Duration of efficacy of treatment of latent tuberculosis infection in HIV-infected adults

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Background: Treatment of latent infection is needed to protect HIV-infected individuals against tuberculosis. A previous report addressed short-term efficacy of three regimens in HIV-infected adults. We now report on long-term efficacy of the study regimens.

Methods: Three daily self-administered regimens were compared in a randomized placebo-controlled trial in 2736 purified protein derivative (PPD)-positive and anergic HIV-infected adults. PPD-positive subjects were treated with isoniazid (INH) for 6 months (6H), INH plus rifampicin for 3 months (3HR), INH plus rifampicin and pyrazinamide for 3 months (3HRZ), or placebo for 6 months. Anergic subjects were randomized to 6H or placebo.

Results: 6H initially protected against tuberculosis in PPD-positive individuals; however, benefit was lost within the first year of treatment. Sustained benefit was observed in persons receiving 3HR and 3HRZ. In a Cox regression analysis, the adjusted relative risk for tuberculosis compared with placebo was 0.67 [95% confidence interval (CI), 0.42–1.07] for 6H, 0.49 (95% CI, 0.29–0.82) for 3HR, and 0.41 (95% CI, 0.22-0.76) for 3HRZ. When the rifampicin-containing regimens were combined, the adjusted relative risk for tuberculosis compared with placebo was 0.46 (95% CI, 0.29–0.71). Among anergic subjects, a modest degree of protection with 6H was present (adjusted relative risk, 0.61; 95% CI, 0.32–1.16). Treatment of latent tuberculosis infection had no effect on mortality.

Conclusion: Six months of INH provided short-term protection against tuberculosis in PPD-positive HIV-infected adults. Three month regimens including INH plus rifampicin or INH, rifampicin and pyrazinamide provided sustained protection for up to 3 years. © 2001 Lippincott Williams & Wilkins

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Introduction

HIV infection is the greatest known risk factor for progression of primary tuberculosis infection and reactivation of latent tuberculosis infection. The development of active tuberculosis in HIV-infected individuals is associated with increased HIV replication, possibly mediated via enhanced cytokine expression [1], and shortened survival [2,3]. Tuberculosis is now the leading cause of death due to an identifiable infectious cause in patients with AIDS worldwide.

Before 1993, there was little information available regarding the efficacy of isoniazid (INH) for the treatment of latent tuberculosis infection in HIV-infected persons. Since that time, seven controlled clinical trials have demonstrated that treatment of latent tuberculosis infection with 6–12 months of self-administered isoniazid (INH) or 2–3 months of rifampicin and pyrazinamide is effective in the short term in preventing tuberculosis in purified protein derivative (PPD)-positive HIV-infected adults [4–10]. Additional information on the duration of protection from tuberculosis in HIV-infected persons is needed. The optimal regimen and duration of treatment of latent tuberculosis infection also are unknown.

In March 1993 the Uganda–Case Western Reserve Research Collaboration began a randomized, placebo-controlled trial of three regimens for the prevention of tuberculosis in PPD-positive HIV-infected Ugandan adults. The three regimens studied were INH given for 6 months, INH plus rifampicin for 3 months, and INH, rifampicin and pyrazinamide given for 3 months. The study was expanded in October 1993 to include persons who were anergic to both PPD and *Candida* antigens based on studies suggesting an increased risk of tuberculosis in anergic HIV-infected adults [11,12]. Anergic subjects were randomized to treatment with 6 months of INH or placebo. In our initial report with a median follow-up period of 12 months after the initiation of treatment of latent tuberculosis infection, INH for 6 months and the rifampicin-containing regimens were safe and protected against tuberculosis [10]. In May 1996, INH was offered to all subjects enrolled in the placebo arm of the trial. We now report the final follow-up of the cohort and address long-term efficacy, effects on mortality, and drug resistance among subjects who developed active tuberculosis.

Materials and methods

Detailed information regarding the study design, interventions, outcomes and measurements has been published earlier [10]. The study was a randomized, placebo-controlled clinical trial to assess the efficacy of three daily self-administered drug regimens for the prevention of tuberculosis in HIV-infected adults. All subjects gave oral informed consent before screening and enrollment in the study. The study protocol was approved by the institutional review board at Case Western Reserve University and University Hospitals of Cleveland and the Ugandan National AIDS Research Subcommittee.

After initial PPD and *Candida* antigen skin testing, subjects were evaluated by medical history and physical examination, chest radiography, and sputum acid-fast bacilli (AFB) smears and mycobacterial cultures before enrollment in the study. HIV testing, complete blood count, and measurement of serum creatinine and aspartate aminotransferase also were performed.

Eligible subjects included 18–50-year-old HIV-infected males and non-pregnant females with a positive ($\geqslant 5$ mm induration) PPD skin test or anergy (no induration) to PPD and *Candida* antigens, and a Karnofsky performance scale score > 50. Subjects with a history of previous tuberculosis or tuberculosis treatment, an abnormal chest X-ray, total white blood cell count $< 3 \times 10^9$/l, hemoglobin level < 80 g/l, serum creatinine > 160 μM, serum aspartate aminotransferase > 90 IU/l, a positive sputum AFB smear or culture, residence > 20 km from a project clinic and pregnant women were excluded from study participation.

PPD-positive subjects were randomly assigned to receive either placebo (ascorbic acid 250 mg) daily for 6 months; INH 300 mg daily for 6 months (6H); INH 300 mg plus rifampicin 600 mg daily for 3 months (3HR); or INH 300 mg plus rifampicin 600 mg and pyrazinamide 2 g daily for 3 months (3HRZ). Randomization in blocks of six subjects was used to assign subjects to a study regimen. Treatment was self-administered. Treatment assignment was concealed by means of sequentially numbered sealed, opaque envelopes, which were opened after a subject was determined to be eligible for the study. Anergic subjects were randomly assigned to receive either placebo (ascorbic acid 250 mg) or INH 300 mg daily for 6 months by a separate but identical process.

Due to the different number of tablets and duration of the regimens and the discoloration of body fluids by rifampicin metabolites in subjects receiving the multiple drug regimens, subjects and medical officers could not be masked to treatment assignment; however, treatment assignments were not listed on study forms and medical officers were asked to perform the clinical evaluations without referring to the treatment code. Chest X-rays, sputum smears and cultures and other laboratory tests were interpreted without knowledge of treatment assignment.

Subjects received education concerning side-effects, the need for compliance, and symptoms of active tuberculosis. Subjects were followed monthly during study therapy and every 3 months thereafter. Study drugs were dispensed monthly. Compliance with study treatment was assessed by attendance at scheduled visits, urinary testing for INH metabolites, and self-report. Subjects who failed to attend scheduled visits were contacted by home health visitors. Subjects with suspected tuberculosis at any visit were evaluated with sputum smears and cultures, chest X-ray and other diagnostic studies such as lymph node biopsy or thoracocentesis if clinically indicated. For subjects who died, the date of death and symptoms at the time of death were recorded from the subject's caregivers. Autopsies were not performed.

The main study outcomes were the development of active tuberculosis, all-cause mortality, and drug resistance in patients who developed tuberculosis at any time during follow-up. Two independent chest physicians who were blinded to treatment assignment reviewed all cases of suspected tuberculosis. Cases were classified as definite (culture-confirmed) tuberculosis, probable tuberculosis, possible tuberculosis or unlikely to be tuberculosis using predefined criteria [10]. *Mycobacterium tuberculosis* isolates from subjects who developed active tuberculosis were stored at $-70°C$ for later drug susceptibility testing against INH, rifampicin, pyrazinamide, ethambutol and streptomycin using standard BACTEC (Becton Dickinson, Sparks, Maryland, USA) radiometric methods [13]. The critical concentrations used were INH, 0.1 μg/ml; rifampicin, 2.0 μg/ml; streptomycin, 2.0 μg/ml; ethambutol, 2.5 μg/ml and pyrazinamide, 100 μg/ml. Standard quality control *M. tuberculosis* strains ATCC 27294 (susceptible to all drugs), ATCC 35822 (resistant to isoniazid), ATCC 35838 (resistant to rifampicin), and ATCC 35828 (resistant to pyrazinamide) were included in each batch of tests.

Statistical analysis

The intent-to-treat approach was used to analyze the study for the primary endpoints of tuberculosis and survival. The incidence rate of tuberculosis or death was estimated using the person-years method for each study arm. Failure-time distributions for incident tuberculosis and death were estimated for each arm using the Kaplan−Meier method and compared using the log-rank test. For the primary analysis of tuberculosis, subjects were censored at the time of death or on the date of their last visit prior to the end of the study (8 August 1998). The sample size was calculated separately for the PPD-positive (410 subjects in each arm) and anergic (500 subjects in each arm) cohorts to achieve 80% power to detect a 67% decrease in the incidence of tuberculosis in the active treatment arms compared to the placebo group with a type I error of 5% and allowance for loss to follow-up, interim analyses and mortality. Interim analyses were reviewed by the investigators and sponsor.

The efficacy of drug treatment was estimated using the relative risk for tuberculosis in the active treatment arms compared to the placebo arm. To assess the role of rifampicin-containing 3 month regimens for treatment of latent tuberculosis infection, data from the 3HR and 3HRZ arms were combined. To determine the relative efficacy of rifampicin-containing regimens versus the 6H regimen, the relative risk of tuberculosis was estimated by comparing the incidence of tuberculosis in the rifampicin-containing regimens to 6H. Unadjusted and adjusted estimates of relative risk were calculated using Cox proportional hazards regression analysis after testing the proportional hazards assumption. A series of regression models was developed to identify and adjust for potential confounding variables. These variables included age, sex, Karnofsky performance status, hemoglobin, body mass index, and period of enrollment (first or second half of the enrollment period) into the study. Of these variables, only hemoglobin, body mass index, and period of enrollment into the study were found to be significant in all models and were used in the regression models to estimate the adjusted relative risks. Separate models were developed for two types of outcomes: definite and probable tuberculosis and definite tuberculosis only. To assess whether the INH-resistant strains altered the results of the regression analyses, models were developed excluding the drug-resistant cases, as if the subject were infected with a drug resistant strain at the time of enrollment. A Poisson regression model was used to model trends in the annual incidence rates of tuberculosis over time and to compare rates among study arms.

Results

Study population and follow-up

Between March 1993 and April 1995, 2736 subjects were enrolled into the study (Fig. 1). Of these, 2018 were PPD-positive and 718 were anergic to both PPD and *Candida* antigens. The treatment groups were balanced at baseline in terms of demographic factors, Karnofsky status, and clinical features [10]. Compliance with follow-up appointments and study drug treatment was comparable between treatment arms [14]. Information regarding baseline characteristics of the subjects, adverse drug reactions during treatment of latent tuberculosis infection and adherence with treatment is described in the initial study report [10].

At the second interim analysis, 6H treatment was found to decrease the risk of tuberculosis by 67% in PPD-positive subjects after an average duration of follow-up
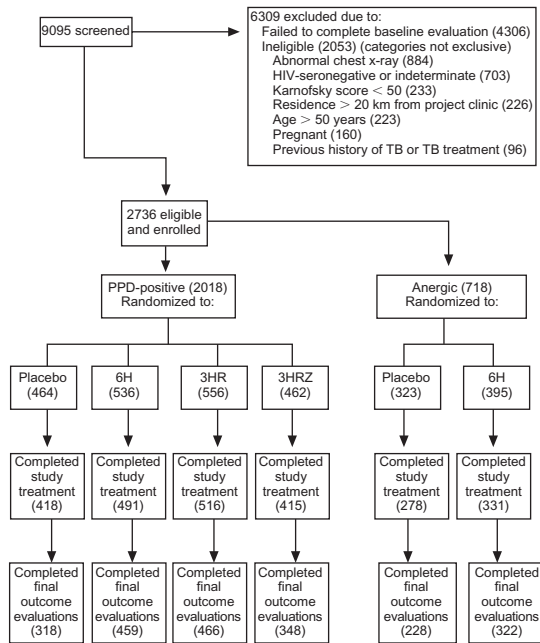
Fig. 1. Subjects screened and enrolled in the study.

of 12 months. An increased rate of minor side-effects also was noted in the 3HRZ arm compared to the other treatment arms. Based on these data, enrollment in the placebo and 3HRZ arms was discontinued and treatment of latent tuberculosis infection with 6 months of INH was offered to all available subjects in the placebo arms beginning in May 1996. Of 787 subjects assigned to the placebo arms of the study, 498 subjects were alive, had not developed tuberculosis and had not been lost to follow-up. Of these, 366 (74%) elected to receive 6H treatment. All subjects remained in active follow-up for study endpoints.

During long-term follow-up, a total of 4064 person-years of observation (PYO) (placebo, 1012 PYO; 6H, 1101 PYO; 3HR, 1151 PYO; 3HRZ, 800 PYO) were accrued in the PPD-positive cohort and 1118 PYO (placebo, 525 PYO; 6H, 593 PYO) were accrued in the anergic group (Table 1). Within the PPD-positive and anergic cohorts, the proportion of subjects completing study drug treatment was similar among treatment arms (Fig. 1). The mean duration of follow-up among PPD-positive individuals was 2 years after the initiation of study drug treatment; the mean duration of follow-up was shorter in the 3RHZ arm than in the other arms. The average duration of follow-up in the anergic cohort was 1.6 years and did not differ between groups. The main reasons for censoring subjects in this analysis were death before tuberculosis (n = 560), end of the study (n = 1465), refusal to participate further (n = 357), and loss to follow-up while on therapy (n = 238).

Incident tuberculosis cases
In the PPD-positive cohort, 113 cases of definite or probable tuberculosis developed during follow-up; 84 cases (74%) were confirmed by culture, i.e., definite. Of the 113 cases, 12 (11%) developed within 6 months of enrollment. Of these 12 cases, six cases developed in the placebo arm: one within 3 months of enrollment and five between 4 and 6 months. Of the remaining six cases two occurred in 6H, three in 3RH and one in 3RHZ; four of these cases developed between 4 and 6 months after enrollment. In the anergic cohort, 39 cases of active tuberculosis (62% culture-confirmed) developed during follow-up: 12 cases developed within 6 months of enrollment, six in the placebo arm and six in the 6H arm. Seven of these cases occurred between 4 and 6 months after enrollment. The number of

Table 1. Follow-up of 2736 subjects enrolled in the study according to assigned treatment and purified protein derivative (PPD) and *Candida* skin test reactivity.

	PPD-positive subjects				Anergic subjects	
	Placebo	6H	3RH	3RHZ	Placebo	6H
Subjects (n)[a]						
Year 1	464	536	556	462	323	395
Year 2	343	401	411	319	196	226
Year 3	271	293	304	197	133	145
Year 4	148	127	164	65	39	51
Year 5 or more	22	29	33	11	0	0
Person-years of observation						
Year 1	400	469	474	377	252	300
Year 2	313	347	354	264	164	185
Year 3	216	208	226	127	97	93
Year 4	79	71	92	29	12	15
Year 5 or more	4	6	5	3	0	0
Tuberculosis cases						
Definite and probable	42	34	22	15	22	17
Definite only	32	26	17	9	14	10
Average duration of follow-up (years)	2.2	2.1	2.1	1.7	1.6	1.5
Censored (%)	91	94	96	97	93	96

[a]Number in cohort at the beginning of each year of follow-up after starting study treatment.

incident tuberculosis cases was similar between subjects enrolled during the first half of the enrollment period (before 1 May 1994) and those enrolled during the second half (after 1 May 1994) for all arms except the isoniazid-containing arms. In the PPD-positive subjects, the number of cases increased from 12 among subjects enrolled before 1 May 1994 to 22 among those enrolled after this date; 19 of these 22 cases were confirmed by culture. A similar observation was made in the anergic cohort as the number of cases increased from five to 12 among those enrolled before compared with after 1 May 1994.

Efficacy of treatment of latent tuberculosis infection

PPD-positive individuals

In a Kaplan−Meier analysis, the overall cumulative incidence of tuberculosis 3 years after study enrollment was greater in the placebo arm (11.6%) than in the 3HR arm (5.4%; $P = 0.003$; Fig. 2a) and the 3HRZ arm (5.6 %; $P = 0.01$) but was not different from the 6H arm (9.1%; $P = 0.24$). In pair-wise comparisons of each treatment arm versus the placebo group, the unadjusted relative risks for tuberculosis were 0.76 [95% confidence interval (CI), 0.48−1.20] for 6H, 0.46 (95% CI, 0.48−0.77) for 3HR, and 0.47 (95% CI, 0.26−0.86) for 3HRZ. The two rifampicin-containing regimens reduced the risk of developing tuberculosis (Table 2 and Fig. 2b). The relative risk for tuberculosis in the 3HR arm compared to the placebo group was 0.49 (95% CI, 0.29−0.82), after adjusting for hemoglobin, body mass index, and period of enrollment in a Cox proportional hazards model. Similarly, for the 3HRZ arm, the adjusted relative risk was 0.41 (95% CI, 0.22−0.76). When the rifampicin-containing regimens were combined, the adjusted relative risk for tuberculosis compared with placebo was 0.46 (95% CI, 0.29−0.71). For 6H, the adjusted relative risk was 0.67 (95% CI, 0.42−1.07). The

adjusted relative risk for tuberculosis among subjects receiving 6H increased with time after starting therapy; the adjusted relative risk of tuberculosis for 6H compared to placebo was 0.11 (95% CI, 0.01−0.93) 9 months after beginning therapy, 0.49 (95% CI, 0.16−1.51) 12 months after starting therapy, and 0.68 (95% CI, 0.35−1.34) 24 months after starting therapy. When the analysis was restricted to culture-confirmed cases of tuberculosis, the adjusted relative risks for tuberculosis were lower for each treatment group (Table 2). When the relative risks for tuberculosis were examined from the completion of therapy, excluding 12 cases that occurred during the first 6 months of the observation that may have been due to incubating tuberculosis at the time of enrollment, the adjusted estimates of relative risk were similar to the risk estimates when analyzing follow-up from the start of therapy (data not shown). In comparisons of the rifampicin-containing regimens with the 6H regimen, the rifampicin-containing regimens showed consistent reduction in the risk of developing tuberculosis. Compared to 6H, the relative risk for developing tuberculosis was 0.72 (95%

CI, 0.42−1.25) for the 3HR arm, 0.60 (95% CI, 0.32−1.12) for 3HRZ and 0.67 (95% CI, 0.42−1.08) for both regimens combined. Results were similar when the analysis was performed including only culture-confirmed cases of tuberculosis.

Anergic subjects

After 2 years of observation, the cumulative incidence of tuberculosis in the placebo arm (9.7%) was higher than that in the 6H group (5.1%; $P = 0.24$; Fig. 3), although the difference was not statistically significant. The crude relative risk for tuberculosis was 0.69 (95% CI, 0.36−1.29), but after adjusting for body mass index, hemoglobin and period of enrollment, the relative risk for tuberculosis decreased to 0.61 (95% CI,
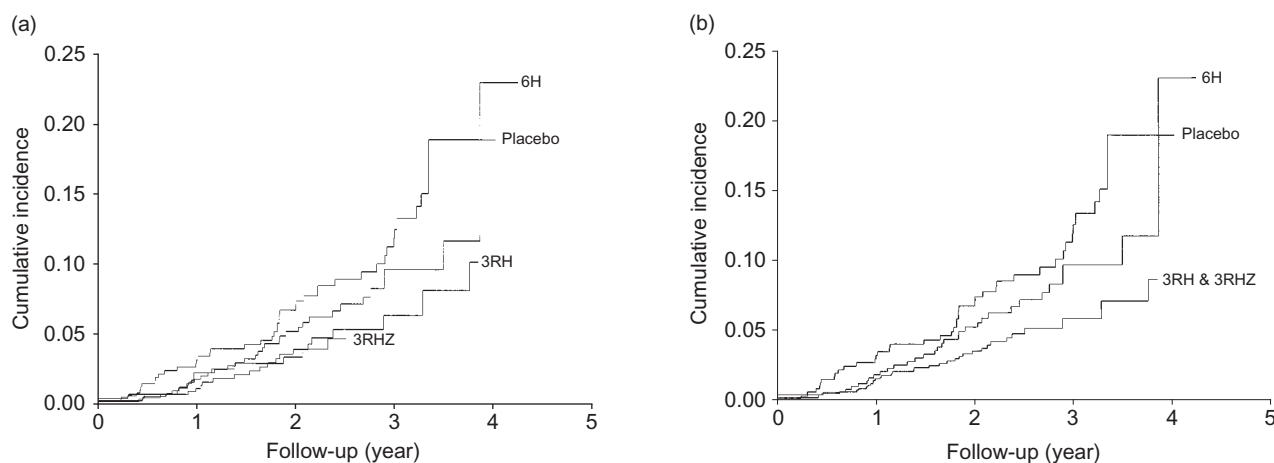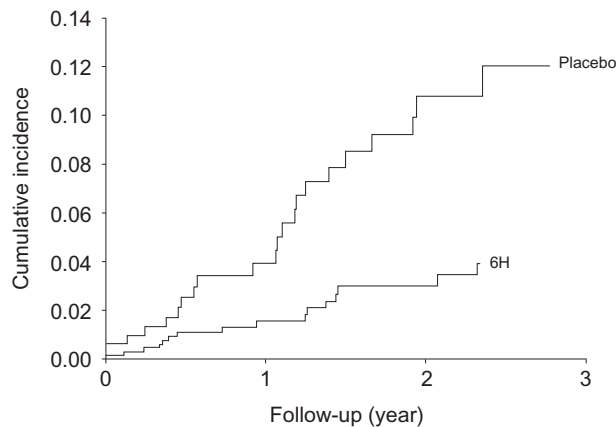

Fig. 2. Cumulative incidence of tuberculosis among PPD-positive subjects for each of the three regimens compared with placebo (a) and combining the 3HR and 3HRZ regimens (b).

Table 2. Incidence of tuberculosis according to study groups and certainty of diagnosis from the time of enrollment into the study and the initiation of therapy.

Study arm	Definite and probable tuberculosis			Definite tuberculosis		
	Adjusted relative risk[a]	95% Confidence interval	P	Adjusted relative risk[a]	95% Confidence interval	P
PPD-positive						
Placebo	1.0	–	–	1.0	–	–
6H	0.67	0.42–1.07	0.09[b]	0.65	0.38–1.10	0.11
3HR	0.49	0.29–0.82	0.007	0.49	0.27–0.89	0.02
3HRZ	0.41	0.22–0.76	0.004	0.34	0.16–0.72	0.005
3HR and 3HRZ	0.46	0.29–0.71	0.006	0.43	0.26–0.72	0.001
6H	1.0	–	–	1.0	–	–
3HR	0.72	0.42–1.25	0.25[c]	0.77	0.41–1.43	0.41
3HRZ	0.60	0.32–1.12	0.11	0.53	0.25–1.15	0.11
3HR and 3HRZ	0.67	0.42–1.08	0.10	0.67	0.38–1.17	0.16
Anergic						
Placebo	1.0	–	–	1.0	–	–
6H	0.61	0.32–1.16	0.13[b]	0.58	0.25–1.32	0.19

[a]The relative risk is adjusted for hemoglobin, body mass index and period of enrollment in a Cox proportional-hazards regression model. [b]P value comparing each treatment group to the placebo arm. [c]P value comparing each treatment group to the 6H arm. PPD, Purified protein derivative.

Fig. 3. Cumulative incidence of tuberculosis among anergic subjects by treatment arm.

0.32−1.16). When the relative risk of tuberculosis was calculated from the time of completion of therapy, the adjusted relative risk was 0.51 (95% CI, 0.24−1.08).

Annual incidence of tuberculosis

In the PPD-positive cohort, the annual incidence rates of tuberculosis were consistently lower throughout the study period for the rifampicin-containing regimens compared to placebo, and, with the exception of the first year, when compared with the 6H arm (Table 3). The annual incidence rate in the 6H arm was lower than the rate in the placebo group in the first year, and in subsequent years, the rates in the 6H arm were comparable with those in the placebo arm. In contrast, in the anergic cohort the annual incidence rate of the 6H arm was lower than that for the placebo arm throughout the follow-up period. In a Poisson regression model, the overall annual incidence rate of

tuberculosis increased significantly over time in the cohort as a whole ($P = 0.03$). Only the rifampicin-containing regimens reduced the incidence rate of tuberculosis over time when compared with placebo ($P = 0.014$); the rifampicin-containing regimens also had lower incidence rates over time when compared with the 6H arm ($P = 0.03$).

Mortality

During the follow-up period, a total of 577 deaths (560 before the development of active tuberculosis) occurred among study subjects, 351 among PPD-positive subjects and 226 among anergic subjects. Survival was greater in the PPD-positive cohort compared to the anergic cohort (Fig. 4; $P = 0.0001$, log-rank test). The 1 year and 2 year survival proportions were 0.81 and 0.66 in the anergic cohort and 0.91 and 0.85 in the PPD-positive cohort. When stratified by the presence of anergy, there was no difference in survival among the treatment arms for both the PPD-positive and anergic cohorts (Fig. 4; $P > 0.7$, log-rank test). Among the 152 subjects who developed tuberculosis, 21 patients died for a cumulative mortality rate of 14%.

M. tuberculosis drug susceptibility testing

Of 152 cases of tuberculosis that occurred during follow-up of study subjects, 108 cases (71%) were culture-confirmed. Isolates were available for drug susceptibility testing for 65 (64%) of the culture-confirmed cases. The proportion of isolates available for susceptibility testing did not differ by study arm. Six (9%) of the isolates were resistant to INH, none was resistant to rifampicin, and one (2%) was resistant to pyrazinamide. No isolate was resistant to more than one drug. INH-resistant isolates were recovered from three PPD-positive and two anergic subjects receiving

Table 3. Incidence rates of tuberculosis according to treatment group and year of study follow-up.[a]

Study arm	Year 1 Cases (n)	Rate[b]	Year 2 Cases (n)	Rate	Year 3 Cases (n)	Rate	Year 4 Cases (n)	Rate	Overall Cases (n)[c]	Rate
PPD-positive										
Placebo	13	3.25	12	3.83	12	5.56	4	5.06	41	4.15
6H	8	1.71	14	4.03	8	3.85	3	4.23	33	3.09
3HR	5	1.05	10	2.82	4	1.77	3	3.26	22	1.91
3HRZ	9	2.39	3	1.14	3	2.36	–	–	15	1.87
Anergic										
Placebo	9	3.57	11	6.71	2	2.06	–	–	22	4.20
6H	8	2.67	5	2.70	4	4.30	–	–	17	2.87

[a]Study subjects in the placebo arms were offered 6H beginning in May 1996. During the first of follow-up one subject received 6H, 19 in the second year, 177 in the third year, 148 in the fourth year, and 22 after 4 years. [b]Per 100 person-years of observation. [c]Two cases of tuberculosis developed after the fourth year of follow-up and are not included in this analysis: one case in the placebo arm, the other in 6H of the PPD-positive cohort. PPD, Purified protein derivative.
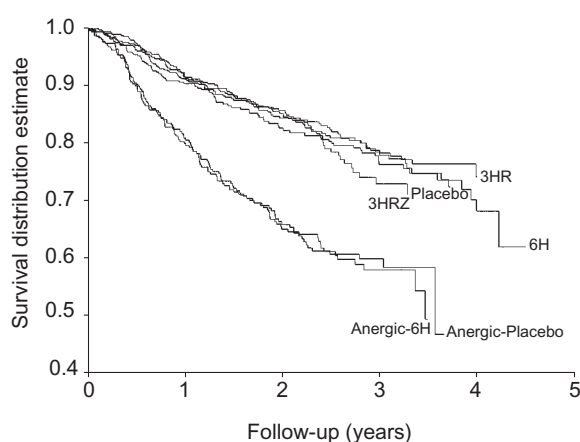
Fig. 4. Cumulative survival among PPD-positive and anergic subjects by treatment arm.

6H and one subject from the anergic placebo arm. The single pyrazinamide-resistant isolate was recovered from a subject in the placebo arm. The proportion of subjects who developed active tuberculosis with INH-resistant isolates did not differ by study arm ($P = 0.17$, Fisher's exact test). After combining treatment groups, five of 41 isolates from patients who received treatment of latent tuberculosis infection (any regimen) were resistant to INH compared to one of 24 isolates from subjects in the placebo arms ($P = 0.40$, Fisher's exact test). After combining the PPD-positive and anergic subjects receiving 6H, five of 20 isolates were resistant to INH compared with one of 24 isolates from the PPD-positive and anergic subjects receiving placebo ($P = 0.19$, Fisher's exact test). Of the 53 isolates from women, six isolates (11%) were resistant to INH, whereas none of 12 isolates from men were resistant to INH ($P = 0.34$, Fisher's exact test). Apart from sex, these six cases were similar to the entire cohort in terms of age, body mass index, Karnofsky performance scale score, and hemoglobin. Subjects who developed

incident tuberculosis with INH-resistant *M. tuberculosis* strains did not differ from subjects with INH-susceptible strains in terms of adherence as measured by self-report, attendance at scheduled appointments, and urine INH metabolite testing results. The estimates of relative risk for tuberculosis were similar when excluding INH-resistant cases from the analysis.

Discussion

In this randomized, placebo-controlled trial, INH administered for 6 months provided some initial protection against tuberculosis in PPD-positive HIV-infected adults in Uganda, a nation with a high prevalence of tuberculosis. This effect was short-lived, however, as limited benefit was evident 12 months after beginning treatment of latent tuberculosis infection. In contrast, treatment with either of the 3 month multidrug regimens containing rifampicin provided protection against tuberculosis for up to 3 years. Treatment of latent tuberculosis infection with INH had marginal benefit in anergic HIV-infected individuals who are more likely to have advanced HIV infection [15] although the direction of effect was to reduce the incidence of tuberculosis in treated persons. The findings from this study were consistent whether we analyzed both definite plus probable cases or only definite cases as the main outcome, whether we analyzed from the time of enrollment or the time of completion of treatment, or whether we excluded or included subjects who developed tuberculosis with an INH-resistant organism.

The optimal treatment regimen and duration of protection against tuberculosis afforded by treatment of latent tuberculosis infection in HIV-infected persons are key issues affecting its implementation. Reactivation of latent tuberculosis infection is believed to be the major

mechanism leading to tuberculosis in HIV-infected individuals [16]; however, recent molecular epidemiologic studies have shown that up to 40% of tuberculosis cases may be due to recent infection and rapid progression to active disease [17,18]. Shorter regimens offer the potential for improved compliance [19] and decreased operational costs whereas extended or lifelong regimens might be needed for persons in areas or situations with a high risk for exogenous reinfection and rapid progression to active tuberculosis. The use of multidrug regimens for the treatment of latent tuberculosis infection also raises concerns regarding the risk of drug resistance to rifampicin and other first-line treatment drugs for subjects who later develop active tuberculosis.

In a preliminary report of a study from Zambia, treatment of latent tuberculosis infection with 6 months of INH significantly decreased the rate of tuberculosis in HIV-infected persons compared to placebo; however, the incidence of tuberculosis increased with the post-prophylaxis interval [20]. Recently, in another study from Zambia, Quigley and colleagues found that the protective benefit from treatment of latent tuberculosis infection declined during follow-up, however, tuberculosis rates during the first 30 months after beginning treatment were lower in subjects receiving 6 months of INH than those receiving placebo [21]. We found that protection from tuberculosis after treatment with INH for 6 months waned by 18 months after starting therapy. It is possible that a longer duration of INH treatment may have more prolonged benefit, although compliance with such regimens is likely to be lower.

Although our study was not designed to compare each regimen separately, our analysis suggests that the multidrug regimens offered longer-lasting protection against tuberculosis in our study than INH alone, despite being given for only 3 months. Multidrug regimens have been studied less extensively than regimens using INH alone. An observational study from the UK suggested that treatment of latent tuberculosis infection for 3 or 4 months with INH and rifampicin was well tolerated and highly effective in preventing tuberculosis in tuberculin-reactive immigrant children who had not received Bacille Calmette−Guerin and in pediatric household contacts of infectious tuberculosis cases [22]. In a study of PPD-positive HIV-infected adults in Haiti with a median follow-up of 30 months, 2 months of twice weekly rifampicin and pyrazinamide was comparable to 6 months of twice weekly INH for the prevention of tuberculosis [6]. Similar results also have been reported recently from a multicenter international trial comparing 12 months of daily INH with 2 months of daily rifampicin and pyrazinamide in more than 1550 HIV-infected, PPD-positive adults [5]. In the study by Quigley noted earlier, the overall incidence

rate of tuberculosis was similar following twice weekly therapy with INH for 6 months (3.0 per 100 PYO) or rifampicin plus pyrazinamide for 3 months (3.7 per 100 PYO) [21]. Both regimens were effective during the first 2.5 years after beginning treatment. Among PPD-positive individuals, however, the protective effect of the INH regimen decreased from 84% during the first 18 months after beginning treatment to 14% during the following year. By contrast, protection of the rifampicin plus pyrazinamide regimen was 69% during the initial 18 months and was sustained for up to 30 months.

Anti-tuberculosis drugs have been hypothesized to have activity against different populations of tubercle bacilli in humans [23]. The number of organisms in individuals with latent tuberculosis infection is believed to be small [19] with many bacilli in a slowly replicating or latent state in which they are effectively contained by host immune responses. Progressive immunosuppression and increasing susceptibility to opportunistic pathogens characterize the natural history of HIV infection. Histopathologic studies have demonstrated depletion of CD4 lymphocytes in granulomas with progressive HIV infection [24] and an inverse relationship between CD4 cell counts and proliferation of tubercle bacilli in the lung parenchyma in HIV-infected patients with active tuberculosis [25]. Therefore, as HIV infection advances, local immune responses previously able to contain small numbers of viable tubercle bacilli walled off in granulomas may be overwhelmed, allowing unchecked microbial replication and the development of active tuberculosis. In patients with recent tuberculosis infection, some of these latent foci may, in fact, represent early subclinical disease that can be eradicated by treatment of latent tuberculosis infection.

The sterilizing capacity of an anti-tuberculosis drug refers to its ability to kill persistent, metabolically inactive bacilli, many of which are believed to be intracellular [23]. In the setting of impaired cell mediated immunity in HIV infection, rifampicin and pyrazinamide, drugs with strong sterilizing capacity and activity against intermittently metabolically active and intracellular organisms [26,27], may play important roles in determining the long-term outcome of treatment of latent tuberculosis infection.

Our results also showed a trend towards a decrease in the incidence of tuberculosis among anergic subjects who received INH for 6 months. When adjusted for important covariates, the protective efficacy of treatment of latent tuberculosis with INH was 40% among the anergic subjects, though this did not reach statistical significance. A similar effect in both magnitude and direction was observed in a trial of INH treatment in anergic HIV-infected adults from the USA [4], but as

in the current study, the results were not statistically significant. In an earlier study in Haiti, no protective effect against tuberculosis was seen in PPD-negative adults [9].

Treatment of latent tuberculosis infection did not alter overall mortality in our study. Only the study by Pape and colleagues in Haiti found that treatment of latent tuberculosis infection decreased mortality in HIV-infected persons [9]. Individual studies of treatment of latent tuberculosis infection, including the current trial, however, have not been designed to assess its effect on overall survival. In a meta-analysis of placebo-controlled trials of treatment of latent tuberculosis infection in HIV-infected adults from the USA, Haiti, Kenya and Uganda (including the initial report for this study), the risk of death was moderately decreased (relative risk, 0.73; 95% CI, 0.57–0.95) among PPD-positive subjects receiving INH; however, no effect on mortality was discernible in PPD-negative persons [28]. Survival was reduced and the risk for tuberculosis was higher among anergic subjects in our study cohort, consistent with earlier studies showing that poor delayed type hypersensitivity skin test responses reflect greater immunosuppression in HIV-infected persons and independently predict progression of HIV infection, risk for opportunistic infections and death [15,29].

In our study, six *M. tuberculosis* isolates from subjects who developed tuberculosis were resistant to INH and one isolate was resistant to pyrazinamide. The proportion of subjects with isolates resistant to INH did not differ by study arm. When treatment arms were combined, the proportion of resistant isolates did not differ from placebo. Although all observed cases of INH-resistant tuberculosis occurred in women, we could not exclude chance alone as reason for the finding. The results of the study are not altered when the drug-resistant cases are excluded as if they were initially infected with a strain of *M. tuberculosis* resistant to INH. For comparison, the prevalence of INH monoresistance was 6% in a tuberculosis treatment trial of 191 previously untreated HIV-infected adults completed in Kampala just before our preventive therapy trial [30] and was 7% in a recent drug resistance survey in Uganda [31]. In four other controlled trials of INH and multidrug regimens for the treatment of latent tuberculosis infection in HIV-infected persons, four isolates from 65 subjects who developed active tuberculosis after receiving treatment were resistant to INH, including one isolate resistant to both INH and streptomycin, compared to one INH-resistant isolate in persons receiving placebo [4,6–8]. In the current study, there is no evidence to suggest that treatment of latent tuberculosis infection is associated with the development of drug resistance in HIV-infected individuals who later develop tuberculosis, but with the small numbers of cases, risk for drug resistance cannot be excluded. In

the context of previous studies, however, the development of drug resistance does not appear to be associated with treatment of latent tuberculosis infection in HIV-infected persons. Our findings are consistent with results from earlier studies in non–HIV-infected persons [32].

The analysis of this study revealed a cohort effect; that is, subjects enrolled during the first half of the enrollment period, before 1 May 1994, exhibited a lower risk for developing tuberculosis than subjects enrolled during the second half. The increased risk for tuberculosis was observed, however, largely in the subjects assigned to the 6H treatment arm in both the PPD-positive and anergic subjects. Of the 34 cases occurring in the 6H cohort enrolled after May 1994, 25 cases (74%) were confirmed by culture. The cohort effect may be produced by a learning effect on the part of the medical personnel or a detection bias as a result of our close-out evaluations for tuberculosis at the end of the study. None of these explanations, however, accounts for the excess incidence of tuberculosis observed in the 6H arm. This raises the question of whether there was bias in the detection of tuberculosis in the 6H arm. The likelihood of a differential detection bias is low for a number of reasons. Although the study was not formally blinded, the medical officers who were responsible for initiating work-ups for tuberculosis were generally not aware of the assigned study arm. A standard protocol for the annual chest radiography and sputum collection was used to screen all study subjects. The standard diagnostic evaluation was followed for all subjects with suspected tuberculosis, regardless of the original treatment assignment. Final classification of incident tuberculosis was done in a blinded manner.

Our study has important implications for the care of HIV-infected individuals. In aggregate, the evidence from large controlled clinical trials shows that treatment of latent tuberculosis infection decreases the short-term risk of developing active tuberculosis by approximately one-half in PPD-positive individuals. The benefits were much less among anergic persons. Provision of 2 or 3 month multidrug regimens for treatment of latent tuberculosis infection, where feasible, may offer greater long-term benefit than longer regimens utilizing INH alone and may promote improved adherence [19]. Treatment of latent tuberculosis infection requires adequate resources to exclude active tuberculosis before beginning preventive therapy. Multidrug regimens for treatment of latent tuberculosis infection offer an additional safeguard against the inadvertent use of monotherapy in persons with occult active tuberculosis not identified during screening.

Early diagnosis and effective treatment of patients with sputum smear-positive pulmonary tuberculosis is clearly the most important priority of national tuberculosis

control programs in both developing and industrialized nations. Decisions regarding the implementation of treatment of latent tuberculosis infection and the regimens and strategies to be used are complex and depend on logistic, economic, and local issues. Treatment of latent tuberculosis infection in developing countries may be most useful in select groups of HIV-infected individuals, such as health care workers, who are at high risk for contact with infectious tuberculosis cases. The integration of tuberculosis screening and treatment of latent tuberculosis infection with other services at HIV voluntary counseling and testing centers and AIDS support organizations is another means to enhance access to tuberculosis prevention, diagnosis and treatment services for both HIV-infected and non-HIV-infected persons [33,34]. New and innovative approaches are needed if the benefits of treatment of latent tuberculosis infection are to be extended to the millions of HIV and *M. tuberculosis* co-infected persons at risk.

Acknowledgements

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