either PA-824 or control. The two treatment arms will be independently compared with the control arm. The statistician performing the analysis will be blinded to the random treatment assignment until database lock.

Baseline data, demographic, clinical and laboratory data will be summarized by the three treatment arms. Continuous variables will be summarized using medians and inter-quartile range and compared using a Wilcoxon rank-sum test. Categorical variables will be summarized by frequencies and compared across treatment arms using a Fisher’s exact test.

Time to culture conversion to negative is the measure of the sterilizing activity of the study regimens in this trial. Results on solid and liquid media will be analyzed separately. Analysis of sputum culture conversion will be per protocol, and also separately by modified intention-to-treat (MITT). The per

protocol analysis will be the primary analysis, although both analyses will be reported. For the per-protocol analysis, patients meeting all of the following criteria will be included: had at least one evaluable culture obtained at the end of intensive phase, had *M. tuberculosis* on baseline culture, were not excluded based on drug-resistance of the baseline culture, and completed the assigned study phase treatment. For the MITT analysis, patients meeting all of the following criteria will be included: had *M. tuberculosis* on baseline culture and were not excluded based on drug-resistance of the baseline culture.

Rate of sputum culture negativity (95% CI) and median time to conversion of sputum cultures to negative after initiation of treatment will first be estimated by the Kaplan-Meier method, and the difference in Kaplan-Meier curves will be compared by the log-rank test at 5% level of significance. An univariable and multivariable analysis using Cox proportional hazard model will be used to determine whether or not treatment arm is independently associated with the time to sputum culture conversion, after adjusting for other variables known to affect time to culture conversion (baseline burden of disease, as measured by smear grade; cavitary disease; HIV; etc.) All adverse events, grade 3 or higher, will be summarized using rates, defined as a ratio of number of events to total person-years of follow-up, and Poisson exact 95% confidence intervals. Unadjusted and adjusted incidence rate ratios of serious adverse events by treatment arms will be estimated using univariable and multivariable Poisson regression models respectively. PK parameters (e.g. AUC_{0-24}, Cmin, Cmax, and Cl/F), will be estimated for PA-824 from semi-intensive sampling data, using a noncompartmental approach using standard PK software, like Phoenix WinNonLin. Using all PK data (sparse and semi-intensive), a PK/PD model will be developed to evaluate the relationship between drug exposures (e.g. Cmax, T>MIC or AUC_{0-24}) and outcomes (e.g. time to culture conversion).

10.3 Sample size

Sample size will be 183 participants based on the following assumptions: a two-sided type 1 error of 5%, power of 90%, target hazard ratio of $\geq$1.9, and 15% inevaluable or lost to follow-up. The target hazard ratio of 1.9 is based on the OFLOTUB Phase 2 trial in which replacement of ethambutol with moxifloxacin resulted in a hazard ratio of 1.7, comparing time to culture conversion in the experimental arm with the control (33). This was considered to be sufficient evidence (alongside other trials) (24) for evaluating this regimen in a Phase 3 treatment-shortening trial. A treatment effect at least this large is expected to be necessary to shorten treatment duration to $\leq$4 months from the current duration of 6 months (25).

11 HUMAN SUBJECTS PROTECTION

11.1 Institutional Review Board Involvement

This research will be conducted in compliance with the requirements of the US DHHS regulations to protect human subjects from research risk (45 CFR Part 46), in compliance with South African regulations protecting human subjects, and with oversight by the IRBs of Johns Hopkins University School of Medicine (FWA #00005752) and the University of Cape Town (FWA #00001637). All study personnel will complete a course in protection of human subjects prior to participating in the study.

The investigator will ensure that the purpose of the study is explained to the patient and that written consent is obtained prior to participation in the study. The patient, investigator or designee, and others as required by local regulatory guidelines will sign the consent prior to entry into the study. The patient will receive a copy of his/her signed consent form. The investigator will retain a copy of the signed consent forms, which may be inspected at the monitor's or auditor's request.

The investigator will promptly report to the Ethics Committee/IRB and regulatory body of all changes in the research activity and all unanticipated problems involving risks to human subjects or others, and will not make changes in the research without Ethics Committee/IRB and regulatory body approval, except where necessary to eliminate apparent immediate hazards to human subjects.

11.2 Informed Consent

Written informed consent will be obtained by trained study personnel prior to screening for inclusion and exclusion criteria. Informed consent is a process that will be initiated prior to the individual's agreeing to participate in the study and will continue throughout the individual's study participation. The informed consent process is described in detail in the "Informed Consent Process" section of "Study Procedures" (Section 8).

11.3 Confidentiality

All study records will be managed in a secure and confidential fashion. Study records will be maintained in locked cabinets, and computer records will be password protected. Access to study records will be restricted to specified study team members. The study monitor(s) or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator.

11.4 Special Populations

There will be no exclusion based on gender or ethnicity. Children and pregnant or breast-feeding women will be excluded from this study because of uncertainties about the safety of PA-824 in these groups. It is anticipated that approximately 40% of study subjects will be women, since, historically, approximately 60% of patients with tuberculosis at clinical sites are males. The gender, ethnicity, and socioeconomic background of study subjects are expected to mirror that of the population of the hospital and clinics served, and that of the population most affected by tuberculosis.

This trial will not enroll persons who are incarcerated. Section 6 "Population" details issues related to follow-up of study subjects who become incarcerated after study enrollment.

11.5 Ethical Issues in Doing This Trial in South Africa

The vast majority of the global TB burden occurs in developing countries. South Africa has an exceedingly high TB case rate of approximately 600/100,000 population/year, and ranks #7 in the world based on number of incident cases. New treatment regimens that substantially improve tuberculosis treatment (shorten and/or simplify therapy) are critically needed in this setting. Therefore, it is appropriate to evaluate a new drug for TB treatment in South Africa.

11.6 Future Use of Stored Specimens

Two types of specimens will be stored: *M. tuberculosis* isolates and plasma. Specimens stored for study purposes will be labeled with study identification number and collection date, and will not be labeled with participant name.

M. tuberculosis isolates: The pretreatment *M. tuberculosis* isolates will be stored frozen, and may be used for confirmation of drug susceptibility testing during the course of the study.

Susceptibility testing will performed on Day 56 and/or Day 84 positive cultures as relevant.

Plasma specimens will be obtained for pharmacokinetic analyses. Plasma specimens will be stored at

-70˚C and may be assayed for concentrations of tuberculosis drugs other than PA-824 during the course of the study.

No samples will be stored for future use or research beyond the study and we confirm that no genetic testing will be conducted on participant DNA.

12 MONITORING AND DATA HANDLING

Site Monitoring will be conducted to ensure that human subject protection, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, CGP/ICH, and regulatory guidelines, and that the study is conducted in accordance with the protocol and sponsor SOPs. Site monitoring will performed according to details in a written monitoring plan.

Data handling and record keeping will be performed according to procedures that will be developed in a detailed Data Management Operations Manual. Each participant will be assigned a unique study ID number. This number will be recorded on each data collection form and clinical specimen. Names and other obvious identifiers will not be used on data collection forms. All study records will be stored in locked files in a secure area and access will be limited to study personnel and designated regulatory personnel. All forms will be reviewed prior to data entry for accuracy, consistency, and completeness by designated study staff.

Source Documents and Access to Source Documents Appropriate records will be maintained for this trial, in compliance with ICH E6 GCP, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. The following will have access to study records: members of the study team; IRBs that review the study, the Office of Human Research Protections, the FDA, and the MCC. Authorized representatives of the sponsor and regulatory agencies will be permitted to examine study records for QA reviews, audits, and evaluation of the study safety and progress.

Data Management Responsibilities The on-site principal investigator and data manager will be responsible for accuracy, completeness, and storage of source records and data collection forms. The study team and data entry staff will review source documents to ensure accuracy and completeness. The site administrator will maintain logbooks to record dates of completed and upcoming clinic visits and specimen collections.

Data Capture Methods Data will be manually entered from the source documents directly into the eCRF.

Reports The timing of reports will be detailed in the Data Management Operations Manual. Briefly, safety data will be reviewed by the DSMB after 50% of the participants have been enrolled and annually thereafter; reports for the DSMB will be prepared for the DSMB according to a schedule determined at the first convened DSMB meeting. Data coding will occur at the time of data collection; ongoing logical data queries will be performed.

Study Records Retention Within 2 years of study completion, identifiers excluding study ID number will be deleted from data files. Study records will be maintained by the investigator following study discontinuation in accordance with all applicable FDA and local South African regulatory requirements. The FDA will be notified prior to destruction of study records.

Protocol Deviations A protocol deviation is any noncompliance with the clinical trial protocol or Manual of Procedures. As a result of deviations, corrective actions are to be developed and implemented promptly. The site will identify and report deviations according to the guidelines of the IND sponsor and IRBs.

Quality Management Procedures for quality management will be detailed in a separate Quality Management Plan. The study site will provide direct access to all trial related facilities, source documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by regulatory authorities. Following written procedures, study monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol and the applicable regulatory requirements.

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