



Daily co-trimoxazole prophylaxis in severely immunosuppressed HIV-infected adults in Africa started on combination antiretroviral therapy: an observational analysis of the DART cohort

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Summary

Background Co-trimoxazole prophylaxis can reduce mortality from untreated HIV infection in Africa; whether benefits occur alongside combination antiretroviral therapy (ART) is unclear. We estimated the effect of prophylaxis after ART initiation in adults.

Methods Participants in our observational analysis were from the DART randomised trial of management strategies in HIV-infected, symptomatic, previously untreated African adults starting triple-drug ART with CD4 counts lower than 200 cells per μL . Co-trimoxazole prophylaxis was not routinely used or randomly allocated, but was variably prescribed by clinicians. We estimated effects on clinical outcomes, CD4 cell count, and body-mass index (BMI) using marginal structural models to adjust for time-dependent confounding by indication. DART was registered, number ISRCTN13968779.

Findings 3179 participants contributed 14 214 years of follow-up (8128 [57%] person-years on co-trimoxazole). Time-dependent predictors of co-trimoxazole use were current CD4 cell count, haemoglobin concentration, BMI, and previous WHO stage 3 or 4 events on ART. Present prophylaxis significantly reduced mortality (odds ratio 0.65, 95% CI 0.50–0.85; $p=0.001$). Mortality risk reduction on ART was substantial to 12 weeks (0.41, 0.27–0.65), sustained from 12–72 weeks (0.56, 0.37–0.86), but not evident subsequently (0.96, 0.63–1.45; heterogeneity $p=0.02$). Variation in mortality reduction was not accounted for by time on co-trimoxazole or current CD4 cell count. Prophylaxis reduced frequency of malaria (0.74, 0.63–0.88; $p=0.0005$), an effect that was maintained with time, but we observed no effect on new WHO stage 4 events (0.86, 0.69–1.07; $p=0.17$), CD4 cell count (difference vs non-users, -3 cells per μL [-12 to 6]; $p=0.50$), or BMI (difference vs non-users, -0.04 kg/m^2 [-0.20 to 0.13]; $p=0.68$).

Interpretation Our results reinforce WHO guidelines and provide strong motivation for provision of co-trimoxazole prophylaxis for at least 72 weeks for all adults starting combination ART in Africa.

Funding UK Medical Research Council, the UK Department for International Development, the Rockefeller Foundation, GlaxoSmithKline, Gilead Sciences, Boehringer-Ingelheim, and Abbott Laboratories.

Introduction

Co-trimoxazole (trimethoprim-sulfamethoxazole) is a widely available, off-patent, low-cost antibiotic that is used in resource-limited settings to treat and prevent community-acquired infections. Although not recommended as malaria prophylaxis, similar to pyrimethamine-sulfadoxine, it also has antimalarial activity.¹ In HIV infection, it is highly effective for treatment of and prophylaxis against *Pneumocystis jirovecii* pneumonia,² *Toxoplasma gondii*,³ and *Isospora belli*.^{4,5} Results of clinical trials and observational studies in HIV-infected, combination antiretroviral therapy (ART)-naive adults and children across Africa have shown that co-trimoxazole prophylaxis reduces mortality, morbidity, and hospital admissions,^{4–10} even in areas of high background bacterial resistance. WHO guidelines recommend that co-trimoxazole prophylaxis is given to all symptomatic adults with

CD4 counts lower than 350 cells per μL in resource-limited settings.¹¹

Routinely used with ART in high-income countries, co-trimoxazole prophylaxis is usually discontinued when CD4 count exceeds 200 cells per μL because the primary goal is prevention of *P jirovecii* pneumonia.^{12,13} Despite WHO guidelines, co-trimoxazole has been poorly used with ART in resource-limited settings; and data for its benefits (in addition to those of ART), toxic effects, and effect on ART adherence are scarce. In only one large retrospective cohort study in Malawi, investigators reported a 41% reduction in mortality during the first 6 months after ART initiation in clinics providing co-trimoxazole prophylaxis.¹⁴

Participants in the Development of Anti-Retroviral Therapy in Africa (DART) trial¹⁵ had variable exposure to co-trimoxazole; prophylaxis was neither routine nor randomised, but was initiated or continued at discretion of the treating clinician. We aimed to estimate the causal

Lancet 2010; 375: 1278–86

Published Online

March 29, 2010

DOI:10.1016/S0140-

6736(10)60057-8

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effect of co-trimoxazole prophylaxis on survival, WHO stage 3 and 4 events, malaria, CD4 cell count, body-mass index (BMI), and haematological indices in adults after initiation of ART.

Methods

Study design and participants

In our observational analysis, we used data from the randomised DART trial¹⁵ comparing laboratory plus clinical monitoring (LCM) with clinically driven monitoring (CDM) of ART, undertaken in two clinical centres in Uganda (the Medical Research Council/Uganda Virus Research Institute Uganda Research Unit on AIDS, Entebbe; and the Joint Clinical Research Centre, Kampala, with a satellite clinic at the Infectious Diseases Institute, Mulago), and one centre in Zimbabwe (University of Zimbabwe, Harare). Participants were symptomatic (WHO stage 2–4) HIV-infected adults (≥ 18 years) with CD4 counts lower than 200 cells per μL who reported no previous ART apart from to prevent mother-to-child transmission.

At enrolment all participants started triple-drug combination ART (coformulated zidovudine-lamivudine [GlaxoSmithKline, Ware, UK] plus tenofovir disoproxil fumarate [Gilead Science, Foster City, CA, USA], abacavir [GlaxoSmithKline, Ware, UK], or nevirapine [Boehringer Ingelheim, Ingelheim, Germany]). Participants attended study clinics every 4 weeks, when nurses administered standard symptom and adherence checklists and dispensed prescriptions. Participants could be referred to a doctor at any time and were asked to return to the clinic if they felt unwell between visits. All participants saw a doctor and had a full blood count, lymphocyte subsets, and liver and renal function tests at weeks 4 and 12, then every 12 weeks. All results for LCM participants were returned to clinicians, whereas for CDM participants, haematology and biochemistry results were returned only if requested for clinical reasons or if grade 4 toxic effects were reported (protocol safety criteria, grades defined in protocol according to minor modifications of the AIDS Clinical Trials Group criteria¹⁶) and lymphocyte subsets were never returned.

Co-trimoxazole was taken once daily (800 mg sulfamethoxazole, 160 mg trimethoprim). Use of other non-ART drugs (prescription and indication) and malaria episodes (clinical or microscopic diagnosis) were recorded at every doctor visit. Structured summaries for all reported WHO 2003 stage 4 events¹⁷ and deaths were reviewed by an endpoint review committee, who were masked to monitoring strategy and CD4 cell count.

137 (4%) participants who entered a non-randomised pilot study of structured treatment interruptions were excluded. A further 813 participants with CD4 counts of 300 cells per μL or higher were randomly allocated at 52 or 76 weeks to interrupted treatment cycles of 12 weeks on and 12 weeks off ART or to stay on

	Off co-trimoxazole prophylaxis at ART initiation (n=1220)*	On co-trimoxazole prophylaxis at ART initiation (n=1959)*	All participants (n=3179)
Centre			
A	550 (57%)	414 (43%)	964 (30%)
B	428 (45%)	514 (55%)	942 (30%)
C	208 (21%)	771 (79%)	979 (31%)
D (satellite clinic)	34 (12%)	260 (88%)	294 (9%)
Randomly allocated to			
Laboratory and clinical monitoring	611 (39%)	969 (61%)	1580 (50%)
Clinically driven monitoring	609 (38%)	990 (62%)	1599 (50%)
Randomly allocated in			
2003	518 (29%)	1244 (71%)	1762 (55%)
2004	702 (50%)	715 (50%)	1417 (45%)
Age (years)	37 (32–42)	37 (32–42)	37 (32–42)
Men	444 (40%)	678 (60%)	1122 (35%)
Women	776 (38%)	1281 (62%)	2057 (65%)
WHO disease stage			
2	263 (41%)	382 (59%)	645 (20%)
3	715 (40%)	1079 (60%)	1794 (56%)
4	242 (33%)	498 (67%)	740 (23%)
WHO stage 3 or 4 event ¹⁷ <4 weeks before ART initiation			
No	792 (38%)	1307 (62%)	2099 (66%)
Yes	428 (40%)	652 (60%)	1080 (34%)
CD4 count (cells per μL)	83 (29–136)	82 (30–137)	83 (29–137)
Haemoglobin (g/L)	114 (103–127)	114 (102–126)	114 (103–127)
Body-mass index			
Median	21 (19–23)	21 (19–24)	21 (19–24)
≤ 18.5 kg/m ²	261 (43%)	340 (57%)	601 (19%)
>18.5 kg/m ²	959 (37%)	1619 (63%)	2578 (81%)
Initial ART			
Combivir plus tenofovir	778 (33%)	1554 (67%)	2332 (73%)
Combivir plus abacavir	173 (58%)	127 (42%)	300 (9%)
Combivir plus nevirapine	269 (49%)	278 (51%)	547 (17%)
Taking co-trimoxazole prophylaxis at screening [†]			
No	1220 (61%)	794 (39%)	2014 (63%)
Yes	0 (0%)	1165 (100%)	1165 (37%)
Weeks of use at screening (in users) [†]	..	12 (3–33)	12 (3–33)

Data are n (%) or median (IQR). 137 of 3316 DART participants randomly allocated to the monitoring strategies who participated in a non-randomised substudy of structured treatment interruptions at week 28 are not included in this analysis. Predictors of use of co-trimoxazole prophylaxis at ART initiation varied by centre. ART=antiretroviral therapy.

*All percentages in on and off co-trimoxazole columns are proportions of the row total in the all participants column.

[†]Participants were screened for entry into the Development of AntiRetroviral Therapy in Africa¹⁵ trial a median 14 days (IQR 13–16) before entry.

Table 1: Characteristics of included participants at ART initiation

continuous therapy.¹⁸ We excluded follow-up after substudy randomisation for interrupted treatment participants and upweighted it in those receiving continuous treatment (ie, continuous treatment participants were given sampling weights (of roughly two) so that they represented interrupted-treatment participants after censoring, in addition to themselves), assuming comparability of treatment groups at substudy randomisation.

	Death* (n=326¶)		New WHO stage 4 events**†		New or recurrent WHO stage 3 or 4 events*‡ (n=1295¶)		New or recurrent malaria*§ (n=1170¶)	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Overall effect 								
Not present user	1.0	..	1.0	..	1.0	..	1.0	..
Present user	0.65 (0.50-0.85)	0.001	0.86 (0.69-1.07)	0.17	0.85 (0.74-0.98)	0.02	0.74 (0.63-0.88)	0.0005
Pattern of co-trimoxazole use								
Never used	1.0	..	1.0	..	1.0	..	1.0	..
Not present user**								
Used >12 weeks ago	1.04 (0.66-1.62)	0.88	0.58 (0.39-0.85)	0.006	1.03 (0.80-1.31)	0.84	1.23 (1.01-1.49)	0.04
Used in past 12 weeks	0.64 (0.35-1.16)	0.14	0.80 (0.37-1.70)	0.56	0.79 (0.55-1.12)	0.18	0.83 (0.62-1.10)	0.19
Present user††								
On for 1-12 weeks	0.46 (0.30-0.71)	0.0005	0.81 (0.60-1.09)	0.16	0.90 (0.75-1.07)	0.23	0.74 (0.60-0.92)	0.007
On for 12-24 weeks	0.68 (0.42-1.10)	0.12	0.70 (0.45-1.09)	0.12	0.89 (0.68-1.17)	0.41	0.80 (0.57-1.13)	0.21
On for >24 weeks	0.74 (0.48-1.12)	0.15	0.63 (0.45-0.89)	0.008	0.77 (0.61-0.97)	0.03	0.80 (0.62-1.02)	0.08
Time on ART								
Not present user	1.0	..	1.0	..	1.0	..	1.0	..
Present user‡‡								
0-12 weeks on ART	0.41 (0.27-0.65)	0.0001	0.74 (0.53-1.02)	0.07	0.86 (0.71-1.05)	0.14	0.76 (0.60-0.98)	0.03
12-48 weeks on ART	0.59 (0.37-0.95)	0.03	0.83 (0.53-1.32)	0.44	0.96 (0.74-1.25)	0.78	0.78 (0.60-1.02)	0.07
48-72 weeks on ART	0.47 (0.20-1.08)	0.08	0.71 (0.37-1.39)	0.33	0.92 (0.63-1.34)	0.66	0.50 (0.33-0.74)	0.001
72-96 weeks on ART	1.28 (0.54-3.03)	0.57	1.10 (0.49-2.46)	0.82	0.92 (0.56-1.51)	0.74	0.84 (0.52-1.34)	0.46
>96 weeks on ART	0.89 (0.57-1.40)	0.61	0.98 (0.67-1.43)	0.91	0.71 (0.54-0.92)	0.01	0.76 (0.55-1.03)	0.08

OR=odds ratio. ART=antiretroviral therapy. *Risk of first event or episode estimated from weighted logistic regression models adjusting for length of time on ART with cubic splines, centre, randomised monitoring group, randomisation year, age, sex, WHO stage, and ART prescription at randomisation together with baseline and time-varying values of CD4 cell count, haemoglobin concentration, body-mass index, a WHO stage 3 or 4 event in the previous 4 weeks or since randomisation, and randomisation into a structured treatment interruption (STI) substudy (never, to continuous therapy [CT]). †Oesophageal candidosis (159), cryptococcosis (115), extrapulmonary tuberculosis (108), cryptosporidia (25), Pneumocystis jirovecii pneumonia (22), chronic mucocutaneous herpes simplex (22), HIV wasting (17), Kaposi's sarcoma (15), toxoplasmosis (13), other (ten or fewer of any event) (19). ‡Severe bacterial infection (365, including pneumonia [280, single occurrences as per WHO 2003 guidelines³⁷], septicæmia [29], pyomyositis [17], meningitis [six], other [33]), oral candida (281), weight loss of more than 10% (133), oesophageal candidosis (115), pulmonary tuberculosis (98), cryptococcosis (87), extrapulmonary tuberculosis (75), oral hairy leukoplakia (36), other (105; ≤20 of any event). §Excluding participants from Harare. ¶Eight deaths, 20 WHO 4 events, 48 WHO stage 3 or 4 events, and 78 malaria episodes occurred after randomisation to CT within the Development of AntiRetroviral Therapy in Africa³⁵ STI/CT substudy and were upweighted in analyses. ||p values for test of heterogeneity between centres were 0.85, 0.37, 0.07, and 0.68, for death, stage 4 events, stage 3 or 4 events, and malaria, respectively. **p values for test of heterogeneity in effect of co-trimoxazole prophylaxis between never users, used more than 12 weeks ago, and used in the past 12 weeks were 0.28, 0.02, 0.36, and 0.02. ††p values for test of heterogeneity in effect of co-trimoxazole prophylaxis between present users by duration of consecutive use (1-12, 12-24, >24 weeks) were 0.21, 0.46, 0.42, and 0.87. ‡‡p values for test of heterogeneity between time periods were 0.06, 0.76, 0.50, and 0.31.

Table 2: Effect of co-trimoxazole prophylaxis on death, new WHO stage 4 events, new or recurrent WHO stage 3 or 4 events, and malaria

Patients gave written consent for screening and, if eligible, enrolment. DART was approved by research ethics committees in Uganda, Zimbabwe, and the UK.

Statistical analysis

All analyses were done with Stata (version 10.0). We used marginal structural models³⁹ to estimate causal effects of co-trimoxazole prophylaxis on outcomes. These models adjusted for time-dependent covariates with inverse-probability treatment weights and were appropriate in the presence of time-dependent covariates (such as BMI) that might be associated with both prescription of co-trimoxazole and outcomes (time-dependent confounders), and could also be affected by past co-trimoxazole prophylaxis (webappendix).

DART was registered, number ISRCTN13968779.

Role of the funding source

The sponsor (UK Medical Research Council) and other funders had no direct role in study design, data

collection, analysis, or interpretation, or writing of the report, either for this observational analysis or the original randomised trial. The corresponding author had full access to all data.

Results

3316 eligible participants were enrolled in DART¹⁵ between Jan 15, 2003, and Oct 24, 2004. We excluded 137 (4%) participants who entered a non-randomised pilot study of structured treatment interruptions 28 weeks after ART initiation. Characteristics of the 3179 participants included are representative of participants in ART programmes in Africa (table 1); all participants had CD4 counts lower than 200 cells per µL before starting therapy. Length of follow-up was median 4.9 years (IQR 4.5-5.3) with only 198 (6%) of 3179 participants last seen alive more than 4 months before Dec 31, 2008 (end of follow-up). Completeness of 4-week nurse (98%) and 12-week doctor (99%) visits was very high.

See Online for webappendix

Participants had variable exposure to co-trimoxazole; prophylaxis was never prescribed during follow-up for 324 (10%) participants, 1959 (62%) were taking it at ART initiation, and 896 (28%) started while on ART, with median 3.5 years (0.9–4.5) total use post-ART in those ever starting. Use at ART initiation varied with centre, year of randomisation, and initial combination treatment. Of 368 participants who died before end of follow-up, only 25 (7%) did not have complete co-trimoxazole history before death and were censored at their last clinic visit. 8128 (57%) person-years of follow-up were spent on co-trimoxazole. Use of prophylaxis differed substantially between the four centres (15%, 72%, 72%, 79%). Only 105 (<1%) person-years of follow-up were spent off ART. Reported ART adherence was high both in participants currently on and off co-trimoxazole, with no missed doses in the past 4 weeks reported at 5234 (83%) and 2426 (78%) visits, respectively, in the first 12 weeks, at 19688 (93%) and 19415 (87%) visits in weeks 12–72, and at 65669 (93%) and 39965 (91%) visits at more than 72 weeks of ART, excluding visits after randomisation to structured treatment interruptions.

Exclusion of follow-up after randomisation to structured treatment interruptions and upweighting of follow-up after continuous treatment gave 326 deaths (including eight upweighted) in 14 214 total person-years (2.3 per 100 person-years) included in analyses. 85 (26%) deaths occurred within 12 weeks of ART initiation (11.8 per 100 person-years). Present co-trimoxazole prophylaxis halved mortality in the first 12 weeks on ART, with no variation between participants on prophylaxis before screening (odds ratio [OR] 0.52, 95% CI 0.30–0.92, adjusted for baseline factors; $p=0.02$) versus those starting prophylaxis at the same time as ART (0.46, 0.25–0.84; $p=0.01$; heterogeneity $p=0.69$) or of variation between centres (heterogeneity $p=0.35$). Overall, using unweighted logistic regression and adjusting for baseline factors, but not controlling for time-dependent confounders, we showed that present co-trimoxazole use was associated with a 27% mortality reduction (0.73, 0.56–0.96; $p=0.02$; between-centre heterogeneity $p=0.07$).

As expected, mortality was higher in participants with low current CD4 cell count, haemoglobin concentration, or BMI, or with a WHO stage 3 or 4 event in the previous 4 weeks or any stage 3 or 4 event since randomisation (data not shown). These factors were also associated with increased probability of co-trimoxazole use, although extent of association varied between centres (data not shown). When we adjusted for these time-dependent predictors and randomisation to continuous treatment as factors within a regression model for mortality, the estimated OR for co-trimoxazole use was 0.62 (0.48–0.80; $p=0.0002$; between-centre heterogeneity $p=0.50$).

In a marginal structural model with inverse-probability treatment weights to control for time-dependent confounders, present prophylaxis reduced overall mortality

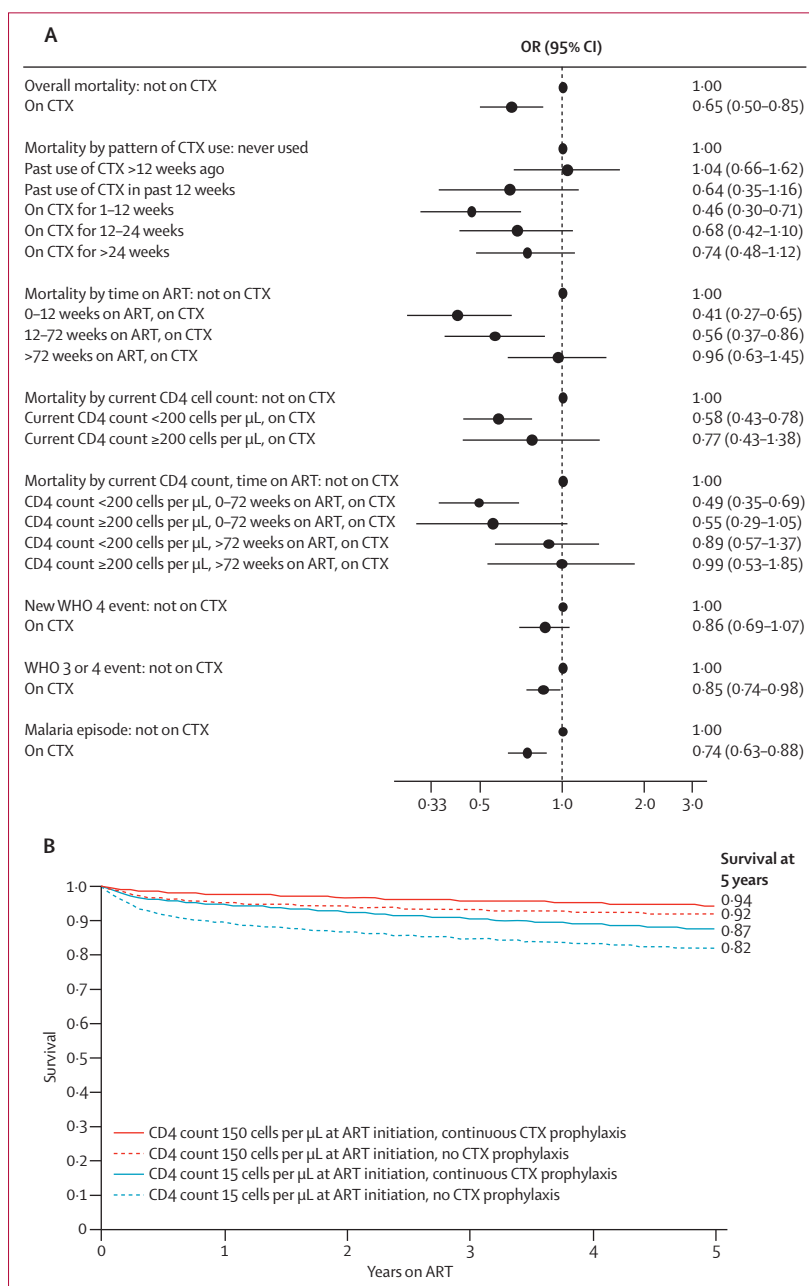


Figure: Effect of co-trimoxazole prophylaxis with ART from weighted models

(A) Clinical outcomes on ART. (B) Predicted survival on ART*. ART=antiretroviral therapy.

CTX=co-trimoxazole. OR=odds ratio. *Assumes co-trimoxazole prophylaxis has different effects in weeks 0–72 (OR 0.49) and after 72 weeks (OR 0.96) on ART. Risks were estimated for a woman aged 30–40 years starting ART (lamivudine-zidovudine plus tenofovir) in 2003 with WHO stage 3 disease, haemoglobin 115 g/L, BMI >21 kg/m², no WHO stage 3 or 4 event in the 4 weeks before starting ART, and CD4 count 15 or 150 cells per μL .

risk by 35% (OR vs no present use 0.65, 0.50–0.85, $p=0.001$; table 2, figure). Benefit did not differ with randomised monitoring group (heterogeneity $p=0.24$). Furthermore, mortality risks did not differ between participants who had stopped prophylaxis and never users, and benefit did not vary with increasing time on co-trimoxazole in present users on ART (table 2, figure).

	0–72 weeks on ART				>72 weeks on ART			
	Deaths/PY*	Rate	OR (95% CI)†	p value	Deaths/PY*	Rate	OR (95% CI)†	p value
Current CD4 count 0–99 cells per μL‡								
Not present user	71/398	17.8	21/235	8.92
Present user	60/563	10.7	0.48 (0.33–0.69)	0.0001	43/474	9.07	0.87 (0.55–1.39)	0.57
Current CD4 count 100–199 cells per μL‡								
Not present user	22/732	3.01	10/643	1.56
Present user	20/884	2.26	0.52 (0.30–0.92)	0.03	16/1330	1.20	0.95 (0.52–1.74)	0.86
Current CD4 count ≥ 200 cells per μL‡								
Not present user	12/881	1.36	20/3177	0.63
Present user	3/695	0.43	0.56 (0.29–1.07)	0.08	36/4202	0.86	1.01 (0.54–1.89)	0.98

ART=antiretroviral therapy. PY=person-years. OR=odds ratio. *Deaths and person-years were excluded after randomisation to structured treatment interruptions in a substudy and upweighted after randomisation to continuous therapy. †OR are relative to no present use of co-trimoxazole prophylaxis and were estimated with history-adjusted marginal structural models, treating every 12-week follow-up visit as a new baseline, and considering mortality during the next 12 weeks (webappendix p 2). ‡CD4 cell count at last 12-week follow-up visit.

Table 3: Effect of co-trimoxazole prophylaxis on death by time on ART and current CD4 cell count†

Benefit of present co-trimoxazole prophylaxis did, however, vary significantly with time on ART (heterogeneity $p=0.04$ in a flexible model for time on ART with cubic splines²⁰), falling from a 58% reduction in the first 4 weeks on ART to a 5% reduction in weeks 68–72 (every additional 4 weeks on ART to 72 weeks increased estimated OR by 1.05 [1.01–1.09]; $p=0.03$), with no effect subsequently ($p=0.67$). When we categorised time on ART to show this variation, mortality reduction was greatest in the first 12 weeks of treatment, sustained from 12–72 weeks, but not evident subsequently (heterogeneity $p=0.02$; figure). Adjustment for time on ART and time on co-trimoxazole simultaneously in present users showed that effect of time on ART was not confounded by duration of prophylaxis; similar mortality risk reductions were reported in the first 72 weeks on ART in participants on co-trimoxazole for fewer than 24 consecutive weeks (OR 0.50, 0.34–0.73) and more than 24 consecutive weeks (0.48, 0.28–0.83), and we noted no reduction in mortality after 72 weeks on ART in either group (0.91 [0.47–1.77], heterogeneity before vs after 72 weeks, $p=0.12$; OR 0.96 [0.63–1.47], heterogeneity before vs after 72 weeks, $p=0.05$ respectively). Estimated average 5-year survival in DART participants starting ART with CD4 counts of 15 or 150 cells per μL increased by 5% and 2%, respectively, with co-trimoxazole prophylaxis (figure).

We estimated effects of prophylaxis on mortality from primary, secondary, or tertiary causes regarded as potentially preventable by co-trimoxazole (92 deaths: septicæmia [39], pneumonia [16], severe brain syndrome as defined by the endpoint review committee [generally representing undiagnosed toxoplasmosis; 13], non-cryptococcal meningitis [8], diarrhoea [5], malaria [4], *P jirovecii* pneumonia [2], acute febrile event [2], toxoplasmosis [1], chronic pulmonary disease [1], or visceral abscess [1]), versus other causes (179 deaths: cryptococcus [31], tuberculosis [26], lymphoma or

Kaposi's sarcoma [16], other [106]) versus unknown cause (55), separately. Present prophylaxis reduced risk of deaths from causes regarded as potentially preventable by co-trimoxazole by 21% (OR 0.79, 0.49–1.27), other deaths by 35% (0.65, 0.45–0.93), and unknown deaths by 48% (0.52, 0.27–1.00). In the first 72 weeks on ART, 66 (35%) deaths were from causes potentially preventable by co-trimoxazole compared with 92 (49%) due to other causes and 30 (16%) unknown; with similar mortality risk reductions associated with co-trimoxazole prophylaxis (OR 0.64 [0.37–1.10], 0.48 [0.30–0.77], and 0.29 [0.12–0.73] respectively). After 72 weeks, 26 (19%) deaths were from causes potentially preventable by co-trimoxazole, 87 (63%) were due to other causes, and 25 (18%) were of unknown cause; again, we found no difference in co-trimoxazole effect (OR 1.56 [0.59–4.14], 0.89 [0.53–1.50], and 0.93 [0.38–2.27], respectively).

All participants began ART with a CD4 count lower than 200 cells per μL ; 2576 (62%) person-years of follow-up in the first 72 weeks and 2678 (27%) subsequently were spent with most recent CD4 count lower than 200 cells per μL . In the first 72 weeks on ART, we estimated similar mortality reductions in participants with current CD4 counts lower than 200 cells per μL and 200 cells per μL or more on co-trimoxazole prophylaxis (figure). After 72 weeks on ART we noted no benefit of present co-trimoxazole prophylaxis in participants with current CD4 counts lower than 200 cells per μL or 200 cells per μL or more (figure). Results were similar for deaths from causes regarded as potentially preventable by co-trimoxazole and for deaths from other causes. Alternative models including an additional CD4 count category of lower than 100 cells per μL (table 3), more than 350 cells per μL , or allowing for a non-linear effect of CD4 cell count also provided no evidence for variation in effect of co-trimoxazole prophylaxis with current CD4 cell count.

	CD4 count (cells per μL)		BMI (kg/m^2)		Haemoglobin (g/L)		Neutrophils $\times 10^9/\text{L}$		Platelets $\times 10^9/\text{L}$	
	Difference	p value	Difference	p value	Difference	p value	Difference	p value	Difference	p value
Change between weeks 0 and 12*†										
Not present user	0	..	0	..	0	..	0	..	0	..
Present user	-2 (-8 to 5)	0.60	-0.06 (-0.17 to 0.05)	0.29	0.2 (-1.2 to 1.5)	0.80	-0.04 (-0.11 to 0.02)	0.18	-4.4 (-10.8 to 2.0)	0.18
Change from week 12‡§¶ (overall effect)										
Not present user	0	..	0	..	0	..	0	..	0	..
Present user	-3 (-12 to 6)	0.50	-0.04 (-0.20 to 0.13)	0.68	0.4 (-0.5 to 1.3)	0.38	-0.05 (-0.10 to 0.00)	0.05	2.0 (-2.5 to 6.5)	0.38
By time on ART‡ 										
Not present user	0	..	0	..	0	..	0	..	0	..
Present user										
12–72 weeks on ART	-13 (-20 to -5)	0.001	-0.10 (-0.27 to 0.07)	0.26	0.1 (-0.9 to 1.1)	0.80	0.02 (-0.03 to 0.07)	0.43	1.6 (-3.0 to 6.3)	0.49
>72 weeks on ART	0.3 (-11 to 11)	0.96	-0.01 (-0.20 to 0.18)	0.89	0.5 (-0.5 to 1.5)	0.32	-0.07 (-0.13 to -0.02)	0.009	2.1 (-2.8 to 7.0)	0.39

Data are effect of co-trimoxazole (95% CI). BMI=body-mass index. ART=antiretroviral therapy. *Estimated from unweighted normal regression models adjusting for centre, randomised monitoring group, randomisation year, age, sex, WHO stage, and ART prescription at randomisation together with baseline values of CD4 cell count, haemoglobin concentration, BMI, and a WHO stage 3 or 4 event in the 4 weeks before randomisation. For platelet and neutrophil outcomes, corresponding baseline values were also adjusted for. †p values for test of heterogeneity between centres were 0.68, 0.56, 0.21, 0.17, and 0.85, for CD4 cell count, BMI, and haemoglobin, and neutrophil and platelet concentrations, respectively. ‡Estimated from weighted repeated measures (every 12 weeks) normal regression models adjusting for length of time on ART with cubic splines, baseline covariates*, and time-varying values of CD4 cell count, haemoglobin concentration, BMI, WHO stage 3 or 4 event in the previous 4 weeks or since randomisation, and randomisation into a structured treatment interruption substudy (never, to continuous therapy). For neutrophil and platelet outcomes, corresponding time-varying values were also adjusted for. §p values for test of heterogeneity between centres were 0.07, 0.47, 0.21, 0.08, and 0.16. ¶By pattern of use no evidence was found for an effect with increasing time on co-trimoxazole prophylaxis or benefit in past users compared with never users for any of the five outcomes (data not shown). ||p values for test of heterogeneity between time periods were 0.01, 0.33, 0.43, 0.0004, and 0.81.

Table 4: Effect of co-trimoxazole prophylaxis on CD4 cell count, body-mass index, and markers of haematological toxic effects

Oesophageal candidosis, cryptococcosis, and extra-pulmonary tuberculosis were the most common WHO stage 4 events, contributing 159 (31%), 115 (22%), and 108 (21%) first events, respectively. Other diagnoses were individually less common. Only 22 (4%) stage 4 events were *P. jirovecii* pneumonia (21 presumptive), of which 13 of 22 were on co-trimoxazole prophylaxis. We noted little evidence for a reduction in first new WHO stage 4 events after ART initiation with co-trimoxazole (table 2). The marginal benefit seen in past users is probably due to chance or incomplete adjustment for confounders in those stopping co-trimoxazole, and results of sensitivity analyses did not suggest that we had missed any benefit in present users. Results did not differ when oesophageal candidosis was excluded (data not shown). The effect of present prophylaxis on WHO stage 3 and 4 events, including recurrences of previous events as outcomes, was significant, but this small effect did not vary with time on ART (table 2). Heterogeneity existed between centres ($p=0.02$), and we were unable to obtain consistent estimates of the effect of co-trimoxazole on pneumonia alone (which accounted for 280 [22%] first WHO stage 3 or 4 events) across all four centres, suggesting variable diagnostic criteria or remaining unadjusted confounders.

In the first 12 weeks on ART, CD4 cell count increased by a median 73 cells per μL (32–128) and BMI by $0.76\text{ kg}/\text{m}^2$ (0.00–1.60). Participants taking co-trimoxazole did not have greater CD4 cell count or BMI increases than did non-users in the first 12 weeks or subsequently (table 4); estimated CD4 increases were slightly reduced with co-trimoxazole, probably because of some residual confounding (difference vs non-users -3 cells per μL [-12 to 6]; $p=0.50$). We showed no effect of co-trimoxazole

on haemoglobin or platelet counts, and observed a small significant reduction in neutrophil count in participants currently on co-trimoxazole after 72 weeks on ART (table 4). Only 22 (3%) of 650 serious adverse events during the trial were judged to be related to co-trimoxazole (ten definite or probable, 12 uncertain relation, all haematological, rash, or hypersensitivity).

Harare has low malaria transmission. In Uganda, 1170 (53%) of 2200 participants had at least one malaria event, with 2362 events in total (260 upweighted; 27 per 100 person-years, 1119 [47%] diagnosed by microscopy, 1243 [53%] clinical). Present prophylaxis was associated with a 26% reduction in risk of first new malaria episode in present users (table 2), with similar estimates if more than one episode per person was included (data not shown) and a weakened effect when restricted to parasite-positive diagnoses (0.85 [0.65–1.11]; $p=0.23$). The reduction in malaria risk associated with co-trimoxazole was maintained throughout follow-up.

Discussion

In this large cohort of HIV-infected symptomatic African adults starting combination ART with low CD4 cell counts, we showed significant reductions in mortality and malaria from daily co-trimoxazole prophylaxis in addition to benefits conferred by ART. Mortality reductions were striking in the first 72 weeks of ART, with, in particular, early (0–12 weeks) mortality on ART being more than halved. The effect on malaria in Uganda was sustained beyond 72 weeks, consistent with reports that co-trimoxazole is an effective agent for malaria prophylaxis in semi-immune adults.²¹

Randomised trials are needed for the highest level of evidence,²² but in their absence patients still have to be managed according to the best available evidence from other studies, particularly those that are large, well done, and have clear, plausible, and consistent results. Since our study was observational, identification and appropriate adjustment for confounders is essential. Participants were in a randomised trial of management strategies, so the most important clinical and laboratory information used by clinicians for participant management was obtained systematically and prospectively, and we were able to do several sensitivity analyses using different models of inverse-probability treatment weights. In addition to the large cohort size, that we had four centres was invaluable for testing of these models since heterogeneity in effect of co-trimoxazole between centres suggested that confounders could remain. In models presented, results were consistent across centres and randomised monitoring strategies. Finally, censoring due to loss-to-follow-up was very low, and although we adjusted for censoring by using additional weighting, the effect was small. Since DART participants, who had advanced immunodeficiency and symptomatic disease, had similar characteristics to those of most patients starting ART in rollout programmes in Africa, our findings should be generalisable. Because our study used observational data we cannot guarantee that the results are free from bias, but with the systematic approaches used the possible direction and magnitude of any remaining bias are difficult to identify.

The survival benefit conferred by co-trimoxazole was restricted to present use, with no variation in benefit with use at or before enrolment or with increasing time on prophylaxis. The roughly 50% mortality risk reduction to 72 weeks after ART initiation is similar to that reported in ART-naive participants in randomised trials in resource-limited countries.^{4,5,7,10} Beyond 72 weeks on ART, we observed no mortality benefits. One limitation of our data was that most present use beyond 72 weeks was in individuals on long-term co-trimoxazole, so we cannot distinguish between time from ART initiation and duration of effects of co-trimoxazole use after this point. Trials in ART-naive participants have typically had little follow-up beyond 72 weeks,^{4,5,7} although Nunn¹⁰ reported a waning effect of co-trimoxazole prophylaxis with time, possibly related to falling adherence, in patients on treatment for concurrent tuberculosis. Stopping of co-trimoxazole prophylaxis after 72 weeks on ART might therefore be a reasonable strategy; randomised trials of different durations of prophylaxis would inform this issue. Although risk of malaria will remain (as for exposed adults not infected with HIV/AIDS), malaria in semi-immune adults is generally not serious and is non-fatal, and co-trimoxazole is not the prophylaxis of choice against malaria. In DART, absolute risk of death was increased at low CD4 cell counts, but we noted no variation in mortality risk reductions according to most

recent CD4 cell count. Results of ART-naive studies have shown no evidence for heterogeneity in effect of co-trimoxazole prophylaxis by baseline CD4 cell count on severe morbidity⁵ or mortality.⁹

What co-trimoxazole prophylaxis is preventing, apart from malaria, is unclear from our data. In two trials of ART-naive adults, co-trimoxazole substantially reduced definitively diagnosed invasive bacterial disease with bacteraemia and malaria.^{4,5} In children, lower respiratory tract infections were significantly reduced, but many died rapidly and most diagnoses were presumptive,^{4,23} as seen in other non-randomised co-trimoxazole studies in African adults. In DART we obtained through structured summaries as much data as possible for deaths, which were then independently reviewed by an endpoint review committee. In 55 (17%) unobserved deaths, no cause could be assigned; in the remainder, primary, secondary, and tertiary causes were assigned with emphasis on confirmation of WHO 4 events because these were the primary endpoint. Although severe bacterial infections (as WHO stage 3 events) were systematically solicited and reported, they were not always microbiologically investigated. Thus, our data for cause of death cannot directly inform about mechanism of action, other than to note a substantial effect of co-trimoxazole prophylaxis on other causes, which could have been because of secondary sepsis.

Recurrent pneumonia diagnoses (individually reported as stage 3 events) have been reviewed by the endpoint review committee, because the revised WHO 2006 guidelines include two pneumonia episodes within 6 months as a stage 4 event.²⁴ However, insufficient events (n=13) were reported for a separate analysis. A key conclusion is that HIV studies in sub-Saharan Africa should document severe bacterial morbidity carefully, especially in patients with WHO stage 3 or 4 events. Although results of a recent study²⁵ suggested that almost all *Mycobacterium tuberculosis* isolates might retain in-vitro sensitivity to co-trimoxazole, we showed no evidence of an effect on pulmonary or extrapulmonary tuberculosis, similar to other WHO stage 3 or 4 events (data not shown).

Even if co-trimoxazole prophylaxis acts by partly or mainly reducing risk of mortality due to bacterial infections, why this mechanism should translate into mortality benefits before but not after 72 weeks is unclear. In particular, although one could postulate that severity of bacterial infections falls with increasing immune restoration, we did not show any variation in effect of co-trimoxazole by current CD4 cell count, and specifically no effect on deaths from causes regarded as potentially preventable by co-trimoxazole or other deaths in participants with low CD4 cell counts after 72 weeks. An alternative explanation, lent support by the absence of effect of prophylaxis on HIV-related disease progression and CD4 cell counts, is that although co-trimoxazole does not prevent HIV-related events, it might reduce mortality

from them, irrespective of CD4 cell count. However, this explanation does not account for why the effect might occur before but not after 72 weeks from ART initiation. One hypothesis is that co-trimoxazole lowers bacterial load in the gut, thus reducing microbial translocation of bacterial lipopolysaccharides from the gastrointestinal tract into the bloodstream. Increased absorption of bacterial lipopolysaccharides raises immune activation,^{26,27} and co-trimoxazole use at ART initiation could increase and accelerate reductions in immune activation, especially before ART has itself affected immune activation. In a few small studies from resource-limited settings, investigators have reported high pre-ART immune activation, which then decreases only gradually during the first 48 weeks of treatment.^{28–30} Peripheral blood mononuclear cells were not stored in DART, so we cannot investigate this immune activation hypothesis further.

Mortality in patients accessing ART programmes in sub-Saharan Africa is very high in the first year on treatment, with 8–26% of patients dying,^{31–33} most in the first 3–6 months. Even when baseline immunodeficiency is allowed for, early mortality is several times higher in resource-limited settings than it is in high-income settings.³⁴ Although co-trimoxazole prophylaxis is recommended by WHO for symptomatic adults initiating ART in resource-limited settings, practice is variable and prophylaxis is often omitted. With no data from Africa, WHO recommendations were based on extrapolation from US studies, and many physicians have judged that co-trimoxazole would both be of little benefit and have the potential to compromise adherence. In DART, adherence was high, and concerns that initiation of both co-trimoxazole and ART together might lead to unacceptably high rates of toxic effects are not substantiated by our data. The mortality benefits, safety, and tolerability, together with the low cost and simplicity of implementation, suggest that co-trimoxazole prophylaxis is cost effective and has a substantial public health effect. Our results reinforce WHO guidelines and provide strong motivation for provision of co-trimoxazole prophylaxis for at least 72 weeks to all adults starting combination ART in Africa.

Contributors

DF, ASW, and AGB developed the first draft of the analysis plan for this analysis, to which all authors then contributed. DF did analyses and wrote the first draft of the report with ASW, AGB, CFG, JHD, and DMG. All authors contributed to interpretation of the data, revised the report critically, and approved the final version.

Conflicts of interest

We declare that we have no conflicts of interest.

Acknowledgments

We thank patients and staff from all centres participating in the DART trial (for the DART Trial Team, see reference 15). DART was funded by the UK Medical Research Council, the UK Department for International Development (DFID), and the Rockefeller Foundation. GlaxoSmithKline, Gilead, and Boehringer-Ingelheim donated first-line drugs for DART, and Abbott provided LPV/r (Kaletra/Aluvia) as part of the second-line regimen. We thank Andrew Copas for helpful discussions and comments on the report, and the reviewers for suggestions that have improved the presentation of our findings.

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