Estimating the Early Transmission Inhibition of New Treatment Regimens for Drug-Resistant Tuberculosis *Purchased*

A. Stoltz[1,a], R. R. Nathavitharana[2,a], E. de Kock,[3], V. Ueckermann[1], P. Jensen[4], C. M. Mendel[5], M. Spigelman[5] and E. A. Nardell[6]

[1]Department of Infectious Diseases, University of Pretoria School of Medicine, Pretoria, South Africa;

[2]Division of Infectious Diseases, Beth Israel Deaconess Hospital, Boston, Massachusetts, USA;

[3]Retrasol, Pretoria, South Africa;

[4]Division of Tuberculosis Elimination, Centers for Disease Control and Prevention, Atlanta, Georgia, USA;

[5]Global Alliance for TB Drug Development, New York, New York, USA; and

[6]Division of Global Health Equity, Brigham and Women's Hospital, Boston, Massachusetts, USA

[a]A. S. and R. R. N. contributed equally to this work.

Presented in part: ATS International Conference, Dallas, Texas, 17–22 May 2019.

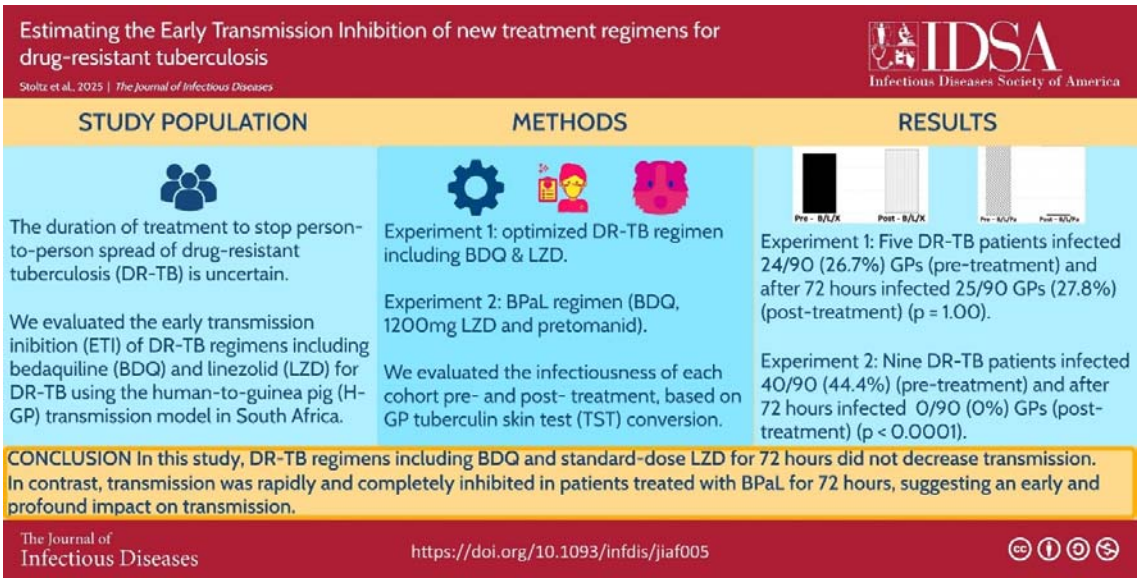
Correspondence: R. R. Nathavitharana, MD, MPH, Division of Infectious Diseases, Beth Israel Deaconess Medical Center, 110 Francis Street, Suite GB, Boston, MA 02215 (rnathavi@bidmc.harvard.edu); E. A. Nardell, MD, Division of Global Health Equity, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115 (enardell@gmail.com)

ABSTRACT

Background: Most drug-resistant tuberculosis occurs due to transmission of unsuspected or ineffectively treated drug-resistant tuberculosis. The duration of treatment to stop person-to-person spread of drug-resistant tuberculosis is uncertain. We evaluated the impact of novel regimens, including BPaL (bedaquiline, 1200-mg linezolid, and pretomanid), on drug-resistant tuberculosis transmission, using the human–guinea pig (H-GP) transmission model.
Methods: In experiment 1, patients initiated an optimized drug-resistant tuberculosis regimen including bedaquiline and linezolid. In experiment 2, patients initiated the BPaL regimen. We measured baseline infectivity for each cohort by exhausting ward air to one of two guinea pig exposure rooms (control group), each containing 90 guinea pigs, for 8 patient-days. Then, after 72 hours of treatment, ward air was exhausted to the second guinea pig exposure room for 8 patient-days (intervention group). The infectiousness of each cohort was compared by performing tuberculin skin tests in guinea pigs at baseline (before treatment) and 6 weeks after the exposure period.
Results: In experiment 1, before treatment, 5 patients with drug-resistant tuberculosis infected 24 of 90 guinea pigs (26.7%) (control group). After treatment (72 hours after drug initiation), the same patients infected 25 of 90 guinea pigs (27.8%) (intervention group) ($P >$ .99). In experiment 2, before treatment, 9 patients with drug-resistant tuberculosis infected 40 of 90 guinea pigs (44.4%) (control group). After treatment (beginning 72 hours after drug initiation), the same patients infected 0 of 90 guinea pigs (0%) (intervention group) ($P <$ .0001).

Conclusions: In this study, drug-resistant tuberculosis drug regimens, including bedaquiline and standard-dose linezolid for 72 hours, did not decrease drug-resistant tuberculosis transmission. In contrast, transmission was rapidly and completely inhibited in patients treated with BPaL for 72 hours, suggesting an early and profound impact on transmission.

GRAPHICAL ABSTRACT



Keywords : tuberculosis, drug-resistant tuberculosis, transmission, early transmission impact, tuberculosis treatment

Drug-resistant tuberculosis represents a major threat to global tuberculosis elimination efforts [1]. It is now recognized that the drug-resistant tuberculosis epidemic is driven by ongoing transmission of unsuspected or ineffectively treated drug-resistant tuberculosis in settings with high tuberculosis incidence [2, 3]. Multidrug-resistant (MDR) tuberculosis is diagnosed in <25% of infected persons, and <20% are started on treatment [1]. Until recently, even those patients with drug-resistant tuberculosis who are started on treatment have had poor outcomes, with average treatment success rates of 50% for MDR and 20% for extensively drug-resistant (XDR) tuberculosis [1]. Evaluations of the cascade of tuberculosis care in India and South Africa revealed that losses throughout the cascade were substantially higher for rifampicin-resistant tuberculosis compared with drug-susceptible tuberculosis, with only 14% and 22% of patients successfully completing treatment, respectively [4, 5]. Improved care delivery of prompt and effective drug-resistant tuberculosis treatment is the most important intervention to decrease transmission of drug-resistant tuberculosis [6].

Establishing the duration of treatment needed to stop person-to-person spread of drug-resistant tuberculosis is critical to guide isolation decisions and ambulatory management. Although a decrease in cough and sputum smear conversion are good signs of a favorable clinical response, patients on effective treatment for drug-resistant tuberculosis often remain sputum smear and culture positive for ≥1 month [7]. Using the human-guinea pig (H-GP) transmission model in the 1960s, Riley et al [8] showed that patients with drug-susceptible tuberculosis who were started on treatment when they were admitted to the experimental ward were 95% less infectious than untreated patients.

Our prior study in South Africa using the H-GP model showed similar findings, retrospectively, for MDR tuberculosis when patients were started on effective treatment [9]. In the absence of XDR tuberculosis, a cohort of 27 patients with MDR tuberculosis newly started on standard South African MDR tuberculosis treatment (before the advent of new and repurposed drugs for drug-resistant tuberculosis) infected only 1% of the exposed guinea pigs [9]. This has been the basis for the active case finding, testing and prompt effective treatment transmission control strategy known as FAST (Find cases Actively, Separate safely, and Treat effectively) [10]. Similarly, in a Russian hospital setting, after MDR tuberculosis was promptly identified by molecular testing and effectively treated through implementation of FAST, there was a 78% odds reduction in the risk of developing MDR tuberculosis in patients being treated for tuberculosis [11].

In 2012, after >50 years without dedicated new drug-resistant tuberculosis drugs, phase 2b trial data demonstrated a decrease in the time to culture conversion when bedaquiline was added to standard treatment for MDR tuberculosis [12]. Bedaquiline was endorsed by the World Health Organization (WHO) [13] and approved by the Food and Drug Administration in the United States [14], followed by delamanid in 2014 [15]. Observational cohort data has since demonstrated the relative safety and efficacy of bedaquiline-containing regimens for drug-resistant tuberculosis compared with standard therapy [16].

This resulted in a change in the South African guidelines in 2017, which recommended that bedaquiline and linezolid, an antibiotic repurposed for the treatment of drug-resistant tuberculosis, should be used in all patients with a diagnosis of MDR tuberculosis [17, 18]. Toward the end of 2018, the WHO also updated their guidelines such that bedaquiline and linezolid are now recommended as part of first-line therapy for patients with this diagnosis [19, 20]. Results from the NiX-TB study in South Africa demonstrated durable cure at 6 months in 89% of patients with XDR tuberculosis, using the all-oral BPaL regimen: bedaquiline, high-dose linezolid (1200 mg daily) and pretomanid (a nitroimidazole, in the same structural class as delamanid) for 6 months [21].

The potential impact of the use of new and repurposed drugs for drug-resistant tuberculosis on transmission has not yet been established. Among patients admitted to a South African drug-resistant tuberculosis referral center for retreatment, we sought to evaluate the very early effects (after 72 hours) of treatment on baseline transmission of two new drug-resistant tuberculosis regimens using the H-GP transmission model. Specifically, we sought to compare the early transmission impact of (1) adding bedaquiline and linezolid to an optimized treatment regimen (based on South African national drug-resistant tuberculosis guidelines) and (2) starting the 3 drug all-oral NiX-TB regimen (without other drugs) in patients with pre-XDR or XDR tuberculosis in South Africa.

METHODS

Airborne Infections Research Facility and Study Overview

This study was performed at the Airborne Infections Research (AIR) facility in eMalahleni, South Africa [22]. The AIR facility consists of a 6-bed inpatient unit, into which high-efficiency particulate air filter–treated air enters at a rate of 12 air changes per hour and is continuously entirely delivered through ventilation ductwork to 2 guinea pig exposure rooms. Animals are maintained under biosafety level 3 conditions at an ambient temperature of 22°C ± 1°C, relative humidity of 50% ± 10% and day/night cycle of 12 hours (these parameters are

tightly controlled in the guinea pig holding rooms and were consistent between experiments 1 and 2). For these experiments, baseline infectivity of the enrolled patients with drug-resistant tuberculosis was measured by exhausting ward air to one of two guinea pig rooms (control group) for an average of 8 patient-days.

In experiment 1, patients were started on an optimized drug-resistant tuberculosis regimen including bedaquiline and linezolid, which reflected the South African national guidelines at that time. In experiment 2, patients were started on the BPaL regimen, consisting of bedaquiline, high-dose linezolid, and pretomanid, which had recently been studied in the NiX-TB trial [21]. After assessment of baseline infectivity, during the initial 72 hours of treatment no ward air was exhausted to either animal room. This was to enable a period for the drugs to achieve a relative steady state, knowing that bedaquiline requires a prolonged loading period. After 72 hours, ward air was exhausted to the second guinea pig room (intervention group) for an average of 8 patient-days. On days when a given guinea pig room did not receive ward air, it received a separate supply of high-efficiency particulate air–filtered, conditioned air that bypassed the patient ward.

Patient Enrollment and Selection

Participants were sequentially selected from patients newly admitted to the adjacent hospital for drug-resistant tuberculosis treatment initiation between 22 June and 5 August 2016 for experiment 1 and between 16 April and 31 July 2018 for experiment 2. Both experiments took place during the dry season (fall/winter). For experiment 1, the participants screened were candidates for treatment with bedaquiline and linezolid (based on the South African MDR treatment guidelines at the time) due to clinical treatment failure with standard MDR tuberculosis treatment regimens. The target study population for both experiments was patients with confirmed or suspected pre-XDR or XDR tuberculosis. Other inclusion criteria for both experiments included age >18 years and being ambulatory and willing to stay at the AIR facility. Patients who were acutely unwell were excluded from both experiments. All participants provided written informed consent.

Guinea Pigs and Tuberculin Skin Testing

Outbred male and female, specific pathogen-free, Dunkin-Hartley guinea pigs (National Health Laboratories Services, South Africa) were used to evaluate patient infectiousness. On receipt from the breeder, animals were randomized to one of the animal rooms and to a specific cage number. Before exposure to air from the ward, animals underwent baseline tuberculin skin testing (TST), as described elsewhere [9, 22] and then again 1 month after the exposure ended. TST indurations ≥6 mm were considered to be positive (indicative of infection). TST readings were performed by 2 independent readers who were blinded to the intervention. Twenty additional guinea pigs were used as positive (BCG-vaccinated) and negative (uninfected, unexposed) controls to validate responses to the skin testing reagent (purified protein derivative).

Sample Size Calculation

Based on historical baseline infection rates [22], with 90 guinea pigs in each exposure room (control and intervention groups), the study was powered to detect a 50% reduction in transmission. Unlike in most clinical trials, our statistical assessment of treatment effectiveness

on transmission is based not on human subject numbers but on guinea pig numbers, just as for environmental interventions [23] or patient masking [24].

Human Subjects and Animal Ethics Approval

This study was approved by the human studies committees of the South African Medical Research Council, the US Centers for Disease Control and Prevention (CDC), and the Brigham and Women's Hospital. Animal care and husbandry was overseen by a licensed laboratory veterinarian, and all protocols were approved by the Animal Use committees of the South African Medical Research Council, the CDC, and Harvard Medical School. For experiment 2, due to use of the BPaL regimen before its being routinely recommended, a drug safety and monitoring board was appointed, and patients were reviewed after being on treatment for 5 days.

RESULTS

For experiment 1, 5 patients (3 female and 2 male) were admitted to the AIR facility. Their median age was 43 years, and all patients had human immunodeficiency virus (HIV). For experiment 2, 9 patients (7 female and 2 male; 2 groups within this cohort) were admitted to the AIR facility. Their median age was 34.4 years, and 5 patients had HIV. All patients in experiment 1 received a regimen that included levofloxacin, 1 g daily, and pyrazinamide, 1.5 g daily, in addition to bedaquiline, 400 mg daily for 2 weeks (loading period) followed by 200 mg 3 times weekly, and linezolid, 600 mg daily (aside from patient 1, who received linezolid 300 mg daily). The other drugs in the regimen were chosen based on genotypic drug susceptibility testing (DST) using molecular testing (Xpert or line probe assay) and determined by their treating clinicians outside of this study. All patients in experiment 2 received the NiX regimen: bedaquiline, 400 mg daily for 2 weeks (loading period) followed by 200 mg 3 times weekly; high-dose linezolid at 1200 mg daily; and pretomanid at 200 mg daily. Table 1 provides details of clinical characteristics and diagnostic test results for both cohorts.

Patient Time Contributed to Study for Assessment of Infectiousness

We calculated the number of hours spent by each study participant in the AIR facility and converted this into patient-days (data displayed in Table 2). Patients were required to be inside the facility for a minimum of 20 hours each day (and were typically there for close to 24 hours). In experiment 1, patient 2 was removed from study after 2.76 patient-days and did not return due to acute tuberculosis-related illness, resulting in death. The 5 patients in experiment 1 contributed a total of 39.89 days before the initiation of the drug regimens indicated in Table 1. Patients were then transferred from the AIR facility to the drug-resistant tuberculosis hospital next door and their regimens were given for 72 hours before their return to the AIR facility, where their treatment continued. Time spent in the facility after treatment initiation was calculated to equal the time before drug exposure for the experiment 1 cohort in the AIR facility. The 4 remaining patients contributed an average of 40.02 days after treatment initiation. On average, each patient in experiment 1 spent 7.91 patient-days before and 8 patient-days after treatment inside the AIR facility.

Table 1. Baseline Clinical Characteristics of Study Populations in Experiments 1 and 2

Patient	Age, y/Sex	HIV Status; CD4 Cell Count, Cells/μL	Symptoms	Smear Result	Chest Radiographic Findings	DST[a]	Clinical Context and Interpretation	Tuberculosis Treatment Regimen (medication doses are daily unless otherwise specified)
Experiment 1								
1	26/F	Positive; 22	Cough (rare but strong), fever, weight loss, appetite loss, chest pain	Scanty	Perihilar infiltrates	Rifampin resistant (Xpert); FQ testing not performed	MDR tuberculosis, retreatment after noncompletion of prior treatment	Pyrazinamide 1.5 g; levofloxacin 1 g; terizidone 500 mg; clofazimine 100 mg; linezolid 300 mg; bedaquiline 400 mg/d for 2 wk then 200 mg 3×/wk
2	29/M	Positive; 16	Cough (strong), fever, weight loss, appetite loss	3+	Diffuse bilateral infiltrate; left upper-lobe fibrosis with cavity	Rifampin resistant (Xpert and rpoBmut3 on MTBDRplus); isoniazid resistant (katGmut1; inhAmut1 on MTBDRplus) kanamycin and ofloxacin resistant	XDR tuberculosis	Pyrazinamide 1.5 g; levofloxacin 1 g; terizidone 500 mg; ethonamide 500 mg; isoniazid 900 mg 3×/wk; capreomycin 500 mg; linezolid 600 mg; bedaquiline 400 mg/d for 2 wk then 200 mg 3×/wk
3	51/F	Positive; 168	Cough (rare, weak), weight loss, appetite loss	2+	Consolidation and cavity in left upper lobe	Rifampin resistant (Xpert, and rpoBmut1 on MTBDRplus); isoniazid resistant (katG, katGWT, katGmut1, inhA, inhAWT2 on MTBDRplus); kanamycin and ofloxacin resistant	MDR tuberculosis, not on ART for HIV	Pyrazinamide 1.5 g; levofloxacin 1 g; terizidone 500 mg; linezolid 600 mg; ethambutol 800 mg; linezolid 600 mg; bedaquiline 400 mg/d for 2 wk then 200 mg 3×/wk
4	51/F	Positive; 198	Cough (rare but strong), fever, weight loss, appetite loss, fatigue	1+	Bilateral patchy consolidation and cavity in left upper lobe	Rifampin resistant (rpoBmut1 and rpoBmut3 on MTBDRplus); isoniazid resistant (katGmut1, inhA, inhAWT2 on MTBDRplus); kanamycin sensitive; ofloxacin resistant	MDR tuberculosis (history of prior treatment)	Pyrazinamide 1.5 g; levofloxacin 1 g; clofazimine 100 mg; terizidone 500 mg; linezolid 600 mg; bedaquiline 400 mg/d for 2 wk then 200 mg 3×/wk
5	58/M	Positive; 274	Cough (frequent, strong), fever, weight loss, appetite loss, fatigue	1+	Bilateral miliary infiltrates (right greater than left)	Rifampin resistant (rpoBmut3 on MTBDRplus); isoniazid and kanamycin sensitive; ofloxacin resistant	Pre-XDR tuberculosis	Pyrazinamide 1.5 g; terizidone 500 mg; levofloxacin 1 g; clofazimine 100 mg; ethonamide 500 mg; capreomycin 750 mg; linezolid 600 mg; bedaquiline 400 mg/d for 2 wk then 200 mg 3×/wk
Experiment 2								
1	26/M	Negative	Cough, night sweats, weight loss, appetite loss, fatigue	Negative	Interstitial infiltrates bilaterally	Rifampin resistant (MTBDRplus); isoniazid result invalid; FQ testing not performed	RR tuberculosis (new diagnosis)	Pretomanid 200 mg; linezolid 1200 mg; bedaquiline 400 mg/d for 2 wk then 200 mg 3×/wk
2	57/F	Positive; 255	Cough (strong), night sweats, weight loss, fatigue	1+	Right upper lobe fibrosis + cavitations, volume loss in right lung, compensatory hyperinflation	Clinical concern for MDR tuberculosis and history of hearing loss prompting use of BPaL	MDR tuberculosis (prior treatment 2 y before study)	Pretomanid 200 mg; linezolid 1200 mg; bedaquiline 400 mg/d for 2 wk then 200 mg 3×/wk

Table 1. Continued

Patient	Age, y/Sex	HIV Status; CD4 Cell Count, Cells/μL	Symptoms	Smear Result	Chest Radiographic Findings	DST[a]	Clinical Context and Interpretation	Tuberculosis Treatment Regimen (medication doses are daily unless otherwise specified)
3	33/F	Positive; 135	Weight loss	Negative	Right-sided pleural effusion, bilateral infiltrates	Rifampin resistant (MTBDRplus); isoniazid, kanamycin, and ofloxacin sensitive	RR tuberculosis (new diagnosis, known hearing loss)	Pretomanid 200 mg; linezolid 1200 mg; bedaquiline 400 mg/d for 2 wk then 200 mg 3×/wk
4	34/F	Positive; 101	Cough (moderate)	3+++	Left upper lobe cavitation; bilateral diffuse alveolar infiltrates	Rifampin resistant (MTBDRplus); isoniazid resistant (katG, inhA on MTBDRplus); SLID resistant; FQ sensitive (MTBDRsl)	Pre-XDR tuberculosis	Pretomani 200 mg; linezolid 1200 mg; bedaquiline 400 mg/d for 2 wk then 200 mg 3×/wk
5	23/M	Positive; 387	Cough (moderate), weight loss, appetite loss	3+++	Bilateral micronodular infiltrates	Rifampin resistant (Xpert and MTBDRplus); isoniazid resistant (MTBDRplus); SLID sensitive; FQ resistant	Pre-XDR tuberculosis	Pretomanid 200 mg; linezolid 1200 mg; bedaquiline 400 mg/d for 2 wk then 200 mg 3×/wk
6	38/F	Positive; 155	Cough (moderate), fever, weight loss Appetite loss, chest pain	2++	Bilateral alveolar infiltrates	Rifampin resistant (Xpert and MTBDRplus); isoniazid resistant (mixed strains; MTBDRplus); SLID sensitive; FQ resistant	XDR tuberculosis	Pretomanid 200 mg; linezolid 1200 mg; bedaquiline 400 mg/d for 2 wk then 200 mg 3×/wk
7	43/F	Negative	Cough (moderate), night sweats Weight loss, appetite loss	3+++	Destroyed left lung, compensatory hyperinflation in right lung	Rifampin resistant (Xpert and MTBDRplus); isoniazid resistant (MTBDRplus); no FQ testing	MDR tuberculosis (known hearing loss)	Pretomanid 200 mg; linezolid 1200 mg; bedaquiline 400 mg/d for 2 wk then 200 mg 3×/wk
8	20/F	Negative	Cough (moderate), fever, weight loss, appetite loss, fatigue	3+++	Right upper lobe cavitations, multilobar infiltrates	Rifampin resistant (MTBDRplus); isoniazid resistant (MTBDRplus); SLID sensitive; FQ sensitive (MTBDRsl)	MDR tuberculosis (on treatment, considered treatment failure)	Pretomanid 200 mg; linezolid 1200 mg; bedaquiline 400 mg/d for 2 wk then 200 mg 3×/wk
9	36/M	Negative	Cough (moderate), fever, weight loss, appetite loss, fatigue	3+	Perihilar infiltrates, hyperinflation	Rifampin resistant (Xpert)	Pre-XDR tuberculosis	Pretomanid 200 mg; linezolid 1200 mg; bedaquiline 400 mg/d for 2 wk then 200 mg 3×/wk

Abbreviations: ART, antiretroviral therapy; BPaL, bedaquiline, 1200-mg linezolid, and pretomanid; DST, drug susceptibility testing; FQ, fluoroquinolone; HIV, human immunodeficiency virus; MDR, multidrug-resistant; RR, rifampin-resistant; SLID, second line injectable drug; XDR, extensively drug-resistant.

[a]DST reflects the programmatic standard of care, which typically represents a combination of Xpert and line probe assay (MTBDRplus for first-line and MDRsl for second-line drugs) and occasionally also phenotypic testing (the latter used more commonly during the time when experiment 1 was performed). As part of routine practice, different laboratories performed genotypic and/or phenotypic DST and typically did not report specific mutations identified.

Table 2. Patient-Days in Airborne Infections Research Facility During Experiments 1 and Experiment 2

Before Drug Exposure		After Drug Exposure[a]	
Patient No.	Duration in AIR Facility, Patient-Days	Patient No.	Duration in AIR Facility, Patient-Days
Experiment 1			
1	13.90	01	11.32
2	2.76	02	0.00
3	8.66	03	11.32
4	8.66	04	11.32
5	5.91	05	6.05
Total duration	39.89	…	40.02
Mean duration	7.91	…	8.00
Experiment 2			
1	5.75	01	5.72
2	5.74	02	5.72
3	4.78	03	5.07
4	2.80	04	3.97
5	5.64	05	3.97
6	3.78	06	3.71
7	5.92	07	4.75
8	3.68	08	4.75
9	0.74	09	0.73
Total duration	38.82	…	38.41
Mean duration	4.31	…	4.26

Abbreviation: AIR, Airborne Infections Research.

[a]Starting 72 hours after drug initiation.

In experiment 2, we similarly calculated the number of preexposure patient-days for the cohort of patients and ensured that the postexposure duration was equivalent. The cohort of patients spent a similar time in the facility before treatment commenced and beginning 72 hours after treatment initiation, contributing a total of 38.82 and 38.41 patient-days respectively. On average each patient in experiment 2 spent 4.31 patient-days before and 4.26 patient-days after treatment inside the AIR facility.

Guinea Pig Infections in the Intervention Compared With the Control Group

In experiment 1 (Figure 1), before treatment, 5 patients with drug-resistant tuberculosis infected 24 of 90 guinea pigs (26.7%) (control group). After treatment (beginning 72 hours after drug initiation), the same patients (excluding 1 who was withdrawn) infected 25 of 90 guinea pigs (27.8%) (intervention group) ($P > .99$). In experiment 2 (Figure 1), before treatment, 9 patients with drug-resistant tuberculosis infected 40 of 90 guinea pigs (44.4%)

(control group). After treatment (beginning 72 hours after drug initiation), the same patients infected 0 of 90 guinea pigs (0%) (intervention group) ($P < .0001$).

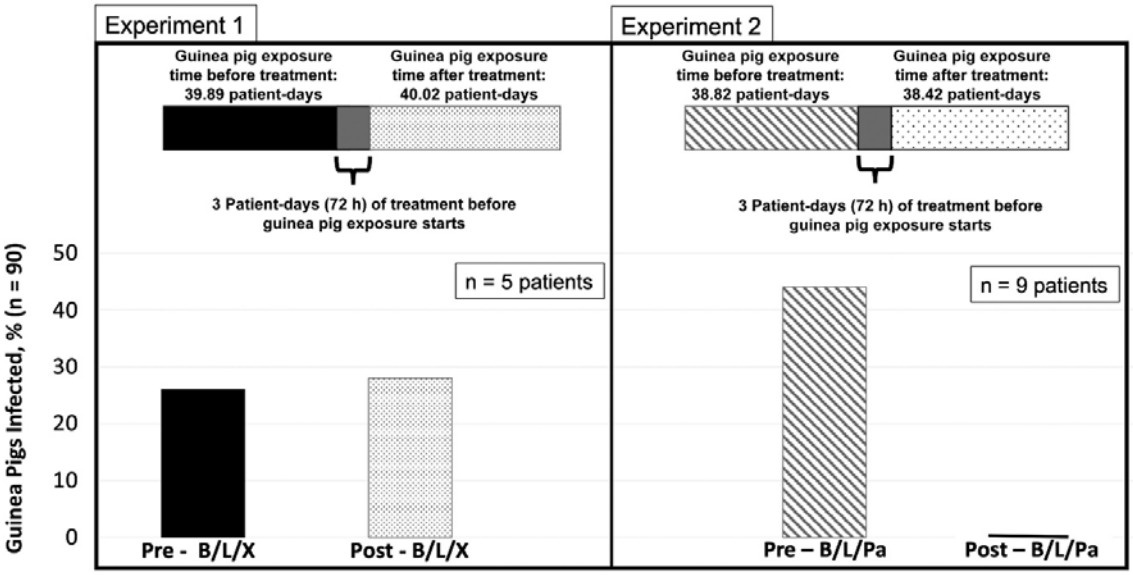


Figure 1. Proportion of guinea pigs with tuberculosis infection before and after treatment regimens for experiments 1 and 2. Abbreviations: B, bedaquiline (200 mg thrice weekly, after 2-week load); L, linezolid (1200 mg/d); Pa, pretomanid (200 mg/d); X, regimen including pyrazinamide (1.5 g/d), levofloxacin (1 g/d), terizidone (500 mg/d), clofazimine (100 mg/d), ethionamide (500 mg/d), isoniazid (900 mg thrice weekly), and capreomycin (500 mg/d).

DISCUSSION

In the current study, using the H-GP model to evaluate transmission, South African drug-resistant tuberculosis drug regimens that included bedaquiline and standard-dose linezolid did not decrease drug-resistant tuberculosis transmission to guinea pigs between 72 hours and 11 days after treatment initiation. In contrast, transmission was rapidly and completely inhibited in patients treated with the BPaL regimen for 72 hours, suggesting an early and profound impact on transmission. Tuberculosis drug development efforts have hitherto relied heavily on the measure known as early bactericidal activity (EBA), which is intended to estimate potential for efficacy and is based on short studies of relatively few patients started on either single agents or drug regimens for a few days or up to 2 weeks [25].

We propose the introduction of a new concept: early transmission inhibition (ETI), which focuses on the ability of new drugs or regimens to inhibit transmission within days of initiation. While EBA may influence ETI, the impact of treatment on transmission has long been understood to occur soon after effective drugs are started, that is, before sputum culture conversion [8, 9]. The exact mechanisms by which effective drugs can affect transmission well before sputum culture conversion are unclear, but this effect on transmission is implicitly acknowledged in practice by the commonly used "2-week rule," whereby patients with drug-susceptible tuberculosis who are responding to therapy are considered noninfectious after 2 weeks [26], even though sputum culture conversion commonly takes ≥1 month [27].

The present studies are the first to prospectively measure ETI of specific regimens and to report major differences between regimens. Developing a standardized model that could be used to

evaluate the ETI of new tuberculosis drugs and regimens found to be effective for tuberculosis treatment could affect practice by informing global guidelines and national tuberculosis programs regarding the duration of infectiousness, the need for isolation, and the need for hospital versus ambulatory treatment.

Retrospective programmatic data from a cohort of 1016 patients with drug-resistant tuberculosis in South Africa demonstrated that regimens including bedaquiline are associated with improved drug-resistant tuberculosis treatment outcomes, including reduced all-cause mortality rates [28]. Studies evaluating linezolid-containing drug-resistant tuberculosis regimens have also demonstrated improved outcomes, safety, and tolerability [29–31]. Our study did not demonstrate a corresponding decrease in ETI with regimens containing standard doses of bedaquiline and linezolid provided under programmatic conditions, in contrast to our prior study using the H-GP model, which demonstrated minimal transmission from patients newly started on older effective MDR tuberculosis regimens [9]. However, we acknowledge several limitations that mean these findings do not provide conclusive evidence of differences in ETI between the 2 bedaquiline- and linezolid-containing regimens received by study patients.

We note that our two experiments were not planned to follow similar protocols. The first looked at the addition of bedaquiline and linezolid as routinely used in South Africa, that is, not following a standardized protocol. The second experiment was specifically designed to test the BPaL regimen and used a recently completed clinical trial protocol. The majority of patients in experiment 2 were treatment naive, compared with experiment 1, in which patients were more likely to be considered to have treatment failure, which may have contributed to the lack of ETI seen for the treatment regimens evaluated. We relied on programmatic DST, which was inconsistently applied to patients (Table 1), and we note that high rates of fluoroquinolone resistance among patients in experiment 1 likely reduced the effectiveness of the regimen. DST was not performed for bedaquiline or linezolid, and thus undetected resistance to bedaquiline [32] and/or linezolid [33] could have decreased the regimen's effectiveness in experiment 1.

An important limitation of the H-GP model as used here is that this only enables only assessment of the infectiousness of a cohort of patients rather than assessment of individual patients. This means that experiments are subject to bias due to the inherent variability in individual patient infectiousness, both before and after treatment. Since we were unable to keep infected guinea pigs until they became sick to enable reliable recovery of *Mycobacterium tuberculosis* from TST-positive animals, with the goal of using DST or whole-genome sequencing to identify which individual patients led to disease in a given guinea pig, we do not know in any given study whether transmission was due to 1, 2, or more patients. It is possible that ≥1 patient in the experiment 1 cohort represented a greater infectious risk than those in experiment 2, for example, due to disease severity or presence of laryngeal or endobronchial tuberculosis, which could potentially have accounted for ongoing infections in its intervention guinea pig group. However, we examined all available chest radiographs and laboratory and clinical data and concluded that the patients appeared to have similar disease severity, based principally on smear status and the extent of infiltrates, including cavitation.

These studies were not designed to compare regimens; rather, by using participants as their own controls before and after treatment, it is reasonable to assume that the same patients infected guinea pigs before and after treatment, enabling assessment of ETI for a given regimen in a cohort. Furthermore, detecting a prompt impact of effective therapy on transmission using the H-GP model is consistent with the findings of Riley et al [8] for drug-susceptible

tuberculosis and Dharmadhikari et al for drug-resistant tuberculosis [9, 22] in studies that included more patients.

While these studies were not set up to evaluate the impact of individual drugs in each regimen, the results suggest that a higher dose of linezolid and/or the use of pretomanid may play a critical role for the regimen to rapidly decrease transmission. Although there is a lack of studies evaluating the relationship between EBA and ETI, understanding the differential EBA of drug-resistant tuberculosis drugs may help in understanding their contribution to ETI. A prior study demonstrated that linezolid has modest EBA, which is focused within the first 2 days of treatment [34]. More recently, data from South Africa show that the EBA of linezolid increases with increasing dose [35], which may support our hypothesis that the dose of linezolid may be critical for both EBA and ETI. A 14-day EBA study of pretomanid demonstrated rapid EBA [12]. The EBA of pretomanid has been shown in a phase 2 trial evaluating the combination regimen of moxifloxacin, pretomanid, and pyrazinamide, which that showed superior bactericidal activity compared with first-line therapy (rifampin, isoniazid, pyrazinamide, and ethambutol) for drug-susceptible tuberculosis and showed similar results in patients with drug-resistant tuberculosis [36]. In contrast, the bactericidal activity of bedaquiline has a delayed onset, with the decline in *M. tuberculosis* colony-forming units not beginning until after 4 days [37].

Transcriptomic studies suggest that antimycobacterial therapy leads to rapid transcriptional responses that may affect infectiousness before smear or culture conversion. Shaikh et al [38] evaluated changes in aerosolized *M. tuberculosis* isolates exhaled by patients before and after treatment initiation (up to 14 days) using RNA sequencing. A distinct change in the overall transcriptional profile, compared with pretreatment, occurred 1 and 3 days after treatment initiation (and continued for 14 days), but only when patients received effective treatment [38]. These included down-regulation of genes associated with cellular activities, cell wall assembly, virulence factors indicating loss of pathogenicity, and a diminished ability to infect and survive in new host cells. These data support past observations demonstrating a decline in the infectiousness of patients with tuberculosis almost immediately after the start of effective treatment, our previous observations on effective MDR treatment [9], and our new observations on the BPaL regimen.

Walter et al [39] evaluated the independent effects of specific drugs and showed that sterilizing drugs like bedaquiline and pyrazinamide and regimens including these drugs have a potent suppressive effect on precursor *M. tuberculosis* ribosomal RNA synthesis within days, in contrast to nonsterilizing drugs or weaker regimens. In this study, the bedaquiline, pretomanid, moxifloxacin, and pyrazinamide (BPaMZ) regimen with the highest sterilizing activity, had the greatest suppression of the RNA synthesis ratio, followed by BPaL, then pretomanid, moxifloxacin, and pyrazinamide (PaMZ) and the first-line rifampin, isoniazid, pyrazinamide, and ethambutol regimen [39]. This may help explain the effect of the BPaL regimen on ETI compared with the other regimens that included bedaquiline and linezolid, since the lower dose of linezolid and lack of pretomanid may delay ETI without necessarily affecting treatment success.

The ZeNix trial evaluating the safety and efficacy of different doses and treatment durations for linezolid (given high rates of peripheral neuropathy and myelosuppression due to the 1200-mg dose), in combination with bedaquiline and pretomanid, demonstrated that using a 600-mg daily dose of linezolid led to similar successful outcomes [40], and this is now recommended by the WHO and CDC. We argue that our approach using the H-GP model to study ETI

combined with transcriptomic data could be obtained in parallel to efficacy trials. This would open up avenues for evaluating the early impact of new treatment regimens on tuberculosis transmission, with implications both for the safety of ambulatory treatment and for the potential impact of new regimens on the epidemic.

In conclusion, these studies provide important preliminary (rather than conclusive) data suggesting different regimens that include bedaquiline and linezolid have differing effects on ETI. We propose that the measurement of ETI is increasingly relevant as new tuberculosis treatment regimens, including treatment-shortening trials for drug-susceptible and drug-resistant tuberculosis, are evaluated and scaled up for wider use. These findings are also important within the current context of efforts to promote high-quality, person-centered care that prioritizes increased access to effective therapy earlier in a patient's disease course, with resultant implications for transmission in facilities and communities.

Acknowledgments. We express our sincere gratitude to the patients who participated in this study.

Disclaimer. The funders had no role in study design, data collection, data analysis, data interpretation, writing of the report, or in the decision to submit for publication. The content is solely the responsibility of the authors and does not necessarily represent the views of the funders.

Financial support. This work was supported by the National Institutes of Health (grant 5R01AI099603–05; principal investigator: A. S.) and the National Institute of Allergy and Infectious Diseases (grant K23 AI132648-05 to R. R. N.).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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