Fourteen-day PET/CT imaging to monitor drug combination activity in treated individuals with tuberculosis

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Early bactericidal activity studies monitor daily sputum bacterial counts in individuals with tuberculosis (TB) for 14 days during experimental drug treatment. The rate of change in sputum bacterial load over time provides an informative, but imperfect, estimate of drug activity and is considered a critical step in development of new TB drugs. In this clinical study, 160 participants with TB received isoniazid, pyrazinamide, or rifampicin, components of first-line chemotherapy, and moxifloxacin individually and in combination. In addition to standard bacterial enumeration in sputum, participants underwent 2-deoxy-2-[18F]fluoro-D-glucose positron emission tomography and computerized tomography ([18F]FDG-PET/CT) at the beginning and end of the 14-day drug treatment. Quantitating radiological responses to drug treatment provided comparative single and combination drug activity measures across lung lesion types that correlated more closely with established clinical outcomes when combined with sputum enumeration compared to sputum enumeration alone. Rifampicin and rifampicin-containing drug combinations were most effective in reducing both lung lesion volume measured by CT imaging and lesion-associated inflammation measured by PET imaging. Moxifloxacin was not superior to rifampicin in any measure by PET/CT imaging, consistent with its performance in recent phase 3 clinical trials. PET/CT imaging revealed synergy between isoniazid and pyrazinamide and demonstrated that the activity of pyrazinamide was limited to lung lesion, showing the highest FDG uptake during the first 2 weeks of drug treatment. [18F]FDG-PET/CT imaging may be useful for measuring the activity of single drugs and drug combinations during evaluation of potential new TB drug regimens before phase 3 trials.

INTRODUCTION

New drugs and improved drug regimens for treating tuberculosis (TB) are urgently needed to combat emerging resistance, decrease mortality and morbidity rates, and potentially shorten therapy (1, 2). The current 6-month, four-drug regimen was defined in a pivotal series of clinical trials by the British Medical Research Council (BMRC) from 1946 to 1976 (3). These trials relied on disease relapse as their primary endpoint, recognizing that patients had to be treated for months after their sputum culture conversion to avoid relapsing with active disease. The standard of care in the 1950s was 18 months of treatment with isoniazid and thiacetazone with daily streptomycin injections for the first 2 months. The first breakthrough in treatment duration occurred when rifampicin or pyrazinamide was added to streptomycin and isoniazid to achieve relapse rates of 3% and 8%, respectively, with only 6 months of therapy (4). Through a series of additional trials, 6-month therapy using both rifampicin and pyrazinamide became established as the standard treatment duration for drug-sensitive TB, with relapse rates around 1%. Regimens that shortened therapy to less than 6 months, even those that combined rifampicin and pyrazinamide, had increasing rates of relapse (5).

Since the BMRC trials more than 40 years ago, this 6-month first-line regimen has not changed in composition or duration, despite the advent of new drugs and resurgence in the global burden of TB. To accelerate identification of clinically effective drug candidates, better methodologies are needed to determine the best combinations of drugs to take forward into lengthy and resource-intensive clinical trials of durable cure. In TB drug development, investigators attempt to monitor bacterial counts quantitatively in the sputum of TB patients as an early indication of drug activity (6) and triage which regimens should proceed to expensive phase 3 trials. The pioneering work of Jindani et al. (7) applied this approach to new combinations of drugs and formalized the methodology called the early bactericidal activity (EBA) study. This methodology has become part of the U.S. Food and Drug Administration official guidance documents for development of new TB drugs (8). Despite the pivotal role of
EBA trials in new drug development, this methodology has limitations. Important sterilizing drugs marked by capacity to shorten therapy and prevent relapse like rifampicin and pyrazinamide have only small 2-day EBA effects, whereas less sterilizing agents like isoniazid consistently perform very well. The only agents to date that have shown 2-day EBA on par with that of isoniazid were the fluoroquinolones, particularly moxifloxacin (9–11). Data from mouse models suggesting strong sterilizing potential (12–14) and some observations of 8-week culture conversion rates in humans (15–17) formed the evidence for launching three phase 3 trials based on using a fluoroquinolone to shorten the duration of TB chemotherapy for drug-sensitive disease to 4 months (18–20). These trials all failed to show noninferiority of 4-month fluoroquinolone regimens to the 6-month standard-of-care regimen of rifampicin, isoniazid, pyrazinamide, and ethambutol based on disease relapse. The inherent limitation in examining only sputum bacterial clearance is that this measure reflects only disease in the airways, not the parenchymal nodules, infected lymph nodes, and other pulmonary disease pathologies characteristic of adult pulmonary TB.

We previously reported 2-deoxy-2-[18F]fluoro-D-glucose (FDG)-posiotron emission tomography (PET)/computed tomography (CT) changes in two cohorts of patients: one small cohort with multidrug-resistant disease imaged at baseline and after 2 months of treatment (21), and one larger cohort in drug-susceptible patients imaged at baseline, 1 month, and end of treatment (22). In both cohorts, we saw large changes in radiologic features, particularly in hard [density from −100 to +200 Hounsfield units (HU)] and total (density from −500 to +200 HU) disease volume on CT and total glycolytic activity (TGA; mean standard uptake values of FDG × total volume) on PET, with successful treatment. In non-human primates experimentally infected with Mycobacterium tuberculosis (Mtba), we distinguished the 6-month sterilizing regimen of isoniazid, rifampicin, pyrazinamide, and ethambutol from a regimen that required 18 months of isoniazid and streptomycin in as little as 2 weeks using FDG-PET/CT changes (23). We therefore postulated that we should be able to see relevant changes in FDG-PET/CT features within a 14-day EBA study that would reveal more about the lesion-specific activity of individual drugs that have been clinically well characterized in TB patients. We conducted an EBA study with the addition of FDG-PET/CT scans (“NexGen EBA”; NCT02371681) before and after 14 days of treatment among 160 pulmonary TB patients randomized across eight arms: four monotherapy arms using isoniazid, rifampicin, pyrazinamide, and moxifloxacin; two dual-agent combination arms: pyrazinamide-isoniazid and pyrazinamide-rifampicin; and two four-drug arms: isoniazid-rifampicin-pyrazinamide-ethambutol and moxifloxacin-rifampicin-pyrazinamide-ethambutol.

**RESULTS**

**Study population and baseline characteristics of participants are comparable across arms**

From December 2015 to September 2017, 178 eligible participants with HIV-negative sputum smear-positive pulmonary TB were enrolled at TASK Applied Science in the Western Cape region of South Africa. Eighteen of the 178 participants were withdrawn from the study (detailed in Fig. 1). The remaining 160 participants completed the study and were included in the overall study analysis. At baseline, there were no differences by study arm in age, sex, body mass index, and sputum bacterial burden as approximated by Xpert MTB/RIF cycle threshold, the number of Mtb colony-forming units (CFU) on culture, or the PET/CT disease burden [analysis of variance (ANOVA), \( P = 0.34 \) to 0.9; table S1]. Total hard lesion volume and cavity airspace volume on CT were comparable by study arm, with large interindividual variability.

**Microbiologic results of EBA**

EBA by sputum CFU enumeration on agar plates (Fig. 2A) and time to positivity (TTP) in broth culture, according to the mycobacterial growth indicator tube system (BACTEC MGIT 960), was highest for the moxifloxacin-rifampicin-pyrazinamide-ethambutol arm and lowest for the pyrazinamide arm (Fig. S1). Among the single-drug arms, moxifloxacin showed the most robust response. There were no differences between the EBA for rifampicin-pyrazinamide, isoniazid-rifampicin-pyrazinamide-ethambutol, isoniazid-pyrazinamide, and moxifloxacin arms or the EBA for the rifampicin and isoniazid arms (Fig. 2A). For the individual participants, large intra-arm variability in the estimated EBA was observed (Fig. 2B) and revealed the largest number of increases in bacterial load among participants...
In (A), the analysis by study arm was estimated using linear mixed-effects (LME) modeling from daily overnight samples plated onto solid growth media. The y axis is the estimated daily log₁₀ decrease in CFU for each arm, and the error bars represent 95% confidence intervals on the estimate by study arm. (B) Intraindividual variation in the estimated drug effect by arm, calculated as the slope of the line connecting log₁₀(CFU) at baseline and log₁₀(CFU) at day 13. Each participant has a unique color coding; the y axis is the log₁₀(CFU) of that participant at baseline. For those participants without day 13 data, we instead calculated the slope.

Analysis of FDG-PET/CT changes in individual TB lesions
We excluded 45 abnormalities (39 lesions and 6 cavitary air) in 34 participants from the analysis due to the following: lesion inactive with no FDG uptake and obvious calcification (n = 13), severe segmental or lobar collapse (n = 13), pleural lesion or effusion (n = 7), new lesion at day 14 that was completely absent from bronchopulmonary segment at baseline (n = 7), rationale explained in the next paragraph), severe reconstruction artifact (n = 4), and accidental duplication of lesion (n = 1) (table S2). Analyses of the PET/CT lesion measures by study arm including change in total lesion volume, TGA, and cavitary air are shown in Fig. 4, A and B, and fig. S3, respectively. Cavity air was the most variable of these measurements across 14 days of treatment, with six of eight arms achieving reductions in air volume of 25 to 45%. In the single-drug arms, rifampicin was the most active (compared to isoniazid, pyrazinamide, and moxifloxacin). All arms containing rifampicin showed large reductions in both lesion volume and inflammation (as measured by TGA). Moxifloxacin as a single agent also performed well. Isoniazid and pyrazinamide by themselves had the least effect, with pyrazinamide actually associated with an increase in mean lesion volume and inflammation over 14 days. Unexpectedly, isoniazid and pyrazinamide were synergistic when combined, with changes similar to rifampicin alone or the combination of isoniazid-rifampicin-pyrazinamide-ethambutol. The combination of rifampicin-pyrazinamide appeared slightly less active than rifampicin alone when analyzed by lesion, although this difference was not different statistically.

New or expanding lesions (≥1 ml) were identified 97 times in 58 participants at day 14. Most of these were progressions of existing lesions into previously uninvolved areas of a bronchopulmonary segment, but in a few cases, new lesions appeared in a previously uninvolved lung segment. These new or expanding lesions occurred in all arms but more frequently in the isoniazid and pyrazinamide arms (fig. S4). In 35 of these participants, there was an obvious direct

randomized to pyrazinamide, relative to the other arms. In six of eight arms, there were individual participants who had increasing sputum CFU over 14 days. This was most prominent in the pyrazinamide monotherapy arm, with 8 of 19 (42%) participants showing an increase.

Quantitative radiologic changes on FDG-PET/CT scans observed over 14 days
Among the 160 participants analyzed, 2 participants were excluded for the radiologic analysis: One had no intrapulmonary abnormalities on baseline PET/CT scan, and the other had FDG incorrectly administered (likely due to intravenous line infiltration) at the day 14 scan. The remaining 158 participants yielded 1122 total PET/CT features including “lesion” features, lung tissue with radiodensity from −500 to +200 HU (N = 802 lesions), and “cavitary air” features, radiodensity within pulmonary cavities from −1024 to −500 HU (N = 320). Lesions were defined as all abnormalities that occurred within a bronchopulmonary segment. If multiple adjacent segments were involved, abnormalities within those segments were considered one lesion. For example, in Fig. 3 (A and B), this participant had three features: one complex lesion involving all five segments of the left superior lobe (green), cavity airspaces within the apical region of that lesion (blue), and one lesion confined to the left inferior lobe segment S6 (yellow). As expected, abnormalities were predominantly located in the upper regions of the lungs, with more than half in either lung involving the apical bronchopulmonary segments S1 and S2 and 20 to 25% involving the apical segment of the lower lobe, S6 (fig. S2). Radiologic data were analyzed in two ways: first from the perspective of individual lesions that were either individual bronchopulmonary segments or combinations of adjacent segments that could not be separated computationally (Fig. 3, A and B) and second by changes within co-registered cubes of voxels as shown in Fig. 3C.
bronchial connection with an active cavity either directly superior to the new or expanding lesion, or in the opposite lung, consistent with direct bronchial spread of existing disease. Endobronchial spread has been well documented in the older pathology literature as a component of the natural history of pulmonary TB (24–29). Seven new lesions were excluded from this analysis (listed in table S2) because they were in a completely new segment, and thus had no baseline reference for comparison. The remainder were included with the lesion from which they expanded in the primary analysis.

Analysis of FDG-PET/CT changes by co-registered sublesion cubes reveals synergistic and antagonistic drug activity

The analysis by TB lesion was also complicated by the heterogeneity of lesions that often spanned multiple bronchopulmonary segments. Even when a lesion was within a single segment, there were often heterogeneous local changes within a lesion. To isolate drug effects on specific subtypes of abnormalities, we computationally divided the dataset into 145,447 cubes of approximately 1 cm³ from the original 802 non-cavity air lesions. We then aligned the baseline and day 14 cubes and calculated the properties of the voxels contained in these cubes at both visits (Fig. 3C and fig. S5). Of these cubes, 18,760 (12.9%) had no density over −500 HU or FDG uptake at baseline. These represented new lesions that emerged during the study and were omitted from this analysis (fig. S6). The remaining 126,687 (87.1%) cubes were used to explore two specific features suggested by the primary analysis—the synergy of isoniazid and pyrazinamide and the antagonism of rifampicin and pyrazinamide.

To determine the interaction between isoniazid and pyrazinamide, we used linear mixed-effects modeling to estimate the change in mean HU for all cubes in the isoniazid, pyrazinamide, and isoniazid-pyrazinamide arms (see Materials and Methods and Supplementary Methods). Isoniazid-pyrazinamide was significantly (P < 0.05) more effective at reducing HUmean than expected from the additive effect of isoniazid and pyrazinamide alone (Fig. 5A). We also analyzed the cubes after stratification for FDG uptake into “hot” [mean standardized uptake value (SUVmean) > 2] and “cold” (SUVmean ≤ 2) categories. Isoniazid and pyrazinamide showed greater than additive decreases in HUmean in both categories of lesion cubes (P < 0.05; Fig. 5B). In the case of rifampicin and pyrazinamide, the effect of combining the two was slightly less than additive (Fig. 5C). This apparent antagonism of rifampicin and pyrazinamide was affected by the baseline FDG uptake of the lesions; lesions that were cold at baseline showed the expected additive value for decreasing HUmean, whereas lesions that were hot at baseline showed significantly (P < 0.05) less than additive decrease in HUmean (Fig. 5D).

Pyrazinamide activity is linked to baseline inflammatory status of lung lesions

Most participants in the pyrazinamide arm had a mixed response, with some lesions progressing, whereas other lesions showed large reductions in volume and FDG avidity. An example is shown in Fig. 6A, where a participant with a left superior lobe cavity showed a large consolidation throughout the upper lobe that resolved 42% of the hard volume and 41% of the TGA after 14 days of pyrazinamide treatment. At the same time, a smaller nodular area of disease in the left inferior lobe worsened by 600% over the same time interval in both hard volume (density from −100 to +200 HU) and TGA. Twelve of the 19 participants in the pyrazinamide arm showed...
signs of progression in lesions from baseline to day 14. Closer inspection of the lesion features that responded to pyrazinamide treatment revealed that response was directly related to the degree of inflammation at baseline. To understand the variability of lesion responses to pyrazinamide, we examined the properties of all cubes embedded in these participants’ lesions. Because this appeared to be related to total lesion density more than simple HUmean, we also calculated the total lesion mass (TLM) by summing the values of individual voxels within cubes after adding 1024 to each [to normalize to positive numbers for negative HU values, with −1024 being the minimum value in the dataset; TLM = lesion volume × (1024 + mean HU of lesion voxels)] as well as calculating TGA for each cube. Changes in those four cube properties were then analyzed, stratified by the baseline SUVmean from 0 to 10. Thus, cubes that had a baseline SUVmean of 0 to 1 (the bottom row of Fig. 6B) worsened in terms of all four properties. We found that cubes that responded to pyrazinamide had baseline SUVmean values above 5, whereas lesions that progressed had SUVmean values below 3 (Fig. 6B). This pattern of response was unique to pyrazinamide because other monotherapies showed no such pattern (Fig. S7).

**DISCUSSION**

Two-week monotherapy studies in participants with active TB are recommended by the U.S. Food and Drug Administration and the European Medicines Agency as a necessary part of the development process for new anti-TB agents (8, 30–32). Our 14-day EBA estimate for isoniazid was 0.1, whereas other studies found it to be 0.19 (33). Our EBA estimate for rifampicin was in line with what has been reported previously and similar to isoniazid in our study. The negligible EBA of pyrazinamide has also been previously confirmed (7, 34). Our estimate for the 14-day EBA of moxifloxacin of 0.14 is slightly lower than the previously reported 0.17 for the 2- to 7-day EBA (10). Our EBA for isoniazid-rifampicin-pyrazinamide-ethambutol was 0.14, in line with a recent meta-analysis of EBA studies where it ranged from 0.1 to 0.2 (35).

Our PET/CT data showed that rifampicin and moxifloxacin were the most effective in achieving reductions in mean lesion volume and TGA. Pyrazinamide and isoniazid, by contrast, had small or negative effects on both lesion volume and TGA. Whereas isoniazid was superior to rifampicin in conventional sputum CFU reduction, its clear inferiority to rifampicin in reducing lesion volume and inflammation radiologically suggests that the radiology results are more in line with the actual clinical performance of these agents.

The superiority of rifampicin alone to all other arms that contain pyrazinamide suggests a potentially unrecognized antagonism between rifampicin and pyrazinamide. The effects of combining isoniazid and pyrazinamide, as well as rifampicin and pyrazinamide, were not directly evaluated in the original BMRC clinical trials because both agents were introduced onto a backbone of isoniazid...
Fig. 5. Radiologic changes by sublesion cubes. Lesions were divided into 1 cm³ cubes, and baseline and day 14 cube sets were computationally aligned to produce 145,447 cube sets. (A) to (D) represent linear mixed-effects modeling estimates of the change in HUmean for all cubes for participants in the indicated arms. (A) and (C) show the results for all cubes, whereas (B) and (D) show the results for hot cubes (SUVmean > 2) and cold cubes (SUVmean ≤ 2). The y axis is the mean decrease in HU observed for all cubes, whereas the vertical bars show the expected value if the individual estimates were summed. *P < 0.05, significant change from baseline; ‡P < 0.05, significant treatment interaction.

and streptomycin and never tested together without isoniazid. However, their effects may be indirectly inferred from additions and subtractions of these drugs to the same background regimen. Specifically, in the 1970 short-course chemotherapy studies in East and Central Africa, the addition of pyrazinamide to streptomycin and isoniazid for 6 months lowered the relapse rate from 29 to 8% compared with 6 months of isoniazid-streptomycin alone, suggesting a potent effect of adding pyrazinamide to isoniazid (4). In addition, while there were no comparisons of rifampicin-isoniazid with rifampicin and pyrazinamide individually in the initial 2 months of therapy, the use of isoniazid-rifampicin-pyrazinamide compared with isoniazid-rifampicin or isoniazid-pyrazinamide in the 4-month continuation phase (with the same 2-month intensive phase regimen isoniazid-rifampicin-pyrazinamide-streptomycin) led to a 16% rate of relapse compared with 11% with isoniazid-rifampicin and 32% with isoniazid-pyrazinamide (36, 37). The lack of activity of pyrazinamide in the continuation phase was notable, and the slightly higher relapse rates when pyrazinamide was used in combination with rifampicin are consistent with our observations that pyrazinamide is uniquely active in the highly inflamed state occurring early in treatment. Mitchison (38) has long argued that the clinical effect of pyrazinamide being limited to the first 2 months of therapy suggests that its activity is directly associated with inflammation. Our lesional analysis proved insufficient to resolve some questions relating to smaller lesion features embedded within the complex TB lesions observed in these participants. To understand the effects of these drugs and combinations, we computationally divided these lesions into cubes of about 1 cm³ and co-aligned these between baseline and day 14. The resulting comparisons offered further support for the observed synergy between isoniazid and pyrazinamide and the observed antagonism between rifampicin and pyrazinamide. It also allowed us to further understand the impact of pyrazinamide on lesions. Cubes within lesions that responded to pyrazinamide monotherapy had notably higher SUVmean than cubes within lesions which did not respond or which worsened. Our understanding of the role neutrophils play in TB disease has altered recently with the demonstration that neutrophils represent the predominant cell type in human sputum (39) and the dominance of a gene signature associated with neutrophils in the peripheral blood of patients with active disease that wanes upon treatment (40). The relationship between lesion reduction with pyrazinamide and areas of high glucose uptake suggests that the inflammation associated with pyrazinamide activity may represent areas rich in neutrophils. The acidic pH required for pyrazinamide activity may then arise from neutrophil myeloperoxidase (41). This may also explain why the activity of pyrazinamide tapers after the first 2 months of treatment, correlating with a decline in neutrophilic and inflammatory burden.

Last, our comparison of the four-drug regimen moxifloxacin-rifampicin-pyrazinamide-ethambutol that failed to shortent the duration of treatment (despite improved sputum bacterial clearance) compared to the standard four-drug treatment isoniazid-rifampicin-pyrazinamide-ethambutol in the REMox trial (18) revealed no advantage of moxifloxacin-rifampicin-pyrazinamide-ethambutol in reducing lesion volume or inflammation. These data are consistent with the clinical data showing that 4 months of treatment with moxifloxacin-rifampicin-pyrazinamide-ethambutol is inadequate to achieve durable cure.

Our study has several important limitations. The 14-day EBA period is intended to precede the effects of drug resistance and tolerance with therapy that develop over time. Therefore, this
methodology cannot be used to identify resistance-proof regimens for TB. FDG-PET/CT remains expensive and has limited distribution globally, which must be balanced against the cost of doing longer phase 2 or 3 studies. The complexity of the data generated by this study also suggests an underlying complexity in disease biology that will require substantial further work to understand.

Our data explain three important clinical observations. First, the observation that pyrazinamide only exerts an effect during the first 2 months of treatment is explained by our finding that the activity of pyrazinamide is limited to lesions with high baseline inflammation. After the first month of therapy, inflammation is substantially reduced, as measured in our previous 1-month PET/CT study (22).

Second, the observation that adding either pyrazinamide or rifampicin to a backbone of isoniazid and streptomycin was enough to shorten therapy to 6 months but adding both did not allow further treatment shortening is explained by the antagonism between pyrazinamide and rifampicin when given together. Third, the failure of moxifloxacin-rifampicin-pyrazinamide-ethambutol to shorten therapy is explained by our data showing that, despite more rapid sputum clearance of bacteria by this combination, the underlying pathology resolves at the same rate as with the standard isoniazid-rifampicin-pyrazinamide-ethambutol.

These observations would not have been possible without detailed analyses of the PET/CT scans, which allowed the heterogeneous responses of different drugs on different TB lesions to be quantitated and teased apart. Our study shows that PET/CT changes, such as lesion volume and PET activity, within a short time frame are a valuable early drug evaluation tool to characterize individual and combinations of agents and understand the differential effects of these agents on specific pathological manifestations of disease. Understanding these effects for new agents and combinations will facilitate the design of rational drug regimens that can achieve sterile cure in a shorter duration.

**MATERIALS AND METHODS**

**Study design**

We performed a partially blinded (laboratory personnel, scan readers, and statisticians were blinded to arm assignment), randomized trial evaluating 14-day EBA, FDG-PET/CT changes, and immunologic and bacterial markers to distinguish responses to standard TB drugs, which have been characterized by clinical outcomes and pharmacokinetic data over their 40 to 60 years of use. HIV-negative individuals with sputum smear microscopy–positive, Xpert MTB/RIF–positive, pulmonary TB, and abnormal chest x-ray findings were enrolled into the study (full inclusion/exclusion criteria in table S3). After informed consent, eligible participants were randomized to one of eight treatment arms, including four single-drug arms (isoniazid, rifampicin, pyrazinamide, and moxifloxacin), two two-drug arms (rifampicin-pyrazinamide and isoniazid-pyrazinamide), and two four-drug arms (moxifloxacin-rifampicin-pyrazinamide-ethambutol and isoniazid-rifampicin-pyrazinamide-ethambutol). The sample size was calculated on the basis of the statistical power to detect changes from baseline to day 14 of treatment in radiologic PET/CT markers.
14 consecutive days in a monitored inpatient setting at the TASK Applied Science Clinical Trial Centre, a registered research hospital, in Cape Town, South Africa. Participants were assigned a study-generated participant identification code ensuring anonymity. Treatment assignment used centrally generated randomization codes in sequentially numbered envelopes maintained by the study pharmacist. All participants underwent a PET/CT scan at pretreatment baseline (day −2 ± 1-day window) and day 14 (±3-day window) of treatment, in addition to daily overnight sputum sample collection for traditional EBA measurements. Safety assessments included daily history, vital signs, physical examination, and monitoring for adverse events; the latter also comprised full blood counts, coagulation studies, clinical chemistry, and urinalysis. The isoniazid-rifampicin-pyrazinamide-ethambutol arm alone was treated to day 28, with additional sputum, blood, and PET/CT scan collected at that time to allow comparison to other studies. Ethics approval was obtained from the National Institute of Allergy and Infectious Diseases institutional review board, the Stellenbosch University, Human Research Ethics Committee, and the South African Health Products Regulatory Authority.

Sputum CFU and TTP
Sputum specimens were collected for 16 hours overnight starting 2 days before treatment initiation and each day afterward. Collections were terminated before administration of the next day’s therapy. Sputum for CFU counts of Mtb and measurement of TTP in liquid culture medium (BACTEC MGIT 960, Becton Dickinson) were subject to laboratory processing centrally at the TASK laboratory. Mtb speciation was done by polymerase chain reaction (PCR). Cultures from baseline and the last available overnight sputum collections were tested for susceptibility to first-line drugs (MGIT SIRE kit, Becton Dickinson) and to moxifloxacin (MGIT 960, Becton Dickinson). CFU data were interrogated using linear mixed-effects modeling. The models, which aimed to quantify the effect of treatment regimen on bacteriological decline, were fit separately within each arm and included a fixed effect term for visit number and a random intercept term for subject. For each participant, his or her measured CFU values at each visit contributed to the estimate of EBA for the arm to which he or she belonged. Results were similar when using standard EBA estimation methods.

FDG-PET/CT
FDG-PET/CT exams were performed before administration of the first dose of study drug and immediately after the 14th daily dose. Participants fasted for at least 6 hours before FDG administration. According to body weight, 185 to 259 megabecquerel of FDG were administered intravenously 60 min before the scan. CT parameters were set at 120 kV, 200 mA (no dose modulation), 0.75-s rotation time, pitch 0.438, and collimation 16 × 0.75 mm. Investigators analyzing the PET/CTs were blinded to treatment arms. PET/CT features were analyzed by grouping pulmonary abnormalities within a bronchopulmonary segment from all participants. We excluded obvious pleural effusions and other extrapulmonary sites of infection. All abnormalities within the thoracic cavity were labeled by the bronchopulmonary segment(s), in which they were located and segmented manually as regions of interest (“ROI”) using the three-dimensional image analysis software Amira (versions 6.4.0 and 6.5.0; Thermo Fisher Scientific). Each abnormality was defined as either cavity air features present within the pulmonary cavity from −1024 to −500 HU or lesion features that represented material with a radiodensity ranging from −500 to +200 HU. If multiple confluent segments were involved, abnormalities within all those segments were considered as one lesion. Every scan was read by two separate readers and then subjected to at least two rounds of quality control editing by a third reader for consistency, accuracy, and removal of normal structures including vasculature. For the sublesion cube analysis, each lesion was enclosed in the smallest axis-aligned rectangular volume of voxels. The voxels were grouped into 11 × 11 × 11 blocks, starting at the top left corner. Lesion voxels within each cube were then used to compute HU- and SUV-based statistics (mean, SD, etc.) for the cube. PET and CT scans were co-registered on the basis of their global coordinates from the scan, and PET values were assigned to CT voxels based on the PET voxel closest to the front, bottom, left corner of each CT voxel. Lesion voxel cubes were then co-registered between the baseline and day 14 scans.

Statistical analysis
Hypothesis testing comparing change from baseline to day 14 across treatment groups was undertaken in the dataset consisting of co-registered cubes. Cube values within a lesion are correlated as are lesions within a subject. To address this possible correlation, we fit linear mixed-effects models with random effects for subject and for lesions nested within subject. Primary comparisons of interest were based on changes of HU mean values for (i) isoniazid and pyrazinamide versus isoniazid-pyrazinamide and (ii) rifampicin and pyrazinamide versus rifampicin-pyrazinamide to evaluate whether the effect of the two-drug combination was synergistic, antagonistic, or additive. Additional comparisons evaluated whether the HU mean changes between arms differed by baseline cube category of SUVhot (SUVmean > 2) versus SUV-cold (SUVmean < 2). Estimation and hypothesis testing were conducted using the LMER and lmerTest packages in R version 3.5.3. P values <0.05 were set to determine statistical significance. Full outputs for the LMER models are in Supplementary Methods.

SUPPLEMENTARY MATERIALS
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Methods
Fig. S1. Estimated EBA effect from TTP of cultures from the cultures in liquid broth medium
Fig. S2. Segmental distribution of annotated lesions (including cavity air).
Fig. S3. Cavity air percent change by study arm.
Fig. S4. Appearance of new or expanded lesions by study arm.
Fig. S5. Sublesional analysis reveals independent treatment response.
Fig. S6. Study arm distribution of cubes that contained no lesion material at baseline.
Fig. S7. Sublesion cube responses to individual agents stratified by baseline SUVmean values.
Table S1. Participant baseline characteristics.
Table S2. Lesions excluded from lesional analysis.
Table S3. Study inclusion and exclusion criteria.

View/request a protocol for this paper from Bio-protocol.

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