

Isoniazid plus antiretroviral therapy to prevent tuberculosis: a randomised double-blind, placebo-controlled trial



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Summary

Background Antiretroviral therapy reduces the risk of tuberculosis, but tuberculosis is more common in people with HIV than in people without HIV. We aimed to assess the effect of isoniazid preventive therapy on the risk of tuberculosis in people infected with HIV-1 concurrently receiving antiretroviral therapy.

Methods For this pragmatic randomised double-blind, placebo-controlled trial in Khayelitsha, South Africa, we randomly assigned (1:1) patients to receive either isoniazid preventive therapy or a placebo for 12 months (could be completed during 15 months). Randomisation was done with random number generator software. Participants, physicians, and pharmacy staff were masked to group assignment. The primary endpoint was time to development of incident tuberculosis (definite, probable, or possible). We excluded tuberculosis at screening by sputum culture. We did a modified intention-to-treat analysis and excluded all patients randomly assigned to groups who withdrew before receiving study drug or whose baseline sputum culture results suggested prevalent tuberculosis. This study is registered with ClinicalTrials.gov, number NCT00463086.

Findings 1329 participants were randomly assigned to receive isoniazid preventive therapy (n=662) or placebo (n=667) between Jan 31, 2008, and Sept 31, 2011, and contributed 3227 person-years of follow-up to the analysis. We recorded 95 incident cases of tuberculosis; 37 were in the isoniazid preventive therapy group (2.3 per 100 person-years, 95% CI 1.6–3.1), and 58 in the placebo group (3.6 per 100 person-years, 2.8–4.7; hazard ratio [HR] 0.63, 95% CI 0.41–0.94). Study drug was discontinued because of grade 3 or 4 raised alanine transaminase concentrations in 19 of 662 individuals in the isoniazid preventive therapy group and ten of the 667 individuals in the placebo group (risk ratio 1.9, 95% CI 0.90–4.09). We noted no evidence that the effect of isoniazid preventive therapy was restricted to patients who were positive on tuberculin skin test or interferon gamma release assay (adjusted HR for patients with negative tests 0.43 [0.21–0.86] and 0.43 [0.20–0.96]; for positive tests 0.86 [0.37–2.00] and 0.55 [0.26–1.24], respectively).

Interpretation Without a more predictive test or a multivariate algorithm that predicts benefit, isoniazid preventive therapy should be recommended to all patients receiving antiretroviral therapy in moderate or high incidence areas irrespective of tuberculin skin test or interferon gamma release assay status.

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Introduction

Tuberculosis is the main cause of morbidity and mortality in people infected with HIV-1. The burden is greatest in sub-Saharan Africa, especially in southern Africa where more than 50% of new tuberculosis cases are co-infected with HIV-1.¹ Findings of a meta-analysis² of randomised controlled trials showed that isoniazid preventive therapy decreased the risk of tuberculosis by 32% in people with HIV-1 who were not on combination antiretroviral therapy. Strong statistical evidence for benefit was only reported in individuals who had positive tuberculin skin tests. However, there was heterogeneity of isoniazid preventive therapy duration and follow-up between the included studies. Antiretroviral therapy independently reduces the risk of tuberculosis by 65%,³ but tuberculosis is more common in people infected with HIV than in people without HIV.⁴

Data from three retrospective observational cohort studies^{5–7} suggested a greater effect of combined antiretroviral therapy and isoniazid preventive therapy on the risk of tuberculosis than antiretroviral therapy alone. The proportion of individuals who received both treatments concurrently was unclear in the Brazilian study,⁶ and isoniazid preventive therapy preceded antiretroviral therapy in the South African study.⁷ In the BOTUSA randomised controlled trial⁸ done in Botswana, isoniazid preventive therapy for 36 months was more beneficial than treatment for 6 months in individuals who started antiretroviral therapy. However, antiretroviral therapy was prescribed in some of the participants at different times during follow-up when participants fulfilled criteria for antiretroviral therapy initiation. Thus, the independent effect of isoniazid preventive therapy in patients on antiretroviral therapy could not be established. In an

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Indian study,⁹ in which the effect of 6 months of ethambutol and isoniazid was compared with 36 months of isoniazid in patients with HIV, some participants started antiretroviral therapy, but the effect of concomitant antiretroviral therapy and isoniazid preventive therapy on efficacy and toxic effects was not reported.

Concurrent isoniazid preventive therapy and antiretroviral therapy could result in shared toxic effects, notably hepatitis and neuropathy. Furthermore, isoniazid inhibits several cytochrome P450 isoenzymes,¹⁰ which metabolise many antiretroviral drugs. This inhibition can cause increased antiretroviral concentrations, which might exceed the toxic threshold. Therefore, whether isoniazid preventive therapy further reduces the risk of tuberculosis in people on antiretroviral therapy is important to establish, and any additional toxic effects should be quantified.

We aimed to assess the effect of isoniazid preventive therapy on the risk of active tuberculosis in people with HIV-1 concurrently receiving antiretroviral therapy. Our secondary objectives were to establish toxic effects of treatment and all-cause mortality.

Methods

Study design and participants

We did a pragmatic individually randomised double-blind, placebo-controlled trial between Jan 31, 2008, and Sept 31, 2011, of isoniazid preventive therapy in people with HIV-1 on antiretroviral therapy at the Ubuntu clinic in Khayelitsha, Cape Town, South Africa.

Adults (≥ 18 years) were recruited among antiretroviral therapy clinic attendees consecutively listed in study screening logs. All participants had baseline tuberculosis symptom screening and sputum mycobacterial culture.¹¹ Exclusion criteria were active tuberculosis or suspicion of active tuberculosis as established by symptom screening, present or previous treatment of latent tuberculosis infection; present treatment with fluoroquinolones or other antibiotics with marked antituberculous activity, history of intolerance to isoniazid; grade 3 or 4 baseline alanine transaminase, grade 3 or 4 peripheral neuropathy, pregnancy, or less than 6 weeks post partum. We used tables from the AIDS Clinical Trials Group¹² to grade toxic effects of treatment for people on antiretroviral therapy.

Ethics approval was obtained from the ethics review boards of the University of Cape Town, Médecins Sans Frontières, and the London School of Hygiene & Tropical Medicine. Written consent or a thumb-print was needed from all participants before screening. Four people on a data safety and monitoring board provided oversight during the study.

Randomisation and masking

Patients were randomly assigned (1:1) to receive daily self-administered isoniazid or matching placebo dosed according to bodyweight (200 mg per day for < 50 kg or 300 mg per day for ≥ 50 kg) together with 25 mg of

pyridoxine for 12 months (could be completed during 15 months). Randomisation was by random number generator software (in Excel) and was stratified by antiretroviral therapy status at baseline: just started antiretroviral therapy (start-antiretroviral therapy) versus established on antiretroviral therapy (on-antiretroviral therapy). Participants, clinicians, and local pharmacy staff were masked to treatment allocations. The appendix gives further details on randomisation and masking.

Procedures

At each visit participants were asked about symptoms of adverse drug events: nausea, vomiting, or right upper-quadrant pain, rashes, and new or worsening peripheral neuropathy. During the intervention phase, we measured alanine transaminase concentration at baseline, every month for the first 3 months, and every 3 months thereafter. Protocol-specified reasons for permanent cessation of study drug because toxic effects were new or worsening peripheral neuropathy of grade 2 or more, grade 3 or 4 raised alanine transaminase concentrations, or clinical hepatitis, new rash grade 2 or more. CD4 lymphocyte counts and viral loads were measured according to clinic protocols (initially every 6 months then every year after the first year on antiretroviral therapy from 2010). Clinic nurses and doctors followed up participants routinely according to regular clinic schedules; schedules were aligned with antiretroviral therapy appointments to aid participant retention. Pharmacy staff dispensed the study drug along with other routine prescriptions. Pharmacy refill records were used to monitor adherence to antiretroviral therapy and the study drug.

A standard tuberculosis symptom screen was done at each clinic visit. Two sputum specimens were obtained from suspected cases of tuberculosis for microscopy by auramine staining and for mycobacterial culture. Species identification and drug sensitivity testing for isoniazid and rifampicin was done for all positive isolates (BACTEC mycobacterial growth indicator tube, Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). Specimens were processed at the National Health Laboratory Service, Cape Town, South Africa. Sputum induction was done on individuals unable to expectorate spontaneously. Needle aspiration biopsy was done in patients who presented with suspected tuberculosis lymphadenitis. Patients suspected to have extrapulmonary tuberculosis and needing further investigation were referred to an HIV specialist clinic (G F Jooste Hospital, Cape Town, South Africa). Urine for acid fast bacilli smear and culture was requested from all participants with suspected extrapulmonary tuberculosis. Infectious disease specialists (GM and RJW) were consulted for difficult cases. Tuberculosis treatment was started for all patients who met the case definition for tuberculosis (definite if compatible clinical features plus culture positive for *Mycobacterium*

See Online for appendix

tuberculosis, probable if based on microscopy, or possible if based only on radiology or clinical features). At each clinic visit, participants were asked about tuberculosis investigations or treatment initiated outside the Ubuntu clinic. The provincial electronic tuberculosis register and the database of the National Health Laboratory Service were searched at study closure for tuberculosis cases to verify completeness of ascertainment and to identify cases diagnosed at other sites in patients who might have left care. Database linking was done via a unique patient number or national identity number. All incident cases were verified before study unmasking.

A tuberculin skin test (2TU RT23 PPD, Statens Serum Institut, Denmark) and interferon gamma release assay (QuantiFERON gold in-tube, Cellestis, Australia) were done as part of a nested study.¹³ These tests were done at the baseline screening visit, before application of specific inclusion and exclusion criteria, and in participants who suggested willingness to return for tuberculin skin test results. Manufacturer's criteria for interferon gamma release assay positivity were used (≥ 0.35 IU/mL); tuberculin skin test induration of 5 mm or more was deemed positive.

Outcomes

The primary endpoint was time to development of incident tuberculosis (definite, probable, or possible) during the study. Secondary endpoints were time to death or the risk of adverse drug reaction. For the primary outcome we calculated person-time at risk from date of randomisation to earliest of tuberculosis (the clinic visit date tuberculosis was diagnosed and registered in the clinic database or notified in the tuberculosis register); death (death ascertainment was by report to the clinic staff and augmented from the national death register); loss to follow-up (participants were defined as lost to follow-up if their last clinic contact was more than 6 months before the date of closure of the study database, which was censored at their last contact date); transferred out (excluding participants transferred out for care at another clinic in the district since outcome data is available on the province-wide patient electronic register and from the national tuberculosis register); or study closure.

Statistical analysis

Results of this trial were reported in accordance with the CONSORT guidelines for reporting pragmatic trials.¹⁴ We did a modified intention-to-treat analysis; we excluded any participants who withdrew from the study before receiving the study drug or those whose baseline sputum culture results suggested prevalent tuberculosis after randomisation. We used the log-rank test to compare survival curves by treatment group. The hazard ratio (HR) for the treatment effect and the associated 95% CIs were calculated by Cox proportional hazards regression. To assess the durability of the treatment effect, follow-up time was split into three time groups (0–11 months,

12–23 months, and ≥ 24 months) and we used the likelihood ratio test to test for effect modification by time interval. At the time of the analysis, we specified the effects of isoniazid by time since randomisation and by tuberculosis infection status at enrolment. Time from randomisation to death was compared by study group and HRs were calculated from the Cox proportional hazards model.

A final sample size of 1368 had 80% power to detect a 35% reduction in the incidence of tuberculosis in the intervention versus control group assuming a rate of 8.5 per 100 person-years in the control group, a type I error of 0.05 and a 30% loss to follow-up in each group (appendix).^{15,16} All analyses were done with STATA (version 12.0).

This study is registered with ClinicalTrials.gov, number NCT00463086.

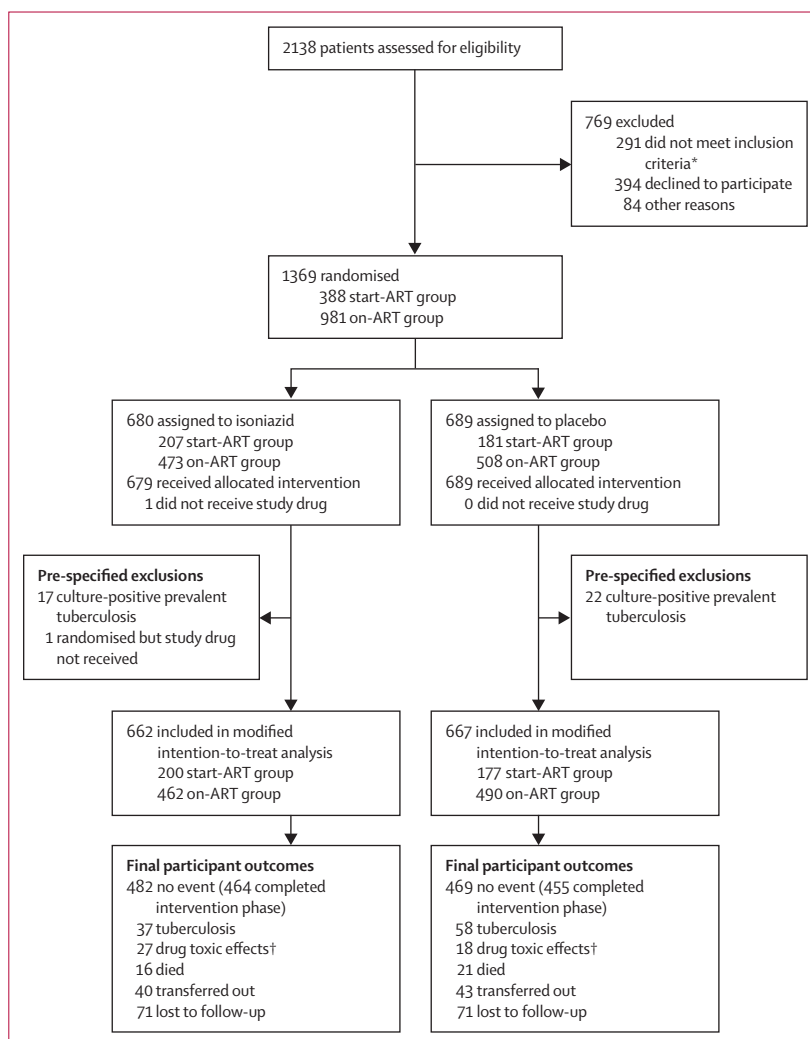


Figure 1: Trial profile

ART=antiretroviral therapy. Start-ART=just started ART. On-ART=established on ART. *Reasons for excluding 291 individuals that did not meet the inclusion criteria: 211 prevalent tuberculosis diagnosed, 13 previous isoniazid preventive therapy, 19 pregnancy, 23 pre-existing grade 3 toxicity (alanine transaminase concentration, peripheral neuropathy, or rash), one younger than 18 years, 24 already on tuberculosis treatment. †Drug toxic effects include: alanine transaminase grade 3 or worse, clinical hepatitis, grade 2 or worse rash or peripheral neuropathy.

Role of the funding source

The funder of the study 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 and had final responsibility for the decision to submit for publication.

Results

Screening started on Nov, 1, 2007, the first participant was randomised on Jan 31, 2008, and the last completed the study drug on Oct 31, 2010, and study closure was on Sept 31, 2011. Of 2138 individuals with HIV assessed for eligibility, 1369 participants were enrolled in the study and randomly assigned to receive either placebo or isoniazid (figure 1). The appendix shows characteristics at screening

of the 478 participants who were screened but not randomly assigned, and the 1369 (64%) who were. The proportion of participants already established on antiretroviral therapy was lower in individuals not randomly assigned and the proportion with previous tuberculosis was higher in those randomly assigned (42.2% vs 34.5%). 39 culture-positive prevalent tuberculosis cases were diagnosed after randomisation, and one person did not receive the study drug, leaving 1329 in the modified intention-to-treat analysis; 667 received placebo and 662 received isoniazid. Baseline characteristics at enrolment for those in the modified intention-to-treat analysis were similarly distributed between the study groups (table 1). The median time on antiretroviral therapy was 357 days (IQR: 139–798 in the on-antiretroviral therapy group and 14 days (IQR: 4–25) in the start-antiretroviral therapy group. The appendix provides reasons for why placebo or isoniazid was stopped during the active intervention phase by study group. The maximum follow-up was 3.7 years, with a median of 2.5 years (IQR 2.1–3.1). Numbers of patients at study closure who transferred out or were lost to follow-up were similar between study groups. The proportion lost to follow-up in each group was 11%; far less than the 30% initially assumed (figure 1).

For participants in each group who completed 12 months of the study drug (n=1100), a median of 360 doses (IQR 330–390) were dispensed during a median of 12 months on the study drug (IQR 11–13 months; appendix). We recorded no difference in median months on study drug and total doses dispensed between study groups.

95 cases of tuberculosis developed during 3226.5 person-years of follow-up (58 on placebo and 37 on isoniazid; 34/95 [36%] were culture-confirmed; table 2). 56 of 95 (59%) cases were diagnosed at the study site, 37 of 95 (39%) at satellite clinics, and 7 of 95 (7%) were identified through linkage to national health laboratory service and provincial electronic tuberculosis notifications data. 72 of 95 (76%) cases developed during the first 2 years of follow-up after randomisation (figures 2–4). The overall rate of tuberculosis was 2.9 per 100 person-years; higher in the placebo group than the isoniazid group (table 2). Adjustment for time-updated CD4 count did not significantly change the overall HR for tuberculosis (HR 0.64, 95% CI 0.42–0.96). Eight of the 95 participants with tuberculosis subsequently died during treatment (two on isoniazid and six on placebo). We did drug sensitivity testing on 25 of the 34 culture-confirmed cases of tuberculosis: four had multidrug-resistant tuberculosis (three on placebo and one on isoniazid), and two had isoniazid mono-resistance (both on isoniazid).

The effect of isoniazid on the risk of tuberculosis was greatest in the first year of follow-up when individuals were still on treatment (unadjusted HR 0.52, 95% CI 0.27–1.01; figure 4). The effect decreased over time (at 12–23 months, HR 0.61, 0.30–1.21, and at 24 months, HR=0.78, 95% CI 0.39–2.0). However,

	Placebo (n=667)	Isoniazid (n=662)	Total (n=1329)
Median age (years)	34 (29–40)	34 (30–40)	34 (30–40)
Women	498 (75%)	500 (76%)	998 (75%)
Established on ART	490 (74%)	462 (70%)	952 (72%)
Median days on ART, on- ART	330 (137–727)	394 (139–889)	357 (139–798)
Median days on ART, start- ART	14 (4–20)	14 (4–25)	14 (4–25)
Median CD4 count (cells per mm ³)*	214 (154–355)	218 (150–373)	216 (152–360)
Previous tuberculosis†	271 (41%)	289 (44%)	560 (43%)
TST			
Positive (≥5 mm)	202 (30%)	190 (29%)	392 (30%)
Negative (<5 mm)	283 (42%)	269 (41%)	552 (42%)
Unknown	182 (27%)	203 (31%)	385 (29%)
IGRA			
Positive	205 (31%)	184 (28%)	389 (29%)
Negative	261 (39%)	274 (41%)	535 (40%)
Indeterminate	38 (6%)	36 (5%)	74 (6%)
Unknown	163 (24%)	168 (25%)	331 (25%)
Discordant or concordant TST/IGRA pairs			
TST+/IGRA+	119 (18%)	115 (17%)	234 (18%)
TST-/IGRA-	190 (29%)	198 (30%)	388 (29%)
TST+/IGRA-	73 (11%)	66 (10%)	139 (11%)
TST-/IGRA+	64 (10%)	54 (8%)	118 (9%)
Unknown	221 (33%)	229 (35%)	450 (34%)
Combination ART regimen			
D4T/3TC/EFV	272 (41%)	253 (38%)	525 (40%)
AZT/3TC/EFV	36 (5%)	53 (8%)	89 (7%)
D4T/3TC/NVP	223 (33%)	212 (32%)	435 (33%)
AZT/3TC/NVP	118 (18%)	130 (20%)	248 (19%)
Other‡	18 (3%)	14 (2%)	32 (2%)
Median baseline alanine transaminase (IQR), IU/mL	28 (21–43)	30 (22–41)	29 (22–42)

Data are median (IQR) or n (%). ART=antiretroviral therapy. TST=tuberculin skin test. IGRA=interferon gamma release assay. Start-ART=just started ART. On-ART=established on ART. D4T=stavudine. AZT=azidothymidine or zidovudine. 3TC=lamivudine. EFV=efavirenz. NVP=nevirapine. *n=602 for placebo, 585 for isoniazid, and 1187 in total. †n=657 for placebo, 652 for isoniazid, and 1309 in total. ‡Other ART regimens: AZT/didanosine/lopinavir/ritonavir (two on placebo), 3TC/EFV/tenofovir (nine on placebo and 12 on isoniazid), D4T/3TC/lopinavir/ritonavir (one on placebo and one on isoniazid), AZT/3TC/lopinavir/ritonavir (two on placebo), 3TC/NVP/tenofovir (three on placebo and one on isoniazid), 3TC/tenofovir/lopinavir/ritonavir (one on placebo).

Table 1: Baseline characteristics at enrolment of individuals included in modified intention-to-treat analysis, by study group

	Overall		Placebo		Isoniazid		Effect HRu* (95% CI)
	Events per person-years	Rate per 100 person-years	Events per person-years	Rate per 100 person-years	Events per person-years	Rate per 100 person-years	
Tuberculosis							
All tuberculosis	95/3226.5	2.9	58/1597.2	3.6	37/1629.3	2.3	0.63† (0.41-0.94)
Definite	34/3226.5	1.1	22/1597.2	1.4	12/1629.3	0.7	0.54 (0.27-1.08)
Probable/possible	61/3226.5	1.9	36/1597.2	2.3	25/1629.3	1.5	0.68 (0.41-1.10)
Deaths							
All causes	37/3579.1‡	1.0	21/1792.8	1.2	16/1786.3	0.9	0.72 (0.34-1.34)

HRu=unadjusted hazard ratio. *HRu for isoniazid vs placebo. †p<0.05. ‡Person-years for death is higher than person-years for tuberculosis: four individuals died after developing tuberculosis.

Table 2: Effect of isoniazid on rate of tuberculosis or death

statistical power was insufficient to show formal interaction by time since randomisation ($p=0.34$, assuming linear effect). The appendix shows tuberculosis rates per study period since randomisation.

Table 3 shows analyses to assess the effect of antiretroviral therapy with isoniazid on time to tuberculosis stratified by tuberculin skin test or interferon gamma release assay status. The effect of isoniazid on tuberculosis incidence was greater in participants with negative tuberculin skin tests or interferon gamma release assay than in those tested positive, but we noted weak statistical evidence that the effects were different (interaction $p=0.58$ for tuberculin skin test and $p=0.24$ for interferon gamma release assay) after adjustment for baseline CD4 count and antiretroviral therapy status on enrolment. Participants who did not accept tuberculin skin test or interferon gamma release assay testing did not differ from those who did with respect to age, sex, CD4 count, previous history of tuberculosis, or antiretroviral therapy status (appendix).

We recorded 37 deaths from all causes during 3579 person-years of follow-up (21 on placebo and 16 on isoniazid; table 2). The overall rate of all-cause mortality was one per 100 person-years; rates were slightly lower in the isoniazid (0.9 per 100 person-years) compared with the placebo group (1.2 per 100 person-years; HR 0.72, 95% CI 0.34-1.34, log-rank $p=0.32$; figure 2). Further details on the 37 deaths were obtained from hospital records or family reports; eight were tuberculosis deaths (two isoniazid and six placebo), 13 were due to non-tuberculosis reasons deemed unrelated to the study drug (six isoniazid and seven placebo; eight happened during the intervention phase), and the rest of the reasons were unknown (eight isoniazid and eight placebo; two happened during the intervention phase). The appendix provides a summary of specific causes of death by study group.

We recorded no difference between study groups in study drug discontinuation due to any adverse event (relative risk [RR] 1.1, 95% CI 0.84-1.42) or presumed toxic effects (any of grade 3 or 4 alanine transaminase

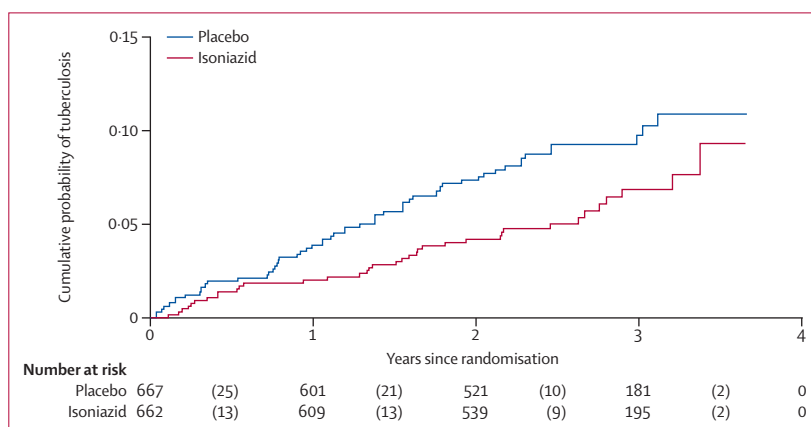


Figure 2: Time to tuberculosis from randomisation

The placebo group was given antiretroviral therapy plus placebo and the isoniazid group was given antiretroviral therapy plus isoniazid. Numbers show the number of participants followed up at each timepoint, and the numbers in parentheses show new tuberculosis cases in each period. Log-rank test p value for equality of survival curves=0.02.

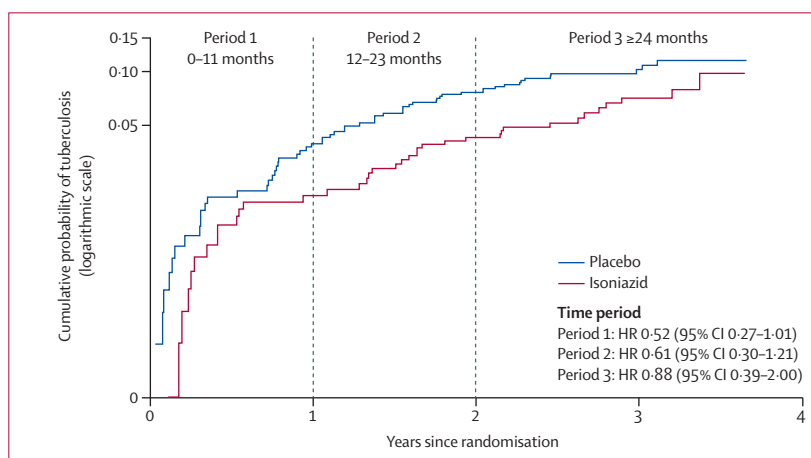


Figure 3: Cumulative hazard plot for antiretroviral therapy versus antiretroviral therapy plus isoniazid preventive therapy effect by time since randomisation

Nelson-Aalen cumulative hazard plot on a logarithmic scale to show proportionality of hazards over time periods. HRs shown are unadjusted. Treatment ended 1 year after participants were randomly assigned. Likelihood ratio test for interaction of treatment group with study time $p=0.61$, and assuming linear trend for study time $p=0.34$. HR=hazard ratio.

concentration, clinical hepatitis, new or worsening grade 2, or more rash or peripheral neuropathy; RR 1.5, 95% CI 0.84–2.7; table 4). 34 participants had grade 3 or more raised alanine transaminase concentration, which resulted in termination of the study drug in 29 participants (in two participants grade 3 alanine transaminase was reported only in month 12 and results were obtained after the study drug was completed, subsequent alanine transaminase in three participants were all <grade 3).

The risk of stopping the study drug because of grade 3 or above raised alanine transaminase alone was 2.9% on isoniazid compared with 1.5% on placebo (RR 1.9, 0.90–4.09). The risk of stopping the drug was 2.8% if on isoniazid and 1.6% on placebo (RR 1.7, 0.72–4.12) for the on-antiretroviral therapy group, and 3.0% and 1.1% for the start-antiretroviral therapy group (RR 2.7, 0.54–12.98; data not shown).

The appendix shows estimates of the numbers needed to treat or harm. 25 individuals on antiretroviral therapy need to be treated with isoniazid to prevent a case of tuberculosis. 100 individuals on antiretroviral therapy need to be treated with isoniazid to result in harm as defined by participants stopping the study drug because of presumed toxic effects.

Discussion

12 months of isoniazid preventive therapy independently reduced the incidence of tuberculosis in participants concurrently on antiretroviral therapy by 37% (panel). The risk-to-benefit ratio was favourable with the number needed to harm four times higher than the number needed to treat to benefit with isoniazid preventive therapy. The greatest benefit from isoniazid preventive therapy seemed to be in the first year; however, evidence was weak for the decreased effect with time. Isoniazid preventive therapy benefit was greater in individuals who had negative tuberculin skin tests and interferon gamma release assays than those who tested positive, although statistical evidence for interaction was weak. Implementation of

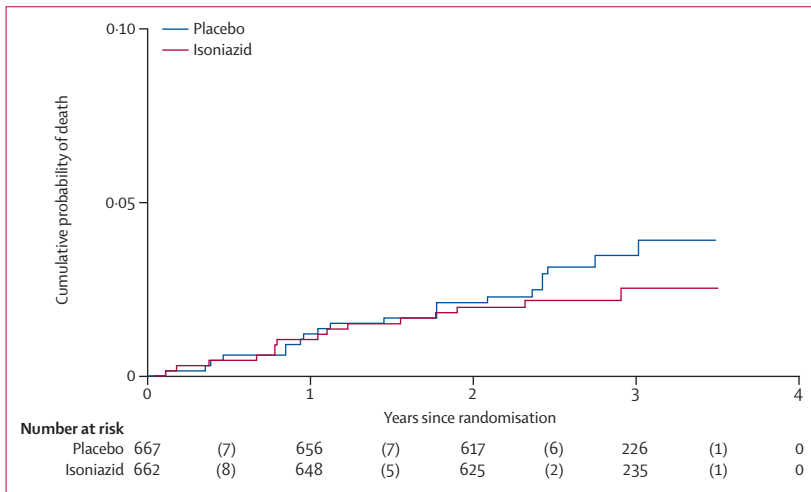


Figure 4: Time to death from randomisation
The placebo group was given antiretroviral therapy plus placebo, and the isoniazid group was given antiretroviral therapy plus isoniazid. Log-rank test p value for equality of survival curves=0.32.

	Placebo			Isoniazid			Effect			
	n	Cases per person-years	Rate per 100 person-years	n	Cases per person-years	Rate per 100 person-years	HRu* (95% CI)	Pi†	HRa‡ (95% CI)	Pi§
Interferon gamma release assay¶										
Negative	261	21/631.9	3.3	274	9/687.9	1.3	0.40 (0.18–0.87)	..	0.43 (0.20–0.96)	..
Positive	205	19/481.7	3.9	184	13/427.3	3.0	0.76 (0.38–1.5)	0.22	0.55 (0.26–1.24)	0.58
Tuberculin skin test**										
Negative	283	27/660.0	4.1	269	11/656.3	1.7	0.41** (0.20–0.83)	..	0.43 (0.21–0.86)	..
Positive	202	14/496.4	2.8	190	12/461.1	2.6	0.92 (0.43–1.97)	0.13	0.86 (0.37–2.0)	0.24

HRu=unadjusted hazard ratio. HRa=adjusted hazard ratio. *HRu comparing isoniazid vs placebo. †Pi=Likelihood ratio test p value for interaction in the unadjusted model. ‡HR comparing isoniazid vs placebo, adjusted for CD4 cell count and antiretroviral therapy status at enrolment. §Pi=Likelihood ratio test p value for interaction in adjusted model. ¶Indeterminate interferon-gamma release assay results included with negatives. ||p<0.05. **Tuberculin skin test negative if induration <5 mm and positive if ≥5 mm. ††p<0.01.

Table 3: Effect of isoniazid on the rate of all tuberculosis stratified by markers of Mycobacterium tuberculosis infection status at enrolment

	Overall		Placebo		Isoniazid		Effect
	Events/n	Risk	Events/n	Risk	Events/n	Risk	RRu† (95% CI)
All reasons*	196/1329	14.7%	94/667	14.1%	102/662	15.4%	1.1 (0.84–1.42)
ALT ≥grade 3	29/1329	2.2%	10/667	1.5%	19/662	2.9%	1.9 (0.90–4.09)
ALT ≥grade 3, clinical hepatitis, ≥grade 2 rash or peripheral neuropathy	45/1329	3.4%	18/667	2.7%	27/662	4.1%	1.5 (0.84–2.72)

Maximum time at risk=3.7 years. RRu=unadjusted risk ratio. ALT=alanine transaminase. *All reasons excluding tuberculosis. †RRu=RRu for isoniazid vs placebo.

Table 4: Effect of isoniazid on the risk of stopping the study drug because of adverse events

isoniazid preventive therapy in an antiretroviral therapy clinic will probably be easier than in pre-antiretroviral therapy care because individuals on antiretroviral therapy receive regular drugs and have more frequent follow-up visits. A strength of our study was the use of sputum cultures to exclude tuberculosis at baseline. Some of the effect of isoniazid preventive therapy reported in studies that did not rigorously exclude tuberculosis might have arisen as a result of the effect of isoniazid monotherapy on subclinical disease.²⁰

The benefit of isoniazid preventive therapy in individuals on antiretroviral therapy seemed to decrease gradually over time rather than rebound rapidly soon after cessation. However, our study was underpowered to assess duration of benefit beyond the study period. The risk of active tuberculosis decreases over time because of improvements in CD4 counts mediated by antiretroviral therapy,³ which might have masked loss of effect of isoniazid preventive therapy after completion. Findings of studies of isoniazid preventive therapy in pre-antiretroviral therapy cohorts have shown that 6 months of isoniazid preventive therapy has a short-term benefit.^{21,22} A similar, but more pronounced, loss of effect was seen in the 6 month isoniazid preventive therapy group of the BOTUSA study,⁸ in which 45% of the participants started antiretroviral therapy according to need during the study period.⁸ Longer courses (>6 months) of isoniazid preventive therapy for all individuals with HIV are now recommended by WHO.²³ Our findings, together with those of the BOTUSA study, support the use of longer-term isoniazid preventive therapy in individuals on antiretroviral therapy living in moderate or high incidence areas. However, in routine clinical practice, the risk of non-adherence and treatment discontinuations might be higher in individuals prescribed extended isoniazid preventive therapy than in those prescribed shorter courses.¹⁷

In our study participants on antiretroviral therapy with negative tuberculin skin test results benefited more from isoniazid preventive therapy than participants with positive tuberculin skin tests. We recorded similar trends in effect by interferon gamma release assay status. This finding was unexpected because results of a meta-analysis² of randomised controlled trials of isoniazid preventive therapy without antiretroviral therapy showed significant benefit only in those with a positive tuberculin skin test. It is not clear why tuberculin skin test positive individuals not on antiretroviral therapy might benefit more from isoniazid preventive therapy than do individuals with tuberculin skin test negative results. Individuals who test negative might not benefit as much because they are not latently infected with *M tuberculosis*. However, in areas with a high prevalence of tuberculosis, many of the negative tuberculin skin test results in individuals with HIV are likely to be false negatives, especially in those with low CD4 counts.²⁴ Isoniazid preventive therapy might be effective in antiretroviral

therapy-naive patients with a positive tuberculin skin test because effective isoniazid preventive therapy needs some acquired immunity to *M tuberculosis*, for which tuberculin skin test is a crude measure. Isoniazid modifies the immune response to tuberculosis, potentially by releasing mycobacterial antigens.²⁵ In individuals who have positive tuberculin skin tests, antiretroviral therapy is likely to augment pre-existing

Panel: Research in context

Systematic review

We searched PubMed for systematic reviews, meta-analyses, and randomised controlled trials of the efficacy of tuberculosis preventive therapy on incident tuberculosis in adults with HIV on combination antiretroviral therapy published between Jan 1, 1996, and Jan 1, 2014. We included the following search terms: "antiretroviral therapy" OR "HIV" AND "tuberculosis" AND "isoniazid" OR "preventive therapy". We identified one recent meta-analysis,² and four subsequent trials that were not included in the meta-analysis,^{8,9,17,18} one was a cluster randomised trial of a complex intervention.¹⁸ We did not identify any randomised controlled trials of tuberculosis preventive therapy in adults on concurrent antiretroviral therapy.

Interpretation

Findings of the meta-analysis showed that preventive therapy reduced incident tuberculosis in adults with HIV with positive tuberculin skin tests, but not in those with negative tuberculin skin tests.² However, the placebo-controlled trials included in the meta-analysis were all from the pre-antiretroviral therapy era, or were done in areas before antiretroviral therapy access. Measurement of the efficacy and safety of tuberculosis preventive therapy in people on antiretroviral therapy is important because antiretroviral therapy also reduces the risk of tuberculosis, toxic effects of preventive therapy might be enhanced, and because 25.9 million of the estimated 35.3 million living with HIV in 2012, qualified for antiretroviral therapy according to 2013 WHO guidelines.¹⁹ Three subsequent trials compared longer duration isoniazid preventive therapy with other preventive therapy regimens.^{8,9,17} The three trials of longer duration isoniazid preventive therapy were done in antiretroviral therapy-naive individuals (except for 2% of participants in the BOTUSA study⁸), with participants starting antiretroviral therapy according to clinical need during follow-up. 36 months isoniazid preventive therapy was more effective than 6 months in the BOTUSA study, with the benefit restricted to those who were tuberculin skin tests positive,⁸ but no benefit was recorded in the other two trials with smaller sample sizes.^{9,17} In the BOTUSA study, antiretroviral therapy for 1 year reduced tuberculosis incidence by 50%. A cluster randomised trial¹⁸ found that improved tuberculosis screening and isoniazid preventive therapy reduced incident tuberculosis, which was independent of the effects of concomitant antiretroviral therapy at baseline.

12 months of isoniazid preventive therapy reduced the incidence of tuberculosis in individuals on antiretroviral therapy, was easy to implement in an antiretroviral therapy clinic, and was well tolerated. Unlike other studies, we found that participants on antiretroviral therapy with negative tuberculin skin test or interferon gamma release assay benefited more from isoniazid preventive therapy than test-positive participants. Until a more predictive test for latent tuberculosis infection or a multivariate algorithm that predicts benefit in the context of antiretroviral therapy is available, isoniazid preventive therapy should be recommended to all patients receiving antiretroviral therapy in moderate or high incidence areas irrespective of tuberculin skin test status. Future studies should explore improved diagnostics for latent tuberculosis infection, large-scale implementation of longer duration of isoniazid preventive therapy, or more sterilising preventive therapy regimens, in people on antiretroviral therapy.

immunity to *M tuberculosis*, and the incremental benefit of isoniazid preventive therapy might be small or non-existent. By contrast, in individuals with negative tuberculin skin tests results, antiretroviral therapy improves acquired immunity to *M tuberculosis* in individuals who have been exposed to *M tuberculosis*, as shown by positive results on repeat skin test. Investigators of a recent Ugandan study²⁶ reported a high negative to positive tuberculin skin test conversion rate of 30.9 per 100 person-years during the first 6 months after antiretroviral therapy initiation. Isoniazid preventive therapy might be effective in this setting of early restoration of acquired immunity. In patients on antiretroviral therapy, the efficacy of isoniazid preventive therapy might not be strongly linked to tuberculin skin test or interferon gamma release assay status.

More participants in the isoniazid group than the placebo group stopped taking the study drug because of adverse events, with a relative risk similar to that reported in the Cochrane meta-analysis² of isoniazid preventive therapy trials in patients not yet started on antiretroviral therapy (RR 1.5, 95% CI 0.84–2.7 vs 1.66, 1.09–2.51). Additionally, we did not find strong evidence for increased risk of cessation of study drug because of toxic effects in individuals newly starting antiretroviral therapy. These data suggest no additive toxic effects of isoniazid preventive therapy in patients concurrently receiving antiretroviral therapy.

Our study had some limitations. First, the study did not have sufficient statistical power to show clear differences in effect estimates by tuberculin skin test or interferon gamma release assay status, or to establish the duration of benefit of isoniazid preventive therapy in individuals on antiretroviral therapy, for which a larger sample size or longer duration of follow up would have been needed. Second, although we aimed to confirm as many cases of incident tuberculosis as possible with culture at the study clinic, 39% of cases were diagnosed at satellite clinics where diagnostic confirmation might not have been as rigorous. Thus, the incidence of culture-confirmed tuberculosis might have been underestimated. Third, we did not repeat tuberculin skin tests and interferon gamma release assays early in the study. The prospective usefulness of one test result obtained at screening might be reduced with increasing study follow-up and time on antiretroviral therapy. Repeat testing after several months of antiretroviral therapy might have allowed more accurate measurement of the presence of latent tuberculosis infection.^{26–31} Fourth, the rate of tuberculosis in the placebo group was lower than the rate assumed, which we had estimated from two local cohort studies^{32,33} available to us when the study started. Possible explanations for the lower than expected tuberculosis incidence are high case ascertainment by screening with sputum cultures, which decreases tuberculosis incidence after start of antiretroviral therapy,³⁴ and

increased CD4 counts at antiretroviral therapy initiation,³⁵ which is the most important risk factor for incident tuberculosis after antiretroviral therapy initiation.³⁶ However, person-years were higher than we had assumed because loss to follow-up was lower than expected and duration of follow-up was longer than planned because of slow recruitment. Our study had 80% statistical power for the recorded effect size of 37% of isoniazid preventive therapy for the primary endpoint, suggesting that the longer period of observation compensated for the lower than expected tuberculosis incidence. Fifth, findings of our study showed a more modest effect than previously shown in mostly observational studies.^{5–7} Issues with methods in previous studies prevented an independent assessment of the effect of isoniazid preventive therapy with antiretroviral therapy.^{5–8} The small effects described, and the resultant high rate of tuberculosis in the intervention group, suggest antiretroviral therapy plus isoniazid preventive therapy alone might not be adequate to control tuberculosis at the population level. Sixth, our study results might not be generalisable to settings of low tuberculosis incidence where background rates of *M tuberculosis* exposure are low. Finally, we did a pragmatic trial in a busy antiretroviral therapy and tuberculosis integrated clinic; study procedures would probably have been more rigorous in a dedicated fully staffed study clinic, but doing the study in a busy antiretroviral therapy clinic with minimum additional staffing allowed us to assess implementation of isoniazid preventive therapy.

In summary, 12 months of isoniazid preventive therapy reduced the incidence of tuberculosis in individuals infected with HIV-1 established on antiretroviral therapy or newly starting antiretroviral therapy and seemed well tolerated. In this high incidence setting, individuals on antiretroviral therapy who have negative tuberculin skin test or interferon gamma release assay might also benefit from isoniazid preventive therapy. Implementation of isoniazid preventive therapy is feasible in busy antiretroviral therapy clinics in settings with high rates of HIV and tuberculosis comorbidity.

Contributors

MXR, RJW, AB, GVC, KAW, EG, and GM conceived and designed this evaluation. MXR, GVC, RG, and SM enrolled participants and collected the data. JRG and KF advised on analysis. MXR analysed and wrote the first draft and JRG, KF, AB, GM, RJW, GvC, and KAW gave input. All authors interpreted the data and helped to revise the paper.

Declaration of interests

We declare that we have no competing interests.

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