Efficacy of a 6-month versus 9-month Intermittent Treatment Regimen in HIV-infected Patients with Tuberculosis
A Randomized Clinical Trial

Soumya Swaminathan[1], Gopalan Narendran[1], Perumal Venkatesan[1], Sheik Iliayas[1], Rameshkumar Santhanakrishnan[1], Pradeep Aravindan Menon[1], Chandrasekharan Padmapriyadarsini[1], Ranjani Ramachandran[1], Ponnuraja Chinnaiyan[1], Mohanarani Suhadev[1], Raja Sakthivel[1], and Paranji R. Narayanan[1]

[1]Tuberculosis Research Center, Indian Council of Medical Research, Chetput, Chennai, India

Rationale: The outcome of fully intermittent thrice-weekly antituberculosis treatment of various durations in HIV-associated tuberculosis is unclear.

Objectives: To compare the efficacy of an intermittent 6-month regimen (Reg6M: $2EHRZ_3/4HR_3$ [ethambutol, 1,200 mg; isoniazid, 600 mg; rifampicin, 450 or 600 mg depending on body weight <60 or ≥ 60 kg; and pyrazinamide, 1,500 mg for 2 mo; followed by 4 mo of isoniazid and rifampicin at the same doses]) versus a 9-month regimen (Reg9M: $2EHRZ_3/7HR_3$) in HIV/tuberculosis (TB).

Methods: HIV-infected patients with newly diagnosed pulmonary or extrapulmonary TB were randomly assigned to Reg6M (n = 167) or Reg9M (n = 160) and monitored by determination of clinical, immunological, and bacteriological parameters for 36 months. Primary outcomes included favorable responses at the end of treatment and recurrences during follow-up, whereas the secondary outcome was death. Intent-to-treat and on-treatment analyses were performed. All patients were antiretroviral treatment–naive during treatment.

Measurements and Main Results: Of the patients, 70% had culture-positive pulmonary TB; the median viral load was 155,000 copies/ml and the $CD4^+$ cell count was 160 cells/mm^3. Favorable response to antituberculosis treatment was similar by intent to treat (Reg6M, 83% and Reg9M, 76%; P = not significant). Bacteriological recurrences occurred significantly more often in Reg6M than in Reg9M (15 vs. 7%; $P < 0.05$) although overall recurrences were not significantly different (Reg6M, 19% vs. Reg9M, 13%). By 36 months, 36% of patients undergoing Reg6M and 35% undergoing Reg9M had died, with no significant difference between regimens. All 19 patients who failed treatment developed acquired rifamycin resistance (ARR), the main risk factor being baseline isoniazid resistance.

Conclusions: Among antiretroviral treatment–naive HIV-infected patients with TB, a 9-month regimen resulted in a similar outcome at the end of treatment but a significantly lower bacteriological recurrence rate compared with a 6-month thrice-weekly regimen. ARR was high with these intermittent regimens and neither mortality nor ARR was altered by lengthening TB treatment.

Clinical Trials Registry Information: ID# NCT00376012 registered at www.clinicaltrials.gov.

Keywords: tuberculosis; HIV; short-course chemotherapy; recurrence; acquired rifamycin resistance

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

There is limited information about the outcome of HIV-infected patients with tuberculosis (TB) treated by short-course intermittent (thrice-weekly) regimens, and the efficacy of extending treatment to 9 months has not been studied. Further, acquired rifamycin resistance has been described with once- and twice-weekly TB treatment in the presence of HIV-associated immunodeficiency, but limited data are available on thrice-weekly regimens.

What This Study Adds to the Field

Our study shows that extending TB treatment to 9 months instead of 6 months did not improve favorable outcome at the end of treatment or mortality, but reduced bacteriological recurrences over 30 months. A high rate of acquired rifamycin resistance at the time of failure was noted irrespective of the length of treatment, with this thrice-weekly fully intermittent regimen in patients with advanced HIV disease, suggesting the need for daily treatment at least in the intensive phase.

Tuberculosis (TB) and HIV are the two leading infectious causes of death worldwide. TB, the commonest and often the first opportunistic infection to occur among HIV-infected persons, is associated with high mortality and morbidity (1–3). Current recommendations for the treatment of HIV-associated TB are similar to those for HIV-uninfected adults, using standardized short-course chemotherapy regimens, but, in general, daily treatment (at least in the intensive phase) is the preferred option (4, 5). In fact, the American Thoracic Society and Centers for Disease Control and Prevention recommendations were modified after reports of acquired rifamycin resistance in patients with low $CD4^+$ cell counts who received highly intermittent therapy in the intensive phase (5). In India, the recommended treatment for newly diagnosed smear-positive and seriously ill smear-negative pulmonary TB (PTB) or extrapulmonary TB (EPTB), including patients known to be HIV infected, is a fully intermittent thrice-weekly short-course regimen (category I, $2EHRZ_3/4HR_3$: ethambutol, 1,200 mg; isoniazid, 600 mg; rifampicin, 450 or 600 mg depending on body weight <60 or ≥ 60 kg; and pyrazinamide, 1,500 mg for 2 mo; followed by 4 mo of isoniazid and rifampicin at the same doses) (6). However, certain issues remain unanswered; namely, the optimal length of treatment and the safety and efficacy of using intermittent dosing of medications for HIV-associated TB (7).

Two previous studies using 6-month rifampicin-containing regimens among HIV-infected patients have reported cure rates at the end of treatment of 59 and 81%, respectively (8, 9). Recurrence rates described in various studies have ranged from 3 to 20% (10–13). However, differences in study design, measured outcomes, and follow-up times as well as lack of strict diagnostic criteria for TB recurrence suggest that these could be underestimates (13).

Our own experience with a pilot study of patients with advanced HIV and TB treated with the category I regimen documented poor outcomes with a cure rate of 72% at the end of treatment, a mortality rate of 35% and a TB recurrence rate of 39% during 2 years of follow-up (14). These patients were treated under program conditions, had advanced HIV disease, and no access to antiretroviral therapy (ART). We hypothesized that in this situation, lengthening treatment duration for TB would improve outcome by increasing cure and reducing recurrences. We therefore undertook a randomized controlled clinical trial to compare the efficacy of the standard 6-month versus a 9-month intermittent antituberculosis treatment (ATT) regimen among HIV-infected persons having pulmonary or extrapulmonary (lymphadenitis or pleural effusion) tuberculosis. Some of the results of these studies have been previously reported in the form of an abstract (15).

METHODS

The study was an open label, parallel arm, randomized controlled clinical trial conducted at the Tuberculosis Research Center (Chetput, Chennai, India) clinics in Chennai and Madurai, southern India with intake between February 2001 and September 2005. HIV-infected patients with symptoms and signs suggestive of TB, aged 15 years or above, not moribund and not pregnant were considered for enrollment. Baseline investigations included a chest X-ray (posteroanterior view), three sputum specimens (two overnight and one spot collection) for acid-fast bacilli (AFB) smear and mycobacterial culture and pleurocentesis or fine needle aspiration cytology/biopsy of lymph nodes when indicated. Sputum smears were stained with auramine–rhodamine and examined by fluorescence microscopy, processed by modified Petroff's method, and cultured on Lowenstein-Jensen medium (16). Positive cultures of *Mycobacterium tuberculosis* were graded as 1–20 colonies, 1+ (20–100 colonies), 2+ (>100 discrete colonies), or 3+ (>100 colonies forming a confluent mass). Species identification was done by high-performance liquid chromatography and drug susceptibility tested by the minimal inhibitory concentration method for isoniazid (H), rifampicin (R), and ethambutol (E) and by the resistance ratio method for streptomycin (S) (17, 18). Cultures were stored for subsequent DNA fingerprinting in the event of failure or recurrence; this was done by three molecular methods: IS*6110* analysis, mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing, and spacer oligonucleotide typing (spoligotyping). To avoid cross-contamination, extrapulmonary and sputum samples were processed separately, and only one tube was opened at a time with the remaining medium and specimen containers kept at least 12 cm away from aerosol-generating activities. A sudden increase in positive cultures, particularly among smear-negative samples with consecutive laboratory numbers, prompted a request for additional DNA fingerprinting.

Chest X-rays were read by a panel of three doctors and a consensus reading was determined. A tuberculin skin test was performed with 1 tuberculin unit of purified protein derivative of the RT23 strain (1 TU PPD RT23) and read after 48–72 hours (19). HIV testing was offered after pretest counseling by a social worker and after obtaining written informed consent. National guidelines concerning HIV testing (positive on three different tests) and related issues with respect to informed consent and confidentiality issues were followed. Other blood investigations included liver and renal function tests, plasma glucose, $CD4^+$ cell count, viral load, and complete blood counts. The diagnosis of pulmonary TB was based on a positive sputum smear or a radiographic lesion persisting for more than 14 days after antibiotics.

Extrapulmonary TB was diagnosed on the basis of cyto/histopathological (for lymph node) or biochemical (for pleural effusion) parameters, with or without a positive AFB smear. Patients admitted on clinical grounds were all retained in the trial and analyzed.

Patients diagnosed as having HIV and TB, and who met the clinical and sociological eligibility criteria as well as laboratory criteria of hemoglobin greater than 7 g/L, granulocyte count greater than 1.1×10^9/L, platelet count greater than 100×10^9/L, serum alanine aminotransferase concentration less than 2.5 times the upper limit of normal, serum creatinine concentration less than 1.1 mg%, and random blood sugar less than 140 mg/dl were enrolled. The randomized allocation sequence was generated by a computer. Block randomization was done, stratified by $CD4^+$ cell counts (>200 and ≤200 cells/mm³) and by smear grade (0, 1+ and 2+, 3+). The allocation codes, prepared by an independent statistician, were concealed in sealed, opaque envelopes and opened at a site away from the patient care facility. The study was approved by the institutional ethics committee and written informed consent was obtained from each patient.

Treatment and Follow-Up

All patients were treated with isoniazid (600 mg), rifampicin (450/600 mg for body weight < or ≥60 kg), pyrazinamide (1,500 mg), and ethambutol (1,200 mg), the same doses used in the national program. Treatment was initiated using all four drugs for the first 2 months (intensive phase), following which patients were randomized to receive either 4 or 7 months of H and R (same doses) in the continuation phase. Drugs were prescribed for thrice-weekly administration throughout. Hence, the two regimens used were Reg6M: $2EHRZ_3/4HR_3$ and Reg9M: $2EHRZ_3/7HR_3$. Patients were reviewed clinically every month and three sputum specimens (two overnight and one spot) were collected for smear and culture. Co-trimoxazole prophylaxis was given to all patients with $CD4^+$ cell count less than 250 cells/mm³. Patients were antiretroviral therapy naive at study entry and during TB treatment.

Monitoring Treatment and Adherence

The taking of all doses in the intensive phase was directly observed by study staff. During the continuation phase patients attended the clinic once per week, when they took drugs under supervision. Two doses were then handed over for self-administration and patients were counseled and motivated to take them regularly. Treatment cards were maintained by study nurses who signed when the patient took drugs under their supervision. If the patient defaulted for treatment, he/she was visited by a health visitor the same day. If he/she still did not attend, they were visited by a social worker and then by a physician, in that order. Attempts were made at each visit to understand the reason for default and corrective action was undertaken.

Study staff made surprise visits to patients' homes at least once in 15 days to do a pill count and to collect a urine sample for acetyl isoniazid testing whenever possible. If extra medications were found at home, this was recorded in the treatment card and counted as missed doses. Adherence was calculated, taking into account the pill count and home visit reports. If a patient complained of any symptoms, he/she was asked to see the study physician, who completed an adverse event form if indicated. Management, especially change of treatment as well as retreatment for TB, was decided by a panel of doctors (any three of S.S., G.N., I.S., S.R.K., P.A.M., and C.P.), statisticians, and bacteriologists who reviewed the case history and results of all the relevant investigations. All patients were followed up for a period of 36 months from study entry. Further details of methodology, investigations, and laboratory procedures during treatment and follow-up are provided in the online supplement.

Statistical Methods

The study was designed to test the hypothesis that the 9-month regimen would be superior to the 6-month regimen and would reduce unfavorable outcomes (failure and recurrence) from 20 to 10%. The estimate of 20% was obtained from a review of existing literature of short-course treatment of HIV/TB at the time of protocol development (8, 9, 13). With 80% power and 5% significance and taking in to account

a dropout of 10% per year, the number of patients required was estimated to be 150 per arm.

The outcome of treatment was considered "favorable" if all available (n = 6) sputum cultures were negative during the last 2 months of treatment, for culture-positive pulmonary TB. For extrapulmonary and culture-negative pulmonary TB, outcome was based on resolution of symptoms and signs and regression of lymph nodes and/or radiographic clearance, respectively.

Unfavorable outcomes during treatment included failure (bacteriological and clinical), death (all-cause mortality), and default (>1 mo)—whichever occurred first. Bacteriological failure was defined as at least two positive cultures during the last 2 months of treatment (at least one with a grade of >1+). Persistence or reappearance of symptoms such as fever, weight loss, and cough with or without radiographic deterioration prompted an evaluation of the case by a panel of doctors. Any permanent change of anti-TB treatment (including due to drug toxicity) was considered a "clinical failure." Recurrences were similarly classified as bacteriological or clinical based on sputum culture results. Recurrences were classified as exogenous reinfection if two or more differences in bands/spots/peaks were observed by any of the three genotypic methods used. An increase in size of a lymph node or extent of radiographic involvement without evidence of drug resistance or associated infection was called a paradoxical reaction.

Analysis

Primary analysis was by intent to treat (ITT), and efficacy (on-treatment) analysis was also performed (including only patients who had received more than 80% of prescribed chemotherapy). Patients in the two arms were compared for baseline demographic and laboratory characteristics. The chi square test was used to compare proportions, a *t* test (paired/unpaired) was used to compare means, and Wilcoxon's test was done to compare medians. Survival analysis was done by the Kaplan-Meier method and the log-rank test was used to compare the survival distribution. Univariate, followed by multivariate logistic regression analysis using the stepwise backward elimination method, was done to identify independent risk factors for acquired rifamycin resistance during treatment.

RESULTS

Of 857 patients screened for suitability to study, 334 patients (173 from Chennai and 161 from Madurai) were enrolled and randomized to one of the study regimens. There were seven exclusions after randomization: four patients had primary multidrug-resistant (MDR) TB, one had *Mycobacterium xenopi*, and two initiated nevirapine-based ART during treatment and had their TB regimen changed. The demographic profile of the 327 patients included in the modified ITT analysis is shown in Table 1 and that of 212 sputum culture–positive patients included in the on-treatment analysis in Table E1 (in the online supplement)—the subgroups analyzed separately had similar profiles. Most patients presented with cough (78%), fever (71%), and 42% had enlarged superficial lymph nodes. Two hundred and twenty-seven patients had *Mycobacterium tuberculosis* isolated from sputum; for 220 drug susceptibility results were available. Whereas 88% had organisms sensitive to all first-line anti-TB drugs, H resistance was present in 23 patients (10%) and E resistance in 13 patients (6%). Outcomes are presented separately for patients with culture-positive, culture-negative, and extrapulmonary TB (Figure 1) and also for patients with isoniazid-susceptible and -resistant *M. tuberculosis* at baseline (Table 2).

Intent-to-treat Analysis

Figure 1 shows the progression of cases from recruitment to end of follow-up (36 mo). One hundred and thirty-eight of 167 patients (83%) in Reg6M and 122 of 160 (76%) in Reg9M had a favorable outcome (risk ratio [RR], 1.08; 95% confidence interval [95% CI], 0.97–1.21; *P* = 0.15). Details of bacteriological failures in the two regimens are provided in Table E2 (in the online supplement) With ATT, patients improved clinically and there was a statistically significant increase in body weight and hemoglobin at the end of treatment, while the CD4$^+$ cell

TABLE 1. PRETREATMENT CHARACTERISTICS OF PATIENTS IN INTENT-TO-TREAT ANALYSIS

Characteristic of Study Subjects	Reg6M (*n = 167*)	Reg9M (*n = 160*)
Median age, years (IQR)	33 (29–38)	33 (29–39)
Median weight, kg (IQR)	44 (39–50)	44 (39–50)
Median CD4$^+$ cells/mm^3 (IQR)	152 (80–304)	167 (88–280)
Median viral load, copies/ml (n = 213)	94,300 (n = 100)	168,000 (n = 113)
Males, %	79	75
CD4$^+$ cell count <200 cells/mm^3, %	63	64
Mantoux ≥5 mm, %	48	53
Mantoux >10 mm, %	41	46
Pulmonary TB (n = 299)		
Culture positive	117 (78%)	110 (74%)
Culture negative	34	38
Extrapulmonary TB (n = 28)		
Culture positive	4	2
Culture negative	12	10
Drug Susceptibility Pattern	(*n = 112*)	(*n = 108*)
Susceptible to all first-line drugs, n (%)	99 (88)	95 (88)
Resistant to isoniazid alone, n (%)	5 (4)	5 (5)
Resistant to isoniazid and ethambutol or streptomycin, n (%)	8 (7)	8 (7)
Radiographic Features in Sputum Culture–positive Pulmonary TB (n = 227)*	(*n = 117*)	(*n = 110*)
Normal, n (%)	16 (14)	13 (12)
Parenchymal opacities, n (%)	69 (59)	70 (64)
Pleural effusion, n (%)	17 (15)	13 (12)
Hilar adenopathy, n (%)	29 (25)	15 (14)
Miliary TB, n (%)	7 (6)	7 (6)
Cavities, n (%)	19 (16)	20 (18)
Others, n (%)	10 (9)	7 (6)

Definition of abbreviations: IQR = interquartile range; Reg6M = 6-month regimen; Reg9M = 9-month regimen; TB = tuberculosis.
Number of patients (n) = 327.
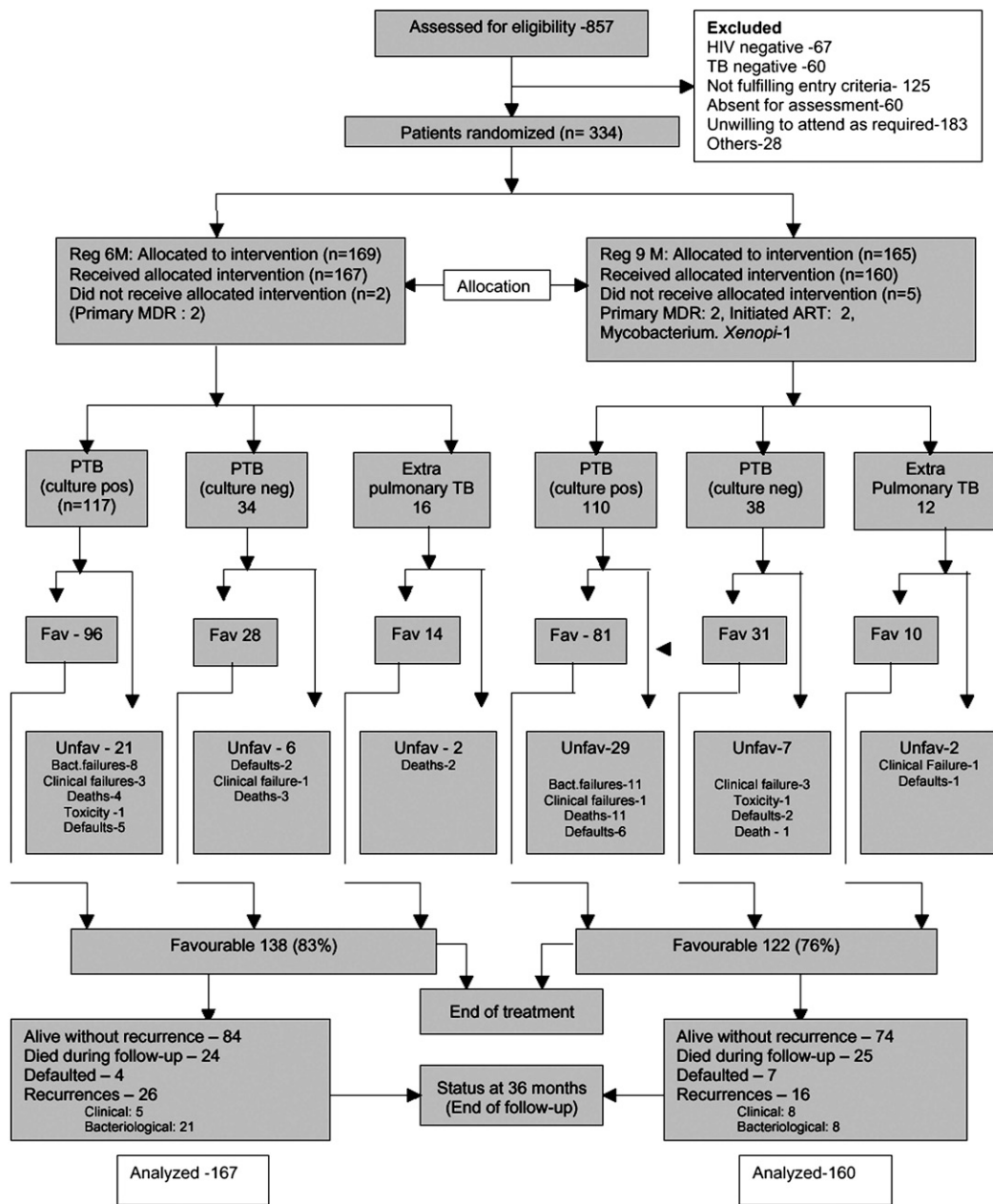* Some patients had more than one type of lesion.

Figure 1. Trial profile. ART = antiretroviral therapy; Bact. = bacteriological; Fav = favorable; MDR = multidrug resistant; PTB = pulmonary tuberculosis; TB = tuberculosis; Unfav = unfavorable.

count and viral load did not show any significant change. Table 2 shows the details of failures among culture-positive pulmonary TB by regimen and baseline drug susceptibility status. One patient in Reg6M and three patients in Reg9M had exogenous reinfection, and three isolates in Reg6M and one isolate in Reg9M were not typed. Among the 220 patients with baseline drug susceptibility results available, 81% of patients with isoniazid-susceptible organisms had a favorable outcome compared with 44% of those with isoniazid resistance (odds ratio [OR], 5.6; 95% CI, 2.11–15.15; $P < 0.001$).

Among patients who had a favorable outcome at the end of treatment, there were 21 (15%) bacteriologically confirmed recurrences in Reg6M and 8 (7%) in Reg9M, with recurrence rates of 7.99/100 and 3.85/100 person-years, respectively (RR, 2.07; 95% CI, 1.33–3.23; $P = 0.03$). The detailed profile of

patients with bacteriologically confirmed recurrences in the intent-to-treat population is described in Table 3. Figure 2 is a Kaplan-Meier curve showing recurrence-free survival over 27–30 months of follow-up among patients who successfully completed treatment. Twenty-one recurrences (72%) occurred within 12 months of treatment completion and 6 were with rifampicin-resistant isolates. Overall, 65% of typeable recurrences were due to exogenous reinfection (4 of 5 in Reg9M and 7 of 12 in Reg6M; Table 4). Further, there were five recurrences diagnosed clinically (one PTB, four EPTB) in Reg6M and eight in Reg9M (four PTB, four EPTB) and details of these are provided in Table E3 (in the online supplement). When overall recurrence rates were compared, there was no significant difference between regimens (Reg6M, 19% vs. Reg9M, 13%; $P = 0.2$). Most patients either had low initial CD4^{+} cell counts

TABLE 2. OUTCOME (INTENT TO TREAT) AT END OF TREATMENT OF ALL PATIENTS WITH CULTURE-POSITIVE ISOLATES AND DRUG SUSCEPTIBILITY RESULTS, BY REGIMEN AND BY BASELINE ISONIAZID SUSCEPTIBILITY STATUS

			Reg6M (n = 112)		Reg9M (n = 108)		Total (n = 220)	
	Reg6M (Overall) (n = 112)	Reg9M (Overall) (n = 108)	H Sensitive (n = 100)	H Resistant (n = 12)	H Sensitive (n = 97)	H Resistant (n = 11)	H Sensitive (n = 197)	H Resistant (n = 23)
Favorable	91 (81%)	79 (73%)	85	6	75	4	160 (81%)	10 (44%)
Unfavorable	21 (19%)	29 (23%)	15	6	22	7	37 (19%)	13 (56%)
Bacteriological failure	8*	11*	3	5	7	4	10	9
Clinical failure	3	1	3	0	1	0	4	0
Death	4	11	3	1	10	1	13 (6.5%)	2 (8.7%)
Toxicity	1	0	1	0	0	0	1	0
Defaulted	5	6	5	0	4	2	9	2

Definition of abbreviation: H = isoniazid.
Number of patients (n) = 220.
* One patient in Reg6M and three in Reg9M had exogenous reinfection confirmed by DNA fingerprinting: DNA fingerprinting data were not available for three patients in Reg6M and one patient in Reg9M.

or demonstrated a fall at the time of recurrence (Table 3; and *see* Table E3).

On-treatment Analysis

For the efficacy (on-treatment) analysis, there were 27 exclusions (7 specified earlier, 1 death within 15 d, and 19 with <80% drug adherence). One hundred and fifty-seven patients in Reg6M and 150 in Reg9M qualified, with a mean drug adherence of 97 and 96%, respectively. Of the 212 patients with culture-positive TB who qualified for this analysis, sputum smear conversion at the end of the second month was observed

in 67% and culture conversion in 87%. There was no difference in favorable outcome at the end of treatment between the two regimens (Reg6M, 85% and Reg9M, 78%; P = not significant). Among those with culture-negative and extrapulmonary TB, 87% each in Reg6M and 86 and 91% in Reg9M had a favorable response, respectively. Overall, of 307 patients, 135 (86%) in Reg6M and 121 (81%) in Reg9M had a favorable response, which was not significantly different (RR, 1.07; 95% CI, 0.96–1.18; P = 0.2).

There were a total of 19 bacteriological failures during treatment—8 in Reg6M and 11 in Reg9M. All of them had

TABLE 3. PROFILE OF PATIENTS WITH BACTERIOLOGICALLY CONFIRMED RECURRENCES AMONG THOSE IN INTENT-TO-TREAT ANALYSIS

Subject No.	Regimen	Age (yr)	Weight (kg)	Overall Adherence (%)	Month of Recurrence	CD4+ Cell Count at Baseline (cells/mm³)	CD4+ Cell Count at Recurrence (cells/mm³)	Viral Load at Baseline (copies/ml)	Drug Resistance Pattern at Baseline	Drug Resistance Pattern at Recurrence	DNA Fingerprint Pattern	Outcome of Recurrence
1	Reg6M	27	56	86	12	195	56	540,000	Nil	Nil	Exo	Died
2	Reg6M	23	41	100	17	56	144	44,600	Nil	Nil	NA	Cured
3	Reg6M	42	59	100	1	260	150	NA	Nil	H	NA	Cured
4	Reg6M	43	34	99	2	24	68	NA	Nil	Nil	Exo	Died
5	Reg6M	32	38	99	12	63	126	31,300	Nil	HR	NA	Died
6	Reg6M	35	45	94	7	420	242	32,400	Nil	Nil	NA	Died
7	Reg6M	25	34	100	8	343	66	135,000	H	H	Endo	Died
8	Reg6M	30	48	100	27	135	66	NA	Nil	Nil	Exo	Died
9	Reg6M	29	52	100	10	132	91	242,000	Nil	Nil	Exo	Died
10	Reg6M	35	50	100	16	187	147	426,000	H	H	Endo	Cured
11	Reg6M	27	44	100	2	286	290	NA	Nil	Nil	Exo	Cured
12	Reg6M	37	37	99	7	75	192	NA	Nil	HR	NA	Died
13	Reg6M	30	55	97	24	308	NA	NA	Nil	Nil	Endo	Cured
14	Reg6M	30	46	100	18	357	NA	NA	Nil	Nil	Exo	Died
15	Reg6M	36	30	97	4	82	138	617,000	Nil	R	NA	Died
16	Reg6M	32	54	100	15	243	242	16,000	Nil	Nil	Endo	Cured
17	Reg6M	29	47	97	6	479	104	76,900	Nil	Nil	Endo	Cured
18	Reg6M	35	52	100	5	484	107	NA	Nil	Nil	NA	Died
19	Reg6M	38	40	89	5	82	148	95,300	Nil	R	NA	Cured
20	Reg6M	29	41	100	11	225	168	14,800	Nil	Nil	Exo	Cured
21	Reg6M	30	44	97	5	224	49	NA	NA	HR	NA	Died
1	Reg9M	27	42	96	8	189	60	750,000	Nil	H	Exo	Died
2	Reg9M	33	39	94	22	105	64	96,400	Nil	Nil	Exo	Cured
3	Reg9M	63	50	100	9	136	132	15,100	Nil	Nil	Exo	Cured
4	Reg9M	32	39	98	29	154	26	22,300	Nil	Nil	Exo	Died
5	Reg9M	30	34	100	2	154	NA	209,000	Nil	Nil	Endo	Died
6	Reg9M	28	40	94	11	39	45	342,000	S	Nil	NA	Cured
7	Reg9M	50	26	100	2	20	130	750,000	Nil	Nil	NA	Died
8	Reg9M	40	41	100	5	173	102	166,000	Nil	R	NA	Cured

Definition of abbreviations: NA, not available; R = rifampicin; S = streptomycin.
Number of patients (n) = 327.
Note: Culture grading is described in Methods. DNA fingerprint pattern: Exo = exogenous reinfection; Endo = endogenous reactivation.
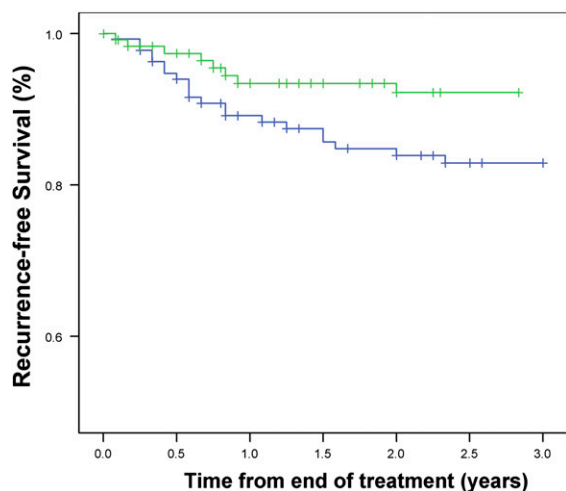
Figure 2. Kaplan-Meier plot showing time to bacteriological recurrence up to 36 months for patients in the 6-month regimen (Reg6M; *blue line*) and in the 9-month regimen (Reg9M; *green line*); $P = 0.03$. Data censored at 36 months.
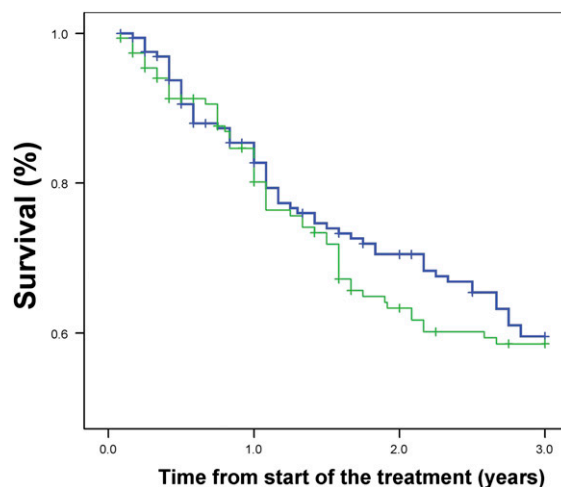

Figure 3. Kaplan-Meier plot showing time to death for patients in the 6-month regimen (Reg6M; *blue line*) and in the 9-month regimen (Reg9M; *green line*); $P = 0.17$ (not significant). Data censored at 36 months.

acquired rifamycin resistance—15 patients had developed MDR TB, whereas 4 had monoresistance to rifampicin. Nine of these patients had isolates with initial isoniazid resistance. DNA fingerprinting revealed exogenous reinfection in 4 of the 19 failures—all were hospitalized due to advanced HIV for a prolonged period, possibly leading to cross-infection. Outcome of retreatment was poor, with only three patients getting cured. Table 5 shows that patients who developed ARR had more advanced HIV disease, a higher rate of baseline H resistance, and suboptimal adherence. Multivariate logistic regression analysis showed that the only significant risk factor for development of ARR was baseline isoniazid resistance (Table 6).

Bacteriological recurrences were observed in 19 patients in Reg6M (20%) and in 7 patients in Reg9M (9%) (RR, 2.23; 95% CI, 1.04–5.27; $P = 0.03$), with recurrence rates of 10.67/100 and 5.12/100 person-years, respectively. *M. tuberculosis* isolates from 17 of these patients (65%) were drug sensitive.

Deaths

Overall, 116 deaths (35%) occurred among 327 patients (including death as the primary event as well as patients dying after failure or recurrence). In Reg6M, 15 deaths occurred during treatment (5 TB, 10 non-TB) and 45 during follow-up (12 TB, 33 non-TB). In Reg9M, there were 19 deaths during treatment (9 TB, 10 non-TB) and 37 (10 TB, 27 non-TB) during the follow-up phase. Apart from patients with death as the first event shown in Figure 1, 15 of 20 defaulters, 15 of 19 failures, and 16 of 27 recurrences also eventually died. Deaths were classified as non-TB if there was no bacteriological proof or clinical suspicion of TB and an alternative HIV-associated condition/opportunistic infection was identified. Causes included neurological complications of HIV, *Pneumocystis jiroveci* pneumonia, chronic diarrhea, and AIDS wasting syndrome. There was no statistically significant difference in overall mortality between the study regimens: 36 and 35% of patients (Figure 3). However, there was a significant difference in survival by immune status. Patients with initial CD4$^+$ cell count less than 200 cells/mm^3 had a mortality of 25.2/100 person-years compared with 7.1/100 person-years among the greater than 200 cells/mm^3 group (RR, 3.54; 95% CI, 2.15–5.77; $P < 0.001$).

Toxicity

Sixty-seven patients, out of 327, experienced 76 adverse drug reactions with no additional toxicity due to the extended regimen (Table 7). Two patients (one in each regimen) had a permanent change of regimen due to cutaneous intolerance (one in Reg6M attributable to Z and one in Reg9M because of Z and R). Four patients had treatment temporarily modified because of abnormal liver function tests and received streptomycin, ethambutol, and ofloxacin daily until the liver function parameters returned to normal, when the trial regimen was reintroduced (within 1 mo). One patient had a reduction in dose of Z to 1,000 mg because of gastritis. Sixty patients had minor adverse reactions: gastrointestinal (45%), cutaneous (28%), peripheral neuropathy (12%), and others (15%). All these were symptomatically managed without any alteration in the trial regimen.

Antiretroviral Therapy

All patients in analysis were ART naive during TB treatment. Suspected paradoxical reactions were observed in three patients, presenting as enlarging lymphadenitis in two and radiographic deterioration in one. During follow-up, 24 patients in Reg6M and 22 in Reg9M began ART with a three-drug generic fixed-dose combination containing zidovudine or stavudine with lamivudine and nevirapine. The median month of ART initiation after TB treatment was 16 months in Reg6M and 17 months in Reg9M and the person-years of time spent on ART was similar in the two arms. None of the patients who had a recurrence were receiving ART at that time.

TABLE 4. COMPARISON AND CATEGORIZATION OF CULTURE-CONFIRMED RECURRENCES BY STUDY ARM IN SPUTUM CULTURE–POSITIVE PATIENTS

	Reg6M (n = 117)	Reg9M (n = 110)
Favorable outcome at end of treatment	96	81
Bacteriological recurrences	19 (20%)	7 (7%)
Exogenous reinfection	7 (9%)	3 (4%)
Endogenous reactivation	4 (4%)	1 (1%)
DNA fingerprinting not available	8	3

Number of patients (n) = 227.

TABLE 5. PRETREATMENT CHARACTERISTICS OF PATIENTS WITH AND WITHOUT ACQUIRED RIFAMPICIN RESISTANCE AT FAILURE

	Without ARR (*n* = 193)	With ARR (*n* = 19)	*P* Value
Weight (kg), mean ± SD	43.4 ± 8.2	43.3 ± 9.7	0.96
Median CD4$^+$ cell count, cells/mm^3 (IQR)	165 (86–297)	101 (54–185)	0.054
Hemoglobin (g%), mean ± SD	10.0 ± 2.5	9.0 ± 2.4	0.13
Median viral load, copies/ml (IQR)	144,000 (33,825–500,000)	355,000 (192,000–629,000)	0.009
Sputum smear grade 2+ or more, %	29	42	0.257
Pretreatment H resistance, %	6	47	0.000
Adherence <90%, %	15	47	0.000

Definition of abbreviation: ARR = acquired rifampicin resistance.
Number of patients (n) = 212.

DISCUSSION

In this randomized comparison of 6-month and 9-month thrice-weekly antituberculosis regimens among ART-naive patients coinfected with HIV and TB, favorable outcomes at the end of treatment and death rates were similar but culture-confirmed recurrences were fewer among patients receiving the longer regimen. Whereas the standard 6-month regimen had a favorable outcome of 83% at the end of the treatment, patients in the 9-month regimen had a slightly lower response of 76%. Although the reasons for this are not entirely clear, these patients had an additional 3 months of time at risk for events unrelated to the efficacy of TB treatment and more related to AIDS-associated morbidity and mortality. Unfavorable outcomes to treatment in our study were due mainly to deaths (7%) and failures (9%). Although weight and hemoglobin improved with ATT, as in previous studies, we observed no significant change in CD4$^+$ cell count or viral load (14, 20).

The ideal duration of ATT among HIV-infected patients is still a debatable issue and has policy implications for several countries including India. Current recommendations for TB treatment among individuals with HIV/AIDS are for standard 6-month regimens with extension of treatment to 9 months for patients with extrapulmonary disease or a delayed response (4, 5, 21). Several retrospective cohort analyses have documented higher recurrence rates with rifampicin-containing regimens of less than 6 months' duration and when ATT drugs are given intermittently during the intensive phase (22–24). However, no trial has previously compared 6-month and 9-month fully thrice-weekly regimens. Our trial establishes that extending the treatment duration reduces bacteriological recurrences by half among HIV-infected patients, even though favorable outcomes at the end of treatment did not improve.

Recurrences of tuberculosis in HIV-infected patients are due to higher rates of both reinfection and reactivation; the relative proportion of each is influenced by the background rate of TB in the community, socioeconomic factors, as well as the adequacy of the regimen (24). In the present trial, about two thirds of recurrences (that could be fingerprinted) were due to exogenous reinfection. Extending treatment appears to have

reduced reactivation and most recurrences in the 9-month arm were due to reinfection, but we could not explain why they occurred early after stopping treatment. We did note that patients who developed a recurrence also had a fall in CD4$^+$ cell count—whether this was a cause or effect of TB is difficult to determine. We have described our experience with DNA fingerprinting among HIV-infected and uninfected patients with TB in more detail separately, highlighting the differences between the two groups, but it is clear that the majority of recurrences among HIV-infected patients in this region are due to reinfection (25). Similar findings of reinfection accounting for 69% recurrences among HIV-infected gold miners have been reported from South Africa (26). Strategies to reduce recurrence rates include longer treatment regimens, posttreatment isoniazid prophylaxis, and antiretroviral therapy to improve immune status (22, 27, 28).

Mortality was similar between the two regimens, with 35% of the cohort dead by 36 months, many due to AIDS-related illnesses rather than TB. Extending treatment by 3 months did not improve survival. Patients who defaulted, failed treatment, or had a recurrence had higher mortality. Only one fifth of the patients in this trial had access to ART, as it became freely available through the national program only after April 2004. That immune status has a major impact on survival is well known and is also demonstrated by the 3.5-fold higher death rate among patients with baseline CD4$^+$ cell count less than 200 cells/mm^3 in our study (9, 22, 29). The use of ART during TB treatment has been associated with faster sputum conversion and lower mortality and is now recommended for all TB/HIV patients with CD4$^+$ cell counts less than 350 cells/mm^3 (22, 30).

Our observation that all 19 patients with bacteriological failure during treatment had emergence of rifampicin resistance is of great concern. The main risk factor was pretreatment isoniazid resistance; suboptimal adherence to treatment also played a role. Although the dosage and schedule of adminis-

TABLE 6. MULTIVARIATE LOGISTIC REGRESSION ANALYSIS OF RISK FACTORS FOR ACQUIRED RIFAMPICIN RESISTANCE

Risk Factor	Exp (B)	Significance	Adjusted Odds Ratio	95% CI
H resistance	2.13	0.002	8.43	2.19–32.47
CD4$^+$ cell count <150 cells/mm^3	0.812	0.241	2.25	0.58–8.78
Viral load >30,000 copies/ml	0.998	0.370	2.71	0.30–23.98
Adherence <90%	1.155	0.107	3.17	0.89–14.38

Definition of abbreviations: Exp (B) = exponential of beta; 95% CI = 95% confidence interval.

TABLE 7. TOXICITY PROFILE DURING ANTITUBERCULOSIS TREATMENT, REGIMEN-WISE

Type of Toxicity	Reg6M (*n* = 167)	Reg9M (*n* = 160)
Arthralgia	3	6
Elevated liver enzymes	2 (2*)	0
Jaundice	3 (2*)	0
Abdominal pain/nausea/vomiting	18	13 (1[†])
Peripheral neuropathy	3	5
Rashes, itching	8 (1[‡])	13 (1[§])
Dizziness	2	0
Any toxicity	39 (23%)	37 (23%)

Number of patients (n) = 327.
* H, R, and Z (pyrazinamide) temporarily withheld.
[†] Z dose reduced.
[‡] Z permanently discontinued.
[§] Z and R permanently discontinued.

tration used in this study were those recommended by the Indian TB control program, the presence of HIV enteropathy and malabsorption leading to subtherapeutic levels could have been contributory factors (31). ARR has been reported in HIV-infected patients given highly intermittent (once- or twice-weekly) rifamycin regimens for treatment or prophylaxis (23, 32, 33). In view of this, recommendations are to treat patients who have $CD4^+$ cell counts less than 100 cells/mm^3 with daily or thrice-weekly treatment (5, 23). Ours is the first prospective study to report the development of ARR in patients treated with thrice-weekly rifampicin. When outcomes were analyzed by baseline drug susceptibility status, it was clear that patients with pretreatment isoniazid resistance had much higher failure rates, a phenomenon described in HIV-uninfected patients as well (34). Thus, for HIV-infected patients without access to ART, it appears preferable to treat tuberculosis with a daily regimen, at least during the intensive phase. With baseline isoniazid resistance levels of 15–20% in the community as well as the advanced state of immunodeficiency in which most patients present, the risk of MDR TB increasing in this population is real (35).

Both study regimens were well tolerated and had similar rates and profiles of adverse reactions. One quarter of our study patients experienced an adverse reaction but 92% of the adverse events were mild and manageable without any modification of ATT. Although some earlier studies found no difference in adverse events between HIV-infected and un-infected patients, some did suggest that the incidence or the grade of adverse reactions was higher in the former (9, 10, 22, 36).

The strengths of our study include a representative patient population with detailed characterization, excellent treatment compliance and follow-up. Further, both ITT and on-treatment analyses gave consistent results. The major limitation was the nonavailability of ART, partly accounting for the high mortality and recurrence rates observed and limiting the generalizability of our findings in the ART era. Further, patients were fairly heterogeneous in nature and those with clinical suspicion of TB were given the benefit of doubt, started on ATT, and considered as clinical recurrences. In addition, physicians who made these decisions were aware of the study assignment. DNA finger-printing results were not available for all patients with failure or recurrence, limiting our ability to analyze this fully. Intake to the study took a little over 4 years because of the strict inclusion and exclusion criteria and relatively low prevalence in this region of HIV among patients with TB (2–8%). Inadequacy of "thrice-weekly" regimens among HIV-infected patients with TB had not been unequivocally demonstrated at the time this trial was ongoing and the study was periodically reviewed by the institutional ethics committee. Other limitations include the use of multiple comparisons within the trial and the limited power of the regression analysis to assess all risk factors for ARR. Finally, because we wanted to mimic the Revised National TB Control Program (RNTCP) regimen, only one of three doses in the continuation phase was supervised; however, we were able to ascertain compliance in all patients.

In conclusion, we have shown that a 9-month intermittent thrice-weekly regimen halved bacteriological recurrences compared with a 6-month regimen but did not increase favorable outcomes at the end of treatment or survival at 36 months. Weakness of intermittent regimens in HIV-infected patients was demonstrated by the emergence of ARR, particularly in those with baseline isoniazid resistance. Recurrences were caused predominantly by reinfection and approximately three fourths occurred within 12 months of treatment completion. Our findings have implications for programs using short-course,

intermittent regimens for the treatment of TB. Although a longer duration of ATT could provide protection against TB recurrences, daily treatment at least in the intensive phase is required to prevent the development of drug resistance in patients without access to ART.

References

1. Swaminathan S, Ramachandran R, Bhaskar R, Ramanathan U, Prabhakar R, Datta M. Risk of development of tuberculosis in HIV-infected individuals in India. *Int J Tuberc Lung Dis* 2000;4:839–844.
2. Kumarasamy N, Solomon S, Flanigan TP, Hemalatha R, Thyagarajan SP, Mayer KH. Natural history of human immunodeficiency virus disease in southern India. *Clin Infect Dis* 2003;36:79–85.
3. Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, Dye C. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Intern Med* 2003;163:1009–1021.
4. Onyebujoh PC, Ribeiro I, Whalen CC. Treatment options for HIV-associated tuberculosis. *J Infect Dis* 2007;196:S35–S45.
5. American Thoracic Society, Centers for Disease Control and Prevention, Infectious Diseases Society of America. Treatment of tuberculosis. *Am J Respir Crit Care Med* 2003;167:603–662; reprinted in *J Infect Dis* 2007;196:S35–S45. *MMWR Recomm Rep* 2003;52:1–77.
6. Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare. TB India 2008—RNTCP status report. Available from http://www.tbcindia.org/pdfs/TB-India-2008.pdf (accessed December 27, 2009).
7. Havlir DV, Getahun H, Sanne I, Nunn P. Opportunities and challenges for HIV care in overlapping HIV and TB epidemics. *JAMA* 2008;23:423–430.
8. Kassim S, Sassan-Morokro M, Ackah A, Abouya LY, Digbeu H, Yesso G, Coulibaly IM, Coulibaly D, Whitaker PJ, Doorly R, et al. Two-year follow-up of persons with HIV-1 and HIV-2 associated pulmonary tuberculosis treated with short course chemotherapy in West Africa. *AIDS* 1995;9:1185–1191.
9. Chaisson RE, Clermont HC, Holt EA, Cantave M, Johnson MP, Atkinson J, Davis H, Boulos R, Quinn TC, Halsey NA. Six-month supervised intermittent tuberculosis therapy in Haitian patients with and without HIV. *Am J Respir Crit Care Med* 1996;154:1034–1038.
10. Perriëns JH, St Louis ME, Mukadi YB, Brown C, Prignot J, Pouthier F, Portaels F, Willame JC, Mandala JK, Kaboto M, et al. Pulmonary tuberculosis in HIV infected patients in Zaire. *N Engl J Med* 1995;332:779–784.
11. Driver CR, Li MJ, Kundamal N, Osahan SS. Relapse in persons treated for drug susceptible tuberculosis in a population with high co-infection with human immunodeficiency virus in New York City. *Clin Infect Dis* 2001;33:1762–1769.
12. Sonnenberg P, Murray J, Glynn JR, Shearer S, Kambashi B, Godfrey-Faucett P. HIV-1 and recurrence, relapse and reinfection of tuberculosis

after cure: a cohort study in South African mineworkers. *Lancet* 2001; 358:1687–1693.

13. El- Sadr WM, Perlman PC, Denning E, Matts JP, Cohn DL. A review of efficacy studies of 6-month short course therapy for tuberculosis among patients infected with human immunodeficiency virus: difference in study outcomes. *Clin Infect Dis* 2001;32:623–632.

14. Swaminathan S, Deivanayagam CN, Rajasekaran S, Venkatesan P, Padmapriyadarsini C, Menon PA, Ponnuraja C, Dilip M. Long-term follow up of HIV-infected TB patients treated with 6-month intermittent short course chemotherapy. *Natl Med J India* 2008;21:3–8.

15. Swaminathan S, Iliayas S, Padmapriyadarsini C, Rajasekaran S, Mohan V, Ponnuraja C, Venkatesan P, Ramachandran R, Paramasivan C, Kumar RS, *et al.* Randomized clinical trial of 6-month versus 9-month anti-tuberculosis treatment in HIV⁺ individuals with pulmonary tuberculosis [Abstract 141]. Presented at the 12th Conference on Retroviruses and Opportunistic Infections, February 22–25, 2005, Boston, Massachusetts.

16. Petroff SA. A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and faeces. *J Exp Med* 1915; 21:38–42.

17. Allen B, Baker FJ. Mycobacteria: isolation, identification and sensitivity testing. London: Butterworth; 1968.

18. Canetti G, Fox W, Khomenko A, Mahler HT, Menon NK, Mitchison DA, Rist N, Smelev NA. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. *Bull World Health Organ* 1969;41:21–43.

19. Chadha VK, Jagannatha PS, Vaidyanathan PS, Jagota P. PPD RT23 for tuberculin surveys in India. *Int J Tuberc Lung Dis* 2003;7:172–179.

20. Morris L, Martin DJ, Bredell H, Nyoka SN, Sacks L, Pendle S, Page-Shipp L, Karp CL, Sterling TR, Quinn TC, *et al.* Human immunodeficiency virus-1 RNA levels and CD4 lymphocyte counts, during treatment for active tuberculosis, in South African patients. *J Infect Dis* 2003;187:1967–1971.

21. Pozniak AL, Miller RF, Lipman MCI, Freedman AR, Ormerod LP, Johnson MA, Collins S, Lucas SB. BHIVA treatment guidelines for TB/HIV infection, February 2005. Available from http://www.bhiva.org/documents/Guidelines/TB/TB_HIV_FINAL2005.pdf (accessed December 27, 2009).

22. Nahid P, Gonzalez LC, Rudoy I, de Jong BC, Unger A, Kawamura LM, Osmond DH, Hopewell PC, Daley CL. Treatment outcomes of patients with HIV and tuberculosis. *Am J Respir Crit Care Med* 2007;175:1196–1206.

23. Li J, Munsiff SS, Driver CR, Sackoff J. Relapse and acquired rifampin resistance in HIV-infected patients with tuberculosis treated with rifampin- or rifabutin-based regimens in New York City, 1977–2000. *Clin Infect Dis* 2005;41:83–91.

24. Korenromp EL, Scano F, Williams BG, Dye C, Nunn P. Effects of human immunodeficiency virus infection on recurrence of tuberculosis after rifamycin based treatment: an analytical review. *Clin Infect Dis* 2003;37:101–112.

25. Narayanan S, Swaminathan S, Supply P, Shanmugam S, Narendran G, Hari L, Ramachandran R, Locht C, Jawahar MS, Narayanan PR. Impact of HIV infection on the recurrence of tuberculosis in South India. *J Infect Dis* (In press)

26. Charalambous S, Grant AD, Moloi V, Warren R, Day JH, van Helden P, Hayes RJ, Fielding KL, De Cock KM, Chaisson RE, *et al.* Contribution of reinfection to recurrent tuberculosis in South African gold miners. *Int J Tuberc Lung Dis* 2008;12:942–948.

27. Churchyard GJ, Fielding K, Charalambous S, Day JH, Corbett EL, Hayes RJ, Chaisson RE, De Cock KM, Samb B, Grant AD. Efficacy of secondary isoniazid preventive therapy among HIV-infected southern Africans: time to change policy? *AIDS* 2003;17:2063–2070.

28. Fitzgerald DW, Desvarieux M, Severe P, Joseph P, Johnson WD Jr, Pape JW. Effect of post-treatment isoniazid on prevention of recurrent tuberculosis in HIV-1-infected individuals, a randomized trial. *Lancet* 2000;356:1470–1474.

29. Harries AD, Hargreaves NJ, Jindani A, Enarson DA, Meher D, Salaniponi FM. Deaths from tuberculosis in sub-Saharan African countries with a high prevalence of HIV-1. *Lancet* 2001;357:1519–1523.

30. WHO 2006: Antiretroviral therapy for HIV infection in adults and adolescents. Accessed on 31 December 2008. Available from http://www.who.int/hiv/pub/guidelines/artadultguidelines.pdf

31. Gurumurthy P, Ramachandran G, Hemanth Kumar AK, Rajasekaran S, Padmapriyadarsini C, Swaminathan S, Bhagavathy S, Venkatesan P, Sekar L, Mahilmaran A, *et al.* Decreased bioavailability of rifampicin and other antituberculosis drugs in patients with advanced human immunodeficiency virus disease. *Antimicrob Agents Chemother* 2004; 48:4473–4475.

32. Burman W, Benator D, Vernon A, Khan A, Jones B, Silva C, Lahart C, Weis S, King B, Mangura B, *et al.*; Tuberculosis Trials Consortium. Acquired rifamycin resistance with twice weekly treatment of HIV-related tuberculosis. *Am J Respir Crit Care Med* 2006;173:350–356.

33. Vernon A, Burman W, Benator D, Khan A, Bozeman L. Acquired rifamycin mono-resistance in patients with HIV-related tuberculosis treated with once weekly rifapentine and isoniazid. *Lancet* 1999;353:1843–1847.

34. Lew W, Pai M, Oxlade O, Martin D, Menzies D. Initial drug resistance and tuberculosis treatment outcomes: systematic review and meta-analysis. *Ann Intern Med* 2008;149:123–134.

35. Paramasivan CN, Venkataraman P. Drug resistance in tuberculosis in India. *Indian J Med Res* 2004;120:377–386.

36. Breen RA, Miller RF, Gorsuch T, Smith CJ, Schwenk A, Holmes W, Ballinger J, Swaden L, Johnson MA, Cropley I, *et al.* Adverse events and treatment interruption in tuberculosis patients with and without HIV co-infection. *Thorax* 2006;61:791–794.