



6-month versus 36-month isoniazid preventive treatment for tuberculosis in adults with HIV infection in Botswana: a randomised, double-blind, placebo-controlled trial

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Summary

Background In accordance with WHO guidelines, people with HIV infection in Botswana receive daily isoniazid preventive therapy against tuberculosis without obtaining a tuberculin skin test, but duration of prophylaxis is restricted to 6 months. We aimed to assess effectiveness of extended isoniazid therapy.

Methods In our randomised, double-blind, placebo-controlled trial we enrolled adults infected with HIV aged 18 years or older at government HIV-care clinics in Botswana. Exclusion criteria included current illness such as cough and an abnormal chest radiograph without antecedent tuberculosis or pneumonia. Eligible individuals were randomly allocated (1:1) to receive 6 months' open-label isoniazid followed by 30 months' masked placebo (control group) or 6 months' open-label isoniazid followed by 30 months' masked isoniazid (continued isoniazid group) on the basis of a computer-generated randomisation list with permuted blocks of ten at each clinic. Antiretroviral therapy was provided if participants had CD4-positive lymphocyte counts of fewer than 200 cells per μL . We used Cox regression analysis and the log-rank test to compare incident tuberculosis in the groups. Cox regression models were used to estimate the effect of antiretroviral therapy. The trial is registered at ClinicalTrials.gov, number NCT00164281.

Findings Between Nov 26, 2004, and July 3, 2009, we recorded 34 (3.4%) cases of incident tuberculosis in 989 participants allocated to the control group and 20 (2.0%) in 1006 allocated to the continued isoniazid group (incidence 1.26% per year vs 0.72%; hazard ratio 0.57, 95% CI 0.33–0.99, $p=0.047$). Tuberculosis incidence in those individuals receiving placebo escalated approximately 200 days after completion of open-label isoniazid. Participants who were tuberculin skin test positive (ie, ≥ 5 mm induration) at enrolment received a substantial benefit from continued isoniazid treatment (0.26, 0.09–0.80, $p=0.02$), whereas participants who were tuberculin skin test-negative received no significant benefit (0.75, 0.38–1.46, $p=0.40$). By study completion, 946 (47%) of 1995 participants had initiated antiretroviral therapy. Tuberculosis incidence was reduced by 50% in those receiving 360 days of antiretroviral therapy compared with participants receiving no antiretroviral therapy (adjusted hazard ratio 0.50, 95% CI 0.26–0.97). Severe adverse events and death were much the same in the control and continued isoniazid groups.

Interpretation In a tuberculosis-endemic setting, 36 months' isoniazid prophylaxis was more effective for prevention of tuberculosis than was 6-month prophylaxis in individuals with HIV infection, and chiefly benefited those who were tuberculin skin test positive.

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Introduction

Tuberculosis is one of the most common causes of morbidity and mortality in people with HIV infection worldwide.¹ Isoniazid preventive therapy significantly reduces tuberculosis in these patients who have a positive tuberculin skin test but not in those with a negative test.² In 1999, WHO recommended a 6-month course of isoniazid for people with HIV infection and a positive skin test, and extended this recommendation to patients living in areas where tuberculin skin testing was not feasible if the prevalence of latent tuberculosis infection was more than 30%.³ In response to rapidly increasing rates of tuberculosis in people with HIV infection, the government of Botswana initiated a national isoniazid preventive treatment

programme that, consistent with these WHO recommendations, did not require tuberculin skin testing.⁴

Antiretroviral therapy profoundly reduces incidence of tuberculosis in people with HIV infection and with continued use risk of tuberculosis progressively declines.⁵ Two retrospective analyses^{6,7} concluded that the benefit of combining isoniazid and antiretroviral therapy was additive. Shortly after beginning the national rollout of isoniazid preventive treatment, Botswana's Government began a nationwide programme to provide free antiretroviral therapy.⁸

Although a 6-month course of isoniazid prevented tuberculosis in people with HIV infection in two trials in sub-Saharan Africa,^{9,10} this benefit was lost within

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6–18 months of completion of prophylaxis in these cohorts.^{11,12} Whether the short-lived response was due to reinfection with *Mycobacterium tuberculosis* or to inadequate treatment of latent infection is unclear. Other studies in Africa reported that 69–77% of tuberculosis recurrences were caused by reinfection with new strains^{13,14} and that 42–79% of cases were clustered.^{15–18} Such reports emphasised the need to establish whether continuous treatment beyond 6 months is beneficial for people with HIV infection in tuberculosis-endemic countries. We aimed to assess such extended treatment in a large population of people with HIV infection in Botswana using the proxy of 36 months' isoniazid compared with 6 months of isoniazid plus 30 months' placebo. We also aimed to estimate the effect of antiretroviral therapy and tuberculin skin test status on tuberculosis prevention, and frequency of adverse effects and isoniazid resistance.

Methods

Study design and participants

In our randomised, double-blind, placebo-controlled trial, we enrolled adults who were 18 years or older with HIV infection who attended one of eight government clinics in Gaborone and Francistown, Botswana, that provided antiretroviral therapy and isoniazid preventive treatment between Nov 26, 2004, and July 20, 2006. The enrolment process is described in detail elsewhere.¹⁹ Potential participants of any CD4 lymphocyte count or tuberculin skin test status were eligible for inclusion. Reasons for exclusion included cough, weight loss, night sweats, other acute illnesses, previous isoniazid preventive treatment, tuberculosis treatment within the previous 3 years, neutrophil count of fewer than 1.0×10^9 cells per L, or an abnormal chest radiograph without antecedent tuberculosis or pneumonia.¹⁹

All participants provided signed informed consent forms and received transportation reimbursements. The protocol was approved by the Botswana Human Research Development Committee and the US Centers for Disease Control and Prevention institutional review board. A four-member independent data and safety monitoring Board monitored the study's progress four times every year. Two ten-member community advisory boards and two participant advisory groups were regularly briefed and consulted.

Randomisation and masking

Participants were randomly allocated to treatment groups at enrolment. All participants received open-label isoniazid for 6 months and subsequently received either 30 months of continued isoniazid prophylaxis (continued treatment group) or placebo (control group). After completion of the first 6 months' open-label isoniazid, participants and clinical trial staff were masked to treatment assignment. One of ten codes was assigned to every patient identifier in a computer-generated

randomisation list before enrolment began. An independent study statistician (US Centers for Disease Control and Prevention, Atlanta, GA, USA) prepared the randomisation list in permuted blocks of ten (balanced allocation 1:1) within every clinic. Participants were assigned identifiers in sequential order as they were screened in clinics. The sequential order was always adhered to. Participants were enrolled and followed up by study staff. Only the data and safety monitoring board, the statistician (NS), and the drug-packaging company (which was uninvolved in the rest of the trial) were aware of treatment assignment.

Procedures

During the initial 6-month phase and continued isoniazid treatment the dose of isoniazid was 300 mg per day for individuals weighing 30–49 kg, or 400 mg per day for those weighing 50 kg or more, supplemented with 25 mg vitamin B6. On April 28, 2005, national guidelines for isoniazid were changed and from Jan 1, 2006, all study participants were given 300 mg per day. Participants who completed the open-label isoniazid phase continued to receive vitamin B6.

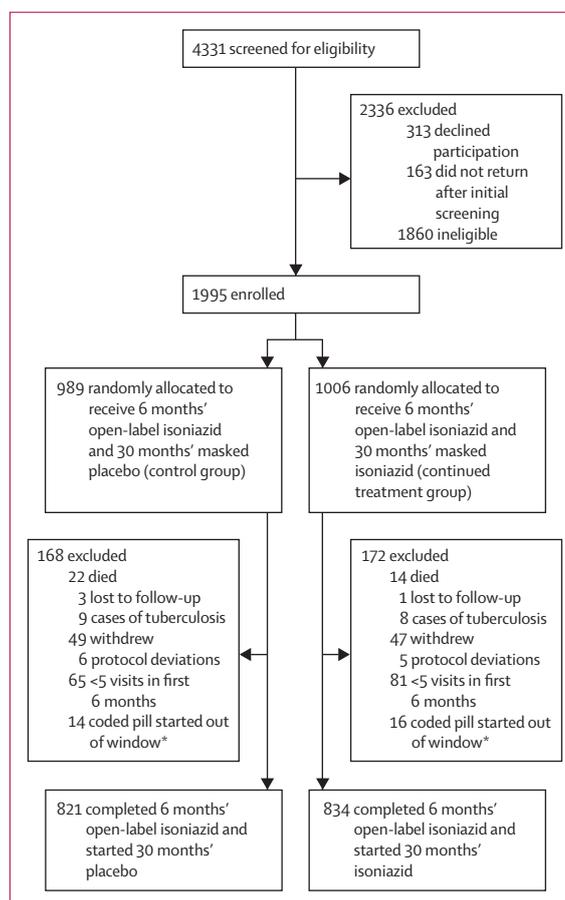


Figure 1: Trial profile

*Participants started treatment more than 21 days before or 28 days after ideal initiation date (180 days after randomisation).

	Control group (n=989)	Continued isoniazid group (n=1006)	Overall (N=1995)
Women	721 (73%)	715 (71%)	1436 (72%)
Age (years)	Median 32 (28–38); mean 34 (8)	Median 32 (28–39); mean 34 (8)	Median 32 (28–39); mean 34 (8)
Body-mass index*			
Underweight	178 (18%)	158 (16%)	336 (17%)
Normal	518 (52%)	538 (53%)	1056 (53%)
Overweight	157 (16%)	171 (17%)	328 (16%)
Obese	89 (9%)	85 (8%)	174 (9%)
Referral source			
Voluntary counselling and testing centre	340 (34%)	343 (34%)	683 (34%)
Local clinic	552 (56%)	555 (55%)	1107 (55%)
Other	97 (10%)	108 (11%)	205 (10%)
Education			
None	70 (7%)	65 (6%)	135 (7%)
Primary	270 (27%)	281 (28%)	551 (28%)
Secondary or higher	645 (65%)	657 (65%)	1302 (65%)
Employment			
Service worker or sales	159 (16%)	163 (16%)	322 (16%)
Labourer	223 (23%)	223 (22%)	446 (22%)
Unemployed	311 (31%)	325 (32%)	636 (32%)
Other	296 (30%)	295 (29%)	591 (30%)
History of tuberculosis	31 (3%)	43 (4%)	74 (4%)
Years of tuberculosis	13 (10)	10 (8)	11 (9)
BCG scar present†	773 (78%)	787 (78%)	1560 (78%)
CD4 lymphocyte count (cells per µL)‡	Median 288 (170–440); mean 331 (220)	Median 307 (176–457); mean 335 (220)	Median 297 (172–449); mean 333 (220)
<100	111 (11%)	130 (13%)	241 (12%)
100–199	193 (20%)	169 (17%)	362 (18%)
200–499	491 (50%)	498 (50%)	989 (50%)
≥500	178 (18%)	196 (20%)	374 (19%)
Tuberculin skin test§			
<5 mm (negative)	729 (74%)	722 (72%)	1451 (73%)
≥5 mm (positive)	216 (22%)	252 (25%)	468 (23%)
Abnormal chest radiograph			
Yes	35 (4%)	46 (5%)	81 (4%)
No	954 (96%)	960 (95%)	1914 (96%)
Haematology			
Haemoglobin (g/L)	126 (19)	126 (21)	126 (20)
White-blood-cell count (10 ⁹ /L)	4.9 (1.5)	4.8 (1.5)	4.8 (1.5)
Antiretroviral therapy			
Before enrolment	19 (2%)	32 (3%)	51 (2%)
By month 36	463 (47%)	483 (48%)	946 (47%)
Initiation month	Median 4 (2–16); mean 9 (11)	Median 5 (2–17); mean 9 (12)	Median 4 (2–16); mean 9 (12)
CPT (any time during study)	304 (31%)	313 (31%)	617 (31%)

Data are n (%), median (IQR), or mean (SD), unless otherwise stated. CD=cluster of differentiation. CPT=co-trimoxazole preventive therapy. *Data were missing for 47 participants in the control group and 54 in the continued isoniazid group. †Data were missing for 52 participants in the control group and 46 in the continued isoniazid group. ‡Data were missing for 16 participants in the control group and 13 in the continued isoniazid group. §Data were missing for 44 participants in the control group and 32 in the continued isoniazid group.

Table 1: Characteristics of participants

Participants attended follow-up visits once every 30 days for drug refills and assessments for symptoms of tuberculosis and drug side-effects by research nurses. Participants were counselled to return to the clinic if they felt unwell and to stop taking study drugs

if they thought they were making them sick. Attempts were made to contact participants immediately after any missed visit. Such participants were contacted first by mobile phone and then by a visit to their city dwelling or home village. If visits were unsuccessful, a

trained health worker traced participants and encouraged them to return to clinic.

Non-study personnel from the Government of Botswana established participants' HIV statuses on the basis of two positive ELISA tests (Ortho Kit HIV-1 and HIV-2 [Ortho Clinical Laboratories, Raritan, NJ, USA] or Abbott Murex [Murex Biotech, Dartford, UK]) or two positive rapid ELISA tests (Determine Assay [Abbott Diagnostics Abbott Park, IL, USA], Unigold [Trinity Biotech, Bray, Ireland], Capillus [Cambridge Diagnostics, Galway, Ireland], or Orasure [Orasure Technologies, Bethlehem, PA, USA]) run in parallel. Non-nucleoside reverse-transcriptase inhibitor-based antiretroviral therapy was offered according to Botswana national guidelines for participants with CD4 cell counts of fewer than 200 cells per μL or WHO clinical stage 3 or 4 disease.

We did tuberculin skin tests during screening by placing 5 tuberculin units (0.1 mL) of purified protein derivative (RT/23 Statens Serum Institut, Copenhagen, Denmark) subcutaneously; the reaction was read by study nurses within 48–72 h. 5 mm or more induration was regarded as positive.

Endpoints

We assessed participants with cough of any duration, weight loss, nocturnal sweats, or lymphadenopathy for incident tuberculosis and took sputum samples or

biopsy specimens for microscopy and mycobacterial culture. The primary endpoint was incident tuberculosis (definite, probable, and possible tuberculosis) and the secondary endpoint was death. Incident tuberculosis was defined as a clinical presentation consistent with tuberculosis and response to anti-tuberculosis therapy. Incident disease was categorised as definite if one or more culture was positive for tuberculosis and speciated as *M tuberculosis* or if two or more sputum smears were positive for acid-fast bacilli; probable if one sputum smear or one biopsy specimen was positive for acid-fast bacilli; and possible if smears and cultures were negative or not done. A death defined as possibly related to tuberculosis was one that had clinical or verbal autopsy evidence consistent with tuberculosis as the proximate cause of death. We asked participants who had severe (grade 3 or worse)²⁰ adverse events or who chose to stop study drugs to remain under observation to establish endpoints. Participants who had severe adverse events stopped taking the study drugs and were not rechallenged. Study doctors assessed whether severe adverse events were attributable to isoniazid on a scale ranging from unrelated to definitely related. We report severe adverse events in our analysis if they were at least possibly related to isoniazid. Severe hepatitis was defined as serum concentrations of aspartate or alanine aminotransferase more than

	All randomised participants (N=1995)			Participants starting masked treatment (N=1655)†		
	Control group (n=989)	Continued isoniazid group (n=1006)	Hazard ratio (95% CI)	Control group (n=821)	Continued isoniazid group (n=834)	Hazard ratio (95% CI)
Overall follow-up (100 person-years)	26.9	27.7	..	19.5	19.8	..
Tuberculin skin test status positive, negative, or unknown						
Tuberculosis (definite, probable, and possible)	34 (1.26)	20 (0.72)	0.57 (0.33–0.99)*	25 (1.29)	12 (0.61)	0.47 (0.24–0.94)*
Tuberculosis (definite and probable)	23 (0.85)	16 (0.58)	0.68 (0.36–1.28)	18 (0.93)	10 (0.51)	0.55 (0.25–1.18)
Tuberculosis‡ and possible tuberculosis deaths	40 (1.48)	24 (0.87)	0.58 (0.35–0.97)*	28 (1.44)	15 (0.76)	0.53 (0.28–0.99)*
Tuberculosis‡ and all deaths	72 (2.67)	59 (2.13)	0.80 (0.57–1.13)	41 (2.11)	37 (1.87)	0.89 (0.57–1.39)
All deaths	38 (1.41)	39 (1.41)	1.00 (0.64–1.56)	16 (0.82)	25 (1.27)	1.54 (0.82–2.88)
Tuberculin skin test status positive only						
Tuberculosis (definite, probable, and possible)	13 (2.22)	4 (0.57)	0.26 (0.09–0.80)*	11 (2.53)	1 (0.19)	0.08 (0.01–0.61)*
Tuberculosis (definite and probable)	12 (2.05)	4 (0.57)	0.28 (0.09–0.87)*	10 (2.30)	1 (0.19)	0.09 (0.01–0.67)*
Tuberculosis‡ and possible tuberculosis deaths	16 (2.73)	4 (0.57)	0.21 (0.07–0.63)*	13 (2.98)	1 (0.19)	0.07 (0.01–0.50)*
Tuberculosis‡ and all deaths	26 (4.43)	9 (1.29)	0.29 (0.14–0.62)*	20 (4.59)	4 (0.78)	0.17 (0.06–0.50)*
All deaths	13 (2.22)	5 (0.71)	0.32 (0.11–0.90)*	9 (2.07)	3 (0.58)	0.28 (0.08–1.03)
Tuberculin skin test status negative only						
Tuberculosis (definite, probable, and possible)	20 (1.01)	15 (0.76)	0.75 (0.38–1.46)	13 (0.92)	11 (0.78)	0.86 (0.38–1.89)
Tuberculosis (definite and probable)	10 (0.51)	11 (0.56)	1.10 (0.47–2.59)	7 (0.50)	9 (0.64)	1.29 (0.48–3.46)
Tuberculosis‡ and possible tuberculosis deaths	23 (1.16)	19 (0.96)	0.83 (0.45–1.52)	14 (0.99)	14 (0.99)	1.00 (0.48–2.10)
Tuberculosis‡ and all deaths	45 (2.28)	47 (2.37)	1.04 (0.69–1.57)	20 (1.42)	32 (2.27)	1.60 (0.92–2.80)
All deaths	25 (1.26)	32 (1.62)	1.28 (0.76–2.15)	7 (0.50)	21 (1.49)	2.99 (1.27–7.04)*

Data are number of cases (rate per 100 person-years) unless otherwise stated. * $p < 0.05$. †Participants completed 5 or more months of open-label isoniazid and began to receive masked treatment; participants did not develop tuberculosis, voluntarily withdraw, deviate from the protocol, or die in the initial open-label 6-month isoniazid preventive treatment period. ‡Tuberculosis definition includes definite, probable, and possible cases.

Table 2: Rates of tuberculosis and death in participants receiving 6 months' open-label isoniazid followed by 30 months' masked placebo (control group) or isoniazid (continued isoniazid group)

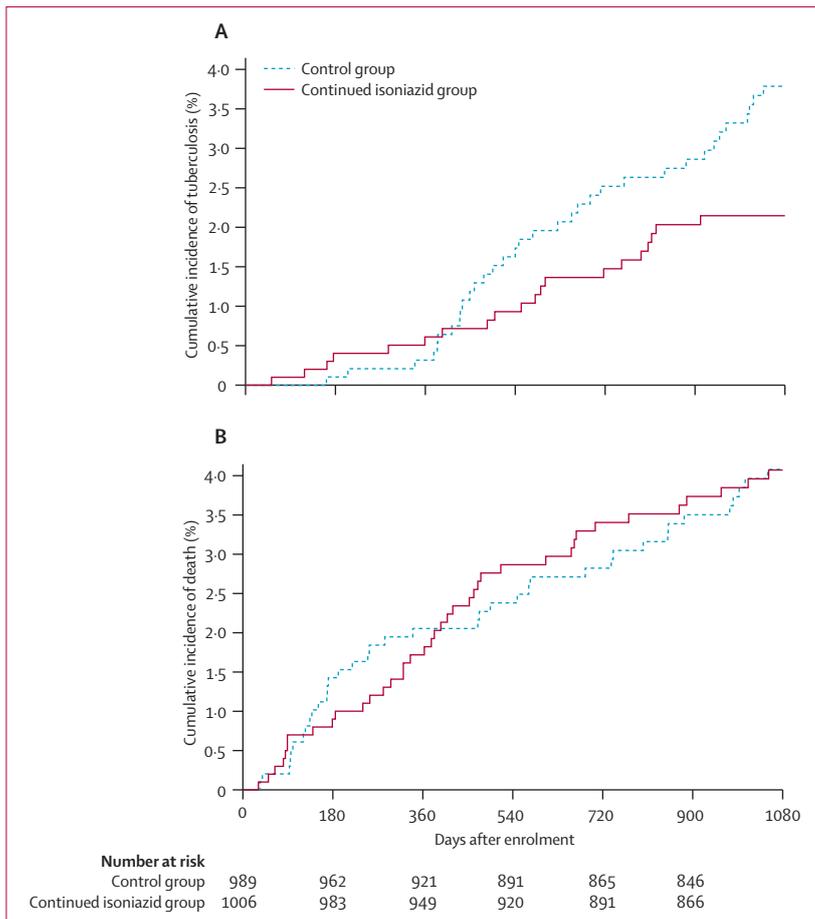


Figure 2: Cumulative incidence of tuberculosis (A) and death (B) in participants receiving 6 months' open-label isoniazid and 30 months' masked placebo (control group) or isoniazid (continued isoniazid group)

See Online for webappendix

five-times the upper limit of normal, irrespective of symptoms. We drew blood 2 weeks after enrolment and before initiation of antiretroviral therapy to assess hepatic function, or if clinically indicated. Incident cases of tuberculosis, deaths, and severe adverse events were reviewed by an independent committee of four experienced clinicians who were masked to treatment assignment.

Statistical analysis

Assuming an incidence of tuberculosis of 10% during the study in controls and a 50% reduction in tuberculosis incidence in participants continuing isoniazid,²¹ we calculated the sample size to be 474 participants per group at 5% significance and power of 80%. This number was inflated by dividing by 0.79 for an expected attrition of 21% and by 0.60 for an expected 40% negative rate¹⁰ on tuberculin skin test, yielding 2000 participants for both groups.

We analysed incidence of tuberculosis and death in two cohorts: the first was all enrolled participants (ie, the randomised population) and the second was all

participants who started masked treatment after the first 6 months of open-label isoniazid. To be included in the second cohort, participants had to have completed 5 months or more of open-label isoniazid without developing tuberculosis, voluntarily withdrawing, deviating from the protocol, or dying. The date of incident tuberculosis was the date when anti-tuberculosis therapy was started. We used the log-rank test to establish differences between the control and continued isoniazid treatment groups, and $p < 0.05$ was regarded as significant. We used unadjusted Cox regression analyses to calculate hazard ratios (HRs).

Duration of antiretroviral therapy was defined as the number of days from initiation to the endpoint (or date of study completion) minus 60 days. This 60-day rule was applied because all participants starting antiretroviral therapy less than 60 days before anti-tuberculosis treatment had previously shown symptoms of tuberculosis, but were started on antiretroviral therapy because treating clinicians suspected AIDS progression before diagnosis of tuberculosis. Because we intended to examine the preventive effect of antiretroviral therapy on tuberculosis, we discounted previous antiretroviral therapy for five such cases of tuberculosis. Since antiretroviral therapy was initiated at different times for different participants, we used a multivariable Cox regression model to assess its effect on incidence of tuberculosis. We initially included treatment group, baseline CD4 cell count (cutoff 200 cells per μL), baseline tuberculin skin test status, antiretroviral therapy as a time-dependent variable, and interaction terms in the models. Without prespecified stopping rules, we applied backward and forward model selection procedures with all interaction terms. We used Akaike information criterion values to review all generated models (webappendix pp 6–8). The final model included the four main variables (treatment group, CD4 cell count, tuberculin skin test status, and antiretroviral therapy) and an interaction term between treatment group and tuberculin skin test status.

To establish the proportion of cases of isoniazid-resistant tuberculosis that would be expected during preventive treatment for comparison with the proportion reported in the study, we did two-by-two table analyses crossing tuberculosis incidence (cases of tuberculosis or no tuberculosis) with the prevalence of isoniazid-resistant latent infection (webappendix pp 16–17). We assumed a background prevalence of 10% isoniazid resistance in latent cases of tuberculosis.²² Tuberculosis incidence was adjusted by the reported efficacy of isoniazid preventive treatment. Statistical analyses were done with Microsoft Excel 2007.

The trial is registered at ClinicalTrials.gov, number NCT00164281.

Role of the funding source

The US Centers for Disease Control and Prevention and the US Agency for International Development (USAID)

funded the study, but had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study, has responsibility for the integrity of the data and the accuracy of the data analysis, and had final responsibility for the decision to submit for publication.

Results

Figure 1 shows the trial profile and table 1 lists characteristics of enrolled participants. Eight participants in the control group and three in the continued isoniazid group were lost to follow-up, and 85 and 91 participants voluntarily withdrew, respectively, during the 36 months. 1548 (78%) of participants attended 80% or more of monthly pharmacy refill visits.

In the enrolled cohort, there were 54 incident cases of tuberculosis; 33 were classed as definite, six probable, and 15 possible (table 2). The causes of ten deaths that were possibly attributed to tuberculosis were very uncertain, and per protocol, were not included as tuberculosis endpoints. 34 (3.4%) of 989 participants in the control group and 20 (2.0%) of 1006 in the continued isoniazid group had incident tuberculosis. Incidence was 1.26% per year in the control group compared with 0.72% per year in the continued isoniazid group (HR 0.57, 95% CI 0.33–0.99, $p=0.047$). Tuberculosis incidence in the two groups diverged about 200 days after completion of the initial 6 months' isoniazid prophylaxis, suggesting that the benefit of the initial treatment was lost by this time (figure 2). Tuberculin skin test status strongly modified the effectiveness of continued isoniazid prophylaxis (table 2, figure 3). For participants with a positive tuberculin skin test, 13 (6.0%) of 216 controls developed tuberculosis compared with four (1.6%) of 252 in the continued isoniazid group (HR 0.26, 95% CI 0.09–0.80, $p=0.02$; table 2). For participants with a negative tuberculin skin test, 20 (2.7%) of 729 controls developed tuberculosis compared with 15 (2.1%) of 722 in the continued isoniazid group (HR 0.75, 0.38–1.46, $p=0.40$; table 2).

Strongest adherence to continued isoniazid treatment in participants with a positive tuberculin skin test was associated with the largest decrease in tuberculosis incidence. Compared with all enrolled participants with a positive tuberculin skin test, those participants who started masked treatment and were tuberculin-skin-test positive had three-times lower rates of tuberculosis if they received continued therapy (table 2). A greater efficacy of continued isoniazid was associated with those who were tuberculin-skin-test positive, started masked treatment, had no protocol deviations, and were 80% or more adherent to monthly pharmacy refill visits: eight (5%) cases of tuberculosis were reported in 156 controls compared with none in 173 from the continued isoniazid group (HR 0.00, 0.00–0.29, $p=0.0023$). There were fewer deaths in all enrolled participants who had positive tuberculin skin tests and were allocated to continued

isoniazid treatment than there were in those allocated to placebo ($p=0.03$; table 2).

We restricted antiretroviral therapy efficacy analyses to the cohort that began masked treatment because only 2% of participants were receiving antiretroviral therapy at enrolment and the additional 45% who started such therapy later did so at a median of 4 months after enrolment (table 1). Of 946 participants starting antiretroviral therapy, 415 (44%) received zidovudine, lamivudine, and nevirapine; 406 (43%) received zidovudine, lamivudine, and efavirenz; 52 (6%) received stavudine, lamivudine, and nevirapine or efavirenz; and 31 (3%) received tenofovir, emtricitabine or lamivudine, and nevirapine or efavirenz. Timing of antiretroviral therapy initiation after enrolment did not differ between groups (webappendix p 6). Cox regression modelling suggested that starting of antiretroviral therapy at enrolment led to greater protection from tuberculosis than did initiation 180 days after enrolment; participants who did not receive antiretroviral therapy had the highest incidence of tuberculosis (figure 4).

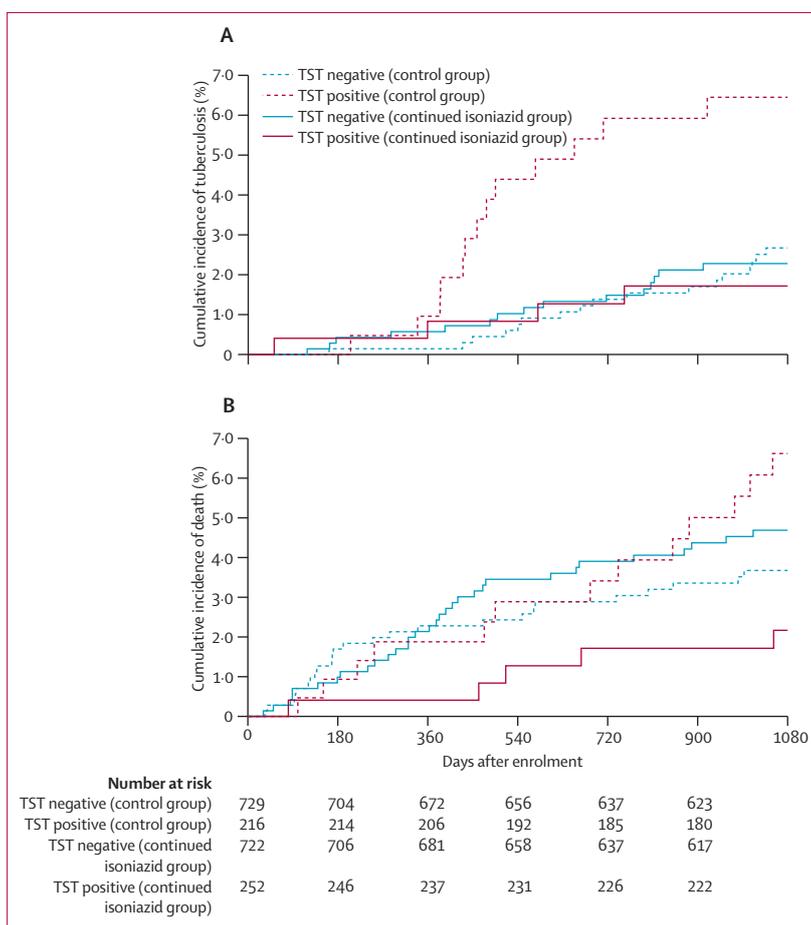


Figure 3: Cumulative incidence of tuberculosis (A) and death (B) in participants receiving 6 months' open-label isoniazid and 30 months' masked placebo (control group) or isoniazid (continued isoniazid group), by TST status
TST=tuberculin skin test.

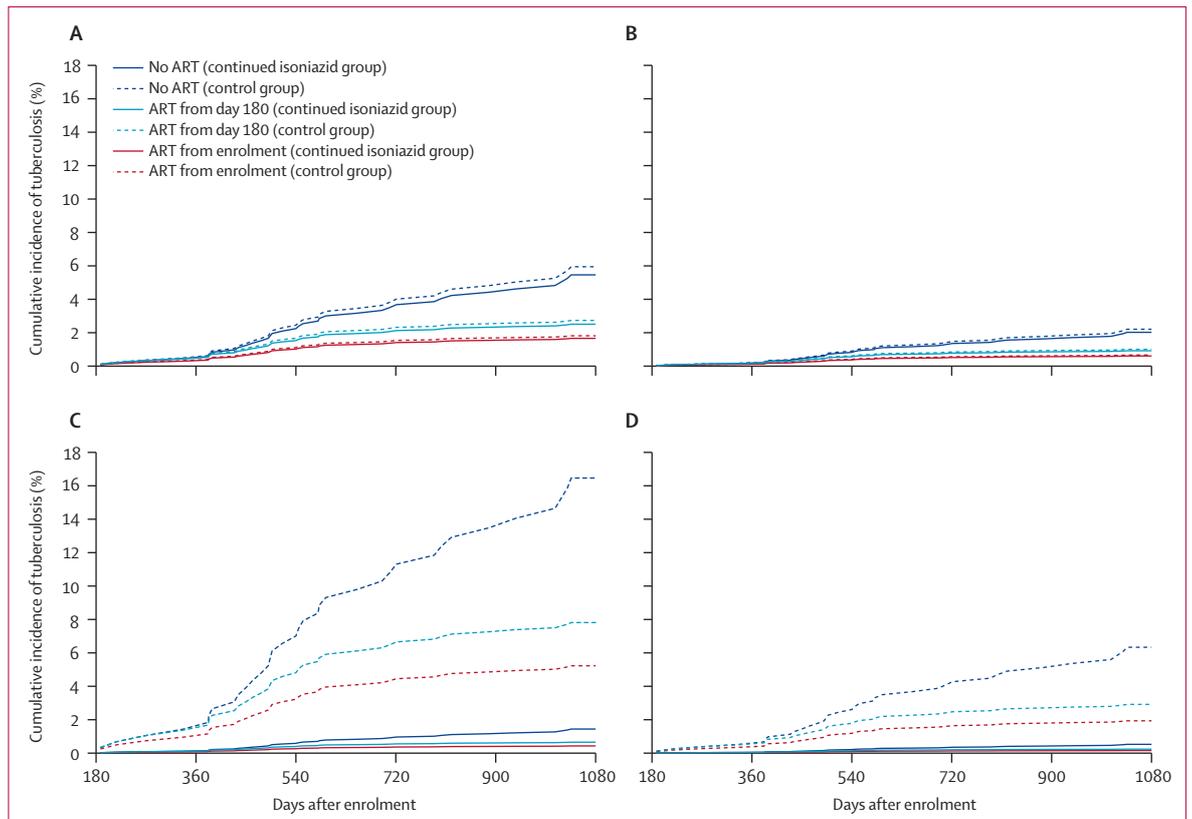


Figure 4: Modelled cumulative incidence of tuberculosis in participants starting 30 months' masked placebo (control group) or isoniazid (continued isoniazid group) after 6 months' open-label isoniazid, by time of antiretroviral therapy initiation, baseline CD4 lymphocyte count, and tuberculin skin test status (A) Negative tuberculin skin test, CD4 cell count of fewer than 200 cells per μL . (B) Negative tuberculin skin test, CD4 cell count of 200 cells or more per μL . (C) Positive tuberculin skin test, CD4 cell count of fewer than 200 cells per μL . (D) Positive tuberculin skin test, CD4 cell count of 200 cells or more per μL . We used a Cox regression model to create these graphs, including the four main variables (treatment group, CD4 cell count, tuberculin skin test status, and antiretroviral therapy as a time-dependent variable) and an interaction term between treatment group and tuberculin skin test status. Webappendix p 9 shows population at risk for every timepoint. ART=antiretroviral therapy.

Antiretroviral therapy progressively reduced the risk of tuberculosis. After 360 days of antiretroviral therapy, the adjusted HR of tuberculosis compared with participants who did not receive antiretroviral therapy was 0.50 (95% CI 0.26–0.97) for controls (table 3). Compared with controls, participants with a positive tuberculin skin test benefited substantially from continued isoniazid treatment, with a possible further reduction in tuberculosis incidence if they also received antiretroviral therapy for 360 days (table 3). Conversely, in participants with negative tuberculin skin tests, continued isoniazid treatment did not reduce the risk of tuberculosis, though antiretroviral therapy did (table 3).

Drug-susceptibility testing results were available for 29 cases of tuberculosis in all enrolled participants. One *M tuberculosis* isolate (3%) was resistant to rifampicin and streptomycin and five (17%) were isoniazid resistant (four resistant only to isoniazid and one resistant to isoniazid, rifampicin, and streptomycin). The proportion of any isoniazid resistance did not differ from the expected proportion of 18% (webappendix p 16).

In the 6-month open-label isoniazid administration phase, we noted severe adverse events associated with

study drugs in 14 (1.4%) of 989 controls and 16 (1.6%) of 1006 participants who continued isoniazid. There were ten cases of hepatitis in each group (one with jaundice in the control group; two with jaundice in the continued isoniazid group); two cases of rash and two seizures in the control group; and three cases of rash, one of seizure, one of headache, and one of tinnitus in the continued isoniazid group.

Seven (1%) of 821 participants who continued in the study after 6 months and were started on placebo had severe adverse events associated with study drugs, compared with 11 (1.3%) of 834 participants who continued isoniazid ($p=0.36$). There were six cases of hepatitis (one with jaundice) and one case of rash in controls and nine cases of hepatitis (one with jaundice), one case of rash, and one case of peripheral neuropathy in the continued isoniazid group. The jaundiced participant in the continued isoniazid group died of hepatic encephalopathy 9 months after enrolment with concomitant jaundice, disseminated herpes zoster, and lymphocytic pleiocytosis on cerebrospinal fluid analysis; she stopped taking isoniazid 3 days after becoming

	Tuberculin skin test negative		Tuberculin skin test positive	
	Adjusted hazard ratio (95% CI)	Reduction (%)	Adjusted hazard ratio (95% CI)	Reduction (%)
Antiretroviral therapy (control group)				
None	1.00 (referent)	..	1.00 (referent)	..
180 days	0.76 (0.58–0.99)	24%	0.76 (0.58–0.99)	24%
270 days	0.61 (0.39–0.98)	39%	0.61 (0.39–0.98)	39%
360 days	0.50 (0.26–0.97)	50%	0.50 (0.26–0.97)	50%
540 days	0.33 (0.11–0.94)	67%	0.33 (0.11–0.94)	67%
Antiretroviral therapy (continued isoniazid group)				
None	0.92 (0.40–2.1)	8%	0.08 (0.01–0.63)	92%
180 days	0.69 (0.29–1.64)	31%	0.06 (0.008–0.49)	94%
270 days	0.56 (0.22–1.44)	44%	0.05 (0.006–0.40)	95%
360 days	0.45 (0.16–1.30)	54%	0.04 (0.005–0.35)	96%
540 days	0.30 (0.08–1.15)	70%	0.03 (0.003–0.27)	97%

We calculated adjusted hazard ratios using the same Cox regression model as is described in figure 4.

Table 3: Multivariable analysis of the effect on tuberculosis incidence of antiretroviral therapy

jaundiced. The participant with jaundice in the control group developed symptoms 16 months after enrolment while receiving placebo; she had a positive serum hepatitis B virus antigen test and was receiving medicines from a traditional healer, but recovered completely after stopping use of all drugs. A second death from hepatic encephalopathy that was attributed to isoniazid occurred during the open-label period and was previously described.²³ From enrolment, there were 29 cases of severe isoniazid-associated hepatitis in more than 3100 person-years of isoniazid use: eight (28%) cases had symptoms (including the two cases of hepatic encephalopathy), four (14%) had jaundice, and 26 (90%) occurred during the first 9 months of therapy. 29 (1%) of the 1995 participants enrolled had severe isoniazid-associated (biochemical) hepatitis (rate 14.5 per 1000 participants), eight had clinical hepatitis (4.0), and two had hepatic encephalopathy (1.0).

After exclusion of 11 participants who were lost to follow-up by 36 months, mortality was 1.3% per year (77 deaths in 1984 participants). In participants starting antiretroviral therapy, mortality was 2.4% in the first year after initiation, 1.0% in the second year, and 1.3% in the third year. Mortality did not differ between study groups for all enrolled participants, but for participants with a positive tuberculin skin test mortality was three-times lower in the continued isoniazid group than in the control group ($p=0.03$; table 2). All-cause mortality was higher in the subpopulation that started masked treatment and had negative tuberculin skin tests and continued to receive isoniazid than it was in equivalent controls ($p=0.01$; table 2). With the exception of the one case of hepatic encephalopathy that occurred in month 9, the 20 other deaths in these participants with negative tuberculin skin tests were not associated with jaundice: five were caused by gastroenteritis, four by respiratory illness unlikely to be tuberculosis, three by possible

tuberculosis, two by suicide, one by homicide, one from hypertensive crisis, one from Kaposi's sarcoma, one from cervical cancer, one from sepsis, and one from severe anaemia.

Discussion

Compared with 6-month isoniazid prophylaxis, continuation of isoniazid preventive treatment for 36 months can reduce incidence of tuberculosis by 43% in adults with HIV infection living in areas that are highly endemic for tuberculosis.

The benefit of initial 6-month isoniazid prophylaxis was lost about 200 days after completion of therapy in the control group, as reported previously.¹² The benefit of continued isoniazid treatment was most striking for participants with a positive tuberculin skin test, who had a 74% reduction in tuberculosis incidence. Similarly, a preliminary as-treated analysis of a smaller clinical trial²⁴ from South Africa showed lower rates of tuberculosis in people with HIV infection and positive tuberculin skin tests receiving 36 months' isoniazid preventive treatment than participants receiving 6 months' isoniazid.

Although people with HIV infection and negative tuberculin skin tests did not benefit significantly from short-course prophylaxis in previous studies,^{9,10,25–27} it was anticipated that they would benefit from continuous prophylaxis. Unfortunately, as in the previous studies that provided 6 months or 12 months of isoniazid, we noted that such participants derived no significant benefit from 36 months' treatment. This absence of observable benefit might have been attributable to inclusion of some participants who did not have latent *M tuberculosis* infection at the time of enrolment and others who, although truly infected, did not have a tuberculosis-specific immune response capable of synergising with isoniazid's bacteriostatic effect on *M tuberculosis*. This postulation is supported by reports^{28–30} that anergic

patients with tuberculosis are less likely to have tuberculosis-specific lymphocyte proliferation and type 1 responses (eg, interferon- γ) and are more likely to have type 2 responses (eg, interleukin-10) compared with patients with tuberculosis and positive tuberculin skin tests. In addition to latent tuberculosis infection, a positive skin test might also be regarded as a marker of immunological ability to react to *M tuberculosis* antigens.³¹ The rate of positive response to tuberculin skin testing declined with declining CD4 cell count in our cohort (webappendix p 1), and participants with CD4 cell counts of 200 cells per μ L or more benefited more from continued isoniazid treatment than did those with CD4 cell counts of fewer than 200 cells per μ L (webappendix p 5). Taken together, these findings suggest that people with HIV infection need to be identified early in its course when they are most likely to be tuberculin skin test positive and benefit from isoniazid preventive treatment.

Extended isoniazid treatment might provide benefit through improved eradication of latent tuberculosis that was present at enrolment,³² but we believe, for two reasons, it was probably due to prevention of new infections. First, short-courses of isoniazid preventive treatment have a durable protective effect for people with HIV and positive tuberculin skin tests in areas of low tuberculosis incidence.³³ Second, high rates of clustering and reinfection of successfully treated cases of tuberculosis have been reported in Botswana and other endemic countries.^{13–18}

That participants with negative tuberculin skin tests did not substantially benefit from continued isoniazid treatment was surprising, since they presumably had a high risk of infection. One possible reason is that most participants with negative skin tests were truly uninfected with *M tuberculosis* at baseline, so a substantially larger sample size would be needed to show a treatment effect. Alternatively, they might have had a lower epidemiological risk of infection after enrolment than did skin-test positive participants or there could have been imbalances in adherence to isoniazid preventive treatment or to antiretroviral therapy use for this subpopulation within the two treatment groups.

In our study, increasing use of antiretroviral therapy conferred a progressively greater reduction in the risk of tuberculosis and was additive to the protective effect of isoniazid preventive treatment. However, our reported reduction of tuberculosis incidence by ART of 50% is somewhat lower than the 59–81% range reported in other studies.^{6,7,34–36} We believe this was a result of differences between our cohort and those in other studies. We analysed the effect of antiretroviral therapy on tuberculosis incidence by comparison of incidence between participants who were eligible for therapy and those who were not. Ineligibility was defined by a high CD4 cell count, so this group inherently had a lower risk of tuberculosis than did those eligible for antiretroviral therapy.⁵ In other studies, the effect of antiretroviral therapy on tuberculosis incidence has been compared between populations that

all had advanced HIV disease and were thus at high risk for tuberculosis. Several studies^{37–39} of Africans with HIV infection who were starting antiretroviral therapy reported an incidence of tuberculosis of 9–22% per year in the first few months after initiation—possibly a result of unmasking of immune reconstitution. We noted a 1.9% yearly incidence of tuberculosis in the first 6 months of antiretroviral therapy. The reasons for this low incidence compared with previous studies might have been not only the study enrolment criteria but also eradication of *M tuberculosis* in asymptomatic participants with positive tuberculin skin tests when isoniazid preventive treatment precedes antiretroviral therapy.⁷ Participants with negative skin tests benefited substantially and primarily from antiretroviral therapy.

The rate of severe isoniazid-associated hepatitis that we reported was below or similar to rates with shorter courses of isoniazid.^{10,27} Cases occurred almost entirely during the first 9 months of treatment. The rate was low despite daily use of isoniazid for more than 3100 person-years in a region with other risk factors for hepatitis such as antiretroviral therapy, hepatitis B virus co-infection, alcohol dependence, and traditional medicines. We previously reported²³ that antiretroviral therapy did not significantly increase the risk of isoniazid-associated hepatitis during the 6-month open-label period.

Overall, mortality was equivalent between the two groups in our study although participants with a positive tuberculin skin test derived a survival advantage from continued isoniazid treatment. Although our report is the first to show this reduction after 36 months' treatment in people with HIV accessing antiretroviral therapy, such a benefit has been reported in a clinical trial²⁶ that provided 12 months' isoniazid preventive treatment in the pre-antiretroviral therapy era, and a retrospective analysis⁴⁰ of an antiretroviral therapy programme that concomitantly provided 6 months of isoniazid. Nevertheless, patients and health workers should maintain vigilance because a small risk of clinical hepatitis and hepatic encephalopathy remains. The two cases of hepatic encephalopathy at months 6 and 9 (both in skin-test-negative participants) are concerning but rates are not significantly higher than those expected on the basis of established statistics.⁴¹

Our study had some limitations. As in other tuberculosis prophylaxis studies,^{9,10} only 33 (61%) of 54 cases of tuberculosis were culture-positive. This outcome was expected because many people with HIV infection have paucibacillary disease; they probably benefited from early detection of tuberculosis in this prospective study. The use of antiretroviral therapy was not controlled in the study's design and, therefore, the strength of our conclusions for antiretroviral therapy is much the same as that for observational cohorts. Our findings might not be generalisable to populations that do not undergo equivalent screening procedures. We used the same screening algorithm as the Botswana

Panel: Research in context**Systematic review**

We searched the PubMed database without language restriction for articles published up to Dec 14, 2010, with the search terms “tuberculosis” AND “isoniazid” AND “HIV” AND “prevention” OR “prophylaxis”. A comprehensive review by Akolo and colleagues² summarised and assessed quality of evidence for tuberculosis preventive therapy in people with HIV infection. Because there were no published clinical trials testing the benefit of tuberculosis preventive therapy beyond 12 months or in combination with antiretroviral therapy, the authors concluded that “trials evaluating...the optimal duration of TB preventive therapy...and combination of antituberculosis chemoprophylaxis with antiretroviral therapy are needed.”²

Interpretation

Chemoprophylaxis for tuberculosis in people with HIV infection was previously shown to reduce the incidence of the disease, but the limited duration of benefit suggested the need to investigate continuous prophylaxis. Our study has shown that extended prophylactic treatment is beneficial in the setting of concomitant antiretroviral therapy in a tuberculosis-endemic country. Continued isoniazid prophylaxis mainly benefited people with HIV infection and positive tuberculin skin tests.

government’s isoniazid preventive treatment programme, but added laboratory and chest radiograph criteria. Compared with people with HIV who would have been enrolled in the government’s programme, our cohort probably had higher neutrophil counts and fewer abnormal chest radiographs.

We showed that, in Botswana (a country where tuberculosis is endemic and where antiretroviral therapy is provided without charge to people with HIV infection), isoniazid prophylaxis for 36 months is better for prevention of tuberculosis than is 6 months’ treatment, especially for individuals who are positive on tuberculin skin tests (panel). Although optimum approaches to implementation of tuberculin skin testing in resource-constrained settings need to be established, our study provides public health officials in tuberculosis-endemic countries with important efficacy and safety information about extended administration of isoniazid preventive treatment.

Contributors

EAT, CDW, TLM, HJM, PHK, KGC, LB, NS, and TSa designed the study. TA, LB, SN, ZT, TSi, BM, and TSa implemented the trial. EAT, TA, BM, TLM, OIM, MKD, PHK, CDW, and TSa did the regulatory support. TA, BM, MKD, KGC, PHK, CDW, and TSa did the administration. TA, SN, ZT, TSi, BM, and TSa did the data collection. TSa and NS analysed the data. TSa wrote the first draft. TA, ZT, TSi, TLM, HJM, EAT, OIM, MKD, KGC, CDW, PHK, and TSa contributed to the interpretation of the data. All authors reviewed and approved the final report.

Conflicts of interest

We declare that we have no conflicts of interest.

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