ORIGINAL CONTRIBUTION

Efficacy and Safety of a 4-Drug Fixed-Dose Combination Regimen Compared With Separate Drugs for Treatment of Pulmonary Tuberculosis

The Study C Randomized Controlled Trial

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Context Fixed-dose combinations (FDCs) of drugs for treatment of tuberculosis have been advocated to prevent the emergence of drug resistance.

Objective To assess the efficacy and safety of a 4-drug FDC for the treatment of tuberculosis.

Design, Setting, and Patients The Study C trial, a parallel-group, open-label, noninferiority, randomized controlled trial conducted in 11 sites in Africa, Asia, and Latin America between 2003 and 2008. Patients were 1585 adults with newly diagnosed smear-positive pulmonary tuberculosis.

Interventions Patients were randomized to receive daily treatment with 4 drugs (rifampicin, isoniazid, pyrazinamide, ethambutol) given as an FDC (n=798 patients) or separately (n=787) in the 8-week intensive phase of treatment.

Main Outcome Measure Favorable treatment outcome, defined as negative culture result at 18 months post randomization and not having already been classified as unfavorable. Noninferiority was dependent on consistent results from a per-protocol and modified intention-to-treat analysis, using 2 different models for the latter, classifying all changes of treatment or refusal to continue treatment (eg, bacteriological failure/relapse, adverse event, default, drug resistance) as unfavorable (model 1) and classifying changes of treatment for reasons other than therapeutic outcomes according to their 18-month bacteriological outcome if available (post hoc model 2). The prespecified noninferiority margin was 4%.

Results In the per-protocol analysis, 555 of 591 patients (93.9%) had a favorable outcome in the FDC group vs 548 of 579 (94.6%) in the separate-drugs group (risk difference, –0.7% [90% confidence interval {CI}, –3.0% to 1.5%]). In the model 1 analysis, 570 of 684 patients (83.3%) had a favorable outcome in the FDC group vs 563 of 664 (84.8%) in the separate-drugs group (risk difference, –1.5% [90% CI, –4.7% to 1.8%]). In the post hoc model 2 analysis, 591 of 658 patients (89.8%) in the FDC group and 589 of 647 (91.0%) in the separate-drugs group had a favorable outcome (risk difference, –1.2% [90% CI, –3.9% to 1.5%]). Adverse events related to trial drugs were similarly distributed among treatment groups.

Conclusions Compared with a regimen of separately administered drugs, a 4-drug FDC regimen for treatment of tuberculosis satisfied prespecified noninferiority criteria in 2 of 3 analyses. Although the results do not demonstrate full noninferiority of the FDCs compared with single drugs given separately using the strict definition applied in this trial, use of FDCs is preferred because of potential advantages associated with the administration of FDCs compared with separate-drug formulations.

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DESPITE THE AVAILABILITY OF A highly effective 6-month chemotherapy regimen, worldwide control of tuberculosis is severely impeded by poor treatment completion rates that threaten the emergence of multidrug resistance.[1,2] It is essential to ensure maximum adherence and avoid inappropriate or selective drug intake, especially during the 2-month intensive phase of treatment, when the risk of emergence of drug resistance is greatest. Fixed-dose combinations (FDCs) of drugs have been advocated as a way of preventing the emergence of drug resistance attributable to inappropriate drug intake.[3,4] In addition, they can reduce the risk of incorrect dosage, simplify drug procurement, and aid in ensuring adherence.

Several studies have been conducted to assess the bioavailability, acceptability, or microbiological effi-

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Table 1. Doses of Drugs Used in the Trial

Phase	Dose by Body Weight, No. of Tablets			
	30-37 kg	38-54 kg	55-70 kg	>70 kg
Intensive				
Combined tablet				
Rifampicin (150 mg) / Isoniazid (75 mg) / Pyrazinamide (400 mg) / Ethambutol (275 mg)	2	3	4	5
Separate tablets				
Rifampicin (150 mg)	2	3	4	5
Isoniazid (100 mg)	1.5	2.5	3	3.5
Pyrazinamide (400 mg)	2	3	4	5
Ethambutol (400 mg)	1.5	2	3	3.5
Continuation, both groups				
Rifampicin (150 mg) + isoniazid (150 mg) combined tablet	2	3	4	5

cacy of rifampicin and isoniazid with or without pyrazinamide administered in a fixed combination for daily or intermittent use.[5-8] These studies have shown that 2- and 3-drug FDCs are generally well tolerated, with proportions of adverse effects similar to those for separate formulations and no difference in acquired drug resistance. A randomized trial conducted in Hong Kong showed that a 3-drug FDC (rifampicin, isoniazid, and pyrazinamide) had an efficacy similar to that of a separate-drugs regimen and showed some advantages in terms of acceptability to patients.[9]

Few randomized trials have been conducted to assess the feasibility, safety, and efficacy of a 4-drug (rifampicin, isoniazid, pyrazinamide, ethambutol) FDC for the treatment of tuberculosis. To this end, the International Union Against Tuberculosis and Lung Disease (the Union) launched the present multicenter randomized controlled clinical trial to evaluate the efficacy and safety of an FDC given in the initial intensive phase of treatment of patients with newly diagnosed smear-positive pulmonary tuberculosis.

METHODS

Design and Procedures

The Study C trial was a parallel-group, open-label, noninferiority, randomized controlled trial conducted between 2003 and 2008 in 11 clinical trial sites situated in 9 countries in Africa, Asia, and Latin America (listed at the end of this article). The study protocol, case report forms, patient information sheet, and informed consent forms were translated from English into French, Spanish, Portuguese, and Vietnamese and submitted to the Union ethics advisory group and to the national ethics committees or institutional review boards in each collaborating site or country for approval before the start of the trial.

Before study enrollment, the conditions of the study were explained to the patients according to information contained in a patient information sheet. This information sheet and the consent form were translated into the local vernacular language. Literate patients were asked to read the information sheet and the consent form. Illiterate patients had the content of these documents explained to them by the local coordinator or a senior treatment supervisor. Patients were given the opportunity to discuss the information sheet and the consent form with the medical officer or treatment supervisor. Once the medical officer or treatment supervisor was satisfied that the patient understood the information sheet and the consent form, the patient was asked to sign the consent form in the presence of a witness. The top copy was sent to the local coordinator, and the duplicate was filed with the patient's study folder.

Patients with newly diagnosed smear-positive pulmonary tuberculosis who had provided written informed consent were randomly assigned to receive either a test or control regimen. The test (FDC) regimen consisted of an initial intensive phase of 8 weeks of daily rifampicin, isoniazid, pyrazinamide, and ethambutol in FDC tablets followed by 18 weeks of rifampicin and isoniazid FDC tablets 3 times weekly, The control (separate-drugs) regimen consisted of the same drugs in separate formulations administered daily in the initial intensive phase, followed by 18 weeks of rifampicin and isoniazid FDC tablets 3 times weekly.

The doses were given according to recommendations from the World Health Organization (WHO) and the Union (TABLE 1), based on the weight of the patient in kilograms at the time of starting treatment without adjustment for weight change during treatment. In addition, every patient received 50 mg of pyridoxine together with the antituberculosis drugs throughout treatment. All drugs used in the trial were manufactured by Svizera (New Delhi, India), an identified supplier of drugs to the Global Drug Facility at the time of the trial, and supplied in bulk to the sites by the Union.

Random allocations were computer generated at the Medical Research Council Clinical Trials Unit, London, United Kingdom. Sealed opaque envelopes containing an allocation slip with a serial number and details of the regimen to which the patient was to be allocated were sent to an independent person in each center. This person had to be contacted by the local trial physician whenever a patient was eligible for enrollment into the trial. Thus, the trial physician was not aware of treatment allocation before enrollment.

Patients were required to attend the treatment facility daily during the initial intensive phase (first 8 weeks) of chemotherapy and then 3 times weekly during the continuation phase. Every treatment dose was to be taken under supervision of a member of the medical staff (ie, as directly observed

therapy). In the majority of the trial centers, treatment was fully supervised for a minimum of 6 days a week, which is currently common practice in the majority of national tuberculosis program centers worldwide. In centers closed on Sundays, treatment was unsupervised for the seventh day. In that case, treatment intake was checked by health workers through unplanned visits to patients' homes and pill counts.

All collected information was recorded on duplicate case report forms. Before data entry, these forms were to be reviewed carefully by the local trial physician. Initially, the forms were entered locally in a specifically prepared Epi-Info database and reentered by the trial data manager at the Medical Research Council Clinical Trials Unit. However, because of problems with timely data entry at some sites, it was decided that all case report forms should be sent in batches by mail to the Clinical Trials Unit every 2 months for data entry. Queries were sent back to the sites for resolution.

Eligibility Criteria

Patients with newly diagnosed pulmonary tuberculosis were admitted to the study if they were 18 years or older, had 2 sputum specimens positive for acid-fast bacilli on direct-smear microscopy, had received either no previous antituberculosis chemotherapy or less than 4 weeks of chemotherapy for the current disease episode, had a firm home address readily accessible for visiting and intended to remain there during the entire study period, and had provided written informed consent to participate in the study.

Patients were not eligible if they were considered unlikely to survive the initial weeks of treatment; had tuberculous meningitis, other extrapulmonary disease, insulin-dependent diabetes, chronic liver or kidney disease, blood disorders, or peripheral neuritis; were known to be pregnant or were breast feeding; had a history of psychiatric illness or alcoholism; or had any contraindication to any medications used in the study. Patients with no positive culture result at entry or rifampicin resistance before treatment were excluded post randomization.

Recruitment

Two sputum specimens were collected before the start of treatment for examination by microscopy and culture. A chest radiograph was obtained and kept for independent assessment. Patients were required to provide a blood sample to test for human immunodeficiency virus (HIV) infection after pretest counseling; the result was communicated to patients if they wished to receive it, and posttest counseling was provided. Those who were HIV-infected were referred to the appropriate local HIV care services. Information was collected on antiretroviral treatment in addition to treatment for tuberculosis.

Follow-up

Patients were seen at the end of the second, third, fifth, and sixth months during treatment and then at 8, 10, 12, 15, 18, 24, and 30 months in the follow-up phase. At each visit during treatment they were asked about any adverse events that may have occurred since they were last seen. Patients who missed an appointment were contacted through a home visit or telephone call by a trial assistant and asked to return to the study clinic. Visits were made to each site twice a year to monitor trial procedures and check all patients' case report forms.

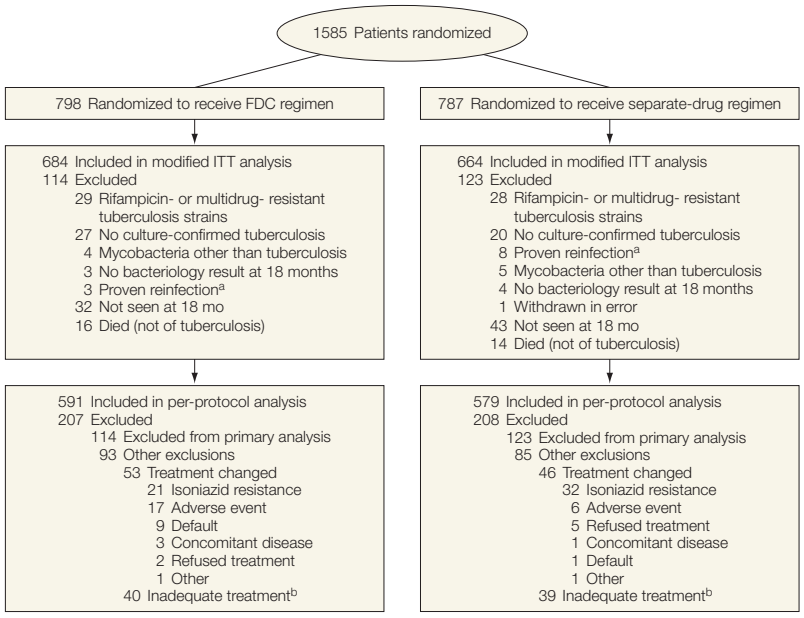
Microbiology

Two sputum samples were collected at each visit and examined either by Ziehl-Neelsen microscopy or fluorescence microscopy for the presence of acid-fast bacilli. To obtain comparable sputum-culture data from all laboratories and minimize variation in sensitivity and specificity resulting from the complexity of the culture procedure, we used a modified version of the simple culture technique in which decontaminated specimens (in closed systems) are directly inoculated into acid-buffered egg-based medium without centrifugation.[10] All positive cultures (pretreatment and follow-up) that grew 5 or more colonies at the site laboratories were subcultured, with 2 subcultures stored at the center's laboratory and a third shipped to the Supranational Reference Laboratory at the Institute of Tropical Medicine in Antwerp, Belgium, for quality control, strain identification, and drug susceptibility testing. Susceptibility to isoniazid, rifampicin, streptomycin, and ethambutol was tested using the proportion method. Sequencing analysis of relevant drug-related genes was performed on any isolates with uncertain results. In addition, spoligotyping and MIRU-VNTR (mycobacterial interspersed repetitive unit–variable-number tandem repeat) typing (15 loci) were applied for fingerprinting of isolates to differentiate true relapse from reinfection.[11] Fingerprinting was performed on both the baseline isolate and the isolate obtained from the patient on the first day of suspected treatment failure or disease recurrence.

Sample Size

Under optimal controlled trial conditions, standard tuberculosis treatment (daily rifampicin, isoniazid, pyrazinamide, and ethambutol for 2 months, followed by 4 months of daily rifampicin and isoniazid) is highly effective, with a 95% or higher success rate.[12] Although it is possible that combined preparations might prove more effective than separate drugs, it would require an impractically large study to demonstrate superiority. For this reason, the study was designed as a noninferiority trial, testing the hypothesis that the FDC regimen was not inferior to the separate-drugs regimen. A 90% (1-sided 95%) confidence interval (CI) was used to determine whether the difference in rate of failure and relapse lay within a prespecified margin of noninferiority[13]; the results are also presented using a 95% CI. The margin of noninferiority (ie, the lowest limit of the CI for the difference from the control regimen that we were prepared to accept) was set at 4%. This margin of non-

Figure 1. Study Flow



ITT indicates intention-to-treat; FDC, fixed-dose combination.
[a] Using fingerprint analysis.
[b] Defined as missing 2 weeks or more of the initial intensive phase or missing 4 weeks or more of treatment in total.

inferiority was selected by the investigators based on current knowledge and expert opinion regarding the expected rate of failure and relapse for the control regimen in a randomized controlled clinical trial.

To achieve 90% power to demonstrate noninferiority as defined required 412 patients in each treatment group. Assuming that 15% of the patients would be excluded because of negative or rifampicin-resistant cultures before treatment, that 10% of patients would not comply with their treatment, and that a further 20% would be lost to follow-up and unassessable after treatment, 749 patients were required in each treatment group to ensure adequate numbers of participants for the per-protocol analysis.

Statistical Analysis

In accordance with the standard approach to noninferiority trials, the analysis was conducted both on per-protocol and modified intention-to-treat (ITT) populations. The modified ITT population was composed of all randomized patients who received study medication on at least 1 occasion, excluding those without culture-confirmed tuberculosis and those found to have either rifampicin- or multidrug-resistant disease at enrollment. Patients who died without any evidence that tuberculosis contributed to the cause of death, those considered (through fingerprint test) to have been reinfected after cure, and those unassessable at 18 months were also excluded.

The per-protocol population was defined as all patients included in the ITT analysis, excluding those who did not receive the regimen as prescribed. These were patients who received less than 6 weeks of treatment (42 days of daily treatment or 36 days of 6-days-a-week treatment) or more than 9 weeks of treatment (63 days of daily treatment or 54 days of 6-days-a-week treatment) in the intensive phase and those who received less than 42 doses (ie, 4 weeks of missed treatment) or more than 60 doses (ie, 2 weeks of extra treatment) in the continuation phase (the protocol requirement is that patients receive 18 weeks of 3-times-weekly treatment, ie, 54 doses). Also excluded were patients whose treatment was modified for reasons other than bacteriological failure or relapse (including patients changing treatment for adverse drug reactions, following return after default, or attributable to concomitant HIV infection).

Efficacy Analysis

The primary efficacy analysis end point was the combined proportion of patients with an "unfavorable" outcome, defined as any of the following: (1) bacteriological failure or relapse by 18 months after start of treatment, defined as a culture of at least 20 colonies' growth or 2 cultures of 10 or more colonies' growth at the end of treatment or in the follow-up phase, not identified as a reinfection through MIRU-VNTR typing; (2) patients whose treatment was changed after month 5 because of 2 positive sputum smear results or a clinical or radiographic deterioration in the absence of bacteriological confirmation; and (3) patients whose cause of death was definitely or possibly attributable to active tuberculosis.

A "favorable" outcome was defined as having a negative culture result at 18 months (or 24 months if the 18-month result was unavailable) and not having been already classified as unfavorable. Patients were considered "unassessable" if they could not be assessed at 18 months and had not already been classified as having an unfavorable outcome, provided there was no evidence to suggest they might be relapsing.

In the modified ITT analysis, patients who refused treatment or had received inadequate treatment (defined as missing ≥2 weeks of the initial intensive phase or as missing ≥4 weeks of treatment in total) or who were not assessable at 18 months were classified as having an "unfavorable" outcome. Two approaches were used for

classifying patients who changed treatment. The first (modified ITT model 1) was defined in the original analysis plan and classified all changes of treatment or refusal to continue treatment for whatever reason (eg, bacteriological failure/relapse, adverse event, default, drug resistance) as "unfavorable." The second (modified ITT model 2) was recommended post hoc by the trial steering committee on the grounds that it represented a more realistic assessment of the long-term outcome and classified changes of treatment for reasons other than therapeutic outcomes according to their 18-month bacteriological outcome if available.

In all 3 analyses, the difference in combined outcome between study groups was tested with a 90% CI (1-sided 95% CI). In addition, the effect of site was also tested (test for homogeneity). Differences in subgroups were tested using an interaction test in logistic regression. All analyses were conducted using Stata version 10.[14]

Safety Analysis

The primary safety analysis end point was the proportion of patients presenting with adverse events during the first 2 months of treatment. All adverse events were reported, including details of the signs and symptoms, assessment of their severity and potential relationship with the treatment, and the ensuing action taken. Adverse events were carefully reviewed by 2 medical officers on the team, both blinded to treatment allocation; queries, clarifications, or both were requested as necessary to the site physicians. Death reports were reviewed blinded to the allocated treatment to assess causal link with tuberculosis and were classified as "not related to tuberculosis," "possibly related to tuberculosis," and "most likely related to tuberculosis."

RESULTS

A total of 1585 patients were randomized (798 in the FDC group, 787 in the separate-drugs group). Of these, 237 (114 FDC group, 123 separate-drugs group) were excluded from the modified ITT analyses, including patients with no positive culture result at entry or rifampicin resistance pretreatment (n = 56 and n = 48, respectively) (FIGURE 1). An additional 178 patients (93 FDC group, 85 separate-drugs group) were excluded from the per-protocol analysis. There remained 1170 patients (591 FDC group, 579 separate-drugs group) included in the per-protocol analysis. Baseline characteristics were similar in the 2 groups (TABLE 2).

In the per-protocol population, culture results were available at 2 months for 569 of 591 patients in the FDC group and 549 of 579 in the separate-drugs group. Among these, 521 patients (91.6%) in the FDC group and 501 (91.3%) in the separate-drugs group had a negative culture result at 2 months.

The per-protocol analysis shows that, at 18 months after start of treatment, 555 of 591 patients (93.9%) in the FDC group had a favorable outcome vs 548 of 579 (94.6%) in the separate-drugs group (TABLE 3), a difference of –0.7% (90% CI, –3.0% to 1.5%), which is within the predefined margin of noninferiority (FIGURE 2). There was no effect of trial site ($P = .29$ for homogeneity).

The modified ITT model 1 analysis shows that 570 of 684 patients (83.3%) in the FDC group had a favorable outcome, compared with 563 of 664 (84.8%) in the separate-drugs group (TABLE 4), a difference of –1.5% (90% CI, –4.7% to 1.8%), which falls outside the prespecified margin of noninferiority ($P = .07$ for between-site homogeneity). In the post hoc modified ITT model 2 analysis, 591 of 658 assessable patients (89.8%) in the FDC group had a favorable outcome, compared with 589 of 647 (91.0%) in the separate-drugs group (Table 4), a dif-

Table 2. Baseline Characteristics of Per-Protocol Population

Characteristic	No. (%) FDC (n = 591)	No. (%) Separate Drugs (n = 579)
Sex		
Men	393 (66.5)	387 (66.8)
Women	198 (33.5)	192 (33.2)
Age, mean (SD), y	33.8 (13.4)	34.2 (13.5)
Weight, mean (SD), kg	51.4 (9.4)	50.6 (8.7)
BMI, mean (SD)[a]	16.6 (5.8)	16.1 (5.1)
HIV test result[b]		
Positive	39 (6.6)	38 (6.6)
Negative	549 (93.4)	541 (93.4)
Radiographic findings[c]		
Bilateral	181 (55.4)	191 (61.5)
Unilateral	145 (44.3)	118 (38.2)
No opacities seen	1 (0.3)	1 (0.3)
Cavitation		
Present	227 (69.4)	218 (70.3)
No cavitation	100 (30.6)	92 (29.7)
Largest cavity diameter, mean (SD), mm	33.9 (15.8)	33.9 (13.7)
Drug susceptibility test results[d]		
Fully sensitive organisms	508 (88.2)	497 (88.9)
Non-MDR isoniazid-resistant isolates	65 (11.3)	62 (11.1)
Smoking		
Current	54 (9.1)	60 (10.4)
Former	207 (35.0)	202 (34.9)
Never	330 (55.8)	317 (54.7)

Abbreviations: BMI, body mass index; FDC, fixed-dose combination; HIV, human immunodeficiency virus; MDR, multidrug resistant.
[a] Calculated as weight in kilograms divided by height in meters squared. Height not available for 1 patient (separate-drugs group).
[b] Result not available for 3 patients (FDC group).
[c] Based on 637 patients (327 FDC, 310 separate drugs) with assessable radiographs.
[d] Based on 1132 patients (573 FDC, 559 separate drugs) with drug sensitivity test results.

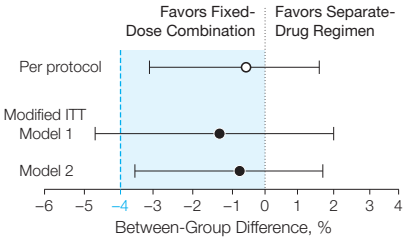
ference of –1.2% (90% CI, –3.9% to 1.5%) (P=.30 for between-site homogeneity). Thus, the 95% CIs are consistent with up to a 4.7% inferior outcome with the FDC regimen compared with the separate-drugs regimen in the model 1 analysis and with up to a 3.9% inferior outcome in the model 2 analysis (Figure 2).

Table 3. Per-Protocol Analysis at 18 Months

Response	No. FDC (n = 591)	No. Separate Drugs (n = 579)
Favorable response		
Culture-negative		
At 18 mo	534	528
At 24 mo	22	20
Total, No. (%)[a]	555 (93.9)	548 (94.6)
Unfavorable response		
Treatment failure		
Culture-confirmed	3	4
Smear-confirmed	5	4
Pleural effusion and chest radiograph deterioration	1	0
Relapse		
Fingerprint-confirmed	16	14
Culture-confirmed	6	3
Smear-confirmed	1	2
Death		
Most likely due to tuberculosis	2	2
Possibly due to tuberculosis	2	2
Total, No. (%)	36 (6.1)	31 (5.4)

Abbreviation: FDC, fixed-dose combination.
[a] Between-group difference in response: –0.7% (95% confidence interval, –3.4% to 1.9% [90% confidence interval, –3.0% to 1.5%]).

Figure 2. Evidence for Noninferiority From the 3 Methods of Analysis



Blue dashed line indicates noninferiority margin; blue-tinted region to the right of between-group difference = –4 indicates values for which a fixed-dose combination (FDC) regimen would be considered noninferior to a separate-drugs regimen. Error bars indicate 90% confidence intervals for between-group differences (FDC regimen vs separate-drugs regimen) in favorable outcome. Model 1: All patients who changed treatment or refused to continue treatment for whatever reason (eg, bacteriological failure/relapse, adverse event, default, drug resistance) were classified as having an unfavorable outcome. Model 2 (post hoc): All patients who changed treatment for reasons other than therapeutic outcomes were classified according to their 18-month bacteriological outcome, if available. ITT indicates intention-to-treat.

In the per-protocol population, the risk of an unfavorable outcome was higher in patients having strains initially monoresistant to isoniazid compared with patients having fully susceptible strains (11/127 [8.7%] vs 52/1005 [5.2%] for both regimens combined, P=.10). There was no evidence, however, of a difference in outcome between the 2 groups when analyzed by sensitivity status (P=.57 for interaction). The risk of an unfavorable outcome was more than 3 times higher in HIV-infected than in HIV-uninfected patients (13/77 [16.9%] and 54/1090 [5.0%], respectively; P<.001). There was no evidence of a difference in outcomes between the 2 groups when analyzed by HIV status (P=.49 for interaction).

There was no difference in acquisition of resistance among patients treated in each group. Among patients with treatment failure, the only patient with initial isoniazid resistance in the separate-drugs group (and for whom a failure culture sample was available) went on to develop rifampicin resistance, whereas the 1 patient in the FDC group with initial isoniazid resistance did not. Among the 13 patients in the FDC group with fully sensitive organisms pretreatment who relapsed and for whom a culture sample was available, 1 developed isoniazid resistance, compared with none of the 14 in the separate-drugs group. Of the 2 patients with initial isoniazid resistance in each regimen who relapsed and for whom a culture sample was available, none developed additional resistance.

Safety Analysis

A total of 1581 patients were evaluable for safety assessment in the first 2 months; of these, 67 (31 in the FDC group, 36 in the separate-drugs group) reported at least 1 adverse event, which was considered to be probably or possibly related to their antituberculosis treatment. The majority of adverse events were dermatologic, rheumatologic, hepatic, or gastrointestinal disorders and were mostly of mild or moderate severity (TABLE 5). They were similarly distributed among the treatment groups (P=.10). The type of action taken by the trial physician, however, differed according to regimen: while 10 patients were removed from the study regimen in the FDC group compared with only 3 in the separate-drugs group, no specific action was taken in 15 patients in the FDC group compared with 27 in the separate-drugs group (P=.03, χ^2_2).

COMMENT

The results of this trial show, using a strict definition of noninferiority, that a 4-drug FDC regimen may be noninferior to a regimen of separately administered drugs in terms of efficacy for treatment of tuberculosis. Our results are consistent with the findings of a recently conducted randomized controlled trial investigating the efficacy, safety, and tolerability of a 4-drug FDC in comparison with separately administered drugs for treatment of tuberculosis, although results of that trial were based on sputum smear conversion only, and the efficacy was measured for 2 different end points (cure at the end of treatment and relapse post treatment), with different assumptions for δ (4% and 10%, respectively).[15]

Our results are also generally in line with findings from an observational study in Indonesia showing better tolerance of 4-drug FDCs compared with separately administered agents,[16] as well as results from earlier studies of 3-drug FDCs.[9,17] Of note, the results of the subanalyses according to the initial susceptibility of the organisms or patients' HIV status are consistent with the main findings.

To our knowledge, this is the first trial conducted to evaluate a treatment for tuberculosis according to Good Clinical Practice standards that uses a noninferiority design and applies the latest recommendations from regulatory authorities for the evaluation of new treatments

for tuberculosis: (1) the use of a combined failure/relapse end point, (2) the need to exclude individuals with proven reinfection, and (3) a follow-up period of at least 18 months post randomization.[18,19]

The definition of noninferiority used in this study was a lower level of the 2-sided 90% CI for the difference in outcome of no less than –4%. Whereas the results of the per-protocol analysis completely satisfy this definition, the modified ITT analysis defined in the analysis plan does not; the post hoc modified ITT analysis (model 2) does satisfy the definition (Figure 2). According to the Committee on Proprietary Medical Products, "similar conclusions from both the ITT and [per-protocol] analysis are required" to declare noninferiority,[20] to reduce the possibility of wrongly declaring a regimen to be noninferior.[21,22] However, there was no evidence of difference in the 2 groups in terms of culture negativity at 2 months, which would support noninferiority of the FDC regimen; it also should be noted that inclusion of the patients with recurrence attributable to reinfection would have reduced the difference between the 2 groups.

Treatment-related adverse events were few and of similar frequency in both regimens, and most reflected the expected undesirable effects of antituberculosis agents: gastrointestinal disorders (nausea, vomiting, anorexia, diarrhea), skin reactions (rash, pruritus), and musculoskeletal disorders (arthralgia, myalgia).[23] Of interest, however, is that the action taken by the treating clinician when confronted with adverse events differed significantly between the study groups: patients treated with FDCs were more likely to be removed from the trial drugs than those treated with drugs administered separately. Because drugs in FDCs cannot be separated, it is difficult to identify the drug within the combination that is potentially responsible for an adverse event, leading to a complete stoppage of treatment; conversely, drugs administered separately can be interrupted and reintroduced progressively. For this reason, trial physicians were more likely to remove the patient fully from treatment when treated with FDCs than when treated with separately administered drugs and were more likely to administer separate drugs than to restart FDCs when the adverse event was resolved. The excess of patients removed from trial drugs in the FDC group, which contributed to the differ-

Table 4. Modified Intention-to-Treat Analysis at 18 Months

Response	No. FDC	No. Separate Drugs
Model 1[a]	(n = 684)	(n = 664)
Favorable response		
Culture-negative		
At 18 mo	548	543
At 24 mo	22	20
Total, No. (%)[b]	570 (83.3)	563 (84.8)
Unfavorable response		
Failure		
Culture-confirmed	5	4
Smear-confirmed	5	4
Pleural effusion and chest radiograph deterioration	1	0
Relapse		
Fingerprint-confirmed	17	14
Culture-confirmed	8	5
Smear-confirmed	1	2
Treatment change due to		
Adverse event	17	6
Resistance	21	32
Default	9	1
Refusal to take study regimen	2	5
Other[c]	4	2
Inadequate treatment	20	19
Death		
Most likely due to tuberculosis	2	3
Possibly due to tuberculosis	2	4
Total, No. (%)	114 (16.7)	101 (15.2)
Model 2[d]	(n = 658)	(n = 647)
Favorable response		
Culture-negative		
At 18 mo	565	564
At 24 mo	26	25
Total, No. (%)[e]	591 (89.8)	589 (91.0)
Unfavorable response		
Failure		
Culture-confirmed	5	4
Smear-confirmed	5	4
Pleural effusion and chest radiograph deterioration	1	0
Relapse		
Fingerprint-confirmed	17	16
Culture-confirmed	10	5
Smear-confirmed	1	2
Inadequate treatment	20	19
Death		
Most likely due to tuberculosis	2	4
Possibly due to tuberculosis	6	4
Total, No. (%)	67 (10.2)	58 (9.0)

Abbreviations: CI, confidence interval; FDC, fixed-dose combination.
[a] Treatment changes for any reason were classified as unfavorable outcome.
[b] Between-group difference in response: –1.5% (95% confidence interval, –5.4% to 2.5% [90% confidence interval, –4.7% to 1.8%]).
[c] Includes treatment extended for concomitant human immunodeficiency virus disease and concomitant diabetes.
[d] Patients who changed treatment for reasons other than bacteriological failure/relapse were classified, where possible, according to their bacteriological status at 18 months.
[e] Between-group difference in response: –1.2% (95% confidence interval, –4.4% to 2.0% [90% confidence interval, –3.9% to 1.5%]).

Table 5. Distribution of Adverse Events Among Trial Participants by Clinical Category and Severity According to Study Group

	No.	
	FDC (n = 797)[a]	Separate Drugs (n = 784)[a]
Patients with adverse events in months 1 or 2 (probably or possibly drug-related)	31	36
Action taken		
None	15	27
Interruption	6	6
Stopped study drugs	10	3
Adverse events by type		
Rheumatological	7	11
Stopped study drug	0	0
Dermatological	16	15
Stopped study drug	7	2
Hepatic	5	1
Stopped study drug	2	0
Gastrointestinal	6	11
Stopped study drug	1	1
Other	3	4
Stopped study drug	0	1
Adverse event severity[b]		
Mild	18	23
Moderate	9	8
Severe	4	5

[a] Total number of patients evaluated for safety, based on those who received at least 1 dose of study medication.
[b] For patients with more than 1 adverse event the maximum is given. Mild indicates that the event does not interfere in a significant manner with the patient's normal functioning; moderate, that the event produces some impairment in the patient's functioning but is not hazardous to the health of the patient; severe, that the event produces significant impairment or incapacitation of functioning and may be hazardous to the health of the patient; and life-threatening, that the event causes extreme impairment of functioning requiring hospitalization and if left untreated could result in the death of the patient. Severe and life-threatening adverse events are designated as serious adverse events.

ence between the 2 groups in the modified ITT analysis, is therefore more likely to represent patients removed from drug formulation (and treated with separately administered drugs after the adverse event was resolved) than a true removal from trial regimens. This has important consequences for the recommendation of FDCs in national tuberculosis control programs, because stocks of single-drug tablets would need to be made available for patients with severe adverse reactions to drugs,[4] and specific training of medical personnel is needed to address the issue of drug-related adverse events, particularly the reintroduction of treatment after their resolution.

A potential limitation of the study is that we used rifampicin and isoniazid FDC tablets during the continuation phase in both groups, which may have decreased the possibility of observing a difference in efficacy between the 2 groups. We decided to investigate the difference between FDCs and loose tablets only in the initial 2-month intensive phase of treatment for 2 reasons. First, the intensive phase is the most critical part of the treatment of tuberculosis, when bacterial load is at its highest and when poor adherence or selective drug intake could lead to the emergence of drug resistance or treatment failure—hence the potential strongest effect of FDCs. Second, in pragmatic terms, the 2 drugs used in the continuation phase of treatment, rifampicin and isoniazid, are presently given as FDCs in the large majority of national tuberculosis control programs worldwide.

One of the main advantages of FDCs is that patients have to take considerably fewer pills (3-4 instead of 9-16 per day in the intensive phase), thus making treatment easier, aiding adherence, and eliminating the risk of developing drug resistance attributable to selective drug intake.[4] We did not observe a difference in emergence of drug resistance between the regimens in our study—probably because, within the conditions of the trial, strict directly observed therapy was being applied. The use of FDCs, however, does not remove the need for directly observed therapy, which remains essential for efficient global tuberculosis control. FDCs should therefore be promoted for tuberculosis control as an integral part of good service delivery, ensuring good quality of drugs and proven rifampicin bioavailability. These are absolute requirements, relying on a mechanism of appropriate prequalification of FDCs, as set up by the Global Drug Facility.[24]

FDCs are a full part of the recently revised WHO treatment guidelines.[25] The uptake of FDCs in tuberculosis control programs globally is gaining momentum, but challenges remain. While a majority of countries have now incorporated FDCs into their national treatment guidelines, there is some evidence that the proportion of patients living in countries where FDCs are incorporated is substantially lower than that in countries with no FDCs incorporated in national guidelines.[26] Furthermore, even within a given country the use of FDCs is variable, with wide differences in use of FDCs compared with separately administered drugs, in both the public and the private sectors. The uptake and acceptance of FDCs is primarily affected by doubts about the efficacy of FDCs, questions of access and quality, advantages over other formulations or packaging, lack of political will at the country level, and the conflicting policies of funders.

Although the results of this study do not demonstrate full noninferiority of the FDCs with single drugs using the strict definition applied in this trial, the results do support the WHO recommendations for use of FDCs because of the potential advantages associated with their administration compared with separate-drug formulations.[25] For efficient tuberculosis control worldwide, it is essential that quality-assured FDCs are made available.[4,25] While new regimens or drugs are being developed for the treatment of tuberculosis, it is essential that strategies are developed for their introduction in national tuberculosis control programs, which includes the protection of these new drugs within established and quality-assured FDCs.[27]

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REFERENCES

1. Raviglione MC. The Global Plan to Stop TB, 2006-2015. *Int J Tuberc Lung Dis*. 2006;10(3):238-239.

2. Zignol M, Hosseini MS, Wright A, et al. Global incidence of multidrug-resistant tuberculosis. *J Infect Dis*. 2006;194(4):479-485.

3. International Union Against Tuberculosis and Lung Disease. Antituberculosis regimens of chemotherapy: recommendations from the Committee on Treatment of the International Union Against Tuberculosis and Lung Disease. *Bull Int Union Tuberc Lung Dis*. 1988;63(2):60-64.

4. Blomberg B, Spinaci S, Fourie B, Laing R. The rationale for recommending fixed-dose combination tablets for treatment of tuberculosis. *Bull World Health Organ*. 2001;79(1):61-68.

5. Ellard GA, Ellard DR, Allen BW, et al. The bioavailability of isoniazid, rifampin, and pyrazinamide in two commercially available combined formulations designed for use in the short-course treatment of tuberculosis. *Am Rev Respir Dis*. 1986;133(6):1076-1080.

6. Geiter LJ, O'Brien RJ, Combs DL, Snider DE Jr. United States Public Health Service Tuberculosis Therapy Trial 21: preliminary results of an evaluation of a combination tablet of isoniazid, rifampin and pyrazinamide. *Tubercle*. 1987;68(2)(suppl):41-46.

7. Acocella G, Nonis A, Gialdroni-Grassi G, Grassi C. Comparative bioavailability of isoniazid, rifampin, and pyrazinamide administered in free combination and in a fixed triple formulation designed for daily use in antituberculosis chemotherapy, I: single-dose study. *Am Rev Respir Dis*. 1988;138(4):882-885.

8. Singapore Tuberculosis Service/British Medical Research Council. Assessment of a daily combined preparation of isoniazid, rifampin, and pyrazinamide in a controlled trial of three 6-month regimens for smear-positive pulmonary tuberculosis. *Am Rev Respir Dis*. 1991;143(4, pt 1):707-712.

9. Hong Kong Chest Service/British Medical Research Council. Acceptability, compliance, and adverse reactions when isoniazid, rifampin, and pyrazinamide are given as a combined formulation or separately during three-times-weekly antituberculosis chemotherapy. *Am Rev Respir Dis*. 1989;140(6):1618-1622.

10. Kudoh S, Kudoh T. A simple technique for culturing tubercle bacilli. *Bull World Health Organ*. 1974;51(1):71-82.

11. Supply P, Allix C, Lesjean S, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit–variable-number tandem repeat typing of *Mycobacterium tuberculosis*. *J Clin Microbiol*. 2006;44(12):4498-4510.

12. Jindani A, Nunn AJ, Enarson DA. Two 8-month regimens of chemotherapy for treatment of newly diagnosed pulmonary tuberculosis: international multicentre randomised trial. *Lancet*. 2004;364(9441):1244-1251.

13. Machin D, Campbell MJ. *Statistical Tables for the Design of Clinical Trials*. Oxford, UK: Blackwell Scientific Publications; 1987.

14. *Stata Statistical Software Release 10*. College Station, TX: StataCorp; 2007.

15. Bartacek A, Schütt D, Panosch B, Borek M; Rimstar 4-FDC Study Group. Comparison of a four-drug fixed-dose combination regimen with a single tablet regimen in smear-positive pulmonary tuberculosis. *Int J Tuberc Lung Dis*. 2009;13(6):760-766.

16. Gravendeel JM, Asapa AS, Becx-Bleumink M, Vrakking HA. Preliminary results of an operational field study to compare side-effects, complaints and treatment results of a single-drug short-course regimen with a four-drug fixed-dose combination (4FDC) regimen in South Sulawesi, Republic of Indonesia. *Tuberculosis (Edinb)*. 2003;83(1-3):183-186.

17. Su WJ, Perng RP. Fixed-dose combination chemotherapy (Rifater/Rifinah) for active pulmonary tuberculosis in Taiwan: a two-year follow-up. *Int J Tuberc Lung Dis*. 2002;6(11):1029-1032.

18. Briefing information for the June 3, 2009, meeting of the Anti-Infective Drugs Advisory Committee. US Food and Drug Administration Web site. http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/Anti-InfectiveDrugsAdvisoryCommittee/ucm161856.htm. Accessed September 3, 2010.

19. Concept paper on preparation of an addendum to the guideline on development of antibacterial agents to specifically address the clinical development of new agents to treat tuberculosis (EMEA/CHMP/EWP/122355/2007). European Medicines Agency Web site. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003396.pdf. 2007. Accessed March 2, 2011.

20. Guideline on the choice of the non-inferiority margin (EMEA/CPMP/EWP/2158/99). European Medicines Agency Web site. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003636.pdf. July 2005. Accessed March 2, 2011.

21. Nunn AJ, Phillips PP, Gillespie SH. Design issues in pivotal drug trials for drug sensitive tuberculosis (TB). *Tuberculosis (Edinb)*. 2008;88(suppl 1):S85-S92.

22. Piaggio G, Elbourne DR, Altman DG, Pocock SJ, Evans SJ; CONSORT Group. Reporting of noninferiority and equivalence randomized trials: an extension of the CONSORT statement. *JAMA*. 2006;295(10):1152-1160.

23. Forget EJ, Menzies D. Adverse reactions to first-line antituberculosis drugs. *Expert Opin Drug Saf*. 2006;5(2):231-249.

24. Global Drug Facility. Stop TB Partnership Web site. http://www.stoptb.org/gdf/drugsupply/quality_sourcing_process.asp. Accessed September 3, 2010.

25. Treatment of tuberculosis: guidelines. 4th ed. World Health Organization Web site. http://www.who.int/tb/publications/tb_treatmentguidelines/en/index.html. 2009. Accessed September 3, 2010.

26. Lin Y. Progress and challenges in addressing the fixed-dose combination (FDC) bottleneck in TB control: summary of selected data. Presented at: Pacific Health Summit; Seattle, WA; June 18, 2009.

27. Lienhardt C, Vernon A, Raviglione MC. New drugs and new regimens for the treatment of tuberculosis: review of the drug development pipeline and implications for national programmes. *Curr Opin Pulm Med*. 2010;16(3):186-193.