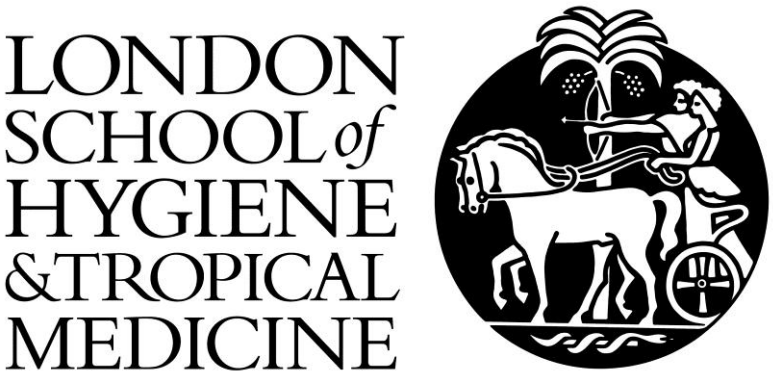

Effect of cotrimoxazole prophylaxis on malaria in HIV-infected adults on antiretroviral therapy

Ronnie Kasirye

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University of London

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Department of Infectious Disease Epidemiology

Faculty of Epidemiology and Population Health

London School of Hygiene and Tropical Medicine

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Statement of own work

I, Ronnie Kasirye, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

13.03.2016

Signature Date

Abstract

Background: It is unknown whether in malaria-endemic areas, cotrimoxazole (CTX) prophylaxis can be stopped in HIV-infected patients on antiretroviral therapy (ART) who have regained immune competence. The aims of this thesis were to: review the literature on the effect of CTX on malaria; investigate the effect of stopping CTX on malaria incidence in a randomised trial; and assess the effect of CD4 count and ART regimen on malaria.

Methods: (i) The literature was systematically searched for relevant papers. (ii) Data from the recently completed COSTOP trial were used to examine the effect of stopping CTX on malaria incidence among HIV-infected Ugandan adults on ART. Participants with CD4 count ≥250 cells/μl were randomised (1:1) to continued CTX or placebo. CD4 counts were determined at ART initiation, enrolment and during follow-up. Malaria was defined as fever with parasitaemia. Incidence and rate ratios (RR) were estimated using random effects Poisson regression, accounting for multiple episodes.

Results: (i) Six studies were identified. All reported an increase in malaria following CTX discontinuation. However, all studies were subject to bias and/or confounding. (ii) In COSTOP, 2180 participants were followed for a median of 2.5 years. They experienced 453 malaria episodes. Malaria incidence was 3.5 (95%CI=2.7-4.4) times higher on placebo than CTX. Few cases of severe malaria occurred, and no increase in malaria mortality. CD4 count had no effect on incidence. Partcipants on a protease inhibitor-based regimen experienced malaria significantly less often than those on other regimens.

Conclusion: Among participants with CD4 of ≥250 cells/μl, malaria incidence increased when CTX was stopped. This effect was lower than shown in previous (unblinded) trials. Malaria mortality did not increase. CD4 count had no effect on malaria incidence. These results support current WHO guidelines on CTX use in malaria-endemic areas, but there may be subgroups who benefit from CTX discontinuation.

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List of abbreviations

AIDS	acquired immunodeficiency syndrome
ART	antiretroviral therapy
ARV	antiretroviral
CD4	CD4 cell count
CRFs	case record forms
CROI	Conference on Retroviruses and Opportunistic Infections
CTX	cotrimoxazole
ERC	Endpoints Review Committee
HIV	human immunodeficiency virus
IAS	International AIDS Society
IDMC	Independent Data safety and Monitoring Committee
INSTI	intergrase strand transfer inhibitors
IRS	indoor residual spraying
ITN	insecticide treated bed-net
LPV/R	lopinavir / ritonavir
NDA	National Drug Authority
NNRTI	non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
OI	opportunistic infections
PI	protease inhibitor
RDT	rapid diagnostic test
RNA	ribonucleic acid
SAE	serious adverse event
SEC	Science and Ethics Committee
SSA	Sub-Saharan Africa
TASO	The AIDS Support Organization
TSC	Trial Steering Committee
UNCST	Uganda National Council of Science and Technology
WHO	World Health Organization

Chapter 1: General introduction

1.1 Malaria

Malaria is a disease of humans that is caused by 5 species of protozoa. All these species belong to the genus plasmodium: *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*. Two of these pose the greatest threat: *P. falciparum,* being the most virulent, is responsible for most deaths, and is the most common in Africa. *P. vivax* has a wider geographic distribution than *P. falciparum* because it can develop and survive in cooler climates and also develops a dormant liver stage that can reactivate months and even years after an infection leading to recurrence of symptoms (1).

Malaria is transmitted from person to person by the bite of an infected female anopheles mosquito which bites between dusk and dawn. The life cycle of the malaria parasite is similar for all the 5 species. Part of the cycle takes place in the human host and part in the mosquito, as follows:

The mosquito stage: The female *Anopheles* mosquito takes up male (microgametocytes) and female (macrogametocytes) gametocytes when it feeds on a person infected with malaria. While in the mosquito's gut, the microgametes penetrate the macrogametes generating zygotes. The zygotes develop into motile and elongated ookinetes which invade the mid-gut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture and release sporozoites (the infectious form of the parasite) which migrate to the mosquito's salivary glands. The sporozoites are then inoculated into humans when the mosquito takes a blood meal (2-5).

The human stage: During the blood meal sporozoites enter the human blood stream. Subsequently they enter liver cells and mature into schizonts which rupture and release merozoites that burst out of the liver cells. *P. vivax, and P. ovale* have a dormant stage (hypnozoites (6, 7)) which persists in the liver and causes relapses by invading the blood stream weeks or even years later. Merozoites enter the red blood cells and multiply forming new merozoites or develop into gametocytes (reproductive form).

During the liver stage the patient remains well; blood stage parasites are responsible for the clinical manifestations of the disease. The cycle continues once the gametocytes are ingested by the female anopheles mosquito.(2-5).

Malaria infection in human beings presents with non-specific symptoms which include fever, headache, malaise, joint pains, nausea and vomiting. Identification of plasmodium in blood is usually based on microscopic examination of a peripheral blood smear or reading an immunochromatographic rapid diagnostic test (RDT) (8). For microscopic examination, a drop of the patient's blood is used to make a blood smear on a slide which is then stained with Giemsa or Leishman's stain to give the parasite a distinct appearance. A thick smear is used to quantify the number of parasites relative to the number of white blood cells, expressed as number of parasites per microliter of blood, and a thin smear to identify the species (9, 10). RDTs detect parasite-specific antigens or enzymes in blood (8). Polymerase chain reaction (PCR) tests which detect parasite nucleic acids tests in blood can also be used to identify plasmodium. These tests are more sensitive and specific than microscopy or RDTs; however they require specialised and costly equipment and reagents. Finally, serological tests which detect anti-plasmodium antibodies can also be applied, but these detect past rather than current infection, and are usually used for screening purposes (8, 11).

Malaria is classified as acute uncomplicated if in addition to the above-mentioned symptoms and a positive parasitological test, there are no signs of severity. Convulsions, mental confusion, severe anaemia and other signs of end-organ failure are indicative of severe malaria (8, 12).

In areas of high malaria endemicity, children are exposed to repeat infections which leads to development of partial immunity. Therefore, malaria infection among adults usually occurs as asyptomatic parasitaemia or presents as a mild disease. Severe malaria in high endemicity areas is mainly seen in children. In areas of low malaria endemicity, immunity to malaria may not be acquired and even adults may acquire severe malaria (13).

Artemesinin based combination therapies (ACTs) are recommended for the treatment of acute uncomplicated malaria. Treatment of severe malaria requires intravenous or intramuscular artesunate until a patient can tolerate oral medication and then treatment with 3 days of an ACT. Parenteral quinine is indicated for treatment of severe malaria when, an effective ACT (based on resistance patterns in the region) is not promptly available. Oral quinine is used in the treatment of uncomplicated malaria, particularly in the first trimester of pregnancy, or also as alternative when an effective ACT is not available (8).

For prophylaxis, sulfadoxine–pyrimethamine is used for pregnant women, doxycycline, mefloquine, and primaquin for travellers from non-endemic areas, and cotrimoxazole (CTX) for HIV-infected individuals (8, 14). The use of CTX is discussed in more detail in section 1.5 below. Other measures used for prevention of malaria include sleeping under an insecticide-treated bednet (ITN), indoor residual spraying (IRS) of houses or dwellings with insecticides, and spraying of breeding places for vector control (8).

1.2 HIV/AIDS

The human immunodeficiency virus (HIV) that causes the acquired immunodeficiency syndrome (AIDS) is a lentivirus, a subgroup of retroviruses. There are two types; HIV 1 and HIV 2 (15). Both are transmitted through contact with body fluids of an infected person. This can occur through sex, sharing needles, breastfeeding or from mother to child during pregnancy. Once acquired, the virus multiplies rapidly and destroys the body's defence mechanisms, mainly the CD4 cells, resulting in opportunistic infections (OI) and increased occurrence of diseases like malaria and malignancies (16-20). HIV infection is identified by use of tests that detect antibodies to the virus, or using PCR to detect viral ribonucleic acid (RNA). There are 6 classes of antiretroviral (ARV) drugs used to treat HIV: non-nucleoside reverse transcriptase inhibitors (NNRTI), nucleoside reverse transcriptase inhibitors (NRTI), protease inhibitors (PI), fusion inhibitors, CCR5 antagonists (CCR5), and integrase strand transfer inhibitors (INSTI) (21). For antiretroviral therapy (ART), these drugs are used in combinations of 3 to 4 drugs (ART

regimens) to maximally suppress virus replication and reduce the risk of the virus developing resistance to individual drugs (22). These drug regimens are used in a sequential way; initially patients start on first-line therapy, then second line, and then third-line/salvage therapy. A patient's regimen is switched due to poor response to treatment either due to occurrence of opportunistic infections, declining CD4 count or viral load >1000 copies/µl (23). In Uganda, the Ministry of Health recommends 2 NRTIs and an NNRTI for first-line, 2 NRTIs and a PI for second-line, and 2 NRTIs, raltegravir (INSTI) and darunavir/ritonavir (PI) for third-line therapy (24). Individual drugs within a regimen may be substituted due to toxicity (23).

These drugs suppress viral replication which allows the immune system, including CD4 cells, to recover (25-27). Some studies suggest that NNRTIs (28) and PIs (29) might also have an antimalarial effect although the evidence for this and for the mechanisms involved is inconclusive .

1.3 Interaction between malaria and HIV infection

HIV impairs T-cell immunity, which is an important component of the antimalarial immune response (30, 31). In areas of stable malaria transmission, HIV infection is associated with more cases of clinical malaria and increased prevalence and density of parasitaemia in non-pregnant adults (32-34). In areas of unstable transmission, HIV is associated with increased risk of severe malaria and death (35, 36). HIV infection has also been associated with impaired response to malaria treatment leading to recrudescence and re-infection; however, with the introduction of the highly efficacious artemesinin combination therapies (ACT) there is less recurrence of malaria (32). Malaria infection is associated with CD4 cell activation and activation of proinflammatory cytokines which facilitates the spread of the virus among CD4 cells and rapid viral replication (37). Following infection with malaria, there is a temporary rise in HIV viral load which can last up to 10 days (38) and it has been suggested that this elevated viral load might lead to faster HIV disease progression and higher risk of HIV transmission (39). Some studies have also reported higher mortality from malaria in HIV-infected

patients (40). As mentioned above (section 1.2), some ART drugs are thought to have antimalarial properties, particularly protease inhibitors (PIs) which should lead to a reduction in malaria incidence among patients taking these drugs. A study done in Uganda found less malaria in HIV-infected patients on ART and CTX compared to patients on CTX only (IRR 0.3 (95%CI=0.2-0.7). Additionally, with the recovery of the immune system on ART, a reduction in the incidence of malaria is expected, however evidence on this association is lacking.

1.4 Public health importance of malaria and HIV disease

There were an estimated 214 million cases of malaria worldwide in 2015 resulting in 438,000 deaths; 88% of these cases and 90% of the deaths occurred in Africa (16).
Overall, 36.9 million people worldwide were estimated to be living with HIV at the end of 2014, with 2.0 million people becoming newly infected globally. Sub-Saharan Africa is the most affected region, with 25.8 million people living with HIV in 2014 and accounts for almost 70% of the global total of new HIV infections. About 1.2 million children and adults were reported to have died from AIDS-related illnesses globally in 2014 (22).

Malaria and HIV are therefore important global health problems and together they are estimated to cause more than a million deaths per year (41). These diseases also have a wide geographical overlap particularly in sub-Saharan Africa, frequently resulting in co-infection and mutual enhancement as mentioned above.

1.5 Chemoprophylaxis

Chemoprophylaxis is used to prevent opportunistic infections in HIV-infected patients particularly those whose immune function has declined, as shown by low CD4 cell counts, but have not yet been started on ART. The highest risk of opportunistic infections has been associated with CD4 count <200 cells/µl (42). Prophylaxis can be either primary or secondary. Primary prophylaxis is to prevent occurrence of an infection, while secondary prophylaxis is given after an infection has occurred and is aimed at preventing recurrence or malaria. Drugs recommended for prophylaxis include: CTX for bacterial, protozoal and fungal infection; isoniazid for TB; and fluconazole for

cryptococcal meningitis (43, 44). CTX has also been found to be effective for prophylaxis against malaria and is recommended for use in malaria endemic areas (14, 45) (CTX use is discussed in detail in section 1.6). In HIV-infected patients whose immune function has been restored, the incidence of opportunistic infections is reduced (26) and prophylaxis may be stopped (except for CTX in malaria-endemic areas) (14, 43). Increasingly HIV-infected adults are being started on ART at high CD4 counts and many countries are now using the WHO recommended test-and-treat approach whereby HIV-infected individuals are started on treatment as soon as they are diagnosed (46). The role of prophylaxis in HIV-infected adults with CD4 counts >350 cells/µl is therefore not clear.

1.6 Cotrimoxazole

Among HIV-infected patients, the daily prophylactic use of CTX reduces mortality and morbidity from OIs (47-54) such as toxoplamosis, *Pneumocystis jiroveci* pneumonia, recurrent non-typhoidal salmonella bacteraemia and isosporiasis (Appendix 1). In addition CTX has been shown to reduce the incidence of malaria in HIV-infected patients (45, 50, 55) as well as among HIV-exposed uninfected children (56).

CTX is a broad spectrum anti-microbial agent comprised of trimethoprim and sulfamethoxazole. It is active against a wide range of bacteria(57), fungi (58) and protozoa(59). Both drugs act by inhibiting the folate metabolism in these organisms (60). Resistance to CTX is usually caused by mutations in the folate pathway involving the dihydrofolate reductase and dihydropteroate synthetase enzymes and is common in areas with widespread CTX use for prophylaxis by HIV-infected patients (61, 62).

In Uganda it is recommended that CTX prophylaxis should be given to all HIV-infected adults and children regardless of whether they are on ART or not (63).The recommended dose of CTX for adults is one double-strength tablet or two single-strength tablets once daily: the total daily dose is 960 mg (800 mg sulfamethoxazole and 160mg trimethoprim) (64).

The World Health Organization (WHO) recommends CTX prophylaxis for anybody with a CD4 cell count <350 cells/µl or who is in clinical stages 3 or 4 of HIV infection, irrespective of their CD4 count. In settings where malaria and/or severe bacterial infections are highly prevalent, CTX prophylaxis should be initiated regardless of CD4 cell count or WHO stage (14). The possible preventive effect on malaria was originally not part of the rationale for the use of CTX. Indeed fears about the possible development of cross-resistance against other anti-malarial drugs due to widespread CTX use were expressed by some authors (65-67).

Although CTX is generally well tolerated, its possible side effects include headache, vomiting, diarrhoea and hypersensitivity-related skin reactions. When used for long term prophylaxis, as is the case in HIV-infected patients, it has been associated with neutropenia, thrombocytopenia and anaemia due to haematotoxicity (bone marrow suppression) (68-70) and it increases patients' pill burden and cost of care (71).

Once a patient is started on effective ART, viral replication is suppressed and immune function eventually recovers resulting in fewer OIs and reduced mortality, and therefore some authors recommend stopping CTX once CD4 count has risen to >200 cells/mm3. Stopping CTX has been shown to be safe in studies in industrialized countries (72, 73). Obviously, the epidemiological situation in these countries differs from that in sub-Saharan Africa (SSA) where more bacterial OIs and malaria occur.

In 2006, the Ministry of Health in Uganda noted that the benefit of CTX prophylaxis for adults and children on ART in Africa whose CD4 cell counts were >200 cells/µl or CD4 percentage >15%, respectively, had not been evaluated. It recommended that until more information is available, the decision to discontinue CTX prophylaxis in this group should be made by the provider on an individual basis (74).

In its previous guidelines of 2006, WHO recommended that in resource-limited countries CTX should be continued for life, but discontinuation could be considered among people with evidence of immune recovery (CD4 >350 after at least six months of ART) (64).

At the time when the study underlying this PhD was initiated, it was therefore not clear whether CTX prophylaxis in HIV-infected patients from countries with high incidences of bacterial OIs and malaria could safely be stopped once their immunity has recovered due to ART. If found to be safe, stopping CTX would reduce pill burden and reduce the risk of CTX related side effects, in particular haematotoxic adverse effects. It was also not known whether the severity of malaria in immune competent patients on ART would differ between those taking CTX prophylaxis and those not taking CTX, and whether and in which way this may depend on CD4 levels at the time of CTX cessation.

The aim of my PhD project was to address these questions, in HIV-infected adults using the opportunity of a randomised placebo controlled trial of CTX cessation in Uganda (COSTOP). COSTOP aimed to assess the efficacy and safety of stopping CTX prophylaxis in HIV-infected adults who were stable on ART. Enrolment into the trial started in January 2011 and ended in March 2013. Enrolment was stratified by site (Entebbe and Masaka) and enrolment CD4 count (<500, ≥500). Participants were followed up for up to 3.5 years. Main results of the trial were presented at the Conference on Retroviruses and Opportunistic Infections (CROI) in February 2015 (75). Analysis of the main trial outcomes was based on the per protocol population and showed that with respect to efficacy, the incidence of CTX preventable events (adjusted for site and CD4 stratum) in the placebo arm was 1.57 (90% CI=1.12-2.21) times higher than that in the CTX arm. With respect to safety, the incidence of grade 3 or 4 haematological adverse events (adjusted for site and CD4 stratum) in the placebo arm was 0.70 (95% CI=0.59-0.82) times lower than that in the CTX arm. There was no difference in the number of deaths by trial arm. In conclusion, discontinuing CTX prophylaxis was associated with a significant increase in CTX-preventable events but a reduction in grade 3 or 4 haematological adverse events. There was no statistically significant difference in mortality between those that stopped and those that continued CTX prophylaxis.

The incidence of malaria was a secondary outcome of the COSTOP trial. It was 3.47 (95% CI 2.74 – 4.39) times higher in the placebo arm than in the CTX arm. The detailed investigation of the incidence of clinical and severe malaria were planned sub-studies of the COSTOP Trial and are the subject of this PhD thesis.

Since this PhD research proposal was written, 3 open-label trials have contributed additional data on whether CTX prophylaxis may be safely stopped in HIV-infected patients who are stable on ART (76-78); these studies are also discussed in the systematic review (chapter 3). In December 2014, WHO also revised its guidelines and now recommends that CTX may be discontinued in patients who are clinically stable with evidence of immune recovery and/or viral suppression on ART, but should be continued in countries with high endemicity of malaria and bacterial infections (14).These guidelines were issued shortly before the COSTOP trial results were presented at CROI 2015 (appendix 2) (75).

1.7 Research questions to be addressed by this PhD research

1. What information is available in the current literature on the effect of CTX on malaria in HIV-infected patients on ART and are there other studies underway which aim to address this question?

2. Does the occurrence of malaria increase in HIV-infected patients who are stable on ART when they stop CTX prophylaxis compared to those that continue and if so, is this increase sustained over time?

3. Is there a difference in the severity of malaria between patients on ART who stop using CTX compared to those that continue?

4. Is the occurrence of malaria in patients who stop CTX associated with the extent of their immune status, as indicated by CD4 count?

5. Does the kind of ART regimen used affect the incidence of malaria in HIV-infected patients on ART?

1.8 Overall aim

To determine the effect of CTX on malaria incidence and severity in HIV-infected persons on ART.

1.9 Overall objectives

Using a systematic review;

- To assess the available literature on this association.

Using a randomised placebo controlled trial;

- To describe malaria incidence and severity in adults who stop taking CTX prophylaxis compared to those that continue.
- To describe the effect of the immune status on malaria incidence in patients stopping CTX versus those that continue.
- To assess the effect of antiretroviral regimen on malaria.

2.0 Role of the candidate

2.0.1 COSTOP trial

I am a co-investigator of the COSTOP trial and was the Trial Coordinator from its beginning in July 2010 to September 2012 (when I took up my PhD research at LSHTM). During this time I was responsible for or made substantial contributions to:

- Obtaining approvals from the regulatory bodies (Uganda Virus Research Institute Science and Ethics Committee (SEC), National Drug Authority (NDA), and Uganda National Council of Science and Technology (UNCST)) and their subsequent (annual) renewals
- Developing the manual of operations and protocol amendments
- Developing the study questionnaires and data collection forms

- Creating standard operating procedures (SOPs) for data collection and processing
- Staff recruitment and training
- Setting up study clinics
- Negotiating with ART providers, explaining the study to them and securing their collaboration
- Overseeing screening and enrolment
- Ensuring that the protocol was adhered to
- Supervision of study teams
- Reporting serious adverse events (SAE) to the regulatory bodies (SEC, NDA, and UNCST)
- Reporting to the Trial Steering Committee (TSC) on the progress of the trial
- Compiling case summaries for the Endpoints Review Committee (ERC)
- Carrying out administrative duties related to the trial

After September 2012 during my PhD studies I contributed to the following:

- Data cleaning process
- Developing the close-out manual of operations
- Dissemination of the trial information and results which included the design paper (Anywaine et al, published in 2015) (appendix 3), the COSTOP main Results presentation (an oral presentation by Jonathan Levin in February 2015 at CROI, Munderi et al.) (appendix 2), and the malaria sub-study results (a poster presentation by the candidate in July 2015 at IAS, Kasirye et al.) (appendix 4)

2.0.2 PhD work

My role in the systematic review included:

- Designing the review after discussions with Kathy Baisley (KB) and Heiner Grosskurth (HG).
- Performing the search for the relevant studies.

- Independently screening the abstracts and extracting data from the identified studies; KB served as a second independent reviewer.
- Wrlting the first draft and revising it following comments from KB, HG and Paula Munderi (PM).
- Submitting the final version of the paper for publication. Responding to reviewers' comments.

My role in the experimental part of my PhD research included:

- Trial coordination and planning data collection until September 2012 as described above.
- Identification of the malaria-related research topics at the design stage of the trial and obtaining approval from the TSC to investigate them as part of a PhD programme.
- Preparation of the research proposal for the PhD.
- Data analysis with respect to incidence of clinical and severe malaria by trial arm and effect of ART regimen on malaria under the supervision and guidance of a senior statistician (KB).
- Contribution to the analysis of the effect of CD4 count on malaria incidence. The analysis itself was mainly performed by KB as it involved a more complicated statistical technique (the use of cubic splines to model the effect of CD4 on malaria incidence).
- Writing the first drafts of all papers from the experimental work (listed below). Revision of drafts following comments from supervisors (KB) and (HG), further revision following comments from other COSTOP investigators (PM, Jonathan Levin, Zacchaeus Anywaine, Andrew Nunn and Anatoli Kamali) and submission of final versions for publication.

Papers written as part of the PhD project:

- Kasirye R., Baisley K, Munderi P, Grosskurth H. (2015). Effect of cotrimoxazole prophylaxis on malaria occurrence in HIV-infected patients on antiretroviral therapy in sub-Saharan Africa. Trop Med Int Health **20**(5): 569-580.

- Kasirye R, Baisley K, Munderi P, Levin J, Anywaine Z, Nunn A, Kamali A, Grosskurth H. Incidence of malaria by cotrimoxazole use in HIV infected Ugandan adults on antiretroviral therapy: a randomized placebo controlled study. AIDS. 2015. Epub 2015/11/13.
- Kasirye R, Grosskurth H, Munderi P, Levin J, Anywaine Z, Nunn A, Kamali A, Baisley K: Longitudinal effect of CD4 by cotrimoxazole use on malaria incidence among HIV-infected Ugandan adults on antiretroviral therapy: a randomized controlled study. Submitted to Malaria Journal (March 2016), accepted (July 2016).
- Kasirye R, Grosskurth H, Munderi P, Levin J, Anywaine Z, Nunn A, Kamali A, Baisley K: Effect of antiretroviral therapy on malaria incidence in HIV-infected Ugandan adults: a prospective cohort study. Submitted to AIDS (March 2016).

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Chapter 2: Methods

2.1 Introduction

This chapter provides an overview of the methods used for this PhD research. Two separate studies were conducted to achieve the objectives:

A systematic review:

- To assess the available literature on the effect of CTX on malaria in HIV infected patients on antiretroviral therapy ART.

A randomised placebo controlled study (I conducted a sub-study within the COSTOP trial):

- To describe malaria incidence and severity in adults who stop taking CTX prophylaxis compared to those that continue.
- To describe the effect of the immune status on malaria incidence in patients stopping CTX versus those that continue.
- To assess the effect of antiretroviral regimen on malaria and whether this effect differs by CTX use.

In this chapter, the two studies are described in general. More detailed information on specific methods is presented in the research papers included in subsequent chapters.

2.2 Systematic review

Embase, Pubmed and Medline, Web of Science, Global Health and the Cochrane Library were searched up to 14 April 2014 for abstracts using terms for malaria, HIV and CTX. After screening the abstracts, full text copies of potentially relevant papers were obtained. Studies that had original data on the effect of CTX on malaria in HIV infected patients on ART were included. Data from papers identified by the search were extracted using a standard form. Guidelines on preferred reporting items for systematic reviews and meta-analyses (PRIMSA) (1) were used. Assessment of bias and confounding within studies and the quality of papers was based on PRISMA guidelines

and the Newcastle-Ottawa quality assessment of studies scale (2). A formal meta-analysis was not done owing to the diversity in study methodologies, comparison groups and populations. The results of this work were published in Tropical Medicine and International Health in May 2015.

2.3 The COSTOP Trial

My research was embedded in the COSTOP trial (ISRCTN44723643). Details of the trial's design have been published (appendix 3) (3). COSTOP was a randomised double blind placebo controlled non-inferiority trial to evaluate whether long-term prophylaxis with CTX could be safely discontinued among Ugandan adults on ART who have achieved sustained immune restoration (measured as a confirmed CD4 count ≥250 cells/µl). The trial had two co-primary outcome measures:

(i) The efficacy outcome measure was the time to the occurrence of the first clinical event (pre-defined CTX-preventable opportunistic clinical event (appendix 1) or death).

(ii) The safety outcome measure was the time to the occurrence of the first grade 3 or 4 haematological adverse event.

Incidence, severity and treatments outcomes of malaria infection were among the secondary objectives of COSTOP. Other secondary outcomes of the trial included mean change in CD4 count after 12 months, mean change in haematological indices after 12 months, and incidence of hospitalisation (all causes).

2.3.1 Study setting

The study was conducted by the MRC/UVRI Uganda Research Unit on AIDS at its research clinics located in Masaka district and Entebbe (located in Wakiso district) (Figure 1) which are both situated along the shores of Lake Victoria. The climate in Uganda allows stable, year round malaria transmission with relatively little seasonal variability in most areas. Malaria is highly endemic in 95% of the country, i.e. in areas where about 90% of the Ugandan population lives (4).

Figure 1: Map of Uganda showing location of study sites (Entebbe and Masaka)

2.3.2 Study population

Adult patients meeting the selection criteria listed below were randomised to receive either placebo (one tablet daily) or active CTX (one tablet of 960 mg daily) in place of their usual CTX (Figure 2).

Selection criteria for patients

Inclusion criteria

a) HIV-infected patient with documented intake of CTX for at least 6 months
b) Age 18 years and above
c) Documented intake of ART for at least 6 months

d) Clinically asymptomatic
e) Two CD4 counts (not more than 6 months apart) ≥250 cells/μl the most recent no more than 4 weeks prior to enrolment
f) Able to attend study appointments and report to the study clinic in the event of intercurrent illness

Exclusion criteria

a) Acute illness (OI or other co-morbidity). Patients were eligible for enrolment after resolution of the illness
b) First trimester pregnancy. Pregnant women who reached their second trimester of pregnancy were subsequently eligible
c) Known hypersensitivity to CTX
d) Grade 3 or 4 anaemia (haemoglobin <7.5g/dL), neutropenia (count $<0.75X10^9$/L) or thrombocytopenia (count $<50\ x10^9$/L) at time of enrolment

2.3.3 Drug description

Active CTX (960 mg tablets) and matching placebo tablets were supplied from CIPLA Limited in India. The tablets were similar in appearance but differed slightly in taste. They were supplied in 1000 tablet containers. They were later repacked in containers of 31 tablets and labelled by independent staff of the MRC/UVRI Uganda Research Unit on AIDS under the supervision of an independent clinician and statistician.

2.3.4 Research facilities and set up

The study was based at two research clinics located at Entebbe and Masaka hospital respectively. Each clinic had consultation rooms, a registry for storage of patient files, a pharmacy, a room for phlebotomy and a meeting room (Figure 3). Data entry and laboratory analyses were done at the offices of the MRC Unit in Masaka and Entebbe, both located within 3 km of the clinics. Each clinic had a team of 2 doctors, 2 nurses/counsellors, 1 administrator, 1 study clerk, 1 field worker and 1 driver. The trial was managed by a full time Trial Coordinator under the supervision of the Principal

Investigator, and was overseen by a TSC. I was Trial Coordinator from the beginning in July 2010 until August 2012.

2.3.5 Study procedures

Randomisation

Allocation to one of the two treatment arms was done by an MRC statistician using random permuted blocks of variable size with separate randomisations carried out in four strata. Strata were defined by the four combinations of the study site (Entebbe or Masaka) and baseline CD4 count category (250-499 vs. 500 or above). There were two randomisation registers at each trial site, one for each baseline CD4 category, containing trial numbers pre-allocated to either trial drug or placebo. Randomisation of patients was on individual basis. Three cases were identified of randomised participants belonging to the same household. They as well as all other trial participants they were encouraged to store their drugs individually and instructed not to share their drugs with anybody.

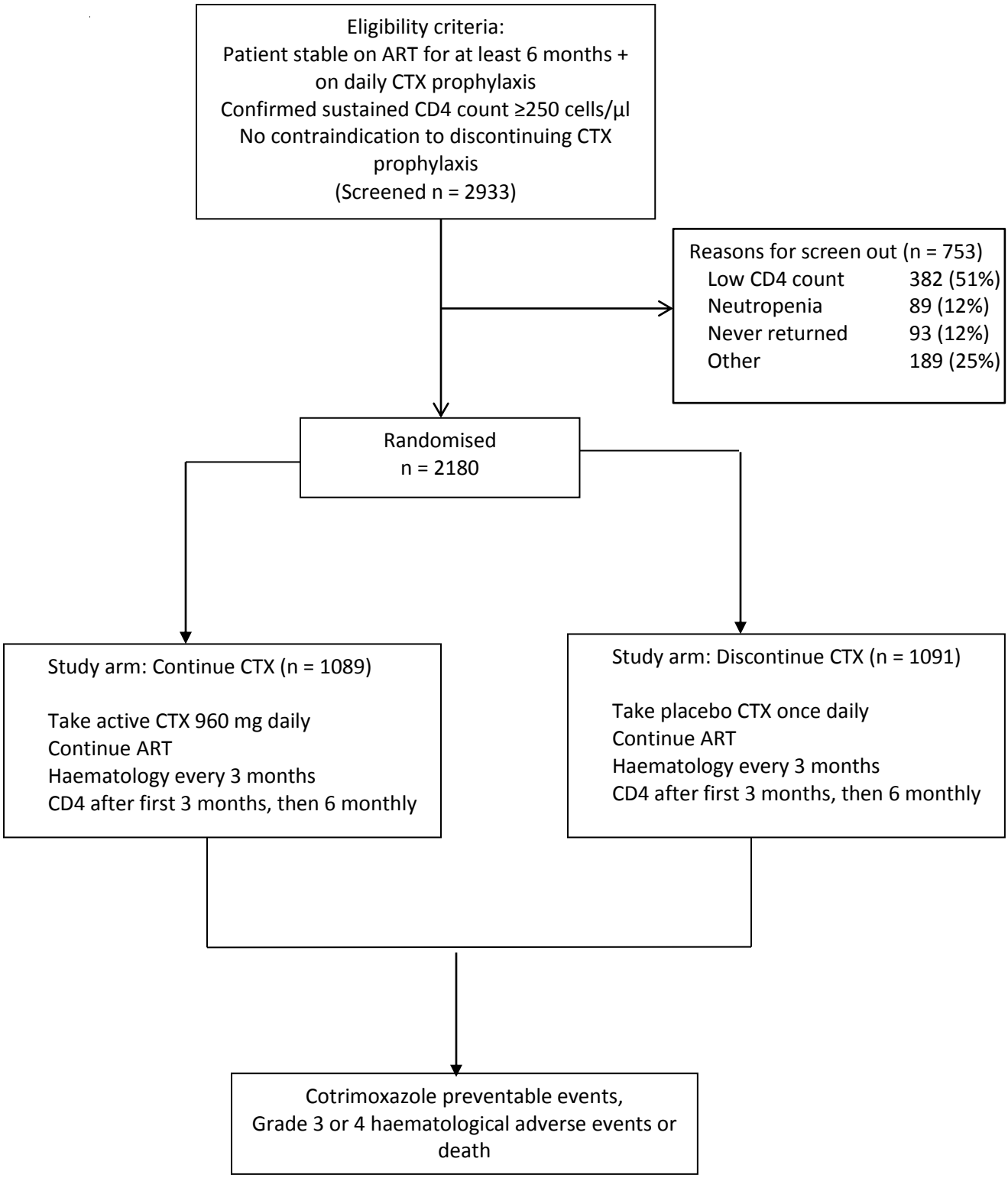

Figure 2: COSTOP trial profile

Figure 3: COSTOP Trial site in Masaka

Screening

The trial teams visited ART provision centres in Masaka (TASO Masaka, Kitovu Mobile, Uganda Cares) and Entebbe (TASO Entebbe, Entebbe Hospital, Kisubi Hospital) and provided information about the study to potential participants. These centres were run by non-governmental organisations or by public or private not-for-profit hospitals. Consent to participate in the screening procedure was obtained from potential participants by a doctor who also recorded their history of OIs, CTX prophylaxis and ART. The doctor also assessed their health and prescribed treatment for concurrent illnesses. Blood specimens were obtained for full blood count and CD4 count from all patients and a urine sample from women to conduct a pregnancy test. This information was recorded on the Screening questionnaire (appendix 5).

Enrolment

Potential participants reported to the study clinics for enrolment within 2-4 weeks of having been screened. They were triaged by a nurse who also recorded information on previous adherence to CTX and ART and social economic details on the Nurse Enrolment questionnaire (appendix 6). A doctor then assessed the patient's health and reviewed their medical history. Any additional or new information on chronic or current illnesses and medication was recorded on the Doctor Enrolment questionnaire (appendix 7). If the doctor then found the patient to be eligible for enrolment, they were asked to provide written informed consent; this information was captured on the Enrolment Consent form (appendix 8). They were then randomised by assigning them the next available number on the trial register based on their baseline CD4; this number was captured on the Randomization form (appendix 9). Blood was collected for plasma storage and for a malaria blood slide. Trial drug was dispensed. The COSTOP study did not provide ART; instead participants continued to receive ART from their ART providers as before. Each participant was issued with an insecticide-treated bed net (ITN) and the need to use it was emphasized.

Follow up

Participants were seen every month (28 days) for the first three months and three monthly thereafter (Table 1). Participants were followed up for a minimum of 12 and a maximum of 38 months depending on date of enrolment.

At every visit the following was done:

- Assessment of adherence to trial drug. Participants were encouraged to bring their drug containers at all visits. They were asked whether they missed any of their scheduled doses, the returned pills were counted and were compared to the expected number that should have been returned. The number of days on which trial drug was missed and the reasons were captured on the Adherence form (appendix 10).

- Assessment of adherence to ART. Participants were asked whether they had missed any ART doses since the previous visit. Information on the drug missed, number of days missed and reasons for missing drug was captured on the Adherence form.

- Adherence to ITN use. Participants were asked whether they had been sleeping under an ITN since the previous visit. The number of days the participant did not use the ITN and reasons were recorded. This information was captured on the Adherence form.

- Physical check-up and recording of intercurrent illness. Participants had their blood pressure, temperature and weight taken by a nurse and were asked whether they had any illnesses or had taken any new medication since the previous visit. This information was captured on a Nurse form (appendix 11).

- Further medical check-up. A doctor reviewed the nurse form, assessed the participant's health and filled the Doctor Follow up form (appendix 12). Information was collected about history of fever, diarrhoea or cough. The participant was examined and any relevant diagnostic tests requested. Any diagnoses made and treatment prescribed were recorded. Information on changes in ART regimen was retrieved from the participant's medical diaries provided by the ART provider and recorded on the Doctor Follow up form as well. If a participant was treated for malaria or had a positive malaria test, this information was captured on a Malaria form (appendix.13). Details of the malaria form are described later in this chapter.

- Provision of trial drug. Trial drug was dispensed to participants in containers with 31 tablets each (3 extra per month).

Blood was drawn for the following laboratory procedures (Table 1);

- Blood slide (thick and thin films), to assess for malaria.

- Full blood count, to detect possible drug related haematological toxicities.
- CD4 count, to monitor immune response to ART.
- Plasma storage, for future studies.

If within two weeks of an appointment a participant had not attended the study clinic, a phone call or home visit was made to the participant by a field worker and/or counsellor. The reason for the missed appointment was established, and if the participant was still interested in participating in the trial, they were reminded about the importance of attending their scheduled visits and a new visit date was agreed. If the participant was no longer interested in taking trial drug or participating in the trial they were advised to sign a Consent Withdrawal form (appendix 14) indicating their choice and their follow up would be stopped or modified accordingly.

Extra / sick visits

Participants were encouraged to come to the study clinic whenever they were ill. Adherence forms, Nurse forms and Doctor follow up forms were filled as described above. The participant was investigated and treated based on their symptoms and signs.

Discontinuation of trial drug

In the event of adverse drug reactions considered to be possibly related to trial drug, medication was discontinued but the blinded allocation of trial drug was maintained, unless unblinding was required in the interest of the participant's safety (for example in the case of a potential CTX related hypersensitivity reaction). Reasons for unblinding a participant were captured on the Unblinding form (appendix 15). The specific event was then managed accordingly. The decision to restart trial drug after resolution of the adverse event was based on the cause of the event.

If a participant's CD4 dropped below 250 cells/µl (and was confirmed by a repeat test), trial drug was discontinued permanently and the participant switched to open label CTX. Such participants continued to be followed until the end of the study. The participant

was investigated for ART treatment failure by ruling out other potential causes of the drop in CD4 count; this was done by assessing adherence to ART and screening for co-infections, for example tuberculosis. This information was shared with the ART provider. Change of a participant's ART regimen due to treatment failure was done by the ART provider and was commonly based on CD4 count for first line ART failure and viral load measurement for second line failure, as recommended by the national treatment guidelines (5). HIV viral load measurements were done when specifically requested by a doctor, mainly for suspected failure on second line ART and not as a routine investigation. Information on starting or stopping trial drug and change in ART regimen was recorded on the Doctor follow up form.

Participants could also be withdrawn from trial drug for the following reasons:

- Participant decision to withdraw consent for study participation
- Intercurrent illness which prevented further treatment.
- Any change in a participant's health condition which justified the discontinuation of treatment in the clinician's opinion.

No additional participants were recruited to replace those who withdraw from the trial. Participants who were withdrawn from the study were still followed up and their clinical data were recorded, unless a participant explicitly also withdrew consent for follow-up

2.3.6 Trial monitoring

The trial was monitored by an Independent Data Safety and Monitoring Committee (IDMC). The IDMC met every 6-12 months. The IDMC reviewed the event rate and assessed whether the study was on schedule to meet its objectives and should continue as planned. The trial was also monitored by an internal (MRC unit) monitor.

2.3.7 Data collection and management

Participants were identified by unique trial and clinic numbers. Individual data was captured in duplicate on case record forms (CRFs). Data was checked at the clinic, double entered using Access into the COSTOP database at the central data and

statistics section in Entebbe. Range and consistency checks were routinely done. Original CRFs were stored centrally but copies were kept to participants' files at the clinic.

2.4 Malaria specific work within COSTOP

2.4.1 Main outcomes

Malaria diagnosis was defined as current fever or a recent history of fever combined with a positive malaria blood slide on microscopy, as is commonly used in studies clinical malaria (6-8). Patients were assessed for parasitaemia at each scheduled visit and when they were ill. If participants were suspected to have malaria, they were triaged by a nurse and assessed by a doctor. Results from routine tests were returned to the doctor within 24 hours and within one hour for a patient suspected to have malaria.

The information captured during a malaria episode was recorded on the Malaria form (appendix 13), and included:

- date of diagnosis, dates of the initial and follow up visits, and highest recorded temperature
- symptoms and signs according to a check list
- laboratory results (whether the blood slide was positive or negative). In the case of a positive slide; the plasmodium species found and the number of parasites per 200 white blood cells.
- treatment given using a check list and source of treatment (options included study clinic, self-medication or other source, e.g. another clinic attended by patients who were unwell during a journey)
- date of resolution of symptoms and results of the follow up blood slide.

If hospitalisation was required, patients were admitted to Entebbe or Masaka hospital where they were followed up by the research team until discharge. A Hospital Admission form (appendix 16) was filled which captured reasons for admission, treatment given, and duration of admission.

2.4.2 Main exposures

Trial drug

The main exposure of interest was trial drug. As mentioned earlier trial arm allocation was by trial number given to a participant at enrolment. This information was recorded on a randomization form and was revealed after unblinding at the end of the trial. A provision was made for unblinding if it was necessary for patient management but no participant was unblinded during the trial. Adherence to trial drug was assessed using self-reports and pill counts and recorded on the Adherence form at all routine visits.

ART regimen

Information on participants' initial and current ART regimens was obtained from records during screening at the ART provision centres and recorded on the Screening questionnaire. Changes in the ART regimen during follow up were recorded on the Doctor Follow up form.

CD4 cell count

CD4 counts for patients before joining the study were obtained from ART provision centres and recorded on the Screening questionnaire. CD4 counts during the study were measured at 3-months then 6 monthly (Table 1) by FACS-count (Becton-Dickinson San Jose). Data were reviewed by a doctor, double-entered and stored as with other CRFs. CD4 count data were thus available for the time of ART initiation, time of enrolment into the trial and during follow-up.

2.4.3 Sample size

2180 patients were recruited into the COSTOP trial. Details of the sample size calculation for the COSTOP trial are covered in chapter 4.

I assessed the power that the sample size of COSTOP would provide to detect an increase in malaria incidence under a variety of assumptions (Table 2). For example, under the assumption that the incidence of malaria in the control arm (with continued CTX prophylaxis) would be 1.4 malaria episodes /100 person years (pyrs) as observed in the study by Campbell et al (9), the COSTOP trial would provide >80% power to detect as significant (at the 5% significance level) a doubling of malaria incidence in those who stop CTX compared to those that continue, assuming a 15% loss to follow up at the end of the study and an average follow up of 2 years.

2.4.4 Analysis

Data was analysed using Stata 12 and 13. For all comparisons by treatment arm, the analysis was by 'Intention to treat', with participants analysed according to the arm to which they were randomised. Detailed analysis methods used for specific study objectives are described in the subsequent chapters presenting papers addressing each objective.

2.5 Ethical considerations and permissions

The studies on malaria specific research questions were a sub- analysis of the main COSTOP trial. The trial was approved by the UVRI Science and Ethics Committee (SEC), the National Drug Authority (NDA) and the Uganda National Council of Science Technology (UNCST). Permission to conduct the malaria related work as PhD research was granted by the COSTOP TSC. Ethical clearance for the PhD project was obtained from the Ethics Committee of the London School of Hygiene and Tropical Medicine (LSHTM) (Appendix 17).

Table 1: Trial procedures

	Trial time							
Trial Procedure	Screening	Enrolment (Wk 0)	Wk 4	Wk 8	Wk 12	Wk 24	3 mthly	6 mthly
Consent for screening	**x**							
Consent for enrolment/ plasma storage		**x**						
History and physical exam	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**
CD4	**x**				**x**	**x**		**x**
Full blood count	**x**				**x**	**x**	**x**	
Pregnancy test	**x**							
Malaria slide		**x**	**x**	**x**	**x**	**x**	**x**	
Plasma storage		**x**				**x**		**x**
Adherence assessment			**x**	**x**	**x**	**x**	**x**	
Study drug prescription/refill		**x**	**x**	**x**	**x**	**x**	**x**	

Table 2: Power calculations (at 5% level of significance) for a given sample size and different scenarios

Sample size (n per arm)	Malaria incidence in control arm (/100 pyrs)	Loss to follow up (%)	Minimum increase in malaria incidence that could be detected (%)	Average follow up (yrs)	Power (%)
1090	1.0	15	50	2	27
1090	1.4	15	50	2	36
1090	5.0	15	50	2	86
1090	1.0	15	100	2	70
1090	1.4	15	100	2	84
1090	5.0	15	100	2	100
1090	1.0	20	50	2	26
1090	1.4	20	50	2	35
1090	5.0	20	50	2	84
1090	1.0	20	100	2	67
1090	1.4	20	100	2	81
1090	5.0	20	100	2	100

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Chapter 3: Effect of cotrimoxazole prophylaxis on malaria occurrence in HIV-infected patients on antiretroviral therapy in sub-Saharan Africa (systematic review).

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

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SECTION A – Student Details

Student	Ronnie Kasirye
Principal Supervisor	Heiner Grosskurth
Thesis Title	Effect of cotrimoxazole on malaria in HIV-infected adults on antiretroviral therapy

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	Tropical Medicine and International Health		
When was the work published?	May 2015		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Yes	Was the work subject to academic peer review?	Yes

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I designed the review after discussions with Kathy Baisley (KB) and Heiner Grosskurth (HG). I performed the search for the relevant studies. I independently screened the abstracts and extracted data from the identified studies. KB served as a second independent reviewer. I wrote the first draft and revised it following comments from KB,

	HG and Paula Munderi. I submitted the final version of the paper for publication. I was the corresponding author and responded to reviewers' and editor's comments and questions.

Student Signature: _____ Date: 30/03/16

Supervisor Signature: _____ Date: 1/4/2016

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Systematic Review

Effect of cotrimoxazole prophylaxis on malaria occurrence in HIV-infected patients on antiretroviral therapy in sub-Saharan Africa*

R. Kasirye[1,2], K. Baisley[1], P. Munderi[2] and H. Grosskurth[1]

1 *London School of Hygiene and Tropical Medicine, London, UK*
2 *MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda*

Abstract

OBJECTIVE To systematically review the evidence on the effect of cotrimoxazole (CTX) on malaria in HIV-positive individuals on antiretroviral therapy (ART).

METHODS Web of Science, PubMed and MEDLINE, EMBASE, Global Health and Cochrane Library databases were searched using terms for malaria, HIV and CTX. Studies meeting the inclusion criteria were reviewed and assessed for bias and confounding.

RESULTS Six studies (in Uganda, Kenya, Malawi, Zambia and Zimbabwe) had relevant data on the effect of CTX on malaria in patients on ART: four were observational cohort studies (OCS) and two were randomised controlled trials (RCTs); two were in children and one in women only. Samples sizes ranged from 265 to 2200 patients. Four studies compared patients on ART and CTX with patients on ART alone; 2 (RCTs) found a significant increase in smear-positive malaria on ART alone: (IRR 32.5 CI = 8.6–275.0 and HR 2.2 CI = 1.5–3.3) and 2 (OCS) reported fewer parasitaemia episodes on CTX and ART (OR 0.85 CI = 0.65–1.11 and 3.6% *vs.* 2.4% of samples $P = 0.14$). One OCS found a 76% (95% CI = 63–84%) *vs.* 83% (95% CI = 74–89%) reduction in malaria incidence in children on CTX and ART *vs.* on CTX only, when both were compared with HIV-negative children. The other reported a 64% reduction in malaria incidence after adding ART to CTX (RR = 0.36, 95% CI = 0.18–0.74). The 2 RCTs were unblinded. Only one study reported adherence to CTX and ART, and only two controlled for baseline CD4 count.

CONCLUSION Few studies have investigated the effect of CTX on malaria in patients on ART. Their findings suggest that CTX is protective against malaria even among patients on ART.

keywords malaria, cotrimoxazole, HIV, antiretroviral therapy

Introduction

Malaria and HIV infection are important global health problems, and these diseases have a wide geographical overlap resulting in frequent co-infection [1–4]. HIV infection is associated with deterioration of the patient's immune system and an increased incidence of opportunistic infections (OI) and of malaria [5–8]. Among HIV-infected patients, the use of daily prophylaxis with cotrimoxazole (CTX) reduces mortality and morbidity from OI [9–15], and it reduces malaria incidence in HIV-infected patients before [12, 16, 17] and after starting antiretroviral therapy (ART)[18, 19], and in children exposed to HIV infection[20].

WHO [21] recommends ART for HIV-infected adults and adolescents with a CD4 count <500 cells/μl or if a person has TB, is pregnant, is breastfeeding, HBV co-infected with severe liver disease or in a sero-discordant partnership. WHO also recommends CTX prophylaxis for anybody with a CD4 count <350 cells/μl, or clinical stage 3 and 4 disease and irrespective of CD4 count or clinical stage in areas of high malaria prevalence and/or severe bacterial infections [22]. This policy aims to reduce OI and all-cause mortality. The possible preventive effect on malaria was not originally part of the rationale for CTX prophylaxis. Indeed, fears about the possible development of resistance against antimalarial drugs owing to

*freely and fully available at www.tmih.com

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wide-spread CTX use were expressed by some authors [23–25].

CTX use increases patients' pill burden and cost of care and is associated with haematological toxicity and hypersensitivity skin reactions. However, adverse reactions to CTX are rare (<2 per 100 person-years of CTX use) and mainly mucocutaneous in nature; they resolve with drug discontinuation [26–28]. With patients on ART being able to regain near normal immune function, some researchers recommend stopping CTX once patients are stable on ART [29, 30]. However, these recommendations are based on studies in industrialised countries which have fewer malaria and bacterial infections than sub-Saharan Africa (SSA). So although HIV-infected patients on ART in SSA may not need CTX for prevention of OI, they might still benefit from its antimalarial effect [31]. A Guideline Development Group on Cotrimoxazole prophylaxis was convened by WHO in 2013. This group recommended that in settings with high malaria prevalence and/or severe bacterial infections, even among patients that are stable on ART, CTX should be continued [22].

Our objectives were (i) to systematically review publications on the effect of CTX on malaria in HIV-infected patients on ART in order to assist policymakers in SSA in taking informed decisions within the context of the epidemiological situation in their area and (ii) to provide background information for an ongoing controlled trial of malaria incidence among HIV-infected patients on ART with and without CTX co-medication.

Methods

Search strategy

The following databases were searched for publications to 14 April 2014: EMBASE, PubMed and MEDLINE, Web of Science, Global Health and the Cochrane Library. The search used terms for malaria, HIV and CTX, without a term for ART, to reduce the chance of missing relevant papers.

A combination of the following MESH terms and free text was used:

- *malaria, malaria incidence, malaria prevalence, malaria severity, malaria outcomes, malaria treatment, Plasmodium* and *parasitaemia* for malaria.
- *cotrimoxazole, trimethoprim/sulfamethoxazole, septrin* and *bactrim* for cotrimoxazole.
- *HIV, human immunodeficiency virus, acquired immunodeficiency syndrome, AIDS* and *immune suppression* for HIV infection.

An example of the search strategy as used in MEDLINE

- malaria OR malaria adj3 (occurrence OR incidence OR prevalence OR treatment OR parasit?emia OR outcomes OR sever*) OR Plasmodium.
- HIV OR human immunodeficiency virus OR acquired immunodeficiency syndrome OR AIDS OR Immune suppres*.
- 1 AND 2.
- cotrimoxazole OR trimethoprim sulfamethoxazole OR septrin OR bactrim.
- 3 AND 4.

The search results were exported to Endnote reference management software (Thomson Reuters, version ×7) and duplicates were removed. All titles and abstracts were screened independently by two authors (RK and KB); inconsistencies were discussed and consensus on potential eligibility reached. Abstracts were checked for studies reporting on a combination of malaria, HIV, cotrimoxazole and antiretroviral therapy. Review articles were excluded but their reference lists were checked for possible additional relevant papers. Reference lists from papers identified from the systematic search were also checked. Full text copies of potentially relevant papers were then obtained. Data requests were sent to authors of studies for which relevant information might have been collected but not reported in their publications. Guidelines on preferred reporting items for systematic reviews and meta-analyses (PRIMSA) were used.

Included studies

We included studies containing original data on the effect of CTX on malaria in HIV-infected patients on ART. No restrictions on area of the world, participant age, language or the date of publication were used.

Data extraction

Data from papers identified by the search were independently extracted by two authors (RK and KB) using a standard form to collect the following information: first author's name, year of publication, type of study, study population, study aim, sample size, follow-up time, study results, how malaria diagnosis was made, number and severity of malaria episodes and the association between malaria and CTX.

Bias

Assessment of bias and confounding within studies and quality of papers was based on PRISMA guidelines[32]

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and the Newcastle-Ottawa quality assessment of studies scale[33]. Studies were assessed on selection bias, adherence to CTX, objectivity of malaria diagnosis, cohort retention, follow-up duration (for assessment of seasonal variation), adjustment for confounding and outcome reporting. A formal meta-analysis was not performed owing to the diversity in study methodologies, comparison groups and populations.

Results

A total of 516 abstracts were retrieved, of which 492 were removed because they were duplicates, were not relevant or did not meet the inclusion criteria (Figure 1). Full text was reviewed for 24 abstracts; only 6 had data on the effect of CTX on malaria in HIV-infected patients on ART and were retained for the final qualitative synthesis. 16 potentially eligible studies did not report on the association of CTX and malaria in patients on ART; authors of 14 papers were contacted where it was felt that relevant data might have been collected but not reported. However, none was able to provide information relevant to this review so the studies were not included.

All six studies included in the final synthesis were conducted in SSA; no relevant studies were found from malarious areas in other continents. Of the six studies, two were randomised controlled trials and four were observational cohort studies; two studies were conducted among children only and four among adults (one in women only) (Table 1). Four studies were conducted in Uganda only (or the analysis of malaria was restricted to Ugandan sites); the other two were multisite studies conducted in Kenya, Malawi, Uganda, Zambia or Zimbabwe. The diagnosis of malaria was based on a patient having history of fever and a positive blood slide or rapid diagnostic test (RDT) (4 studies); clinical features alone or clinical features and a positive blood slide (1 study); and a positive blood slide or positive RDT, or detection

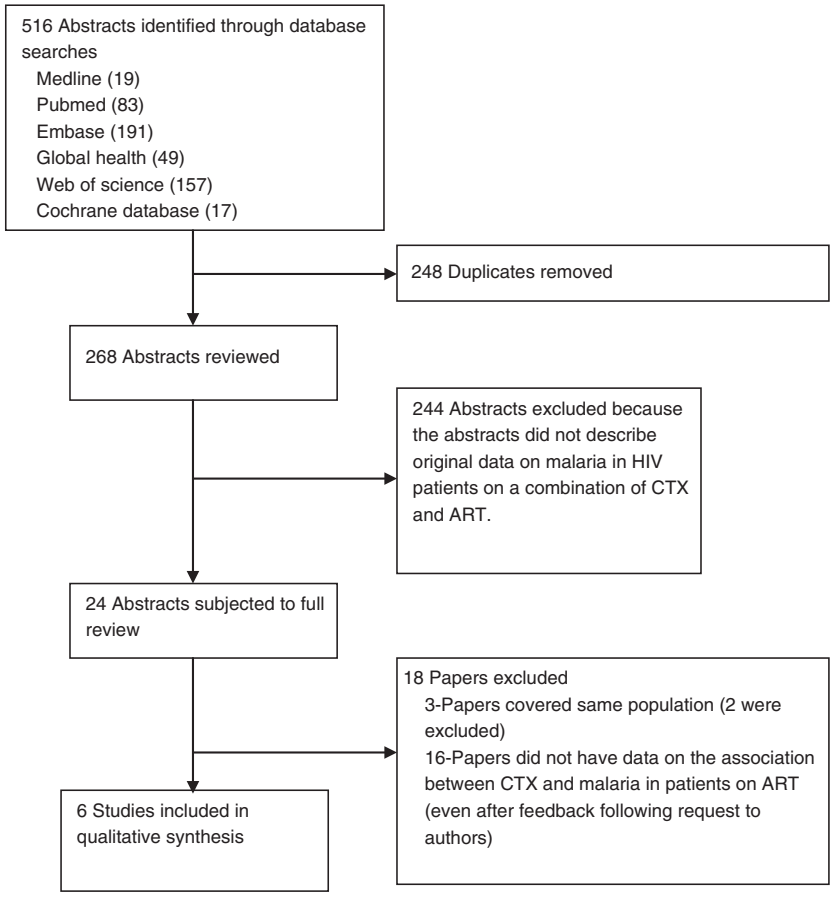

Figure 1 Results of the systematic search.

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of *Plasmodium falciparum* histidine rich protein 2 in plasma (1 study). Study samples sizes ranged from 265 to 2200 patients. Median length of follow-up was not specified for one study and was between 4 months and 4.9 years for the others. A summary of studies in the final synthesis is shown in Table 1.

Summary of study objectives and populations

Bwakura-Dangarembizi *et al.* [34] conducted a randomised, open-label, controlled trial in Uganda and Zimbabwe to assess the effect of stopping *vs.* continuing CTX prophylaxis in HIV-infected children and adolescents on long term ART. The trial enrolled 760 participants with median age of 7.9 years; at enrolment, the median time on ART was 2.1 years and median CD4 T-cell percentage was 33% (nadir-13%).

Campbell *et al.* [35] conducted a cluster randomised (by household) controlled trial in Uganda to investigate the effect of CTX discontinuation on the incidence of malaria and diarrhoea among HIV-infected adults on ART with CD4 > 200 cells/μl. The trial enrolled 836 participants; at enrolment, the median time on ART was 3.7 years, median CD4 was 489 (nadir = 129) cells/μl, and 94% had a viral load <100 copies/μl.

Gasasira *et al.* [12] assessed the protective efficacy of CTX on the incidence of falciparum malaria and on the prevalence of *Plasmodium falciparum* mutations conferring antifolate resistance among children treated for malaria in Uganda, comparing HIV-infected children on daily CTX both on and off ART, and HIV-uninfected children not taking CTX. The study enrolled 899 children (300 HIV infected) with a median age of 7.4 (HIV-uninfected) and 5.7 years (HIV infected) at enrolment. The median CD4 T-cell percentage was 23% (HIV-infected). HIV-infected children contributed 665 person-years of follow-up, of whom 275 were on ART (292 participants).

Mermin *et al.* [19] assessed the effect of ART on malaria and the additive effects of CTX, ART and insecticide-treated bed nets (ITNs) in HIV-infected adults attending clinics of The AIDS Support Organization (TASO) at two sites in Uganda. Study participants had sequential exposure to the intervention divided into four phases:

- Phase 1 – no intervention (466 participants; median CD4 at enrolment = 75 cells/μl)
- Phase 2 – participants started on CTX prophylaxis (399 participants; median CD4 at enrolment = 77 cells/μl)
- Phase 3 – participants continued CTX and started on ART (1035 participants; median CD4 at enrolment = 124 cells/μl)
- Phase 4 – participants continued CTX and ART, and ITNs were provided (985 participants; median CD4 at enrolment = 175 cells/μl)

Skinner *et al.* [36] assessed the effect of a protease inhibitor (PI)-based ART regimen on malaria compared with a nevirapine-based regimen, stratified by CTX use. Patients were part of the Optimal Combination Therapy After Nevirapine Exposure study (OCTANE) [37], a multicentre trial comparing non-nucleoside reverse transcriptase inhibitor (NNRTI) and PI-based regimens for HIV-infected women with a history of nevirapine prophylaxis to prevent mother-to-child HIV transmission. The Skinner *et al.* study evaluated 265 women from the OCTANE trial who at baseline had a median age of 37 years, median CD4 of 121 cells/μl and HIV RNA of 5.2 log10 copies/ml.

Walker *et al.* [18] assessed the effect of CTX on survival, WHO stage, malaria, CD4 count, body mass index (BMI) and haematological indices in HIV-infected patients with CD4 count <200 cells/μl initiating ART in Uganda and Zimbabwe in the Development of Antiretroviral Therapy (DART) trial for Africa which compared ART monitoring strategies in resource limited settings. The trial enrolled 3179 participants; the analysis of malaria incidence was restricted to 2222 participants in Uganda. At enrolment into the DART trial, median CD4 count was 83 cells/μl.

Effect of CTX on malaria in patients on ART

Two of the six studies evaluated the effect of CTX on malaria in HIV-positive participants on ART as their main study objective. The other four studies had different objectives, but the data allowed assessment of the effect of CTX on malaria.

The six studies used different comparison groups: CTX and ART *vs.* ART alone (4 studies); CTX and ART *vs.* HIV negative (1 study); CTX only *vs.* HIV negative (1 study); CTX only *vs.* HIV positive not on treatment (1 study); CTX and ART *vs.* HIV positive not on treatment (1 study); CTX, ART and ITNs *vs.* HIV positive not on treatment (1 study).

All four studies that examined the occurrence of malaria in HIV-positive persons on CTX and ART compared with those on ART alone found a beneficial effect of CTX. Bwakura-Dangarembizi *et al.* [34] found that children and adolescents who discontinued CTX had a higher incidence of malaria (HR 2.21, 95% CI = 1.50–3.25 $P < 0.001$) compared to those who continued CTX prophylaxis. Campbell *et al.* [35] also found strong evidence of a higher malaria incidence in patients on ART

Table 1 Summary of studies on the effect of CTX on malaria in HIV-infected patients on ART

Author/year	Type of study	Study population	Main study aim	Number of participants (median follow-up)	Main study or non-malarial outcome. Ratio (95% CI)	Malaria diagnosis (number of episodes).	Malaria comparison by CTX/ART	Association between malaria and CTX. Ratio (95% CI)
Bwakura-Dangarembizi 2014[34]	RCT	Children on ART (Uganda and Zimbabwe)	Assess the effect of stopping *vs.* continuing CTX in children on ART	758 (2.1 years)	Stopping CTX associated with higher rates of hospitalisation or death. HR 1.64 (1.14–2.37 $P = 0.007$)	Positive smear or RDT (169)	ART only *vs.* CXT/ART	HR 2.21 (1.50–3.25; $P < 0.001$) Median parasite density (221 *vs.* 153) Hospitalisation for malaria (49 *vs.* 21)
Campbell/ 2012[35]	RCT	Adults on ART (Uganda)	Assess effect of stopping CTX on malaria and diarrhoeal incidence	836 (4 months*)	Stopping CTX associated with higher incidence of diarrhoea IRR 1.8 (1.3–2.4, $P < 0.001$)	Smear positive fever (57)	ART only *vs.* CXT/ART	IRR 32.5 (8.6–275.0; $P < 0.001$) Parasite density >1250 parasites/μl (70% *vs.* 100%)
Gasasira/ 2010[12]	Cohort	HIV-infected and uninfected children (Uganda)	Assess protective efficacy of CTX on malaria and prevalence of CTX resistance mutations in *P. falciparum*	517 HIV-uninfected (2.1 years) and 292 HIV-infected (2.4 years)	Prevalence of DHFR and DHPS mutations was >90%. Efficacy of CTX on malaria (HIV infected *vs.* uninfected) was 80% (72–85%)	Smear positive fever (576 total, 65 in HIV positive)	Efficacy† (CTX with ART *vs.* HIV negative; CTX only *vs.* HIV negative)	CTX and ART: efficacy = 76% (63–84%) CTX only: efficacy = 83% (74–89%)
Mermin/ 2006[19]	Cohort	HIV-infected adults (Uganda)	Assess the effect of ART on malaria and additive effects of CTX, ART and ITNs. Had 4 phases; *one*-no intervention (NI), *two*-CTX, *three*-CTX and ART, *four*-CTX, ART and ITNs	Phase; *one* 466 (154 days), *two* 399 (532 days), *three* 1035 (126 days), *four* 989 (560 days)	Adjusted IRR Cumulative (phase *one* as reference) *CTX vs. NI* 0.24 (0.12–0.17) $P < 0.001$ *CTX/ART/ITNs vs. NI* 0.05 (0.03–0.08) $P < 0.001$ Additive effect (the previous phase as the reference) *CTX vs. NI* 0.24 (0.15–0.38) $P < 0.001$ *CTX/ART/ITNs vs. CTX/ART* 0.58 (0.31–1.11) $P = 0.1$	Smear positive fever smear. (166)	Cumulative CTX/ART *vs.* NI Additive CTX/ART *vs.* CTX	Cumulative 0.08 (0.04–0.17) $P < 0.001$ Additive effect 0.36 (0.18–0.74) $P = 0.006$ Similar rates observed when malaria defined as parasitaemia >1250 μl

Table 1 (*Continued*)

Author/year	Type of study	Study population	Main study aim	Number of participants (median follow-up)	Main study or non-malarial outcome. Ratio (95% CI)	Malaria diagnosis (number of episodes).	Malaria comparison by CTX/ART	Association between malaria and CTX. Ratio (95% CI)
Skinner Adams/ 2012[36]	Cohort	HIV-infected women in OCTANE trial‡ (Kenya, Uganda, Malawi, Zambia)	Assess effect of LPV/R compared to nevirapine-based ART on malaria	265§	Samples positive for malaria in subjects receiving LPV/R compared to those receiving NVP-based ART (2.8% vs. 1.8%, $P = 0.13$)	Positive smear, RDT or malaria antigen in plasma (104)	ART *vs.* CTX and ART only	Number of positive samples; Analysing one episode per subject 2.9% *vs.*. 2.2% $P = 0.42$ Allowing multiple episodes per subject 3.6% *vs.* 2.4% $P = 0.14$
Walker/ 2010[18]	Cohort	HIV-infected adults in the DART trial¶ (Ugandan sites)	Assess effect of CTX on survival, WHO stage, malaria, CD4, BMI and haematological indices after initiating ART	2200 (4.9 years)**	Being on CTX *vs.* being off CTX; Mortality (0.65, 0.50–0.85)	2362 events (Clinically 1243, microscopically 1119)	CTX/ART *vs.* ART	Clinical and laboratory diagnosis OR = 0.74 (0.63–0.88) $P < 0.001$. When restricted to parasite positive diagnoses OR = 0.85 (0.65–1.11) $P = 0.23$

RCT, Randomised controlled trial; IRR, incidence rate ratio; DHFR, dihydrofolate reductase; DHPS, dihydropteroate synthetase; BMI, body mass index; OR, odds ratio; LPV/R, lopinavir/ritonavir; RDT, rapid diagnostic test; DART, Development of Antiretroviral Therapy; ITNs, insecticide-treated bed nets.
*Total fup time.
†Protective efficacy (1-IRR).
‡Octane (A5208) trial sites with malaria; Kericho Kenya, Lilongwe Malawi, Kampala Uganda, Lusaka Zambia.
§Prevalence in samples, no follow-up time.
¶Development of Antiretroviral Therapy trial sites with malaria; Kampala and Entebbe, Uganda.
**Median fup is across all sites (Uganda and Zimbabwe).

who discontinued CTX compared to those who continued CTX prophylaxis (IRR 33; 95% CI = 9–275, $P < 0.001$). Walker *et al.* [18] found strong evidence of a reduction in the number of clinical malaria episodes among patients on ART and CTX compared to those on ART alone (OR = 0.74, 95% CI = 0.63–0.88, $P < 0.001$); however, the reduction in the risk of parasitaemia was not statistically significant (OR 0.85; 95% CI = 0.65–1.11, $P = 0.23$). Skinner *et al.* [36] found weak evidence of decrease in detectable parasitaemia in patients who were on CTX and ART compared to those who were on ART alone (3.6% *vs.* 2.4%; $P = 0.14$).

Gasasira *et al.* [12] found a 76% (95% CI = 63–84%) reduction in malaria incidence in children on CTX and ART, and a similar 83% (95% CI = 74–89%) reduction in children on CTX only, when both were compared with HIV-negative children not on CTX.

Mermin *et al.* [19] found that CTX alone was associated with 76% reduction in malaria incidence (RR = 0.24, 95% CI = 0.15–0.38; $P < 0.001$), and CTX and ART reduced malaria incidence by 92% (RR = 0.08, 95% CI = 0.04–0.17; $P < 0.001$), when compared with HIV-positive participants not on CTX or ART. In their sequential comparisons of the additive effects of the interventions in each phase of the study, they found that adding ART to CTX was associated with a 64% (RR = 0.36, 95% CI = 0.18–0.74; $P = 0.006$) reduction in malaria incidence compared to CTX alone.

Four studies reported parasite density. Bwakura-Dangarembizi *et al.* [34] found parasite density to be higher in patients on ART who stopped CTX compared with those who continued CTX (median parasite density per 200 white cells = 221/μl *vs.* 153/μl, $P = 0.004$). Campbell *et al.* [35] found that 70% of the 55 malaria episodes in patients on ART who stopped CTX had parasite densities >1250/μl compared with 100% of the two episodes in patients who continued CTX. Gasasira *et al.* [12] found that geometric mean parasite density was lower for HIV-infected children who were also on CTX (6462/μl) compared to HIV-uninfected children (11 270/μl), although the difference was not statistically significant ($P = 0.40$). Mermin *et al.* [19] evaluated the cumulative and additive effects of CTX, ART and ITNs on malaria parasitaemia >1250/μl; their conclusions were similar to those when malaria was defined as fever with a positive blood slide (as described above).

Risk of bias and confounding

All studies reviewed were of good quality but subject to sources of bias (Table 2). Three studies reported adherence to CTX (Bwakura-Dangarembizi, Campbell and Gasasira *et al.*), and 3 (Bwakura. Mermin and Walker *et al.*) reported adherence to ART. Only the Bwakura *et al.* study reported both CTX and ART adherence.

Campbell *et al.* and Bwakura-Dangarembizi *et al.* were the only studies randomised by CTX use; however, these studies were not blinded. Most of the studies used clinical and/or laboratory based methods to diagnose malaria; Walker *et al.* also used clinical diagnosis alone.

Multivariate analysis to control for potential confounders was not used by Skinner *et al.* due to the small number of malaria cases. Only the Mermin, Bwakura-Dangarembizi and Walker studies controlled for the potential confounding effect of CD4 count at baseline. No study explored the potential confounding effect of socio-economic status. The Walker study was the only study to control for potential time-dependent confounders such as current CD4 count, haemoglobin and BMI levels.

Discussion

Most studies in this review were conducted after 2005, when most developing countries had started to roll out ART to patients, of whom most were on CTX. The search was performed using terms for malaria, HIV and CTX, without using the term for ART, to reduce the chance of missing relevant papers. However, only six studies were identified with data on the effect of CTX on malaria in patients on ART.

Four of the six studies compared malaria occurrence in patients on ART alone with that in patients on ART and CTX, and all found a higher incidence of malaria in patients on ART alone. This is expected given the antimalarial properties of CTX[38], even in areas where malaria parasites have antifolate resistance[12]. The strongest evidence for this beneficial effect was observed in the Campbell (IRR = 32.5) and Bwakura-Dangarembizi (HR = 2.21) studies. The latter study was conducted in children and adolescents and the former in adults. With better immune memory to malaria in adults [39], a smaller difference in malaria incidence between those stopping CTX and those continuing CTX might be expected in adults than in children, but the reverse was observed. The Campbell study was stopped after just 4 months because of increased malaria incidence in the discontinuation arm. As the authors point out, it is not clear whether the increase in malaria after stopping CTX may have been only temporary. It is possible that the larger beneficial effect of CTX on malaria in adults in this study, compared with that in children and adolescents in the Bwakura-Dangarembizi study, is because of the shorter follow-up time (4 months *vs.* 2.1 years, respectively).

Table 2 Risk of bias and confounding within studies

Criterion Assessment of bias	Study Author, year					
	Bwakura-Dangarembizi 2014 [34]	Campbell 2012 [35]	Gasasira 2010 [12]	Mermin 2006 [19]	Skinner 2012 [36]	Walker 2010 [18]
Ascertainment of exposure (adherence to CTX and ART)	No difference between groups in adherence to ART. Self-report: 6% had missed CTX doses during the previous 4 weeks	ART not reported, CTX adherence in cont. CTX group not mentioned	Median level of CTX adherence in HIV-infected population was 100%. ART adherence not mentioned	95% ART adherence, CTX not reported	Not reported. From the main trial: trial 1, 81% in LPV/r AND 83% in NVP arm took 95% of expected doses. In trial 2, adherence to ART at each visit = 84–92% No information about CTX adherence	ART adherence - no missed doses reported at 83% of visits in those on CTX and at 78% of visits in those not on CTX in first 12 wks; 93% and 87% of visits in weeks 12–72 93% and 91% of visits >72 weeks on ART. CTX not reported
Ascertainment of malaria diagnosis	Positive slide/RDT	Fever in past 7 days and positive slide	Fever in past 24 h and positive slide	Fever in previous 2 days and positive slide	Positive slide, RDT or antigen in plasma	Clinically and or microscopically
Randomised by CTX in patients on ART	Yes	Yes	No	No	No	No
Study groups comparable at baseline	Yes	No (mean CD4 higher in stop CTX arm)	No	No	N/A	No
Participants /investigators blinded to CTX use	No	No-only laboratory technicians were blinded	No	No	No	No
Loss to follow-up	7 in stop CTX arm (2%) and 2 in CTX arm (0.5%)	0% (short fup)	13% in HIV neg: 6% in HIV positive (not given by ART status)	<10% all three phases	N/A (data analysed on a cross sectional basis) Main trial: 2.5% in trial 1 and 6% in trial 2	6%
More than 1-year follow-up (seasonal variation)	Yes	No	Yes	Yes, overall and phase 2 and 4 but not in phase 1 and 3	N/A	Yes

Table 2 (*Continued*)

Criterion	Study Author, year					
Assessment of bias	Bwakura-Dangarembizi 2014 [34]	Campbell 2012 [35]	Gasasira 2010 [12]	Mermin 2006 [19]	Skinner 2012 [36]	Walker 2010 [18]
Control for potential confounders						
Baseline CD4 cell count	Study design	No*	No	Analysis	No	Analysis
ITN use	No†	Study design	No	Study design	No	No
Age	Study design	Study design	Analysis	Analysis	No	Analysis
Sex (Gender)	Study design	Study design	No	Analysis	Study design	Analysis
Socio-economic status	Study design	Study design	No	No	No	No
Other	Stratification by randomisation factors	Clustering by household not adjusted for	Breast feeding not controlled for	Season, adjust for in analysis	Multivariate analysis not carried out	Length of time on ART. Used MSM to control for time dependent confounding

N/A, Not applicable; No, confounder not controlled for; RDT, rapid diagnostic test.
*Selection criterion in this study was applied after randomisation giving a difference in CD4 count as baseline.
†ITN use reported to be higher in patients stopping CTX ($P = 0.02$). MSM, marginal structural models.

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The other two studies showed strong evidence of a decrease in episodes of clinical malaria (Walker *et al.* OR = 0.74, 95% CI = 0.63–0.88; $P < 0.001$), and weak evidence of a decrease in malaria prevalence (Skinner et al. 3.6% *vs.* 2.4%, $P = 0.14$), in patients on ART and CTX compared to patients on ART alone. One study showed that HIV-infected children on CTX had 80% (95% CI; 72–85%) lower malaria incidence than HIV-negative children not on CTX.

Only the Bwakura-Dangarembizi and Campbell studies were originally designed to look at the effect of CTX on malaria in patients on ART. This may explain why most studies did not report adherence to CTX and/or ART and did not attempt to address potential bias and confounding related to malaria. Both the Bwakura-Dangarembizi and Campbell studies were randomised by CTX use, but investigators and participants were not blinded. These trials showed that patients stopping CTX prophylaxis had a higher risk of malaria than those who continue CTX. The Bwakura-Dangarembizi study reported more hospitalisations in children stopping CTX than in those who continued. The Campbell study showed no significant difference in adult hospitalisation rates or mortality between the trial arms, and none of the four deaths recorded was related to malaria. Malaria in this study was uncomplicated and the clinical relevance of this malaria is therefore not clear.

Results of a trial 'CTX Prophylaxis Discontinuation Among ART-Treated Adults: A Randomized Non-Inferiority Trial' were presented at the Conference on Retroviruses and Opportunistic Infections in March 2014[40]. These results were not included in this synthesis because some data relevant to the review were not provided and the results are yet to be published in a peer-reviewed journal. This trial was an open-label randomised controlled trial comparing stopping *vs.* continuing CTX prophylaxis among 500 HIV-infected adults in western Kenya on ART >18 months and followed for a year. The authors reported that patients stopping CTX had a 33.2 (95% CI = 4.5–241.0; $P = 0.001$) times higher malaria incidence than those who continued CTX. They also found that combined morbidity and mortality were greater in the group stopping CTX (IRR 2.27; 95% CI = 1.52–3.38, $P < 0.001$) but that this result was driven by malaria morbidity.

Publication of the full results of this trial is awaited but like the Campbell study, this trial was not blinded and therefore susceptible to reporting bias. However, the results suggest that the higher incidence of malaria seen in patients stopping CTX in the Campbell study may be maintained even when patients are followed for longer.

It is clear from this review that patients who are stable on ART and stop taking CTX experience malaria episodes more frequently but a number of questions remain unanswered:

- Given that none of the studies was blinded, how much does reporting bias contribute to the observed increased risk of malaria in patients who stop CTX compared to that in those who continue?
- What is the clinical significance of malaria occurring in HIV-infected adults on ART who do not take CTX prophylaxis?
- Do patients who stop CTX have a higher incidence of malaria than would be observed in HIV-uninfected people?
- How does CTX compare with other antimalaria prophylactic drugs such as chloroquine (which may imply a lower pill burden)?
- Does the background ecological exposure to malaria have an effect on the relationship between malaria and CTX?
- Is CTX prophylaxis still beneficial in patients recently diagnosed with HIV who start ART early, that is with high CD4 counts, for example 500 cells/μl?

Two ongoing randomised controlled trials, one in Uganda (ISRCTN44723643) and one in Malawi (NCT01650558), will help answer some of these questions. These studies investigate the effect of CTX on malaria incidence in HIV-infected patients on ART. The Ugandan trial is double-blind and placebo-controlled, and compares continued CTX prophylaxis with stopping CTX. The Malawian trial compares continued CTX with stopping CTX, but is not placebo-controlled; instead weekly chloroquine (CQ) prophylaxis is substituted for CTX. Results are expected in 2015.

Conclusion

Few studies have investigated the effect of CTX on malaria in patients on ART; these studies show a trend towards a beneficial effect of CTX on malaria. Only 2 of the reviewed studies were randomised and they were the only ones specifically designed to investigate this association. Most of the reviewed studies were subject to bias and confounding, and the clinical relevance of malaria experienced by patients stable on ART who are not on CTX prophylaxis is unclear.

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Corresponding Author **Ronnie Kasirye**, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK. E-mail: ronniekasirye@yahoo.com

Chapter 4: The COSTOP trial

This chapter gives an overview of the COSTOP trial in which the experimental PhD work was embedded.

4.1 Aim

The aim of the COSTOP trial was to assess the safety of discontinuing cotrimoxazole (CTX) prophylaxis in HIV infected adults, whose immunity had been restored on ART. COSTOP was a randomised a placebo controlled trial. The trial had two co-primary outcome measures:

(1) Time to first CTX-preventable event excluding malaria (efficacy outcome)

(2) Time to the occurrence of the first grade 3 or 4 haematological adverse event (safety outcome)

Secondary outcome measures included:

- All-cause mortality
- Incidence of all confirmed malaria episodes
- Severity and outcome of all confirmed malaria episodes
- Mean change in CD4 count after 12 months on the trial
- Mean change in haematological indices after 12 months on the trial
- Incidence of hospitalisation (all causes)

The main COSTOP trial analysis did not include malaria related outcomes; these outcomes were my responsibility from the conception of the trial and were to part of my PhD project.

Non-malarial clinical events occurring during follow up were adjudicated by an end-points review committee (ERC) as to whether they were CTX preventable or not.

4.2 Methods

The study population and screening, enrolment and follow up procedures are described in detail in the Methods section of this thesis (chapter 2) and in the published COSTOP design paper (Appendix 3).

Statistical methods

Sample size

The sample size calculation for the efficacy outcome was based on being able to conclude non-inferiority of placebo, whereas the calculation for the safety outcome was based on superiority of placebo. Led by the trial statistician, Jonathan Levin, different scenarios were assessed at the planning stage of the trial. The trial was designed to have 80% power at the 5% level of significance to detect non-inferiority of placebo to CTX (upper limit of adjusted hazard ratio (aHR) 90% confidence interval (CI) <1.25) with respect to the incidence of CTX preventable effects. Assuming an incidence of CTX preventable effects of 10 per 100 pyrs in the CTX arm, it was calculated that a sample size of 2160 participants followed up for 18 months with a 4% loss to follow up per year would be required to achieve this. For the co-primary endpoint of the time to the first grade 3 or 4 haematological adverse event, a sample size of at least 1000 subjects per arm would have approximately 85% power to detect as statistically significant at the 5% level a true hazard ratio (HR) of 2 for those that continue CTX compared to those that stop, if overall 10% of those in the arm that continues CTX prophylaxis experience such an event. The above assumptions on the incidence of CTX preventable events were derived from other studies conducted in Uganda, in particular a trial that compared ART monitoring strategies (DART) (1, 2).

Intention to treat and per protocol populations

Intention to treat (ITT): This population comprised all participants who took at least one dose of study medication and who had at least one follow-up assessment.

Per protocol (PP): The PP population consisted of all participants whose adherence assessed by pill count or self-report remained above 80% for each period between study visits.

Analysis

The trial statistician developed the analysis plan and performed the main trial analyses. For the time to first CTX-preventable event or death analysis, the statistical test for non-inferiority was used; hence the main analysis was based on the PP population. This analysis is preferred for non-inferiority/equivalence studies because it is conservative i.e. it excludes data on participants who for example had protocol violations, dropped out or crossed over in treatment which would dilute the treatment effect and make it easier to conclude that there was no difference between arms. Time to event methods were applied (Kaplan Meier plots, stratified log rank test and Cox proportional hazards regression). Non-inferiority was tested by estimating the HR for the experimental arm versus the control arm and finding the two-sided 90% CI. The experimental arm was to be considered non-inferior to the control arm if the upper limit of the 90% CI was less than 1.25.

For the time to first grade 3 or 4 haematological adverse event, an analysis based on the ITT population was carried out. This analysis that is preferred for testing for superiority because it is conservative i.e. it includes participants for example who did not take their drugs, had protocol violations or switched drug all of which will dilute the treatment effect as described above making it more difficult to show a difference (superiority). Time to event analysis as described above was used, with superiority tested using the stratified log rank test.

All HR were adjusted apriori for randomisation strata and enrolment site. Similar methods were used for the secondary outcome measures; since these were not analysed as non-inferiority endpoints, the ITT population was used.

4.3 Results

The results presented on the main trial outcomes including the figures and table below were from analyses performed by the COSTOP trial statistician. 2180 patients were enrolled at two sites in Uganda (Entebbe and Masaka); 74% were female. The median age was 41 years, the median CD4 count 518 cells/ul, and the median time on ART prior to enrolment was 48 months. In total 1875 participants completed follow up; 232 (109 CTX) were lost to follow up/ withdrew, 36 participants (18 CTX) stopped trial drug and 37 participants (19 CTX) died.

We documented 115 clinical events that were adjudicated to be CTX preventable; 46 of these occurred in the CTX arm and 69 in the placebo arm. Bronchopneumonia was the most common event with 53 cases (20 CTX arm). In the PP population, a total of 98 adjudicated CTX preventable events occurred (39 CTX arm) (Table 3). The time to the first CTX preventable event was significantly shorter in the placebo arm compared to the CTX arm (P=0.03) (Figure 4). The HR, adjusted for site and CD4 stratum (aHR) was 1.6 (90%CI=1.1-2.2). The number needed to treat with CTX for one year to prevent one event was 113.

Participants experienced 551 grade 3 or 4 adverse events (318 CTX). Participants in the CTX arm were more likely to experience haematological grade 3 or 4 adverse events: the time to the first grade 3 or 4 haematological adverse event was significantly shorter in the CTX arm compared to placebo (P<0.001) (Fig.5). The incidence of the grade 3 or 4 haematological adverse events was lower in the placebo arm, aHR 0.7, (95%CI=0.6-0.8) (Table 3).

We documented a total of 37 deaths ((19 CTX) log rank test P=0.9)). The ERC deemed 6 of them as CTX preventable, of which 4 occurred in the placebo arm.

4.4 Conclusion

In this study population, the discontinuation of CTX led to a significant increase in CTX-preventable clinical events, but is associated with a decrease in grade 3 or 4

haematological adverse events. There was no statistically significant difference in mortality between those that stopped CTX prophylaxis and those that continued.

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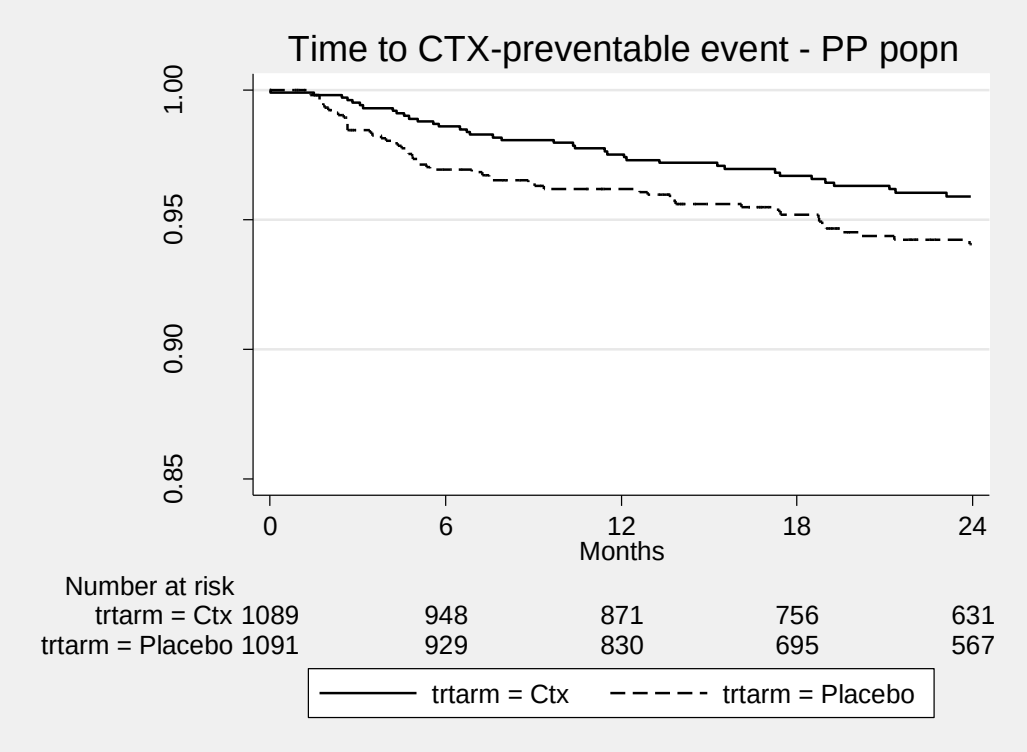

Figure 4: Time to CTX preventable event

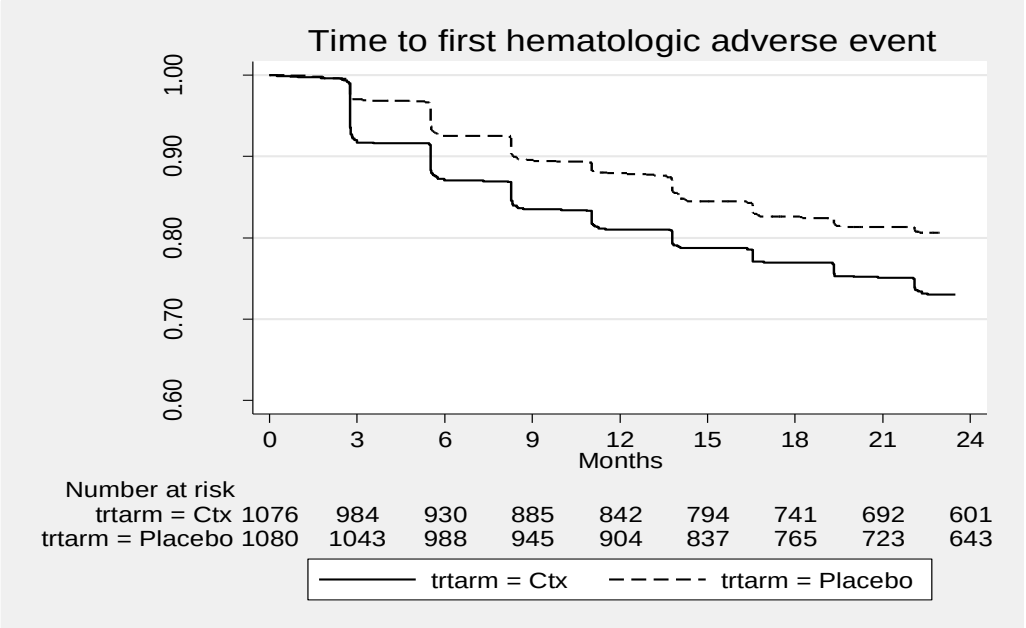

Figure 5: Time to first hematologic grade 3 or 4 adverse event

Table 3: CTX preventable events and grade (3/4) adverse events by trial arm

End point	Treatment arm	Events	Person years	Rate/100 pyrs (CI)[1]	Rate ratio(CI)[1]	P-value
CTX preventable event[2]	CTX	39	21.2	1.8 (1.3-2.5)	1	0.03
	Placebo	59	20.1	2.9 (2.3-3.8)	1.6 (1.1-2.2)	
Grade 3 or 4 adverse events[3]	CTX	318	20.7	15.3 (13.7-17.1)	1	<0.001
	Placebo	233	21.9	10.6 (9.4-12.1)	0.7 (0.6-0.8)	

[1]Adjusted for CD4 stratum and site. [2]CI=90%. [3]CI=95%.

Chapter 5: Incidence of malaria by cotrimoxazole use in HIV infected Ugandan adults on antiretroviral therapy: a randomized placebo controlled study.

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

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SECTION A – Student Details

Student	Ronnie Kasirye
Principal Supervisor	Heiner Grosskurth
Thesis Title	Effect of cotrimoxazole on malaria in HIV-infected adults on antiretroviral therapy

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	AIDS		
When was the work published?	February 2016		
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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I conceived the study after discussions with Paula Munderi (PM), Jonathan Levin (JL) and Heiner Grosskurth (HG). I was the study coordinator from 2010 to 2012. I performed the analysis under the supervision of Kathy Baisley (KB). I wrote the first draft and revised it after comments from KB and HG. I made further revisions based on comments

	from the other COSTOP trial co-investigators. I submitted the paper for publication. I was the corresponding author and responded to reviewers' and editor's comments and questions.

Student Signature: _____ **Date:** 30/03/16

Supervisor Signature: _____ **Date:** 1/4/16

Incidence of malaria by cotrimoxazole use in HIV-infected Ugandan adults on antiretroviral therapy: a randomised, placebo-controlled study

Ronnie P. Kasirye[a,b], Kathy Baisley[b], Paula Munderi[a], Jonathan Levin[a,c], Zacchaeus Anywaine[a], Andrew Nunn[d], Anatoli Kamali[a] and Heiner Grosskurth[b]

Introduction: Previous unblinded trials have shown increased malaria among HIV-infected adults on antiretroviral therapy (ART) who stop cotrimoxazole (CTX) prophylaxis. We investigated the effect of stopping CTX on malaria in HIV-infected adults on ART in a double-blind, placebo-controlled trial.

Methods: HIV-infected Ugandan adults stable on ART and CTX with $CD4^+$ cell count at least 250 cells/μl were randomized (1 : 1) to continue CTX or stop CTX and receive matching placebo (COSTOP trial; ISRCTN44723643). Clinical malaria was defined as fever and a positive blood slide, and considered severe if a participant had at least one clinical or laboratory feature of severity or was admitted to hospital. Malaria incidence and rate ratios were estimated using random effects Poisson regression, accounting for multiple episodes.

Results: A total of 2180 participants were enrolled and followed for a median of 2.5 years; 453 malaria episodes were recorded. Malaria incidence was 9.1/100 person-years (pyrs) [95% confidence interval (CI) = 8.2–10.1] and was higher on placebo (rate ratio 3.47; CI = 2.74–4.39). Malaria in the placebo arm decreased over time; although incidence remained higher than in the CTX arm, the difference between arms reduced slightly (interaction *P* value = 0.10). Fifteen participants experienced severe malaria (<1%); overall incidence was 0.30/100 pyrs (CI = 0.18–0.49). There was one malaria-related death (CTX arm).

Conclusion: HIV-infected adults – who are stable on ART and stop prophylactic CTX – experience more malaria than those that continue, but this difference is less than has been reported in previous trials. Few participants had severe malaria. Further research might be useful in identifying groups that can safely stop CTX prophylaxis.

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Keywords: antiretroviral therapy, cotrimoxazole, HIV, malaria, trimethoprim/sulfamethoxazole

Introduction

Adults with advanced HIV disease who are not on antiretroviral therapy (ART) are at an increased risk of opportunistic infections and malaria [1–3]. Cotrimoxazole (CTX), an antimicrobial agent containing trimethoprim and sulfamethoxazole, reduces the incidence of opportunistic infections, mortality and malaria [4–9]. However,

[a]MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda, [b]London School of Hygiene and Tropical Medicine, London, UK, [c]School of Public Health, University of Witwatersrand, Johannesburg, South Africa, and [d]MRC Clinical Trials Unit at University College London, UK.

Correspondence to Ronnie P. Kasirye, MBChB, MSc, MRC/UVRI Uganda Research Unit on Aids, P.O. Box 49, Entebbe, Uganda.
E-mail: ronniekasirye@yahoo.com
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CTX use is associated with increases in cost of care [10], risk of haematological toxicity [11], hypersensitivity skin reactions [12] and pill burden. Once started on ART, patients' immune function improves and the risk of opportunistic infections reduces. Based on studies from developed countries it has been recommended to stop CTX once patients' immune function has been restored [13,14]; however, this may not be advisable for sub-Saharan Africa where the prevalence of malaria and bacterial infections is often high. Of recent, WHO recommends that CTX may be discontinued in patients who are clinically stable with evidence of immune recovery and/or viral suppression on ART, but should be continued in countries with high endemicity of malaria and bacterial infections [15]. A systematic literature review found that patients who stop CTX prophylaxis experience an increase in malaria episodes, with the strongest evidence provided by randomized-controlled trials (RCTs); however, none of the reported RCTs was blinded so observational or reporting bias cannot be excluded [16]. We investigated the effect of CTX in a blinded, placebo-controlled trial on CTX cessation in HIV-infected adults who are stable on ART (COSTOP; ISRCTN44723643). The main results of this trial have been reported previously. In summary, the trial found that stopping CTX prophylaxis leads to a significant increase in CTX-preventable clinical events [mainly bacterial pneumonias; adjusted hazard ratio (aHR) = 1.57, 90% confidence interval (CI) = 1.12–2.21] and a significant decrease in grade 3/4 haematological adverse events (aHR = 0.70, 95%CI = 0.59–0.82), the co-primary outcomes of the trial. There was no effect on all-cause mortality. The estimated number needed to treat (NNT) for 1 year to prevent one infection (excluding malaria) was 113 [17]. In this article we provide a detailed account on the effect of CTX prophylaxis on malaria, a secondary outcome of the trial.

Methods

COSTOP was a randomized, double-blind, placebo-controlled noninferiority trial conducted in Uganda to determine whether long-term prophylaxis with CTX can be safely discontinued among HIV-infected adults on ART with sustained immune competence (defined as $CD4^+$ cell counts $\geq$250 cells/μl) [17,18]. The trial had two co-primary outcomes: time to occurrence of the first CTX-preventable clinical event (according to a pre-defined list) or death, and time to the occurrence of the first grade 3 or 4 haematological adverse event [17]. COSTOP was conducted by the MRC/UVRI Uganda Research Unit on AIDS (MRC/UVRI) at its research clinics in Masaka and Entebbe in Uganda.

Study procedures

Detailed procedures have been described previously [18]. Briefly, participants were eligible for enrolment if they were HIV infected; aged 18–59 years; clinically asymptomatic; had been taking CTX and ART for at least 6 months; had two $CD4^+$ cell counts not less than 250 cells/μl, the most recent within 4 weeks of enrolment; and were able to attend regular study appointments. Exclusion criteria included pregnancy, grade 3 or 4 anaemia, neutropenia or thrombocytopenia. At an initial screening visit, information was recorded on socio-demographics, medical history, current illness and medication and a physical examination was performed. Laboratory tests included a full blood count, malaria slide and $CD4^+$ cell count.

At enrolment (2–4 weeks after screening), participants were randomized to receive either active CTX (960 mg) or matching placebo once daily in place of their regular CTX. Randomization was stratified by enrolment site and $CD4^+$ cell count ($\leq$250–499 and $\geq$500 cells/μl). Participants were provided with an insecticide-treated bed net (ITN) and educated about the importance of using it. Participants continued to receive ART from their usual providers, but trial staff monitored ART availability to ensure an uninterrupted supply. Enrolment started in January 2011 and was completed in March 2013.

Participants were seen every month for the first 3 months and three-monthly thereafter, and were followed for 12 months to 3.5 years, depending on date of enrolment. At each visit, adherence to trial drug, ART and ITN use was assessed, using a structured questionnaire and returned pill counts (trial drug only). Participants were seen by a doctor who assessed their health, treated any concurrent infections, and dispensed trial drug. Participants were issued with a supply of trial drug to last until their next scheduled visit, along with a 3 day/month buffer stock in case they were late. Blood samples were drawn at scheduled visits for a malaria slide, $CD4^+$ cell count, and full blood count. Participants were encouraged to attend the study clinic if unwell. If the participant was suspected to have malaria, based on a history of malaria associated symptoms (reported fever, headache, chills and rigors, joint aches, muscles aches, vomiting or diarrhoea), a blood slide and other tests deemed necessary were done, and confirmed malaria was treated with arthemeter-lumefantrine according to national guidelines [19]. Participants who reported having been treated for malaria elsewhere (e.g. during a journey) were asked to present documentary evidence of diagnoses and test results. Participants were withdrawn from trial medication and started on open-label CTX if their $CD4^+$ cell count fell below 250 cells/μl at any point during the trial.

Laboratory methods

A blood sample was used to prepare thick and thin films on a glass slide. Specimens were processed using Leishman's stain and examined by microscopy. Thick film specimens were used to record the number of

parasites per 200 white blood cells and thin films to identify the plasmodium species.

For this study we considered two categories of malaria:

(1) clinical malaria, defined by the presence or history (during the previous 2 weeks) of fever and microscopically confirmed malaria parasites;
(2) severe malaria (based on WHO guidelines [20]), diagnosed if a patient had P. falciparum asexual parasitemia, no other obvious cause of symptoms and met any of the following criteria: convulsions, loss of consciousness, hypotension (systolic blood pressure <70 mmHg), admission to hospital due to malaria, laboratory evidence of liver or kidney damage, severe normocytic anaemia (haemoglobin <50 g/dl, PCV<15%), or hyperparasitaemia on blood slide (>5% or 250 000/μl).

Sample size

A total of 2180 participants were recruited and followed for up to 3.5 years. The sample size for the main trial was defined based on the power to demonstrate noninferiority of placebo for the primary efficacy outcome of time to the first CTX-preventable event or death [17,18]. For this study, assuming an incidence of 1.4 clinical malaria episodes/100 person-years (pyrs) in the control (CTX) arm, as observed in a previous study among HIV positive adults on ART in Uganda [21], and an average follow-up of 2 years, we estimated that the COSTOP trial would have more than 80% power to detect as significant (at the 5% level) a doubling of the incidence of clinical malaria in participants who stopped CTX compared with those who continued, assuming a 15% loss to follow-up at the end of the study.

Statistical analysis

Data were double-entered and verified in MS Access and analysed using Stata 12 (Stata Corp, College Station, Texas, USA). All analyses used an intention-to-treat (ITT) approach.

Baseline characteristics were compared between trial arms. Socioeconomic status (SES) was measured by combining data from all trial participants on housing construction and ownership of household items into an asset index score using principal component analysis [22].

Person years at risk were calculated from enrolment until the date last seen or end of trial. After each malaria episode, participants were considered to be not at risk for another episode until the episode resolved (as evidenced by resolution of symptoms and a negative repeat slide at the 14-day follow-up visit), or for 28 days, if a resolution date was not available. Time to first episode of clinical malaria was examined using Kaplan–Meier plots, and compared between trial arms using the log rank test. The incidence of all clinical malaria episodes, the rate ratio for the effect of trial arm, and 95%CI were estimated using random effects Poisson regression to account for multiple episodes within the same participant. The incidence of severe malaria was calculated and compared between arms.

In secondary analyses, follow-up time was divided into 12-month bands and analysis stratified by time to investigate possible effect modification of trial arm with time. Effect modification was assessed by comparing a model with fixed effects for treatment arm and timeband to one with treatment arm, timeband and their interaction, using the likelihood ratio test. In addition, effect modification by enrolment site was investigated.

The effect of trial drug adherence, and of ITN use, on malaria was assessed. The proportion of expected doses of trial drug taken, based on counts of returned pills at each scheduled visit, was calculated as (tablets dispensed − tablets returned)/(days elapsed since pills were dispensed). We defined 'good' adherence as taking 80–105% expected doses, allowing for adherence up to 105% due to possible imprecision in tablet counts. Each participant's overall adherence was categorized as being good at not less than 80% of visits or less than 80% of visits. At each visit, participants were asked if they had always slept under an ITN since their previous visit. Overall ITN use was characterized as having always used an ITN at not less than 90% of visits or less than 90% of visits.

Ethical approval

Approval for the study was obtained from the Science and Ethics Committee of the Uganda Virus Research Institute, the Uganda National Council of Science and Technology, the Ugandan National Drug Authority, and the Ethics Committee of the London School of Hygiene and Tropical Medicine.

Results

A total of 2180 participants were enrolled into the COSTOP trial. Participants' characteristics at baseline were balanced by trial arm (Table 1). Baseline characteristics have been described previously [17]. Overall, mean (SD) age was 41 [8] years; 74% of participants were female; 70% had primary education or less; and 52% had a $CD4^+$ cell count below 500 cells/μl.

Although balanced by trial arm within each site, there were some differences in participant characteristics between the enrolment sites. Participants in Masaka were slightly older, had lower levels of education, and

Table 1. Baseline characteristics by trial arm and site.

	Overall ($N=2180$)		Site					
			Entebbe			Masaka		
	CTX ($N=1089$)	Placebo ($N=1091$)	CTX ($N=501$)	Placebo ($N=501$)	Total ($N=1002$)	CTX ($N=588$)	Placebo ($N=590$)	Total ($N=1178$)
Age								$P=0.002$[a]
<30	85 (7.8)	83 (7.6)	47 (9.4)	38 (7.6)	85 (8.4)	38 (6.5)	45 (7.6)	83 (7.0)
30–34	148 (13.6)	189 (17.3)	75 (15.0)	104 (20.8)	179 (17.9)	73 (12.4)	85 (14.4)	158 (13.4)
35–39	232 (21.3)	235 (21.5)	112 (22.3)	107 (21.3)	219 (21.9)	120 (20.4)	128 (21.7)	248 (21.1)
40–44	262 (24.1)	221 (20.3)	116 (23.1)	100 (20.0)	216 (21.6)	146 (24.8)	121 (20.5)	267 (22.7)
45–49	198 (18.2)	190 (17.4)	87 (17.4)	91 (18.1)	178 (17.8)	111 (18.9)	99 (16.8)	210 (17.8)
≥50	164 (15.1)	173 (15.9)	64 (12.8)	61 (12.2)	125 (12.4)	100 (17.0)	112 (19.0)	212 (18.0)
Mean age (SD)	41.0 (8.0)	40.7 (8.3)			40.0 (8.0)			41.5 (8.3)
$CD4^+$ cell count stratum								$P=0.001$
250–<500 cells/μl	563 (56.2)	577 (49.0)	282 (56.3)	281 (56.1)	563 (56.2)	288 (49.0)	289 (49.0)	577 (49.0)
≥500 cells/μl	439 (43.8)	601 (51.0)	219 (43.7)	220 (43.9)	439 (43.8)	300 (51.0)	301 (51.0)	601 (51.0)
Time on ART (years)								$P<0.001$
<1	73 (6.7)	82 (7.5)	46 (9.2)	43 (8.6)	89 (8.9)	27 (4.6)	39 (6.6)	66 (5.6)
1–2	144 (13.2)	160 (14.7)	71 (14.2)	79 (15.8)	150 (15.0)	73 (12.4)	81 (13.7)	154 (13.1)
2–5	508 (46.7)	480 (44.0)	261 (52.0)	255 (50.9)	516 (51.5)	247 (42.0)	225 (38.2)	472 (40.1)
>5	364 (33.4)	369 (33.8)	123 (24.6)	124 (24.7)	247 (24.6)	241 (41.0)	245 (41.5)	486 (41.3)
Sex								$P=0.520$
Man	286 (26.3)	283 (25.9)	130 (26.0)	125 (25.0)	255 (25.5)	156 (26.5)	158 (26.8)	314 (26.7)
Women	803 (73.7)	808 (74.1)	371 (74.0)	376 (75.0)	747 (74.6)	432 (73.5)	432 (73.2)	864 (73.3)
Education level								$P<0.001$
None	111 (10.2)	113 (10.4)	46 (9.2)	37 (7.4)	83 (8.3)	65 (11.2)	76 (12.9)	141 (11.9)
Primary	649 (59.6)	660 (60.5)	259 (51.7)	281 (56.1)	540 (53.9)	390 (66.3)	379 (64.2)	769 (65.3)
Secondary	276 (25.3)	268 (24.6)	160 (31.9)	157 (31.3)	317 (31.6)	116 (19.7)	111 (18.8)	227 (19.3)
Tertiary	53 (4.9)	50 (4.6)	36 (7.2)	26 (5.2)	62 (6.2)	17 (2.8)	24 (4.1)	41 (3.5)
Socio-economic status								$P<0.001$
Low	486 (44.6)	474 (43.4)	197 (39.3)	213 (42.5)	410 (40.9)	289 (49.1)	261 (44.2)	550 (46.7)
Medium	318 (29.2)	329 (30.2)	117 (23.4)	113 (22.6)	230 (23.0)	201 (34.2)	216 (36.6)	417 (35.4)
High	285 (26.2)	288 (26.4)	187 (37.3)	175 (33.9)	362 (36.1)	98 (16.7)	113 (19.2)	211 (17.9)
Marital status								$P=0.32$
Married/cohabiting	473 (43.4)	465 (42.6)	213 (42.5)	210 (41.9)	423 (42.2)	260 (44.2)	255 (43.2)	515 (43.7)
Divorced/separated/widowed	577 (53.0)	577 (52.9)	271 (54.1)	273 (54.5)	544 (54.3)	306 (52.0)	304 (51.3)	610 (51.8)
Single	39 (3.6)	49 (4.5)	17 (3.4)	18 (3.6)	35 (3.5)	22 (3.7)	31 (5.3)	53 (4.5)

ART, antiretroviral therapy; CTX, cotrimoxazole.
[a]P value for comparison between sites, by χ^2 test.

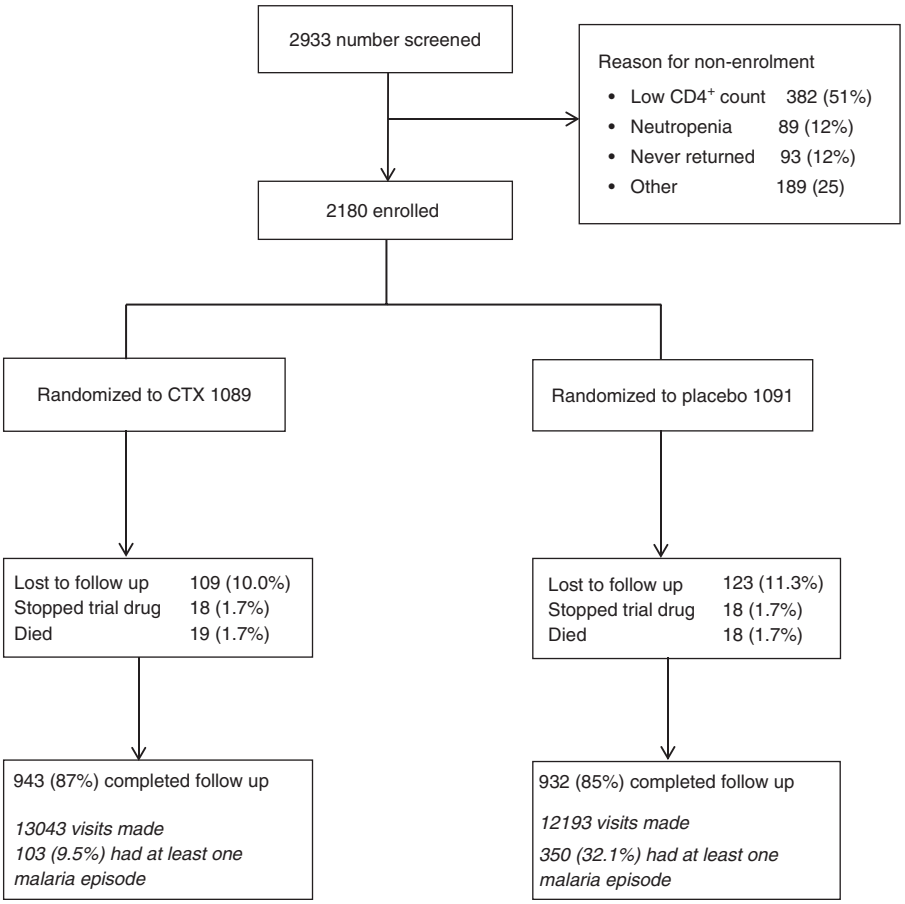

Fig. 1. COSTOP trial profile.

were of lower SES than those in Entebbe (Table 1). They also had higher $CD4^+$ cell counts at enrolment, and had been on ART for longer.

Follow-up

Participants were followed for a median of 2.53 years (interquartile range = 1.86–2.76), with no evidence of a difference between arms (CTX median 2.53; interquartile range 1.96–2.76 years vs. placebo 2.53; 1.84–2.76 years; $P = 0.66$ by Wilcoxon rank-sum test). In total 1875 participants (943 CTX) completed follow-up (Fig. 1); 36 participants were withdrawn from trial drug (18 CTX) and started on open-label CTX; 232 participants were lost to follow-up (109 CTX); and 37 participants died (19 CTX). Only one death was malaria related (CTX) and was due to quinine toxicity. Overall, 13 043 scheduled visits were attended by participants on CTX and 12 913 by those on placebo (Fig. 1).

Effect of stopping cotrimoxazole

There were 453 episodes of clinical malaria experienced by 362 participants (range 1–5) during follow-up; 9% of participants in the CTX arm and 24% of those in the placebo arm had at least one episode of clinical malaria. Time to the first clinical malaria event was significantly shorter in the placebo arm than in the CTX arm (log rank test $P < 0.001$) (Fig. 2).

The overall incidence of clinical malaria was 9.1 (95%CI = 8.2–10.1) episodes/100 pyrs. The incidence of malaria was 3.5 times higher in participants on placebo than on CTX (95%CI = 2.7–4.4; $P < 0.001$; Table 2).

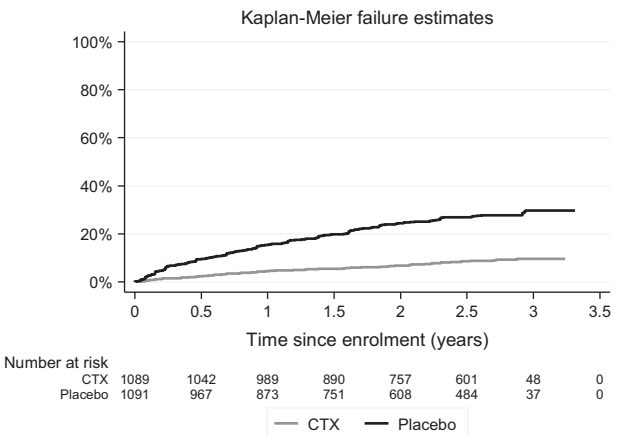

Fig. 2. Time to occurrence of first malaria event by treatment arm.

Table 2. Incidence of malaria by trial arm, follow-up time, trial site, adherence to trial drug and ITN use.

	Trial arm	Episodes	Person-years	Rate/100 person-years (95% CI)[a]	Rate ratio[a]	*P* value (CTX vs. placebo)	*P* value (for interaction)[b]
Clinical malaria							
	CTX	103	2540	4.1 (3.3–5.0)	1	<0.001	
	Placebo	350	2515	14.1 (12.5–15.0)	3.5 (2.5–4.4)		
Severe malaria[c,d]							
	CTX	2	2543	0.08 (0.02–0.31)	1	0.004	
	Placebo	13	2524	0.52 (0.30–0.89)	6.55 (1.48–29.01)		
Stratified analyses							
Stratum variable							
Follow-up time							
1st year	CTX	51	1065	4.8 (3.6–6.3)	1	<0.001	0.097
	Placebo	183	1062	17.3 (14.8–20.2)	3.6 (2.6–5.0)		
2nd year	CTX	29	939	3.1 (2.1–4.5)	1	<0.001	
	Placebo	120	924	13.1 (10.8–15.8)	4.2 (2.8–6.4)		
After 2nd year	CTX	23	536	4.3 (2.8–6.5)	1	0.004	
	Placebo	47	528	9.0 (6.7–12.1)	2.1 (1.3–3.5)		
Site							
Entebbe	CTX	19	1215	1.6 (1.0–2.5)	1	<0.001	<0.001
	Placebo	127	1209	10.6 (8.8–12.8)	6.8 (4.1–11.1)		
Masaka	CTX	84	1325	6.3 (5.1–7.9)	1	<0.001	
	Placebo	223	1306	17.2 (14.8–20.0)	2.7 (2.1–3.6)		
Trial drug adherence							
Good adherence at							
≥80% of visits[e,f]	CTX	57	1724	3.3 (2.5–4.4)	1	0.01	0.25
<80% of visits[e,f]	CTX	46	816	5.6 (4.1–7.6)	1.7 (1.1–2.6)		
≥80% of visits[e,g]	Placebo	205	1617	12.8 (12.0–14.9)	1	0.05	
<80% of visits[e,g]	Placebo	145	897	16.4 (13.6–19.8)	1.3 (1.0–1.6)		
ITN use							
Reported always using ITN at							
≥90% of visits	CTX	86	2124	4.1 (3.3–5.1)	1	$P = 0.98$	0.39
<90% off visits	CTX	17	416	4.1 (2.5–6.7)	1.0 (0.6–1.7)		
≥90% of visits	Placebo	267	2038	13.3 (11.6–15.2)	1	$P = 0.06$	
<90% off visits	Placebo	83	476	17.5 (13.6–22.5)	1.3 (1.0–1.8)		

CTX, cotrimoxazole; ITN, insecticide-treated bed net.
[a]rates and rate ratios adjusted for clustering of multiple episodes within participant using random effects Poisson regression.
[b]*P* value for interaction between stratum variable with treatment arm.
[c]*P. falciparum* malaria with clinical or laboratory features of severity.
[d]Rates and rate ratios from Poisson regression without adjustment for clustering since no participant had more than one episode.
[e]'Good' adherence defined as 80–105% of expected tablets taken, based on pill counts.
[f]Rate ratio and *P* value for comparison in CTX arm.
[g]Rate ratio and *P* value for comparison in placebo arm.

There was some evidence that the effect of stopping CTX on clinical malaria incidence decreased over time: although incidence remained higher in the placebo arm, the difference between arms was less in the third year (rate ratio = 2.1; 95%CI = 1.3–3.5) than in the first year (rate ratio = 3.6; 95%CI = 2.6–5.0; *P* for interaction = 0.10) (Table 2). In the placebo arm, clinical malaria incidence decreased from 17.3/100 pyrs in the first year to 9.0/100 pyrs after the second year (P for trend <0.001; Supplementary Figure, http://links.lww.com/QAD/A828). In the CTX arm, clinical malaria incidence remained similar over time (rate ratio for linear trend in incidence from one year to the next = 0.90, CI = 0.69–1.16, $P = 0.40$).

The relative effect of stopping CTX on clinical malaria was greater in Entebbe (rate ratio = 6.8; 95%CI = 4.1–11.1) than in Masaka (rate ratio = 2.7; 95%CI = 2.1–3.6; *P* for interaction <0.001) (Table 2). In both arms, the incidence of clinical malaria was higher in Masaka than in Entebbe (CTX: 6.3 vs. 1.6/100 pyrs, respectively; placebo 17.2 vs. 10.6/100 pyrs, respectively).

Overall, 15 (2 CTX, 13 placebo) episodes of severe malaria occurred among the 2180 participants (<1%) (Table 2). None of the participants had more than one episode of severe malaria. Reasons for classifying malaria as severe were: high parasitemia [2], loss of consciousness [1], mental confusion [1] and hospital admission [11]. The overall incidence of severe malaria was 0.30/100 pyrs (95%CI = 0.18–0.49); the incidence of severe malaria was 6.5 (95%CI = 1.5–29.0) times higher in the placebo arm than in the CTX arm. Only one participant (CTX arm) died of a malaria-related event.

The NNT with CTX for one year to prevent one malaria episode was 10, and was 233 for severe malaria.

Effect of adherence to trial drug and insecticide-treated bed net use on malaria

Among participants on CTX, the incidence of clinical malaria in those with good adherence at less than 80% of visits was 1.7 (95%CI = 1.1–2.6) times higher than in those with good adherence at not less than 80% of visits ($P = 0.01$) (Table 2). Among participants on placebo, malaria incidence was 1.3 (95%CI = 1.0–1.6) times higher in those with good adherence at less than 80% of visits ($P = 0.05$). The relative outcome of effect of stopping CTX was greater in participants with good adherence than in those with lower adherence (rate ratio = 3.9, 95%CI = 2.8–5.3 vs. rate ratio = 2.9, 95%CI = 2.0–4.2).

Malaria incidence did not differ by reported ITN use among participants on CTX (rate ratio = 1.0; 95%CI = 0.6–1.7; $P = 0.98$). However, malaria incidence was higher in participants on placebo who reported using an ITN at less than 90% of visits than in those who reported ITN use at not less than 90% of visits (rate ratio = 1.3, 95%CI = 1.0–1.8; $P = 0.06$), although there was no evidence of significant effect modification by treatment arm ($P = 0.39$).

Discussion

In this trial, participants on ART who stopped prophylactic CTX had a 3.5-fold higher probability to experience clinical malaria than those who continued. This is expected given the antimalarial properties of CTX [23] and is consistent with other studies in adults on ART who discontinued CTX [21,24–28]. However the rate ratio in our study was much smaller than reported by other randomized trials in adults. Campbell *et al.* [21] conducted an open-label, cluster (household) RCT in Uganda to investigate the effect of stopping CTX on the incidence of malaria and diarrhoea among HIV-infected adults on ART with $CD4^+$ above 200 cells/μl. The trial enrolled 836 participants with median time on ART of 3.7 years and found that participants stopping CTX had a 32.5-fold (8.6–275.0) higher malaria incidence than those that continued. The trial was stopped after 4 months. Polyak *et al.* [28] conducted an open-label RCT comparing stopping versus continuing CTX prophylaxis among 500 HIV-infected adults in western Kenya who had been on ART for more than 18 months. After a year of follow-up participants stopping CTX had a 33.2-fold (95%CI = 4.5–241.0) higher malaria incidence than those who continued CTX.

The contrasting results between these trials and ours could have resulted from the shorter follow-up times (4 months and 1 year in the Campbell and Polyak studies, respectively) and from the smaller number of malaria episodes (55 and 34, respectively) compared with our study, in which 453 episodes of clinical malaria were documented over a median follow-up time of 2.5 years. Another explanation could be that participants in our trial may have used CTX from other sources outside the trial, which may have accounted for the smaller differences between arms. However, exit interviews conducted at the end of our trial did not find any evidence that participants had taken CTX from other sources. We used an ITT approach to the analysis; however, only 18 participants in the placebo arm were withdrawn from trial medication and started on open-label CTX, so this is unlikely to have had a large impact on our results. Importantly, the Campbell and Polyak studies were not blinded, so that investigators or participants might have been more likely to investigate or seek treatment if they felt that stopping CTX might be risky. This source of potential observer bias may have resulted in diagnosing malaria more frequently in participants who had stopped CTX.

Although the incidence of severe malaria was higher in the placebo arm, there was very little severe malaria in our trial (only 1.2% of participants in the placebo arm) and there was no statistical difference in overall number of deaths (due to any cause) between trial arms. This is consistent with findings in the study by Campbell *et al.* [21]. Only one malaria-related death was recorded, and this occurred in the CTX arm.

We found a marked decrease in malaria incidence over time in the placebo arm whereas the incidence in the CTX arm remained stable. This initially higher incidence among participants who had stopped CTX could be a consequence of the detrimental effects that HIV had caused on the innate immune response to malaria and other infections [29]. Whilst participants with low immunity were initially protected against malaria due to CTX, it is possible that the incidence of malaria increased when CTX was discontinued, until participants re-acquired some immunity against malaria. This has been described among children [30].

As expected, participants with good adherence to trial drug in the CTX arm had less malaria than those whose adherence was not as good. Unexpectedly, good adherence was also associated with reduced malaria incidence in the placebo arm. A possible explanation is that participants with suboptimal adherence to trial drug might also be less likely to adhere to other malaria prevention measures like ITN use. There was no association of reported ITN use with malaria in the CTX arm. In contrast, participants on placebo with high reported ITN use had less malaria than those with lower reported ITN use, suggesting that people who discontinue CTX would benefit from general malaria prevention measures.

There were fewer malaria episodes observed in participants at Entebbe than Masaka, in both trial arms and over

time. Participants at Entebbe were generally younger, and had attained a higher education level and SES compared with those in Masaka. If participants in Entebbe were better able to take care of themselves, had better housing or could access treatment more easily this may have resulted in a lower incidence of malaria at the Entebbe site. Another possible explanation is that malaria endemicity and therefore exposure to malaria was higher in Masaka compared with Entebbe. Data on malaria endemicity by region in Uganda are limited; however, both areas are considered to have very high malaria endemicity [31]. Recent reports for the three quarters up to June 2015 showed an incidence of 101, 59, and 106 per 1000 population in Masaka district, and 82, 86 and 56 in Wakiso district, which includes Entebbe [32]. This seems to suggest that background exposure to malaria may be higher in Masaka.

Strengths and limitations

Strengths of our study include its design as a double-blind placebo-controlled randomized trial, its large sample size, and that participants were seen at frequent scheduled visits and sick visits at which screening for malaria was routinely performed and adherence to trial drug and ART assessed. Furthermore, participant retention was high, with more than 85% completing follow-up.

Unfortunately we do not have data on malaria incidence from HIV-negative individuals in our study area. We can therefore not determine the extent to which the incidence of malaria among HIV-infected participants on ART and CTX may have been reduced below normal levels.

Conclusion

In this blinded placebo-controlled trial in Uganda, participants who were stable on ART and stopped taking prophylactic CTX had malaria more frequently and severely than those who continued, but the difference was less than has been reported by earlier studies. Malaria incidence reduced in participants on placebo over time. Few participants experienced severe malaria. A potential decision to stop or continue CTX will have to take into account the main COSTOP trial results that have shown a clear benefit of continued CTX prophylaxis in preventing bacterial infections, but also showed an increase in neutropenia incidence and no reduction in overall mortality [17]. The NNT to prevent one CTX-preventable event or malaria is also worth considering. According to current WHO guidelines CTX may be discontinued in some situations, but should be continued in countries with high endemicity of malaria and bacterial infections [15]. Given the costs and toxicity of CTX and the potential development of wide spread of CTX resistance, further research will be useful in identifying groups and circumstances in which CTX prophylaxis could be safely stopped.

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Contributions: R.K., P.M., J.L., H.G. conceived the study idea; R.K., Z.A., P.M., A.K., A.N. conducted the study; R.K., K.B. did the data analysis; R.K., K.B., H.G. developed the first draft. All authors contributed to the interpretation of the data, revised the article critically and approved the final version.

Conflicts of interest

There are no conflicts of interest.

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Chapter 6: Longitudinal effect of CD4 by cotrimoxazole use on malaria incidence among HIV-infected Ugandan adults on antiretroviral therapy: a randomized controlled study.

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

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SECTION A – Student Details

Student	Ronnie Kasirye
Principal Supervisor	Heiner Grosskurth
Thesis Title	Effect of cotrimoxazole on malaria in HIV-infected adults on antiretroviral therapy

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?			
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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Malaria Journal
Please list the paper's authors in the intended authorship order:	Ronnie Kasirye, Heiner Grosskurth, Paula Munderi, Jonthan Levin, Zacchaeus Anywaine, Andrew Nunn, Anatoli Kamali, Kathy Baisley
Stage of publication	**Submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I conceived the study after discussions with Paula Munderi (PM), Jonathan Levin (JL) and Heiner Grosskurth (HG). I was the study coordinator from 2010 to 2012. I provided input into the analysis which was performed by Kathy Baisley (KB). I wrote the first draft and revised the draft after comments from

	KB and HG. I made further revisions based on comments from the other COSTOP trial co-investigators. I submitted the paper for publication.

Student Signature: _____ **Date:** 30/03/16

Supervisor Signature: _____ **Date:** 1/4/16

Longitudinal effect of CD4 by cotrimoxazole use on malaria incidence among HIV-infected Ugandan adults on antiretroviral therapy: a randomized controlled study.

R.Kasirye[1, 2], H.Grosskurth[1,2], P. Munderi[1], J. Levin[1,3], Z. Anywaine[1], A. Nunn[4], A. Kamali[1], K.Baisley[2]

1 MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda

2 London School of Hygiene and Tropical Medicine, London, UK

3 School of Public Health, University of Witwatersrand, Johannesburg, South Africa

4 MRC Clinical Trials Unit at University College London, UK

Abstract

Introduction

We investigated the effect of CD4 count on malaria incidence in HIV infected adults on antiretroviral therapy (ART) in the context of a randomized controlled trial on the effect of stopping cotrimoxazole (CTX).

Methods

This study presents a sub-analysis of the COSTOP trial (ISRCTN44723643) which was carried out among HIV-infected Ugandan adults stable on ART with CD4 counts ≥250 cells/µl. Participants were randomized (1:1) to continue CTX or stop CTX and receive matching placebo, and were followed up for a minimum of one year (median 2.5 years). CD4 counts were measured at baseline, 3 months and then every 6 months. Clinical malaria was defined as fever and a positive blood slide and considered severe if a participant had one or more clinical or laboratory features of severity or was admitted to

hospital. We assessed the effect of CD4 count (<350,350-499,>500) at enrolment, CD4 count (<100, 100-249, >250) at ART initiation, and CD4 count at infection (<350,350-499,>500) during follow-up on malaria using random effects Poisson regression to account for multiple episodes within participant.

Results

2180 participants were enrolled into the COSTOP trial. The incidence of clinical malaria was approximately 4 episodes/100pyrs in the CTX arm and 14 episodes/100pyrs in the placebo arm, and did not depend on CD4 count at ART initiation, enrolment or during follow up. When compared with participants in the lowest CD4 stratum, rate ratios within each trial arm were all close to 1, and P-values were all above P=0.30. There was no evidence that parasitemia levels differed by CD4 count at infection (P=0.24). There was no effect of CD4 on severe malaria (P=0.14).

Conclusion

The immune status of HIV infected participants who are stable on ART as measured by CD4 count was not associated with malaria incidence and did not modify the effect of stopping CTX on malaria. The decision of whether to stop or continue CTX prophylaxis for malaria in HIV infected individuals who are stable on ART should not be based on CD4 counts alone.

Key words

Malaria, CD4, antiretroviral therapy, cotrimoxazole, HIV

Introduction

In many parts of sub-Saharan Africa (SSA), both malaria and HIV infection are highly endemic. HIV infection enhances malaria acquisition and severity, similarly malaria enhances HIV viral replication (1-5). The effect of HIV infection on malaria incidence seems to be a consequence of the immune suppression that is a characteristic of HIV infection (6). In clinical practice CD4+ cell counts are used to measure the degree of HIV induced immune suppression which guides decisions on antiretroviral therapy (ART) and the need for prophylaxis against opportunistic infections (7, 8).

Decreasing CD4 counts have been associated with higher risk of acquiring malaria. In a study in rural Uganda, HIV infected adults with CD4 counts <200 cells/μl had 6.1 (CI=2.1-17.5) times the risk of clinical malaria compared to those with CD4 counts ≥500 (P=0.002) (9). A study among HIV infected adults in Entebbe, Uganda found that the incidence of clinical malaria due to Plasmodium falciparum increased with decreasing CD4 count; 57, 93, 140 cases per 1000 person years (pyrs) for CD4 counts >500, 200-499, <200 and respectively (P<0.001).(10) Both studies were in ART naïve individuals. Once viral replication is suppressed by ART, CD4 counts increase over time (11, 12). Consequently it may be expected that the risk of malaria will decrease as individuals' CD4 counts increase. However, it is unclear whether this effect continues once the immune system has recovered under ART, or whether there is a CD4 threshold after which malaria incidence stabilizes.

In order to address these research questions, we conducted a sub-analysis of data collected during the recently completed COSTOP trial in Uganda (13-15). These data

also provide an opportunity to investigate whether the effect of CD4 count on malaria incidence differs between individuals on ART who take prophylactic cotrimoxazole (CTX) medication and those who are not on CTX. It is well known that CTX is beneficial to HIV infected individuals as a prophylaxis against malaria and bacterial infections (16-18) even when individuals are on ART (19-21), and CTX is recommended for routine use in areas in which malaria is highly prevalent (8).

Aim

The study aimed to determine among HIV infected adults on ART with CD4 counts ≥250 cells/μl:

(i) the effect of CD4 count on malaria incidence

(ii) whether this effect differs in the presence and absence of CTX medication

Methods

This study used data gathered during the COSTOP trial conducted from 2011 to 2014 in Uganda (ISRCTN44723643). Trial methods have been described previously (13, 15). Briefly, COSTOP was a randomized, double-blind, placebo controlled non-inferiority trial to determine whether long-term prophylaxis with CTX can be safely discontinued among HIV infected adults on ART with sustained immune competence (defined as a confirmed CD4 counts of ≥250 cells/μl). Individuals were eligible for enrolment if they were HIV-infected; aged 18 years or older; clinically asymptomatic; had been taking CTX and ART for at least 6 months; and had 2 CD4 counts (not more than 6 months apart) ≥250 cells/ μl, the most recent no more than 4 weeks prior to enrolment. Exclusion criteria included

pregnancy, grade 3 or 4 anemia, neutropenia or thrombocytopenia. Participants were randomized to receive either active CTX (960 mg) or matching placebo once daily after stopping their regular CTX medication. Randomization was stratified by enrolment site (Entebbe or Masaka, both located in SW Uganda) and CD4 count (≥250-499 and ≥500 cells/µl).

Study procedures

Informed consent for study procedures was obtained at screening and enrolment.

At screening, data were documented on; disease history, duration of prior ART and CTX medication, and CD4 count at time of ART initiation. At enrolment, data were documented on socio-demographic characteristics and each participant was provided with an insecticide-treated bed net (ITN) and educated about the importance of using it. Participants were seen at scheduled follow-up visits every month for the first three months and three-monthly thereafter, and were followed for 12 months to 3.5 years, depending on date of enrolment. At these visits, participants were asked about their health, symptoms suggestive of malaria, adherence to medication and bednet use. Blood samples were drawn; at enrolment, monthly for three months and three monthly thereafter for a malaria slide; at 3 months, 6 months and 6 monthly thereafter for CD4 count; and three monthly for the full blood count. Participants were asked to attend the study clinic at any time they felt unwell; if malaria was suspected, based on a history of malaria associated symptoms (fever, headache, chills and rigors, joint aches, muscles aches, vomiting or diarrhea), a blood slide and other tests deemed necessary were done. Participants who reported having been treated for malaria elsewhere (for example

during a journey) were asked to present documentary evidence of diagnoses and test results.

Laboratory methods

A sample of blood was taken either from the fingertip using a lancet or from a peripheral vein using a syringe, and used to prepare thick and thin films on a glass slide. The specimens were processed using Leishman's stain and examined by microscopy. Thick film specimens were used to record the number of parasites per 200 white blood cells and thin films to identify the plasmodium species. Venous blood samples were taken for CD4 cell counts and measured using a FACS-count system (Becton-Dickinson San Jose) at the MRC/UVRI laboratories in Entebbe and Masaka.

Statistical analysis

Analyses were carried out using Stata 13. Clinical malaria was defined as presence or history (during the previous 2 weeks) of fever and microscopically confirmed malaria parasites. Severe malaria (based on WHO guidelines) (15, 22) was diagnosed if a participant had P. falciparum asexual parasitemia, no other obvious cause of symptoms and met any of the following criteria: convulsions, loss of consciousness, hypotension (systolic blood pressure <70 mmHg), admission to hospital due to malaria, laboratory evidence of liver or kidney damage, severe normocytic anaemia (haemoglobin <50g/dl, PCV<15%), or hyperparasitemia on blood slide (>5% or 250,000/µl).

The CD4 count at enrolment was calculated from the mean of the two most recent pre-enrolment (screening) CD4 counts. Person years at risk were calculated from enrolment

until the date last seen or end of trial. After each malaria episode, participants were considered to be not at risk for another episode until the episode resolved, or for 28 days, if a resolution date was not available. Follow-up data were organized into intervals corresponding with the visit schedule. For time-varying variables during follow-up (e.g. CD4 count at malaria infection, BMI) we used the most recent value measured at the start of each interval. CD4 count values were carried forward for the visits where CD4 counts were not done, until the next recorded CD4 count.

First, we assessed the effect of current (time of infection) CD4 count on clinical malaria incidence during follow up, using random effects Poisson regression to account for the clustering of multiple episodes within the same participant. The analysis was restricted to participants in the placebo arm in order to examine the effect of CD4 counts in the absence of the anti-malaria effects of CTX. We examined the effect of CD4 adjusted for baseline covariates that were considered as potential confounders a priori (enrolment site, age, sex, socioeconomic status (SES) and baseline CD4 count), and then including time-varying variables (time since enrolment, current BMI). SES was measured by combining baseline data from all trial participants on housing construction and ownership of household items into an asset index score using principal component analysis (23). In order to allow for non-linear effects, CD4 at infection, baseline CD4 and age were modelled using restricted cubic splines with 4 knots; this approach provides a flexible way to model the shape of the relationship of a continuous variable with the outcome (24).

Among participants with clinical malaria, we assessed the effect of CD4 count at infection on parasitemia during each malaria episode as the outcome, using random effects linear regression; parasitemia levels were log transformed for analysis. The analysis was adjusted for baseline and time-varying potential confounders as described above. In addition, we assessed whether CD4 count at infection had an effect on severe malaria; since there were only 15 episodes of severe malaria (13 placebo, 2 on CTX) (15), we did not attempt to adjust for potential confounders.

Secondly, we assessed whether the effect of CD4 count at baseline, CD4 count at ART initiation, or CD4 count at infection on clinical malaria differed by treatment arm (CTX or placebo), using random effects Poisson regression. Regression models contained fixed effects for CD4 count group, treatment arm, enrolment site and year since enrolment, and an interaction term between CD4 count group and treatment arm.

Ethical approval

Approval for the COSTOP trial was obtained from the Science and Ethics Committee of the Uganda Virus Research Institute, the Uganda National Council of Science and Technology, and the Ugandan National Drug Authority. Approval for this sub-analysis was obtained from the COSTOP Trial Steering Committee and the Ethics Committee of the London School of Hygiene and Tropical Medicine.

Results

2180 participants were enrolled into the COSTOP trial, 1002 (46%) at the Entebbe site, and 1091 (50%) were allocated to placebo (stopping CTX). Baseline characteristics were well balanced between trial arms (Supplementary Table 1). Mean age at enrolment was 41 years and 74% were female. The median (IQR) CD4 count at ART initiation was 155 (89-199) and 159 (83-214) for Entebbe and Masaka, respectively, and the median (IQR) CD4 count at enrolment was 446 (361-600) and 519 (397-655), respectively. At the Entebbe site, 56 (5.7%) participants were on a protease inhibitor (PI)-containing regimen compared to 30 (2.6%) at Masaka. 239 (24%) participants in Entebbe and 220 (19%) in Masaka site had been on ART for <2years.

Effect of CD4 on malaria

Among participants in the placebo arm, overall clinical malaria incidence was 14.1/100 person-years (95%CI=12.5-15.8). There was no evidence of an effect of CD4 count at infection on clinical malaria (P=0.56; Table 1; Figure 1). Furthermore, there was no evidence that parasitemia levels differed by CD4 count at infection (P=0.24 from random effects linear regression model; Figure 2).

There were 15 cases of severe malaria (13 placebo, 2 CTX). Severe malaria rates decreased with increasing CD4 counts among participants with CD4<400, then remained fairly similar in participants with higher CD4 counts. (P=0.14; Supplementary figure 1).

Effect of CD4 count on malaria by trial arm

The incidence of malaria did not differ significantly between CD4 count strata, neither for CD4 count at infection, CD4 count at ART initiation or CD4 count at enrolment into the study, and this was irrespective of whether participants were in the CTX arm or the placebo arm of the trial. Although malaria incidence was significantly lower in the CTX arm than on placebo (15), compared to participants on the lowest CD4 stratum, rate ratios were all close to 1 and P-values were all above P=0.30 for each of the three CD4 measures and within each arm.(Table 2).

Discussion

Previous studies in HIV-infected adults have reported an increase in malaria incidence with decreasing CD4 counts (9, 10) but these studies were in individuals who were not on ART.

We have previously reported that the incidence of clinical malaria in our study population was lower in participants on CTX than on placebo, and reduced during follow up (15). This reduction over time was primarily driven by reduced incidence in the placebo arm while incidence in the CTX arm remained fairly constant. One possible explanation is that the immune system recovers in individuals on ART and is therefore able to more effectively control malaria infection. In the COSTOP trial, there was evidence of continued recovery of the immune system in HIV-infected participants who are stable on ART as shown by an increase in CD4 counts over time, particularly in participants on placebo (14). However we did not find evidence of the expected

association between CD4 count and the incidence of clinical malaria, or degree of parasitemia. This lack of an effect of CD4 count on malaria was observed for CD4 count at the time of starting ART (considered a measure of the extent of immune damage before starting ART), time of randomization (indicating the immune status at beginning of study) and time of malaria episode. These are surprising findings. One possible explanation is that all participants in the COSTOP trial had a CD4 count >250 cells/µl at enrolment, so participants may have been above the threshold below which CD4 count significantly influences the risk of malaria. An alternative explanation could be that an improvement of CD4 cell quality rather than quantity under ART may be important for malaria containment (25). We found no evidence of an effect of CD4 count on severe malaria; however, because there were so few cases of severe malaria, our power to detect significant associations was poor.

Strengths and limitations of the study

This study made use of a well-documented data set from a large trial of HIV-infected adults on ART. The large sample size and the regular collection of data on exposures (CD4 count), outcomes (clinical malaria and parasitemia) and a variety of potential confounders allowed us to investigate the research questions in great detail.

Our study had some limitations. In spite of the large sample size only a small number of severe malaria episodes occurred which limited the power to detect a potential effect of CD4 count. Also, the study was not a priori designed to address detailed research questions related to malaria, but rather was conducted as a sub-analysis of data

gathered in the context of a randomized trial on the effect of stopping CTX. Although we adjusted for some potential confounders, we cannot rule out residual confounding as a result of imperfectly measured covariates for which we adjusted (e.g. SES) or covariates which we did not measure. Furthermore, we did not measure viral load, which may be a better indicator of immune competence than CD4 count: it has been shown that effective viral suppression reduces the incidence of opportunistic infections (26) and a similar effect might be expected for clinical malaria. Lastly, we were not able to investigate the immune responses to malaria which might have provided insight into why CD4 counts had no apparent effect on malaria incidence.

Conclusion

In this study of HIV-infected individuals on ART with baseline CD4 counts ≥250 cells/μl, the incidence of clinical malaria and the intensity of parasitaemia among patients with clinical malaria were not influenced by CD4 counts at ART initiation, enrolment into the study, or at the time of malaria infection. The finding of no association between malaria and CD4 count was similar among participants randomized to stop prophylactic CTX and those who continued CTX. The decision of whether to stop or continue CTX prophylaxis for malaria in HIV infected patients who are stable on ART should not be based on a patient's CD4 cell count alone.

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Authors' contributions

RK, JL, PM, HG conceived the idea for this analysis, KB and RK did the analysis. RK, KB, HG developed the first draft. All authors contributed to the interpretation of the data, revised the article critically and approved the final version.

Competing interests

The authors declare they have no competing interests.

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Figure 1 a and b:

Association of malaria incidence rate ratios (and 95% confidence intervals) with CD4 count at time of malaria episode as observed during follow up, modelled using restricted cubic splines with 4 knots in a random effects Poisson regression model, unadjusted (a), and adjusted for covariates during baseline and follow up (b). A CD4 count of 200 was used as the reference to calculate the rate ratios.

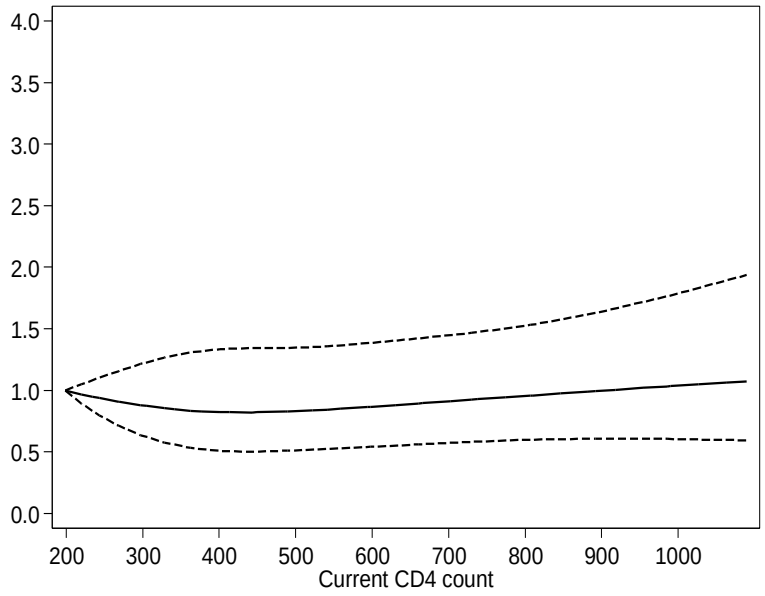


Figure 1a

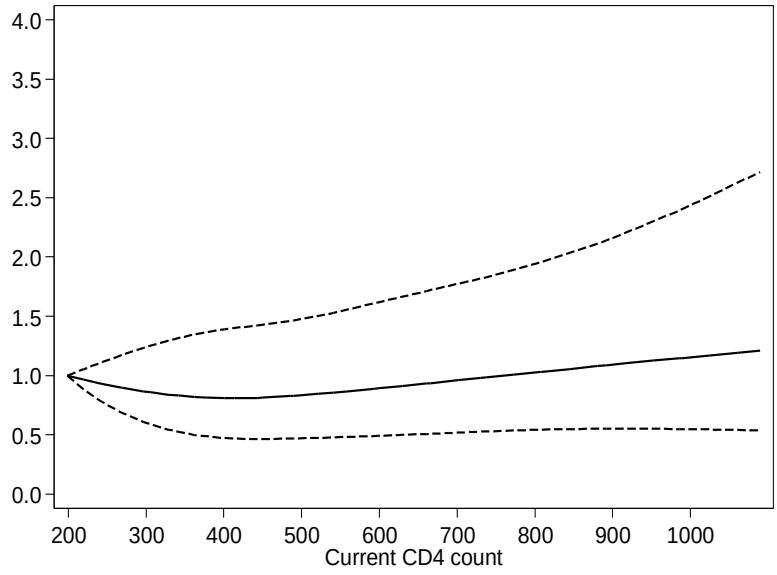


Figure 1b

Figure 2: Parasite counts by CD4 count at infection among participants in the placebo arm with clinical malaria. The central line represents the median; boxes represent 75th and 25th centiles; whiskers represent upper and lower adjacent values and dots represent outside values.

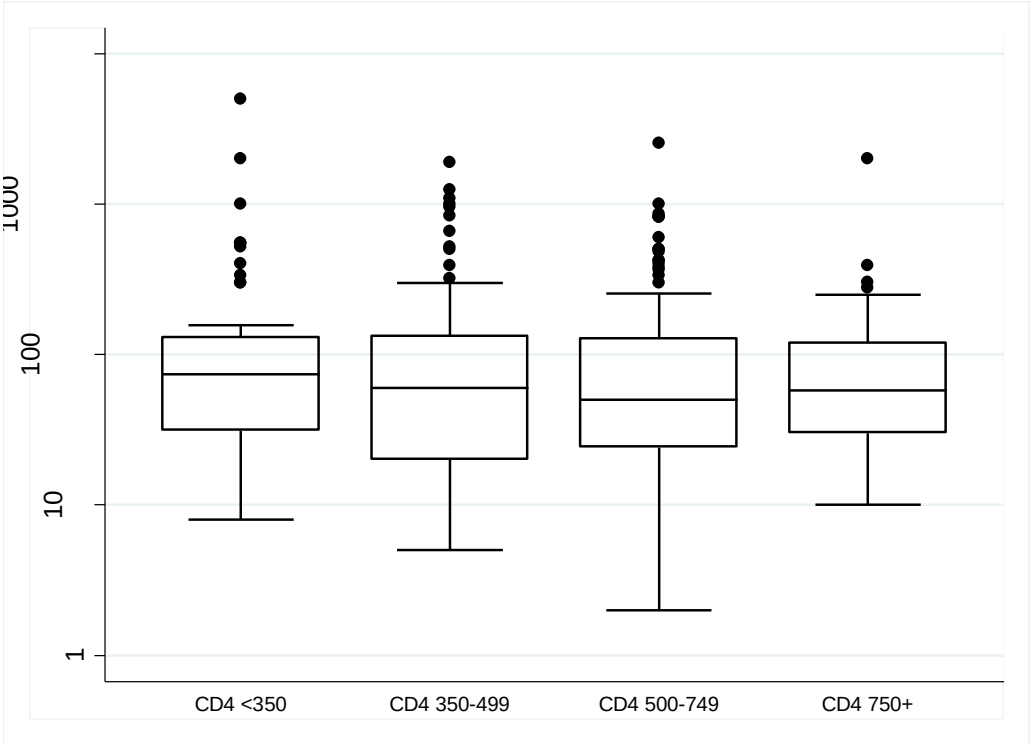


Table 1: Association of CD4 count (at infection) with malaria in the placebo arm

	Median value	Rate/100 person yrs (95% CI) [1]	Crude rate ratio (95% CI)[1]	Adjusted rate ratio (95% CI) [1,2]	Adjusted rate ratio (95% CI) [1,3]
CD4 count at infection[4]					
Clinical malaria					
			P=0.60	P=0.81	P=0.56
<300	262	14.6 (11.2-19.0)	1	1	1
300-399	355	13.4 (11.0-16.3)	0.9 (0.7-1.1)	0.9 (0.7-1.2)	0.9 (0.7-1.2)
400-499	448	13.1 (11.1-15.5)	0.9 (0.7-1.2)	0.9 (0.7-1.2)	0.9 (0.6-1.3)
500-599	547	13.5 (11.5-15.9)	0.9 (0.7-1.3)	0.9 (0.6-1.3)	0.9 (0.6-1.4)
600-699	644	14.2 (11.7-17.1)	1.0 (0.7-1.4)	0.9 (0.6-1.5)	1.0 (0.6-1.6)
≥700	836	15.5 (12.7-19.0)	1.1 (0.8-1.5)	1.0 (0.6-1.7)	1.2 (0.7-1.9)
Baseline factors					
Site			P<0.001	P<0.001	P=0.002
Entebbe		10.6 (8.8-12.8)	1	1	1
Masaka		17.2 (14.9-19.9)	1.6 (1.3-2.1)	1.5 (1.2-1.9)	1.5 (1.1-1.9)
Age[4]			P=0.07	P=0.04	P=0.04
<35 years	31	12.4 (10.0-15.2)	1	1	1
35-44 years	39	15.1 (12.8-17.9)	1.2 (1.0-1.5)	1.2 (1.0-1.5)	1.2 (1.0-1.5)
≥45 years	50	14.0 (11.5-17.1)	1.1 (0.8-1.5)	1.1 (0.8-1.4)	1.1 (0.8-1.5)
Sex			P=0.83	P=0.46	P=0.63
Male		14.4 (11.4-18.1)	1	1	1
Female		13.9 (12.2-16.0)	1.0 (0.7-1.3)	0.9 (0.7-1.2)	0.9 (0.7-1.2)
SES			P<0.001	P=0.002	P=0.003
Low		16.1 (13.6-19.0)	1.8 (1.3-2.4)	1.7 (1.3-2.4)	1.7 (1.2-2.3)
Middle		15.7 (12.8-19.1)	1.7 (1.2-2.4)	1.6 (1.1-2.2)	1.6 (1.1-2.2)
High		9.0 (6.9-11.8)	1	1	1
Baseline CD4 count[4]			P=0.63	P=0.88	P=0.98
<350	310	13.3 (10.4-16.9)	1	1	1
350-499	422	13.1 (10.7-16.1)	1.0 (0.7-1.3)	1.0 (0.7-1.3)	1.0 (0.7-1.4)
≥500	634	14.7 (12.1-18.0)	1.1 (0.8-1.5)	1.0 (0.8-1.4)	1.0 (0.6-1.5)
Factors during follow-up					
Time since enrolment			P<0.001		P<0.001
<1 year		17.3 (14.8-20.1)	1.9 (1.4-2.6)		1.9 (1.4-2.7)
1-2 years		13.1 (10.8-15.7)	1.4 (1.0-2.0)		1.5 (1.0-2.1)
≥2 years		9.0 (6.7-12.1)	1		1
BMI (kg/m^2)			P=0.01		P=0.02
<18.5		10.0 (6.9-14.4)	0.6 (0.4-0.9)		0.6 (0.4-0.9)
18-24.9		15.8 (13.8-18.1)	1		1
≥25		11.6 (8.9-15.0)	0.7 (0.5-1.0)		0.8 (0.6-1.0)
Bednet use			P=0.05		P=0.14
≥90% of visits		13.2 (11.6-15.1)	1		1
<90% of visits		17.5 (13.7-22.3)	1.3 (1.0-1.7)		1.2 (0.9-1.6)

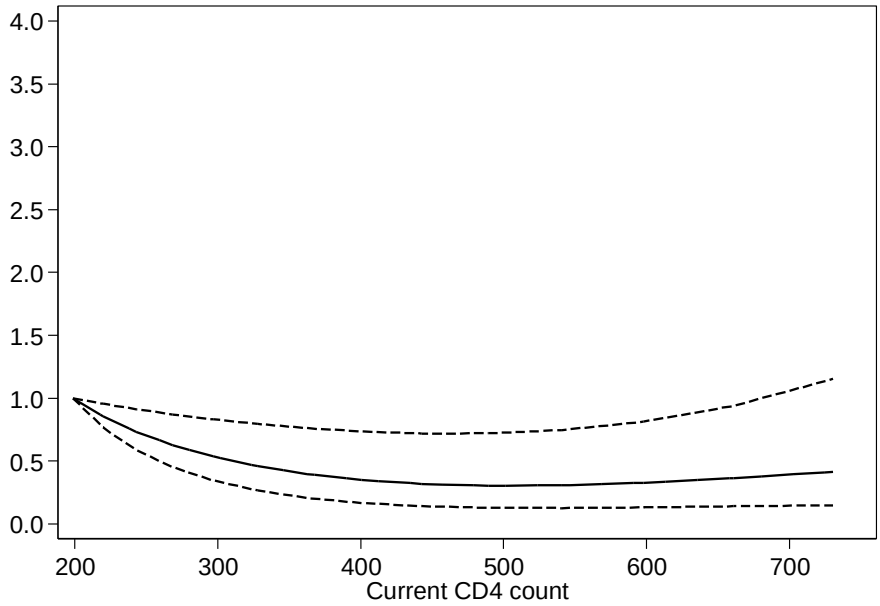
[1]Rates and rate ratios estimated from random effects Poisson regression. [2]Adjusted for enrolment site, age at enrolment, sex, SES and baseline CD4 count. [3]Adjusted for all covariates in footnote 2, and time since enrolment, current BMI and bednet use. [4]Continuous covariates (CD4 count and age) were modelled by restricted cubic splines with 4 knots. Rates are estimated at the median value in each range; the median value in the lowest range is used as the reference to estimate the rate ratios. P-value is for overall association with covariate from likelihood ratio test.

Table 2: Effect of trial drug on malaria, by CD4 count at enrolment, ART initiation and at the time of malaria episode

Trial arm	Stratum	Episodes	Person years	Rate/100pyrs (95% CI) [1]	Rate ratio[2]	P-value[3]
CD4 count at ART initiation						
CTX	<100	30	698	4.0 (2.8-5.9)	1	0.99
	100-249	59	1461	3.9 (3.0-5.1)	1.0 (0.6-1.5)	
	250+	10	229	3.9 (2.0-7.4)	1.0 (0.5-2.0)	
Placebo	<100	92	709	12.4 (9.8-15.7)	1	0.73
	100-249	196	1458	13.4 (11.4-15.7)	1.1 (0.8-1.4)	
	250+	36	212	14.7 (10.1-21.5)	1.2 (0.8-1.9)	
CD4 count at enrolment						
CTX	<350	14	416	3.5 (2.0-5.9)	1	0.51
	350-499	32	900	3.4 (2.4-4.8)	1.0 (0.5-1.9)	
	500+	57	1234	4.3 (3.3-5.7)	1.3 (0.7-2.3)	
Placebo	<350	58	453	13.1 (9.8-17.5)	1	0.98
	350-499	117	850	13.6 (11.1-16.7)	1.0 (0.7-1.5)	
	500+	175	1211	13.4 (11.3-16.0)	1.0 (0.7-1.4)	
CD4 count at infection						
CTX	<350	21	4618	4.4 (2.9-6.9)	1	0.52
	350-499	30	8811	3.3 (2.3-4.7)	0.7 (0.4-1.3)	
	500+	52	1197	4.0 (3.0-5.4)	0.9 (0.5-1.5)	
Placebo	<350	60	390	14.6 (11.1-19.2)	1	0.32
	350-499	98	807	11.8 (9.5-14.6)	0.8 (0.6-1.1)	
	500+	192	1317	14.1 (12.0-16.5)	1.0 (0.7-1.3)	

[1]Marginal means from random effects Poisson regression model with fixed effects for CD4 count stratum, treatment arm and their interaction, and site and year since enrolment. [2]rate ratio for effect of treatment arm in each CD4 count stratum, adjusted for site and year since enrolment, from random effects Poisson regression model. [3]P-values for overall association of CD4 count with malaria incidence within each treatment arm. P-values for interaction between CD4 count and treatment arm: CD4 count at ART initiation P=0.87; CD4 count at enrolment P= 0.60; CD4 count at infection P=0.96.

Supplementary figure 1: Rate ratio and 95% confidence interval for change in incidence of severe malaria with CD4 count at infection during follow-up modelled using restricted cubic splines with 3 knots in a Poisson regression model (Note: no participant had more than one event so random effects not included).



Supplementary Table 1: Baseline characteristics by trial arm and site

	Overall (N=2180)		Site					
			Entebbe			Masaka		
	CTX (N=1089)	Placebo (N=1091)	CTX (N=501)	Placebo (N=501)	Total (N=1002)	CTX (N=588)	Placebo (N=590)	Total (N=1178)
Age								
Mean (SD)	41.0 (8.0)	40.7 (8.3)	40.2 (7.9)	39.9 (8.1)	40.1 (8.0)	41.7 (8.1)	41.3 (8.5)	41.5 (8.3)
Sex								
Male	286 (26.3)	283 (25.9)	130 (26.0)	125 (25.0)	255 (25.5)	156 (26.5)	158 (26.8)	314 (26.7)
Female	803 (73.7)	808 (74.1)	371 (74.0)	376 (75.0)	747 (74.6)	432 (73.5)	432 (73.2)	864 (73.3)
Socio-economic status								
Low	486 (44.6)	474 (43.4)	197(39.3)	213 (42.5)	410(40.9)	289 (49.1)	261 (44.2)	550 (46.7)
Medium	318 (29.2)	329 (30.2)	117 (23.4)	113 (22.6)	230(23.0)	201 (34.2)	216 (36.6)	417 (35.4)
High	285 (26.2)	288 (26.4)	187 (37.3)	175 (33.9)	362(36.1)	98 (16.7)	113 (19.2)	211 (17.9)
CD4 count at enrolment								
<350	181 (16.6)	201 (18.4)	110 (22.0)	110 (22.0)	220 (22.0)	71 (12.1)	91 (15.4)	162 (13.8)
350-499	394 (36.2)	371 (34.0)	182 (36.3)	189 (37.7)	371 (37.0)	212 (36.1)	182 (30.8)	394 (33.4)
≥ 500	514 (47.2)	519 (47.6)	209 (41.7)	202 (40.3)	411 (41.0)	305 (51.9)	317 (53.7)	622 (52.8)
ART regimen at enrolment[1]								
NNRTI	1021 (95.1)	1025 (94.9)	4532 (92.6)	449 (91.5)	902 (92.0)	568 (97.1)	576 (97.8)	1144 (97.4)
NRTI	11 (1.0)	11 (1.0)	11 (2.3)	11 (2.2)	22 (2.2)	0	0	0
PI	42 (3.9)	44 (4.1)	25 (5.1)	31 (6.3)	56 (5.7)	17 (2.9)	13 (2.2)	30 (2.6)
Years on ART[1]								
< 1	73 (6.7)	82 (7.5)	46 (9.2)	43 (8.6)	89 (8.9)	27 (4.6)	39 (6.6)	66 (5.6)
1 to 2	144 (13.2)	160 (14.7)	71 (14.2)	79 (15.8)	150 (15.0)	73 (12.4)	81 (13.7)	154 (13.1)
2 to 5	508 (46.7)	480 (44.0)	261 (52.0)	255 (50.9)	516 (51.5)	247 (42.0)	225 (38.2)	472 (40.1)
>5	364 (33.4)	369 (33.8)	123 (24.6)	124 (24.7)	247 (24.6)	241 (41.0)	245 (41.5)	486 (41.3)
CD4 count at ART initiation[2]								
<100	292 (28.7)	310 (30.2)	135 (28.8)	139 (28.5)	274 (28.6)	157 (28.7)	171 (31.7)	328 (30.1)
100-249	619 (60.9)	621 (60.4)	296 (63.1)	314 (64.3)	610 (63.7)	323 (58.9)	307 (56.8)	630 (57.9)
≥ 250	106 (10.4)	97 (9.4)	38 (8.1)	35 (7.2)	73 (7.6)	68 (12.4)	62 (11.5)	130 (12.0)

[1]Missing data on ART regimen at enrolment for 26 participants (15 on CTX and 11 on placebo). [2]Missing data on CD4 count at ART initiation for 135 participants, 72 on CTX and 63 on placebo.

Chapter 7: Effect of antiretroviral therapy on malaria incidence in HIV-infected Ugandan adults: a prospective cohort study.

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

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SECTION A – Student Details

Student	Ronnie Kasirye
Principal Supervisor	Heiner Grosskurth
Thesis Title	Effect of cotrimoxazole on malaria in HIV-infected adults on antiretroviral therapy

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	AIDS
Please list the paper's authors in the intended authorship order:	Ronnie Kasirye, Heiner Grosskurth, Paula Munderi, Jonthan Levin, Zacchaeus Anywaine, Andrew Nunn, Anatoli Kamali, Kathy Baisley
Stage of publication	**Submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I conceived the study after discussions with Paula Munderi (PM), Jonathan Levin (JL) and Heiner Grosskurth (HG). I was the study coordinator from 2010 to 2012. I performed the analysis under the supervision of Kathy Baisley (KB). I wrote the first draft and revised the draft after comments from KB

	and HG. I made further revisions based on comments from the other COSTOP trial co-investigators. I submitted the paper for publication.

Student Signature: _____ **Date:** 30/03/16.

Supervisor Signature: _____ **Date:** 1/4/16

Concise communication

Effect of antiretroviral therapy on malaria incidence in HIV-infected Ugandan adults: a prospective cohort study.

R.Kasirye[1, 2], H. Grosskurth[1,2], P. Munderi[1], J. Levin[1,3], Z. Anywaine[1], A. Nunn[4], A. Kamali[1], K.Baisley[2]

1 MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda
2 London School of Hygiene and Tropical Medicine, London, UK
3 School of Public Health, University of Witwatersrand, Johannesburg, South Africa
4 MRC Clinical Trials Unit at University College London, UK

Abstract

Introduction

Using the data of a trial on cotrimoxazole cessation (COSTOP), we investigated the effect of different ART regimens on the incidence of clinical malaria.

Methods

During the COSTOP trial (ISRCTN44723643), HIV-infected Ugandan adults who had a CD4 count of ≥250 cells/μl were randomised to receive either cotrimoxazole (CTX) prophylaxis or matching placebo and were followed for a median of 2.5 years. Blood slides for malaria microscopy were examined at enrolment, monthly for the first three months and three-monthly thereafter. Malaria was defined as fever with a positive blood slide. ART regimens were categorized as nucleoside reverse transcriptase inhibitor (NRTI)-only, non-nucleoside reverse transcriptase inhibitor (NNRTI)-containing or protease inhibitor (PI)–containing. The incidence of malaria was calculated using random effects Poisson regression to account for clustering of events.

Results

Malaria incidence in the 3 ART regimen groups was 9.3 (8.3-10.4), 9.9 (3.6-27.4) and 3.5 (1.6-7.6) per 100 person-years (pyrs), respectively. Incidence in the PI group was significantly lower than that in the other groups (adjusted RR 0.4, 95%CI=0.2-1.0, comparing with NNRTI-regimens). Stratification by CTX/placebo use gave

similar results, without evidence of an interaction between the effects of CTX/placebo use and ART regimen.

Conclusion

PI-containing ART regimens were associated with a lower incidence of clinical malaria compared to NNRTI-containing or NRTI-only regimens and this effect was not modified by cotrimoxazole use. This observation may be of importance when selecting ART regimens for patients from malaria endemic areas.

Short title: Effect of ART regimen on malaria

Key words: Malaria, HIV, antiretroviral therapy, cotrimoxazole

Introduction

Antiretroviral therapy (ART) is used to control HIV replication in infected patients (1, 2). In addition, some ART drugs, particularly protease inhibitors (PIs), have shown anti-malaria properties *in vitro* (3-5) and among children (6, 7). However, a study of HIV infected adult women found no beneficial effect of lopinavir/ritonavir compared to nevirapine (NVP) on the incidence of malaria (8). Other studies on PI use in the general adult population are lacking.

Cotrimoxazole (CTX), a broad spectrum antimicrobial agent, is routinely used to prevent opportunistic infections and malaria in HIV infected patients (9). Its use in HIV infected patients who are stable on ART was recently evaluated in the (COSTOP trial; ISRCTN44723643), a placebo controlled trial to investigate the safety of stopping CTX among adults who are stable on ART (10, 11). In the study presented here, we used the COSTOP trial data set to assess whether malaria incidence differed between participants receiving different ART regimens and whether any such effects were modified by CTX use.

Methods

This study was conducted among participants enrolled in the COSTOP trial at two sites in Uganda (ISRCTN44723643). The trial has been described previously (10, 11). In brief, this randomized, double-blind, placebo controlled non-inferiority trial was conducted between 2011 and 2014 to determine whether long-term prophylaxis with CTX can be safely discontinued among HIV infected adults on ART with CD4 counts ≥250 cells/μl. Participants were randomised to receive either CTX or placebo. At enrolment, eligible participants were aged 18 years or older and clinically asymptomatic.

Participants were followed-up every month for the first three months and three-monthly thereafter, for 12 months to 3.5 years, depending on date of enrolment. Participants were encouraged to return also if they felt unwell. At enrolment participants were provided with an insecticide-treated bed net (ITN) and educated about the importance of using it. Blood samples were drawn at all visits for malaria microscopy, and for CD4 count, and full blood count at routine visits. Participants

who had been treated for malaria elsewhere (for example, during a journey) were asked to present documentary evidence of diagnoses and test results.

Information on participants' ART regimens at ART initiation and at enrolment was obtained from the records of the 2 NGOs and 4 hospitals in the study area that provided ART and from where participants were recruited. During the trial, participants continued to receive ART from their usual providers, but trial staff ensured an uninterrupted supply of ART in case of unexpected shortages. Most participants were on a non-nucleoside reverse transcriptase inhibitor (NNRTI)-containing regimen (the recommended first line regimen in Uganda) (12). Some participants were on a 1st line nucleoside reverse transcriptase inhibitor (NRTI)-only regimen of zidovudine/lamivudine/tenofovir (13). A few participants were on PI-containing regimens, which is the recommended second-line therapy (12).

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Blood samples were used to prepare thick and thin films on a glass slide. The specimens were processed using Leishman's stain and examined by microscopy. Venous blood samples were taken for CD4 cell counts and measured using a FACS-count system (Becton-Dickinson, San Jose, USA).

Analysis

Data were double-entered and verified in MS Access and analyzed using Stata statistical software (release 13, College Station, Texas StataCorp LP).

Person years (pyrs) at risk were calculated from enrolment until the date last seen or end of trial. After each malaria episode, participants were considered to be not at risk for another episode until the episode resolved, or for 28 days, if a resolution date was not available. The incidence of malaria and rate ratios comparing ART regimens were calculated using random effects Poisson regression to account for multiple episodes within the same participant. Since we have previously shown that malaria incidence varied between enrolment sites and over time (10), models were adjusted for treatment arm (CTX or placebo), enrolment site, and time since enrolment as a-priori confounders. ART regimens were categorized as follows: NRTI only, if a regimen containing only NRTIs was used; NNRTI containing, if one of the drugs was an NNRTI and none was a PI; or PI containing, if at least one drug was a PI. ART regimen was analysed as a time-updated exposure, to take into account participants

who switched regimens during follow-up. The effect of ART regimen on malaria incidence was examined overall and separately for both treatment arms (CTX and placebo groups); stratified rate ratios were obtained from a model containing a term for interaction between treatment arm and ART regimen.

Results

Baseline characteristics have been described previously (10). Briefly; 2180 participants were enrolled into the trial; with 1002 and 1178 at each site respectively. Half (1089) were randomised to CTX, 382 (18%) had a CD4 count between 250 and 350 cells/μl, and 569 (26%) participants were male. 1721 (79%) participants had been on ART for 2 years or more. At the time of ART initiation, 2114 (97%) were started on an NNRTI-containing regimen, 58 (3%) on an NRTI-only regimen and 3 on a PI-containing regimen; data were missing for 5 participants. At enrolment, 2046 (94%) were on an NNRTI containing regimen, 22 (1%) on an NRTI-only regimen and 86 (4%) were on a PI- containing regimen; for 26 participants (1%) enrolment ART information was missing. Of those on a PI-containing regimen; 75 (87%) were on lopinavir/ritonavir, 4 (5%) on atanazavir and 7 (8%) on another PI. 10 participants changed ART regimen during follow-up (9 NNRTI-containing, 1 NRTI-only); all changes were to a PI-containing regimen. Of the participants who changed to a PI during follow up; 6 changed to lopinavir/ritonavir, 3 to atanazavir and 1 to other PI.

Effect of ART regimen

2154 participants contributed data to the analysis; and 447 malaria episodes were observed in 4989 pyrs of follow up.

In the unadjusted analysis, malaria incidence was similar for participants on NRTI-only compared to participants on the NNRTI-containing regimen, and was lower for participants on a PI regimen compared to participants on an NNRTI (Table). After adjustment for treatment arm, enrolment site and time since enrolment, malaria incidence among participants on an NRTI-only regimen was 1.6 (0.6-4.3) times higher than among those on an NNRTI-containing regimen, whilst that among participants on a PI-containing regimen was 0.4 (0.2-1.0) times lower (P=0.05; Table).

In the adjusted analysis stratified by treatment arm, malaria incidence among participants on an NRTI-only regimen was 1.5 (0.2-11.8) (CTX) and 1.6 (0.5-5.0) (placebo) times higher than those on an NNRTI-containing regimen, whilst that in participants on a PI-containing regimen was 0.5 (0.1-2.3) (CTX) and 0.4 (0.2-1.0) (placebo) times lower, respectively. There was no evidence of interaction between the effect of treatment arm and ART regimen (P=0.95; Table).

Discussion

PI- containing regimens are recommended as second line therapy in Uganda and elsewhere (12, 14) and were used by up to 4% of participants in this study. The most commonly used PI was lopinavir/ritonavir. 1% of participants were on an NRTI-only regimen, an alternative initial regimen recommended at the time (2, 15).

We found that NRTI-only regimens provided the least protection against malaria followed by NNRTI-containing regimens. This is consistent with *in vitro* studies that showed no antimalarial activity from NRTIs and some activity from NNRTIs but at levels which were not achievable in-vivo at standard dosing (3). A PI-containing regimen offered the strongest protection (RR 0.4; 95%CI=0.2-1.0, compared with NNRTI-containing regimens) and this is consistent with some of the previous studies (6, 7). The effect of PIs on malaria was not modified by CTX.

These findings have potential public health implications. It has been suggested that the antimalarial prophylactic effect of PI-containing ART regimens could reduce the cost of care in malaria endemic countries due to a potential reduction in malaria treatment costs (16), could help reduce the prevalence of malaria (17) and could even contribute to malaria eradication because lopinavir inhibits plasmodium falciparum liver stage parasites (18).

Strength and Limitations

Our study benefited from a well described study population that was followed for up to 3 years and routinely assessed for malaria. However, whilst this offered an opportunity to investigate the effect of ART regimen on malaria, the COSTOP trial was not specifically designed to address this question. The number of patients in the different ART groups differed largely, limiting comparability between groups. Due to

the small number of participants on PIs, the observed protective effect just reached statistical significance, and therefore we cannot exclude the possibility that the effects might have occurred due to chance. Adherence to ART in our study population was high (10). However, as we did not determine viral load or serum levels of ART drugs during follow up, our findings may be subject to potential residual confounding resulting from differences in adherence between ART groups.

Conclusion

Among adult HIV infected individuals on ART medication, PI- containing ART regimens were associated with a reduced incidence of clinical malaria compared to NNRTI-containing or NRTI-only regimens. The anti-malarial properties of PIs may have clinical and public health importance.

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Table: Incidence of malaria by ART regimen overall and stratified by treatment arm

	ART regimen[1]	Events	Person years	Rate[2]	Rate ratio[2]	Rate ratio[2,3]
					P= 0.02[4]	P=0.05[2,4]
	NNRTI containing	435	4737	9.3 (8.3-10.4)	1	1
	NRTI only	5	50	9.9 (3.6-27.4)	1.1 (0.4-3.0)	1.6 (0.6-4.3)
	PI containing	7	202	3.5 (1.6-7.6)	0.4 (0.2-0.8)	0.4 (0.2-1.0)
Stratified analysis					P=0.92[5]	P=0.95[5]
					P=0.62[6]	P=0.64[6]
CTX	NNRTI containing	97	2380	4.1 (3.3-5.0)	1	1
	NRTI only	1	25	4.2 (0.5-32.1)	1.0 (0.1-8.0)	1.5 (0.2-11.8)
	PI containing	2	100	2.0 (0.5-8.2)	0.5 (0.1-2.0)	0.5 (0.1-2.3)
					P=0.07[6]	P=0.11[6]
Placebo	NNRTI containing	338	2357	14.5 (12.9-16.4)	1	1
	NRTI only	4	25	15.4 (4.9-48.0)	1.1 (0.3-3.3)	1.6 (0.5-5.0)
	PI containing	5	102	5.0 (2.0-12.4)	0.3 (0.1-0.9)	0.4 (0.2-1.0)

[1]NNRTI containing was defined as a regimen that contained at least one NNRTI and no PI; NRTI only was defined as a regimen containing only NRTIs; PI containing was defined as a regimen in which at least one of the drugs was a PI. [2]estimated from random effects Poisson regression. [3]adjusted for treatment arm, site, and time since enrolment. [4]P-value for effect of ART regimen, from likelihood ratio test (LRT). [5]LRT for interaction between treatment arm and ART regimen.[6]P-value from the Wald test

Chapter 8: Effect of cotrimoxazole on CD4 count (unpublished research result)

8.1 Introduction

CTX use is associated with haematological toxicity (1, 2). We assessed the effect of trial arm (active CTX versus placebo) on CD4 count. This analysis was done as part of the work in chapter 5. However, it was not included in the submitted version of the paper because it was decided within the team of co-investigators to publish these findings in a separate future paper. I was encouraged though to include this analysis in the thesis.

8.2 Methods

The general methods have been described in chapter 2, but specifically for this analysis, the effect of trial arm on current CD4 count as the outcome was assessed using random effects linear regression. CD4 counts were log transformed for analysis. Models were adjusted for enrolment site, age, sex, baseline socioeconomic status (SES), time since enrolment, current BMI and current haemoglobin as a priori confounders. Continuous covariates were modelled using restricted cubic splines with 4 knots.

8.3 Results

During follow up, CD4 count progressively increased from week 12 to week 144 (P<0.001) (Figure 6; Table 4). This increase was significantly lower among participants allocated to continued CTX medication: the geometric mean CD4 during follow up in the CTX arm was 480 cells/μl vs.504 cells/μl on placebo (P<0.001). After adjusting for CD4 count at baseline, participants in Masaka had a lower geometric mean CD4 count during follow up compared to those in Entebbe (P<0.001). There was strong evidence of interaction between enrolment site and treatment arm: the increase in log CD4 counts on placebo was greater in Entebbe than in Masaka (0.07, 95%CI=0.05-0.09 vs 0.03, 95%CI=0.01-0.05, respectively; P-value for interaction=0.004).

8.4 Discussion

CTX has been shown to slow the rate of CD4 decline in individuals who are not on ART (3); however, in the COSTOP trial that enrolled individuals who were stable on ART, we found that participants on CTX had lower CD4 counts during follow up than those on placebo and that the further increase of CD4 counts over time (that continued as expected on ART) was lower in the CTX arm than in the placebo arm. This is consistent with observations made among HIV infected adults enrolled in the DART trial in Uganda and Zimbabwe (4). The most likely explanation for these observations is the known haematotoxic property of CTX (1, 2). We also found a lower average CD4 count increase in participants on placebo at the Masaka site than in Entebbe. Participants at the Masaka site had lower social economic status (which could be associated with poorer nutrition) and a higher incidence of malaria compared to those at the Entebbe site, possibly as a result of increased environmental exposure (5), which may explain some of the difference in CD4 count response.

The clinical implication of this observation at the individual level is not clear. At the population level, the negative effect of CTX seems to be outweighed by the benefits of CTX medication, at least in the COSTOP study population (6).

In summary, prophylactic CTX medication in adults who are stable on ART is associated with lower CD4 counts and a reduced increase of CD4 counts over time, compared to individuals who do not take CTX. This is probably a result of the haematotoxic effects of CTX. The overall benefits of CTX prophylaxis seem to justify CTX continuation. Further research would be needed to determine whether there may be subgroups of HIV infected patients on ART who would fare better without CTX e.g. individuals with evidence of higher degrees of haematotoxic side effects due to ART.

8.5 References

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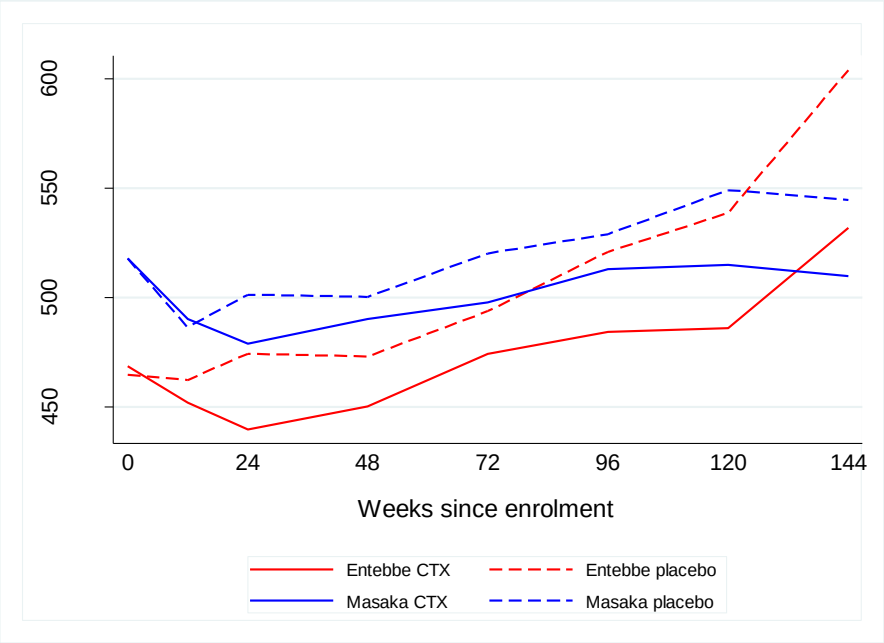

Figure 6: CD4 counts during follow-up, by treatment arm and enrolment site

Table 4: Association between CD4 count and treatment arm, enrolment site and with time since enrolment

	Mean log CD4 (95% CI) [1]	**Geometric mean CD4 (95% CI)** [1]	**Difference in log CD4 (95% CI)** [2]
Treatment arm			
			P<0.001
CTX	6.17 (6.16, 6.19)	480 (475, 485)	Reference
Placebo	6.22 (6.21, 6.23)	504 (499, 509)	0.05 (0.03, 0.06)
Enrolment site			
			P<0.001
Entebbe	6.21 (6.20, 6.23)	500 (494, 506)	Reference
Masaka	6.18 (6.17, 6.19)	485 (480, 490)	–0.03 (–0.05, –0.01)
Weeks since enrolment[3]			
			P<0.001
0-12	6.17 (6.16, 6.18)	480 (475, 485)	0.005 (–0.004, 0.01)
12-24	6.17 (6.16, 6.18)	478 (473, 482)	Reference
24-48	6.18 (6.17, 6.19)	484 (479, 488)	0.01 (0.007, 0.02)
48-96	6.23 (6.22, 6.24)	506 (502, 512)	0.06 (0.05, 0.07)
96-144	6.26 (6.24, 6.28)	523 (515, 532)	0.09 (0.08, 0.11)

[1]marginal means from random effects linear regression model with fixed effects for treatment arm, enrolment site, baseline CD4 count, weeks since enrolment, age, sex, current BMI, current haemoglobin, and SES at enrolment. Continuous covariates (baseline CD4 count, weeks since enrolment, age, and haemoglobin) modelled using restricted cubic splines with 4 knots. [2]estimated from regression model in footnote 1. [3]means are estimated at 12, 24, 48, 96 and 144 weeks, respectively; 24 weeks is used as the reference point for the calculation of the change in log CD4.

Chapter 9: Discussion

9.1 Work that led to this PhD research

Based on studies that showed a reduction in OI and malaria when CTX is used (1-5), CTX has been recommended and is routinely used for the prophylaxis of OI and malaria in ART naïve HIV-infected adults (6). Subsequently, it has also been widely used among patients receiving ART whether or not their health has improved. However, there has been inconclusive evidence as to whether, in patients whose immunity has recovered and are stable on ART, CTX could be stopped (7).

The overall aim of this PhD was to investigate the effect of CTX on malaria incidence in HIV-infected adults who are stable on ART. To assess this association, I conducted a systematic review of the available literature and, using the opportunity provided by a randomised, placebo-controlled trial of CTX cessation (COSTOP); I examined the incidence of malaria and assessed the effect of CD4 count and of ART regimen on malaria by CTX use.

The findings of the studies in this thesis should help improve our understanding of:

- The effect of CTX on malaria in HIV-infected adults on ART; using a systematic review I found that there was limited literature on this effect, the majority of the reviewed studies found CTX use to be associated with reduced occurrence of malaria
- The incidence of malaria in HIV-infected patients that are stable on ART once prophylactic CTX is stopped; using a placebo randomised controlled trial, I found that the incidence of malaria was higher in individuals who stopped CTX, however, the effect of stopping CTX was lower than reported by previous open-label studies.
- The antimalarial effect of ART regimen on malaria in adults and whether there is an interaction with CTX use; I found that malaria incidence was reduced among patients on a PI-containing regimen but this effect did not vary with CTX use

- The effect of CD4 count on malaria and whether it varies with CTX; I found no evidence of an effect of CD4 count on malaria incidence irrespective of CTX use

This chapter summarises the main findings, discusses the strengths and weaknesses of the work done, and provides an overview on on-going and future research and makes concluding remarks.

9.2 Summary of results

9.2.1 Systematic review

I searched the literature using terms for HIV, malaria and CTX only (without a term for ART) to reduce the chances of missing relevant papers on the association between CTX and malaria in patients on ART. However I identified only 6 studies: 4 observational studies and 2 randomised controlled trials (RCTs). Due to the diversity of the studies in the review, a meta-analysis to provide a point estimate of the effect of CTX on malaria in this population could not be done. Instead a qualitative synthesis of the selected studies was done, and the reviewed studies showed a trend towards a beneficial effect of CTX on malaria. The malaria in these studies was mostly mild. All reviewed studies were subject to bias and confounding. The RCTs were the only reviewed studies that were designed to investigate the association between CTX and malaria; however neither of them was blinded, and they were therefore prone to observer and reporting bias. I concluded that the current literature on the effect of CTX on malaria in patients on ART was limited in numbers, that the studies reviewed suffered from bias and confounding, and that their results were inconclusive. Also, the clinical relevance of malaria experienced by patients stable on ART who are not on CTX prophylaxis was not clear.

9.2.2 Experimental work

9.2.2.1 Incidence of malaria

Analyses of data collected in the COSTOP trial showed that participants on ART who stopped prophylactic CTX experienced 3.5 (95%CI=2.7-4.4) times more clinical malaria episodes than those who continued and this is consistent with other studies that found

more malaria episodes in participants who are not on CTX prophylaxis (8-11). However the difference in our study was much smaller than that reported by other randomised trials in adults (Campbell et al. RR 32.5, 95%CI=8.6-275.0 (9) and Polyak et al. RR = 33.0, 95%CI=4.5-241.0 (8)). It was similar to that reported by a study conducted in children (Bwakura-Dangarembizi et al. HR 2.2, 95%CI=1.5-3.3 (10)).

The contrasting results between our trial and the other two trials among adults could have resulted from a difference in follow-up times. This would be the case if there was a higher incidence of malaria during the first few months after stopping CTX (rebound effect) followed by a reduction with time as patients' immune response to malaria improves with increased exposure to malaria after stopping CTX. The Campbell et al. study (9) had a follow-up of only 4 months and the authors hypothesized that the observed high incidence of malaria could have indeed been due to a rebound effect of malaria once CTX had been stopped. Our study had a median follow-up time of 2.5 years and although clinical malaria incidence remained higher in the placebo arm, the difference between the arms was less in the third year (RR= 2.1, 95%CI=1.3-3.5) than in the first year (RR 3.6, 95%CI=2.6-5.0), P for interaction=0.10. Additionally, clinical malaria incidence in the placebo arm decreased from 17.3 per 100 pyrs in the first year to 9.0 per 100 pyrs after the second year (P for trend <0.001). In the Polyak et al. study (8) follow-up was for one year; malaria occurred throughout the study and authors did not find evidence of a rebound in the participants that discontinued CTX. It is, therefore, possible that some of the difference between our study and the others in adults was due to a difference in follow-up time although the evidence is inconclusive.

Another possible explanation might be the smaller number of malaria episodes (55 and 34, in the Campbell and Polyak studies respectively) compared to ours in which 453 episodes were observed. The plausibility of this explanation follows from the fact that in our study, the site with fewer malaria episodes (146 in Entebbe versus 307 in Masaka), in both trial arms and over time, observed a larger effect of stopping CTX (RR=6.8, 95%CI=4.1-11.1 versus RR=2.7, 95%CI=2.1-3.6, respectively), implying that the

number of episodes of malaria (which likely reflects the background prevalence of malaria) may play a role.

Furthermore, the incidence of malaria among participants randomised to stop CTX in our study was similar to that in the Polyak study (14.1 versus 13.0 episodes/100pyrs, respectively), however the incidence among participants randomised to continue CTX was lower in the Polyak study (4.1 versus 0.4 episodes/100pyrs), suggesting that adherence to CTX might have been better in the open-label study. This would have been the case if being on open-label active drug acted as motivation for better adherence, as has been suggested elsewhere (12). Adherence in both trial arms of our study, as measured by pill counts and self-report, was very high. Therefore, differences in adherence to CTX are unlikely to explain the difference in the effect of stopping CTX between our study and the Polyak study. Ascertainment of CTX use is discussed further in subsection 9.3.1.3.

Also, the observed differences in the effect of stopping CTX between our trial and the others in adults could have occurred if participants randomised to placebo took CTX from sources outside the study clinic. The results of exit interviews conducted at the end of the study suggest that this was not the case. This is discussed in more detail in subsection 9.3.1.3.

Importantly, our trial has been the only one so far that was double-blinded and placebo controlled. Unblinded trials may have been subject to bias as the investigators may have diagnosed malaria less frequently in participants who continued CTX, and participants may have reported febrile episodes less often, if either felt that continuing CTX reduced the risk of malaria. This bias may further explain the greater effect reported in the Campbell and Polyak studies.

9.2.2.2 Effect of CD4 count

We found evidence of continued recovery of the immune system in HIV-infected participants who are stable on ART as shown by an increase in CD4 counts over time, particularly in participants on placebo. However, we did not find evidence of the expected association between CD4 count and the incidence of clinical malaria, the degree of parasitaemia, or the incidence of severe malaria. This lack of an effect of CD4 count on malaria was observed regardless of the timing of CD4 counts: we observed it for CD4 count examined at the time of starting ART (considered a measure of the immune damage before ART initiation), for CD4 count at time of randomisation (indicating the immune status at beginning of the study) and for CD4 count at time of malaria episode. One possible explanation is that since all participants in the COSTOP trial had a CD4 count >250 cells/µl at enrolment, they may have been above the threshold below which CD4 count may significantly influence the risk of malaria. An alternative explanation could be that an improvement of CD4 cell quality rather than quantity under ART may be important for malaria containment and that this improvement occurs early once patients are started on ART (13). The lack of an association between malaria incidence and CD4 count was similar among participants randomised to stop prophylactic CTX and those who continued CTX.

9.2.2.3 Effect of ART regimen

Another factor that could potentially affect the incidence of malaria in patients on ART and CTX is the ART regimen (7). PIs are reported to have antimalarial effects (14, 15). This class of drugs is mainly recommended for second-line therapy, in Uganda and elsewhere (16, 17). When comparing PI-containing regimens and NRTI-only regimens with NNRTI-containing regimens, we found that malaria incidence among participants on an NRTI-only regimen was 1.6 (95%CI=0.6-4.3) times higher than among those on an NNRTI-containing regimen, whilst that among participants on a PI-containing regimen was 0.4 (95%CI=0.2-1.0) times lower (P=0.05). This is consistent with previous studies (14, 18). The effect of PIs on malaria was not modified by CTX use (P=0.95). The observed protective effect just reached statistical significance, probably due to the

small number of participants on PIs (4% at enrolment, N=86), and therefore the possibility that the effects might have occurred due to chance cannot be excluded. Although trial arm allocation was adjusted for in the analysis, the comparison by ART regimen was not randomised and so the possibility of residual confounding cannot be ruled out. Furthermore, because most of the participants receiving a PI-containing regimen were on second-line therapy; the immunological condition of participants on such medication is likely to have been worse than that of participants who were not on a PI-containing regimen. It is, therefore, possible that the pharmacological effect of PIs on malaria is stronger than we observed.

9.2.2.4 Non-malarial CTX preventable events and haematological adverse events

In the main COSTOP analysis we found that stopping CTX was associated with a significant increase in CTX-preventable clinical events, but also with a decrease in grade 3 or 4 haematological adverse events (19). We also found that continued CTX use was associated with a slower rise in CD4 cell counts which is likely to be due to the haematological toxicity associated with CTX (20, 21). Other randomised studies investigating the effect of stopping CTX have reported 1.6 (1.1-2.4) times higher rate of hospitalization or death (10), 1.8 (95%=CI 1.3-2.4) higher incidence of diarrhoea (9), but one study found no statistically significant difference between trial arms with respect to the rates of diarrhoea and pneumonia IRR=1.4 (95%=CI 0.8-2.3) and IRR 1.4 (95%=CI 0.5-3.8) respectively (8). Taken together, these results suggest that the advantages and disadvantages of continued CTX use have to be carefully weighed.

The potential public health impact of stopping CTX should also be taken into account.by policy makers when developing guidelines for CTX use. When calculating the number of patients needed to treat (NNT) with CTX prophylaxis, we found that to prevent one CTX preventable infection, one clinical malaria episode and one episode of severe malaria the NNT was 113, 10, and 233 respectively. This suggests that, at least in areas of substantial malaria endemicity such as Southern Uganda, the main benefit of continued CTX prophylaxis is the prevention of mild malaria, whilst the prevention of more serious

outcomes is substantially more cost-intensive. Also, we did not have a comparison with HIV-uninfected individuals therefore it is not clear how malaria incidence in those who stopped CTX compares to that in HIV-uninfected immune competent people.

9.2.2.5 Synthesis of results

Our study findings suggest that the higher the malaria endemicity, the less effect stopping CTX prophylaxis has on malaria incidence. The reason for this is not clear, one possible explanation is that repeat exposure in areas of high malaria endemicity sharpens the immune response to malaria and therefore prophylaxis has less of an impact. The other possible explanation is that high malaria endemicity may lead to widespread CTX use, especially in areas with high HIV prevalence, leading to development of resistance to CTX (antifolate resistance) and hence reducing the impact of stopping CTX. We do not have data on antifolate resistance in our study areas; however, CTX has been shown to be effective even in areas where there is resistance (22).

Another study finding is that among HIV-infected adults who are stable on ART, CD4 count is not a good predictor of the risk of malaria and should not be used in deciding who should stop CTX prophylaxis. This finding also suggests that CD4 count might not be useful when deciding who to put on CTX prophylaxis among individuals who start ART with high CD4 counts as is the case with the new test and treat recommendations (23). Viral load has been shown to have predictive value for opportunistic infections (24, 25). Therefore, viral load changes might give a better reflection of the immune status and better predict the risk of malaria among HIV-infected patients on ART.

Also, our finding that PIs are associated with reduced malaria incidence may have implications for the treatment of HIV in adults. WHO recommends first-line regimens containing two NRTIs and one NNRTI. These regimens are generally less expensive than other regimens, have generic formulations, are often available as fixed-dose combinations, do not require a cold chain and preserve PIs for second-line (26). These benefits, in spite of the antimalarial benefits of PIs, should still make NNRTI-based

regimens the preferred choice for first-line, even in malaria endemic areas. However PI-containing regimen should be considered for individuals, who might be particularly vulnerable for malaria for example non-immune migrants into a malaria endemic area,

Finally, the effects of discontinuing CTX in HIV-infected patients on ART may be considered at individual and population level. At the individual level, as we found in our study, discontinuing CTX leads to a higher incidence of CTX preventable events, lower incidence haematological adverse events and higher rise in CD4 count, this should be useful in patient management, for example, CTX should be stopped in patients with poor CD4 count response or continued in individuals at high risk of malaria. At the population level, widespread use of CTX should result in lower prevalence of malaria due to its antimalarial properties and although CTX use is beneficial even in areas of widespread antifolate resistance (22), with continued large-scale use, the prevalence of antifolate resistance will most likely increase and eventually result in CTX losing most of its potency as an antimicrobial drug.

9.3 Strengths and limitations

9.3.1 Exposure ascertainment

9.3.1.1 CD4 counts

CD4 count at the time of starting ART was extracted from patients' records that we obtained from their ART providers. Whilst we could not be sure that these records were accurate, they offered the best information possible to assess our patients' immune status at that time. Of note, viral load is not measured in any routine HIV care programme in Uganda. CD4 count at enrolment was based on the average of two pre-enrolment CD4 counts that had been done no more than 6 months apart, the most recent of which within 4 weeks of enrolment: this is likely to have been a good reflection of participants' immune status at enrolment. During the course of the trial, participants' CD4 counts were measured at 3 months and then 6-monthly. At the time of a malaria episode, the most recent CD4 count was used to reflect the immune status of a participant at that time with the assumption that this was still a good reflection of the

current situation. This assumption might not have been totally accurate. Any misclassification would have been non-differential, so would have biased the effect towards the null. However, analyses of the effect of CD4 count at enrolment and at ART start gave similar results, with RR very close to 1.

9.3.1.2 ART regimen

The ART regimen at start of treatment and any changes before joining the trial were recorded on case record forms from the ART providers' records. Most of this information was readily available in their records and predictable because first-line ART was generally in line with the recommended national guidelines (17). Some participants had been involved in an earlier (completed) trial comparing antiretroviral therapy monitoring strategies (DART) at the Entebbe site and had been started on a triple nucleoside regimen (27), information on the history of these participants was easily accessible. Any ART changes either for toxicity or for treatment failure during follow-up were made by the ART provider and the ART provider and/or the patient informed the trial team of such changes. Few participants (N=11, 0.5%) changed ART during follow-up and this is consistent with other studies (28). It is, therefore, unlikely that ART regimen was misclassified. There was also good adherence to ART as assessed by self-report. However, we did not monitor viral load during the trial or measure serum levels of ART drugs. Assessment of the effect of ART regimen on malaria was not a randomised comparison; therefore our findings may be subject to potential residual confounding resulting from possible differences in adherence or other differences between ART groups.

9.3.1.3 CTX

There was a risk that any truly existing association between CTX use and malaria incidence might be diluted if patients allocated to active CTX did not take it or if those allocated to placebo took CTX from other sources. The placebo and active drug were similar in colour, size, and shape to reduce participant bias. Although some researchers

felt that there was a slight difference in taste between active drug and placebo, most participants were not aware of a difference, as suggested by the trial exit interviews described below. Through regular talks, participants were encouraged to adhere to the allocated trial drug. Adherence to trial drug was measured by pill count and self-report, both of which have limitations (29-31). Other ways of measuring adherence to trial drug were also explored: application of medication event monitoring system (MEMS) and a test to identify CTX metabolites in either blood or urine. Both methods proved to be too costly. However, at study exit, we retrospectively assessed drug adherence during the trial using two complementary approaches: a questionnaire was administered to all exiting participants (1993 respondents); and the MRC Unit's social science team conducted individual qualitative (in-depth) interviews with a random selection of these participants. Both methods did not provide any evidence showing that adherence to trial medication had been seriously affected. For example, when asked about obtaining open label CTX from outside sources during the trial, only 92/1963 (4.7%) participants said they had done so (32). It is, therefore, unlikely that adherence problems had a significant effect on the results.

All analyses were based on an intention to treat approach. However, only a few participants (1.6% in each arm) had stopped trial drug and switched to open label CTX; usually due to a participants' CD4 count falling below 250 cells/μl, a level below which they were considered to be at an increased risk of OI. Also, for 79% and 76% of participants on CTX and placebo, respectively, adherence based on pill counts was >80% at each visit throughout the trial. Therefore, it is unlikely that an alternative on-treatment analysis would have given substantially different results.

9.3.2 Outcome ascertainment

9.3.2.1 Clinical malaria

Clinical malaria was defined as current fever or a recent history of fever combined with a positive malaria blood slide on microscopy, as is commonly used in other studies of

malaria (1, 22, 33). The personnel reading the slides comprised trained laboratory technicians working at the laboratories of the MRC/UVRI Uganda Research Unit on AIDS, located in Entebbe and Masaka. Microscopy is considered the gold standard for malaria diagnosis as it provides information on both the plasmodium species implicated and the level of parasitaemia (34), neither of which are available from serological tests. However, rapid diagnostic tests (RDTs) would have been of benefit as well because they are more sensitive at detecting plasmodium in blood than microscopy (35). However, doing both blood slides and RDTs would have significantly increased the costs of the study without much additional benefit, and was therefore not considered.

When looking at malaria parasitaemia (with or without malaria-related symptoms), participants who had stopped prophylactic CTX were 2.8 (95%CI=2.3-3.4) times more likely to be diagnosed with malaria than those who continued. When any symptom (whether thought to be malaria-related or not) combined with a positive slide was used to suspect malaria, participants who stopped CTX had 3.2 (95%CI=2.6-4.0) times more malaria episodes than those who continued. So even with alternative definitions of malaria, participants that continued CTX had a lower disease incidence than those who stopped; and rate ratios were similar to those found for clinical malaria.

9.3.2.2 Severe malaria

A diagnosis of severe malaria was made if a patient had *P. falciparum* asexual parasitaemia, no other obvious cause of symptoms and met any of the following criteria: convulsions, loss of consciousness, hypotension (systolic blood pressure <70 mmHg), admission to hospital due to malaria, laboratory evidence of liver or kidney damage, severe normocytic anaemia (haemoglobin <50g/dl, PCV<15%), or hyperparasitaemia on blood slide (>5% or 250,000/µl). We did not proactively investigate all malaria episodes for severity by screening for end organ damage, as this would have been very costly, but participants with symptoms of severity were investigated further. In view of this approach it is possible that some cases of severe malaria were missed. We also classified all malaria patients, irrespective of parasitaemia, that were admitted to

hospital as cases of severe malaria this may have led to misclassification of some cases of non-severe malaria.

9.3.3 Blinding

Our study was based on data from a double-blind placebo controlled trial: both the investigators and the participants were blinded to the allocated trial arm, which should have minimized reporter and observer bias. The intervention (CTX discontinuation) was individually randomised which resulted in a good balance of participants' characteristics between trial arms at baseline with respect to likely confounders and also to unknown confounders.

9.3.4 Sample size

Our trial had a sample size of 2180 participants followed up for a median of 2.5 years; with this large sample size and long observation time it was sufficiently powered to detect any relevant differences in malaria incidence between the trial arms.

9.3.5 Follow up

Participants were seen every month for the first three months and three monthly thereafter and were also asked to come to the study clinics any time that they felt unwell. At scheduled visits participants were screened for malaria (and at sick visits when suspected to have malaria) and had their adherence to ART and trial drug assessed. This allowed us to capture any new malaria episodes and to regularly assess participant's adherence to trial drug.

9.3.6 Study retention

Participant retention was high, with >85% completing follow-up and the loss to follow-up was balanced between trial arms: 10% versus 11% for CTX and placebo respectively. Our results are therefore unlikely to be biased due to differential loss to follow-up, which could have been the case if; for example, there were fewer events in the placebo arm because participants were lost due to illness, or fewer events in the CTX arm because participants on CTX were lost due to drug side effects.

9.3.7 Generalisability

Uganda has high to moderate levels of malaria endemicity (36). The results of this trial might not be generalizable to other parts of sub-Saharan Africa, especially with low intensity of malaria transmission.

9.3.8 Malaria exposure

Due to limited funding we were not able to use Global Positioning Systems to map participants' residences in relation to ecological exposure to malaria, nor to use antibody or antigen tests to measure ecological exposure to malaria e.g. among residents of the surrounding communities. Therefore, differences in malaria incidence between sub-groups of trial participants could possibly have been due to differences in exposure. However, the randomized design of the trial that was stratified by enrolment site and its large sample size should have resulted in a balance in malaria exposure and other potential confounders across trial arms. This applies to the first objective of this study (incidence of malaria by trial arm), but the non-randomised comparisons (effect of CD4 count on malaria and effect of ART regimen on malaria) might have been subject to this limitation.

9.3.9 Comparison with HIV-uninfected adults

We do not have data on malaria incidence in HIV-uninfected individuals from our study area. Our study, like the other randomised trials on the effect of CTX on malaria in patients on ART, compared malaria incidence (most common infection in this population in malaria-endemic areas) in participants who stopped CTX to that in participants who continued CTX. CTX has antimalarial properties and it is expected that individuals on CTX prophylaxis will have fewer malaria episodes. Therefore, it is not clear how malaria incidence in those who stopped CTX compares to that in HIV-uninfected immune competent people. Malaria incidence may not genuinely differ between these two populations; and in that case the continued use of CTX for malaria prophylaxis in immune competent patients may be questionable.

9.4 On-going work and future research

I am aware of only one on-going study in a malaria endemic setting that is investigating CTX discontinuation in HIV-infected adults that are stable on ART. In this trial (NCT01650558), the primary outcome is incidence of severe events (composite of death and WHO stage 3 and 4 illnesses), and secondary outcomes include incidence of detectable viral load, bacterial infections and malaria. The trial has three arms: i) continuing CTX, ii) discontinuing CTX (no placebo), and iii) discontinuing CTX and starting weekly chloroquine. The eligibility criteria include HIV-infected patients aged 18 years and above, with CD4 count >250cells/μl and undetectable virus (<400 copies/ml). The purpose of this study is to determine if there is a benefit of taking CTX as prophylaxis among HIV positive adults with viral suppression and a good clinical response to ART. The study will enrol up to 1500 adults to be followed for 32 to 66 months. This trial is an open-label trial and is, therefore, likely to be affected by reporting and observer bias. Nevertheless, it should shed light on the comparative effects of CTX and antimalarial drugs. In this context, the investigators state that they expect to determine whether among patients with successful viral suppression a classical anti-malarial prophylactic drug may be preferable over continued CTX medication (37).

The trial will collect information on viral load every 6 months, and this will provide an opportunity to investigate whether the degree of viral suppression has an effect on malaria incidence and therefore might help interpret our own finding of a lack of effect of CD4 count level on malaria incidence. Results of this trial are expected in 2019.

A number of questions remain unanswered and require further research:

a) Do patients who stop CTX have a higher incidence of malaria than would be observed in HIV-uninfected people?
b) Are there groups which do not require CTX prophylaxis at all such as recently infected patients who start ART whilst their immune system is still undamaged?

c) As additional studies will be published, what is the point estimate (and its 95%-confidence interval) of the effect of CTX prophylaxis on malaria in HIV-infected patients on ART in malaria endemic areas, based on a meta-analysis?

9.5 Conclusion

To date, the best approach to CTX use in HIV-infected adults living in areas that are endemic to malaria or that have a high bacterial disease burden is still not clear. One consideration is that HIV-infected patients may be regarded as a population that is particularly vulnerable to malaria as is the case with children, pregnant women or the elderly. The other is that continued CTX prophylaxis implies an additional pill burden, additional cost, an extra source of potential adverse drug events and a potentially unnecessary risk for the development of drug resistance in patients in whom CTX prophylaxis could possibly be stopped because their immune function has fully recovered, or who may not require CTX in the first place as their immune system may not have deteriorated.

Our study (the first placebo controlled trial investigating this question) has shown that HIV-infected adults who are stable on ART and stop taking CTX experience malaria episodes (mostly uncomplicated) more frequently, that CD4 count has no effect on malaria incidence and that PI-based ART regimens reduce malaria. A potential decision to stop or continue CTX must of course take into account the main COSTOP trial results that have shown a clear benefit of continued CTX prophylaxis in reducing non-malarial CTX preventable infections, but on the other hand also showed a higher rate of haematological grade 3 or 4 adverse events among participants on continued CTX prophylaxis, as well as a slower increase of CD4 counts. Of note in this context is the fact that overall mortality among those who stopped and those who continued CTX medication was similar (19).

Overall, our findings support the current WHO guidelines on CTX which state that CTX may be discontinued in some situations, but should be continued in countries with high endemicity of malaria and bacterial infections (38). However, this decision is not

straightforward, as other factors such as cost at population level, and ART regimen and risk of toxicity at individual level should be considered. It should also be noted that because of the negative effect of CTX on CD4 count increase, an individual patient's CD4 count alone might not be a sufficient criterion in guiding this decision. Further research might be useful in identifying groups that can safely stop CTX prophylaxis.

9.6 References

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Appendix 1

Events regarded as potentially preventable by cotrimoxazole medication

WHO clinical stage 4 events of HIV infection

Pneumocystis pneumonia

Recurrent severe bacterial pneumonia

Central nervous system toxoplasmosis

Chronic isosporiasis

Recurrent non-typhoidal salmonella bacteraemia

WHO clinical stage 3 events of HIV infection

Unexplained severe weight loss (>10% of presumed or measured body weight)

Unexplained chronic diarrhoea for a duration of more than one month

Unexplained persistent fever (above 37.6°C, intermittent or constant, for longer than one month)

Severe bacterial infections (such as pneumonia, empyema, pyomyositis, bone or joint infection, meningitis or bacteraemia)

Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis

WHO clinical stage 2 events of HIV infection

Moderate unexplained weight loss (<10% of presumed or measured body weight)

Recurrent upper respiratory tract infections (sinusitis, tonsillitis, otitis media, laryngitis and pharyngitis)

Appendix 2 - CROI presentation

MRC | MRC/UVRI Uganda Research Unit on AIDS

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Is it safe to stop Cotrimoxazole in adults on ART: COSTOP a non-inferiority RCT

Paula Munderi, Jonathan Levin, Zacchaeus Anywaine,
Ronnie Kasirye, Anatoli Kamali, Andrew Nunn & Heiner Grosskurth
for The COSTOP Trial Team

Conference on Retroviruses and Opportunistic Infections (CROI 2015), Seattle, USA
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MRC | Medical Research Council

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Jonathan Levin has no financial relationships with commercial entities to disclose

Background

Policy on Cotrimoxazole Preventive Therapy (CPT)

adopted in resource limited settings

follows WHO/UNAIDS recommendations

- Studies in Africa on **ART naïve HIV +ve patients** had demonstrated reduction in HIV-related mortality ranging from 25-46%, in hospitalization 21 – 53% and in malaria up to 72%. Other morbid events not characterized.

Benefit for patients stable on ART remained to be determined

Concerns: pill burden & haematological co-toxicity with ART

- In developed countries, primary CPT is not routinely practiced

Studies on CPT in ART treated populations

Adults on ART for a mean of 3.7 years who discontinued CPT
had a relative risk of malaria of 32.5 (95% CI 8.6–275.0)
and of diarrhea of 1.8 (95% CI, 1.3–2.4)
Campbell JD et al. CID 2012;54(8):1204-11

Adults on ART who continued CPT had a reduction in Malaria IRR = 33.2
no difference in pneumonia and diarrhea
Polyak CS et al. CROI 2014. Oral Abstract 98

ARROW trial, Children who stopped CPT after 96 weeks of ART had higher rates of hospitalisation/death HR=1.57, mainly due to malaria & bacterial RTI.
Bwakura-Dangarembizi et al. NEJM 2014;370:41-53

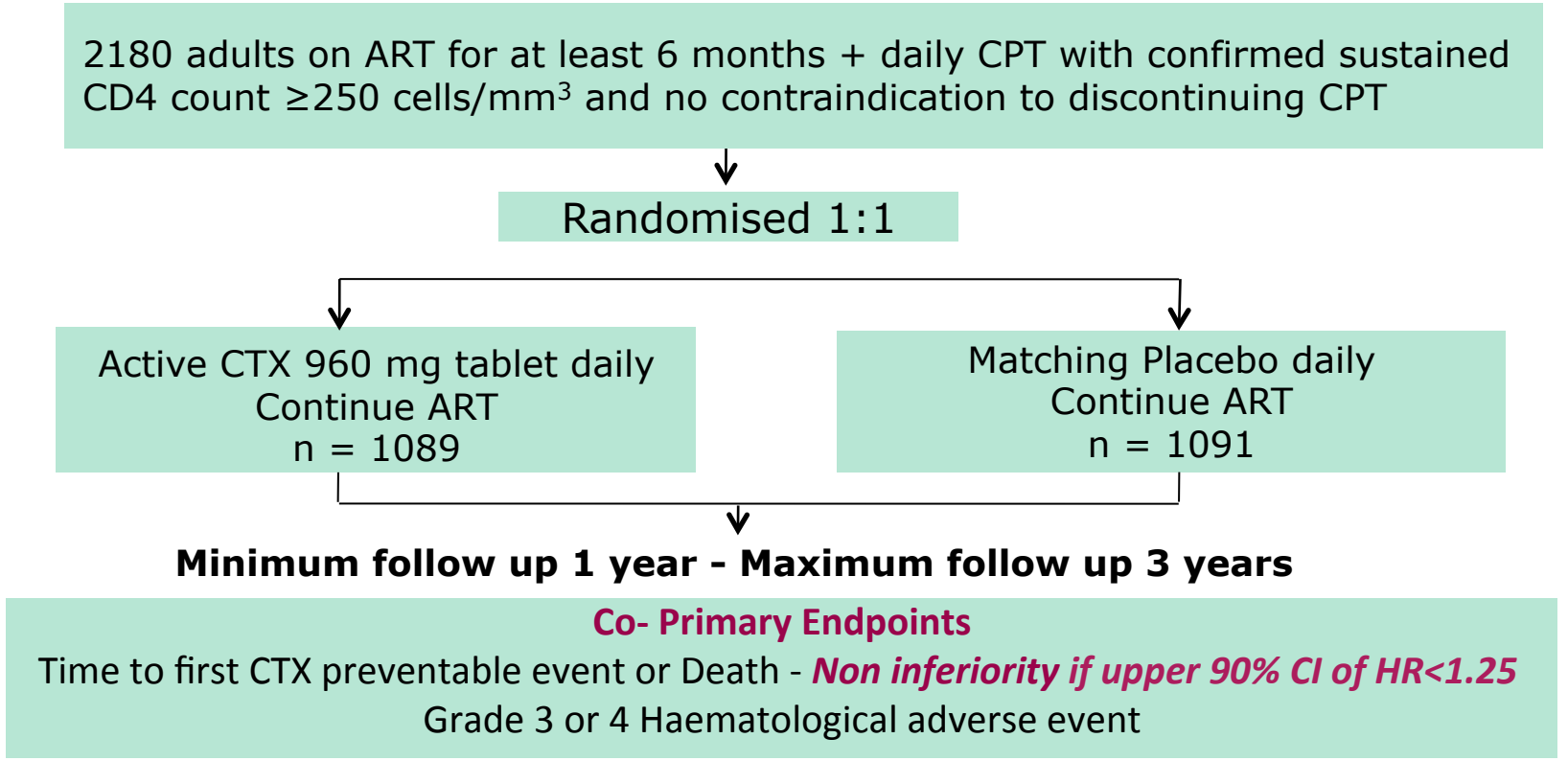
All of these were open-label trials

Objectives of COSTOP

A placebo controlled trial to asses whether,
in patients on ART with CD4 count ≥250cells/mm³,
discontinuation of CPT is

- not inferior to the control regimen in which CPT is continued
- superior with respect to the incidence of haematological adverse events

Study Design



Secondary endpoints

- Incidence of all CTX preventable events
- All cause mortality
- Incidence, severity & outcome of confirmed malaria episodes
  - asymptomatic & symptomatic
- Mean change in CD4 count & haematologic indices
  - after 12 months on the trial
- Incidence of all hospitalisations & SAEs

Baseline Characteristics

	CTX *(n=1089)*	Placebo *(n=1091)*
Entebbe Site	46.0%	45.9%
Masaka Site	54.0%	54.1%
Females	73.7%	74.1%
Age in years- Median (IQR)	41 (36-46)	40 (35-47)
Months on ART - Median (IQR)	48 (27-66)	47 (26-65)
CD4 cells/mm3 - Median IQR	518 (410-696)	519 (411-682)
WHO Clinical Stage III	57.4%	57.6%
WHO Clinical Stage IV	10.6%	9.7%
Sleeps under an ITN	62.1%	63.6%

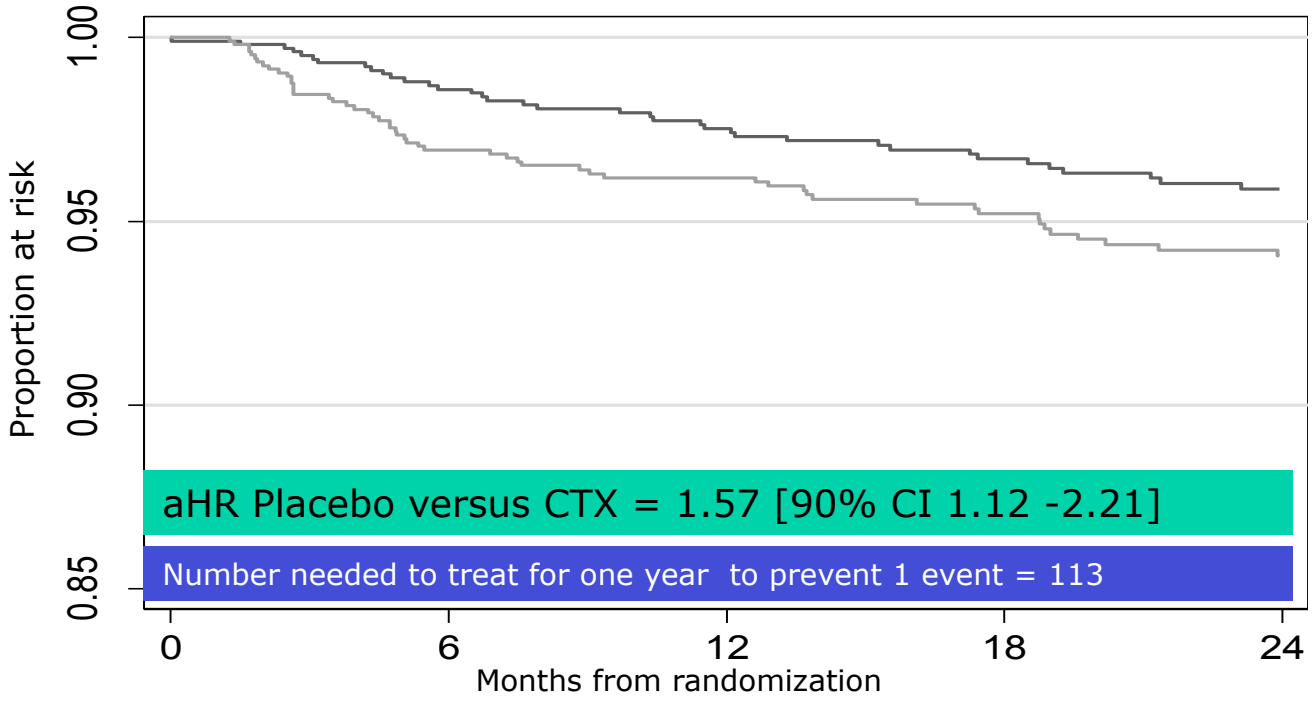
Results: ITT and PP populations

ITT: all participants who took at least one dose of study medication and who had at least one follow-up assessment (*no f/u in 5 participants*)

PP: participants whose adherence assessed by pill count or self-report remained above 80% for each period between study visits

Week	CTX ITT % retained	Placebo ITT % retained	CTX PP % retained	Placebo PP % retained
12	98.3	98.8	90.4	91.0
24	97.5	97.2	88.2	88.4
36	96.0	95.4	85.6	84.2
48	94.4	93.8	82.1	79.9
60	91.3	89.7	77.8	74.5

Time to first CTX preventable event – PP population



CTX arm
39 events (1.84 / 100PY)

59 events (2.94 / 100PY)
Placebo arm

CTX preventable events

The most common CTX preventable events were:

- *Bronchopneumonia (33 P ; 20 CTX)*
- *Recurrent Bacterial URTIs (4 P ; 5 CTX)*

6 deaths were deemed CTX preventable

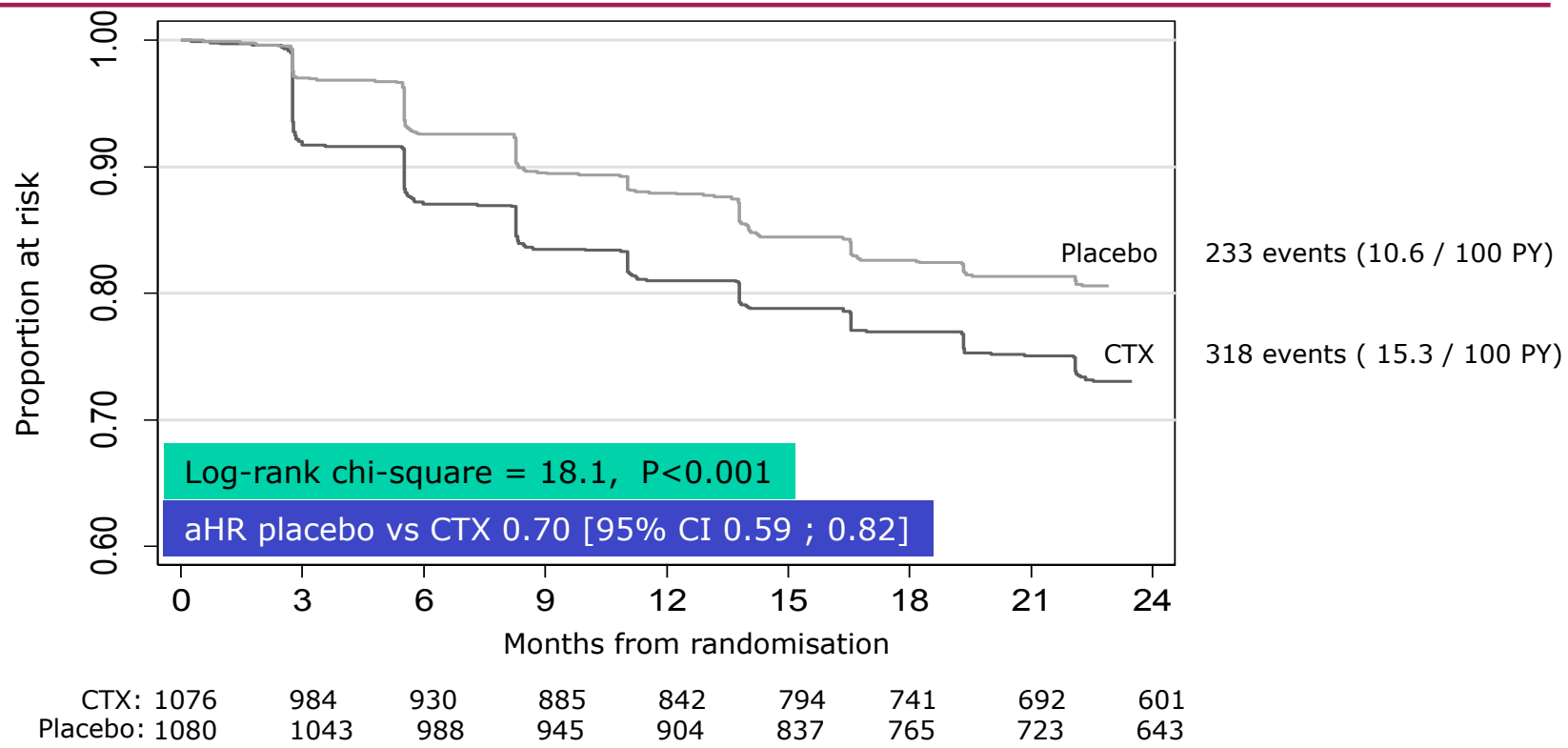
Placebo arm

1. *Klebsiella pneumonia*
2. *Septicaemic shock*
3. *Diarrhoea of unknown cause*
4. *KS with severe sepsis*

CTX arm

1. *Malaria w quinine toxicity*
2. *Pyogenic meningitis*

Time to first grade 3 / 4 haematological adverse event



Haematological adverse events

- Large number of grade 3 / 4 hematological adverse events
  Mainly grade 3 / 4 neutropenia

- Participants who experienced ≥ 1 grade 4 neutropenia
  8.2 % in CTX arm vs 5% in the Placebo arm

- Number of participants with grade 4 anaemia
  or grade 4 thrombocytopenia was very low & similar in two arms

Secondary endpoint: all cause mortality

A total of 37 deaths, 6 were deemed CTX-preventable by ERC, while 31 not CTX-preventable

	CTX	Placebo
Number of deaths	19	18
Stratified log-rank test $p = 0.91$		

Secondary endpoint: symptomatic malaria

In total 362 (16.6%) participants experienced 453 episodes of symptomatic malaria (parasitaemia + fever)

	CTX	Placebo
Number of episodes of symptomatic malaria	103	350
Rate	4.1 / 100 PY	13.9 / 100 PY
Log-rank chi-square = 137.3 ; P<0.001		
aHR placebo vs CTX 3.43 [95% CI 2.69 – 4.38]		

Secondary endpoint: CD4 count at week 48

Adjusting for baseline CD4 count and study site, CD4 count at week 48 was significantly higher in placebo arm than in CTX arm (P<0.001)

	CTX	Placebo
Back-transformed adjusted mean CD4 count at 48 weeks	469.5 cells/mm^3	495 cells/mm^3
% participants with **no** increase in CD4 count at 48 weeks	54.2%	45.7%

Secondary endpoint: hospitalisations

146 participants had a total of 175 hospital admissions

	CTX	Placebo
Number of 1st Hospital admissions	53	93
Rate	2.1 / 100 PY	4 / 100 PY
log rank chi-square = 12.36 P=0.0004		
aHR placebo versus CTX = 1.82 [95% CI 1.30 – 2.5]		

Reasons for admission

	CTX	Placebo
Malaria related	13	34
Anaemia	4	8
Bacterial pneumonia	2	4
Neutropenia	1	0
Unknown cause	9	5

Secondary endpoint: SAE's

155 SAE's reported

	CTX	Placebo
Total number of SAE's reported	61	94
Malaria related SAE's	8	29
Classified as "anaemia with clinical symptoms"	7	7

Time to 1st SAE: 122 participants had at least one SAE

	CTX	Placebo
Number with at least one SAE	47	75
Rate	21.92 / 100 PY	3.18 / 100 PY
log rank chi-square = 7.35 P=0.007		
aHR placebo versus CTX = 1.65 [95% CI 1.15 – 2.38]		

Conclusion

Discontinuing CPT:

- leads to a significant increase in CTX-preventable clinical events, mainly bacterial pneumonias
- significantly increases risk of Malaria and related hospitalisation
- is associated with a decrease in grade 3 / 4 haematological adverse events, mainly neutropenia
- has a small effect on change in CD4 counts on ART
- has no effect on all cause mortality

Implications

- Our results are in line with recently revised WHO guidelines on CPT in resource limited settings
- Number needed to treat with CPT (for one year) is 113 to prevent one event

  A cost effectiveness analysis is pending

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COSTOP Trial Monitors: M Akello & EACCR Monitors.
Independent Trial Steering Committee: EK Mbidde (Chair), A Kambugu, S Watiti, M Roberts (Observer)
Independent Data Monitoring Committee: T Peto (Chair), S Bahendeka, C Lombard.
Independent Endpoint Review Committee: F Semitala (Chair), R Parkes, F Kiweewa, L Ssebuyira.

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Safety of discontinuing cotrimoxazole prophylaxis among HIV infected adults on anti-retroviral therapy in Uganda (COSTOP trial): Design

Zacchaeus Anywaine [a,*], Andrew Abaasa [a], Jonathan Levin [a,d], Ronnie Kasirye [a], Anatoli Kamali [a], Heiner Grosskurth [a,c], Paula Munderi [a], Andrew Nunn [b]

[a] MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda
[b] MRC Clinical Trials Unit at University College London, UK
[c] London School of Hygiene and Tropical Medicine, London, UK
[d] School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

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ABSTRACT

Introduction: Cotrimoxazole (CTX) prophylaxis is recommended by the World Health Organisation for HIV infected persons. However, once HIV infected patients have commenced ART in resource limited settings, the benefits of continued CTX prophylaxis are not known. The few studies that investigated the safety of discontinuing CTX prophylaxis in these settings had limitations due to their design.
Materials and methods: COSTOP is a randomised double blind placebo controlled non-inferiority trial among HIV infected Ugandan adults stabilised on anti-retroviral treatment (ART). Participants with CD4 count of 250 or more cells/mm^3 are randomised to two arms: the intervention arm in which CTX is discontinued and the control arm in which CTX prophylaxis is continued. The study aims to assess whether the intervention regimen is not inferior, with respect to the incidence of pre-defined CTX-preventable events, to the control regimen and superior with respect to the incidence of haematological adverse events.
Discussion: Studies that have previously evaluated the safety of discontinuing CTX prophylaxis among HIV infected adults in resource limited settings have provided moderate to low quality evidence owing in part to methodological limitations. COSTOP is designed and conducted with sufficient rigour to answer this question. The results of the trial will assist in guiding policy recommendations.
Conclusion: This paper describes the design and methodological considerations important for the conduct of CTX cessation studies.

1. Introduction

The use of cotrimoxazole (CTX) as prophylaxis against opportunistic infections among HIV-infected persons is part of the standard of care recommended by the World Health Organisation (WHO) [1,2]. In resource limited settings, once HIV infected patients have commenced ART, the benefits of continued prophylactic CTX medication are not known [1].

A few studies in resource limited settings [3–6] have investigated the effect of providing prophylactic CTX *versus* no CTX among patients concurrently taking ART. All these studies had limitations in that either they were observational [3], had small sample sizes [4,5] or followed participants for short periods as reviewed by Suthar et al. [6]. This systematic review also concluded that "cotrimoxazole significantly increased survival in HIV infected adults on ART. Further research is needed to determine the optimum duration of CTX treatment in these patients". Campbell and colleagues carried out a trial in a home based care setting in rural Eastern Uganda in which 836 patients who had been on ART for a median time of 3.7 years and who had a CD4 count above 200 cells/μl were randomised at household level to continue or discontinue CTX prophylaxis in an open label design [7]. The trial was stopped at the recommendation of the DSMB following the occurrence of significantly higher rates of asymptomatic and symptomatic malaria in the group which stopped CTX (RR = 27.7, 95% CI 6.8, 113.1, $p < 0.001$). There was also a significantly higher rate of self-reported diarrhoea, but no difference between the two arms in the incidence of AIDS-related opportunistic infections and no deaths were reported. Recently, a study conducted in Kisumu—Kenya compared the effect of CTX cessation *versus* continuation on a composite outcome of death, malaria, pneumonia and diarrhoea among HIV infected adults stabilised on ART [8]. None of these studies used a double-blind placebo controlled design.

A WHO Guideline Development Group on CTX Prophylaxis convened in 2013 recommended the continuation of CTX prophylaxis

* Corresponding author.
E-mail addresses: Zacchaeus.Anywaine@mrcuganda.org (Z. Anywaine), Andrew.Abaasa@mrcuganda.org (A. Abaasa), Jonathan.Levin@mrcuganda.org (J. Levin), Ronnie.Kasirye@mrcuganda.org (R. Kasirye), Anatoli.Kamali@mrcuganda.org (A. Kamali), Heiner.Grosskurth@lshtm.ac.uk (H. Grosskurth), Paula.Munderi@mrcuganda.org (P. Munderi), a.nunn@ucl.ac.uk (A. Nunn).

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among patients stable on ART in settings with severe bacterial infections and high malaria prevalence; but that these guidelines should be adapted to 'national context' [2]. There is still uncertainty within resource limited settings and further research is needed to provide evidence based recommendations for or against stopping CTX. Garnering high quality evidence requires studies with robust designs, methodological and ethical considerations.

In this paper we present the design and methods used in the conduct of the CTX prophylaxis cessation trial among HIV infected adults on ART in Uganda (trial registration number: ISRCTN44723643).

2. Materials and methods

COSTOP is a randomised, double-blind, placebo controlled non-inferiority trial among HIV-infected adults in Uganda that have been immunologically stabilised on ART. The objective of the study is to assess whether, in patients with CD4 count of ≥250 cells/mm^3, a regime in which CTX prophylaxis is discontinued is:

(a) not inferior, with respect to the incidence of pre-defined CTX-preventable events to the control regimen in which prophylaxis with CTX is continued and
(b) superior to continuing CTX prophylaxis with respect to reducing the incidence of haematological adverse events.

The pre-defined CTX-preventable events (Table 1) are a subset of the WHO-staging events, namely those that are deemed to be CTX-preventable *a priori*.

2.1. Study setting and population

The study is being conducted in Uganda at the MRC/UVRI Unit clinics in Entebbe and Masaka. Patients on long-term CTX and ART care are recruited from local HIV treatment centres situated near-by.

The eligibility criteria used are as follows:

Inclusion criteria

- HIV-infected patient with documented intake of CTX for at least 6 months;
- age of ≥18 years;
- documented intake of ART for at least 6 months;
- clinically asymptomatic;
- 2 CD4 counts (not more than 6 months apart) of ≥250 cells/mm^3, the most recent no more than 4 weeks prior to enrolment; and
- able to attend study clinics at 3-monthly intervals and in the event of intercurrent illness.

Exclusion criteria

- acute illness (opportunistic infection or other co-morbidity);
- first trimester pregnancy;
- known hypersensitivity to cotrimoxazole; and
- grade 3/4 anaemia, neutropenia or thrombocytopenia.

Table 1
Cotrimoxazole preventable WHO staging events.

Cotrimoxazole preventable events
WHO clinical stage 4
Pneumocystis pneumonia
Recurrent severe bacterial pneumonia
Central nervous system toxoplasmosis
Chronic isosporiasis
Recurrent non-typhoidal salmonella bacteraemia
WHO clinical stage 3
Unexplained severe weight loss (>10% of presumed or measured body weight)
Unexplained chronic diarrhoea for longer than one month
Unexplained persistent fever (above 37.6 °C intermittent or constant, for longer than one month)
Severe bacterial infections (such as pneumonia, empyema, pyomyositis, bone or joint infection, meningitis or bacteraemia)
Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis
Unexplained anaemia (<8 g/dl), neutropaenia (<0.5×10^9 per litre) or chronic thrombocytopaenia (<50×10^9 per litre)
WHO clinical stage 2
Moderate unexplained weight loss (<10% of presumed or measured body weight)
Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media and pharyngitis)

2.2. Ethical approval

Ethical permission was obtained from the Uganda Virus Research Institute Research and Ethics Committee (UVRI REC), the Uganda National Council for Science and Technology (UNCST) and the Ugandan National Drug Regulatory Authority (NDA). The trial is monitored by an Independent Data Monitoring Committee (IDMC).

2.3. Intervention

All participants are required to stop their regular CTX after which they are randomised to receive CTX tablets of 960 mg or a matching placebo tablet. All participants continue to receive ART from their routine providers. Trial medication is dispensed monthly for the first three months and three-monthly thereafter with a fixed number of extra tablets to allow for the possibility of late attendance. Participants are requested to return their trial medication packs with any unused tablets at scheduled clinic visits. Allocated trial treatment is discontinued in the event of the following: confirmed CD4 count drop to below 250 cells/mm^3, participants' consent withdrawal and intercurrent illness preventing further treatment with trial drug. No additional participants are recruited to replace those withdrawn. Participants withdrawn from trial treatment due to a confirmed CD4 count drop to below 250 cells/mm^3 or due to consent withdrawal are prescribed open label CTX. Follow-up of participants withdrawn from the study intervention continue unless the participant explicitly withdraws consent for follow-up.

2.4. Study schedule

A summary of the study schedule of visits and procedures is shown in Table 2. Participants are informed about the trial and provide informed consent before screening by signing the informed consent form. Illiterate participants sign by thumbprint in the presence of an independent literate witness.

At screening, potential participants are assessed for eligibility, sociodemographic and behavioural characteristics, and for their medical history (including ART use and past WHO clinical stage events). A clinical examination is conducted and Laboratory investigations include a full blood count, malaria slide and CD4 count.

The enrolment visit takes place within 2 to 4 weeks of screening; eligibility is confirmed and consent obtained for randomisation into the trial. At enrolment and each follow-up visit, routine trial procedures are performed as indicated in Table 2. Participants are seen monthly for the first three months and 3 monthly thereafter. All participants are provided with an insecticide treated mosquito net (ITN) and educated about the importance of using it. Other medications and investigations are provided as required for the management of the participant's reported disease condition. Participants are encouraged to report to the study clinics whenever they fall sick. All adverse events (AE) are

Table 2
Summary of study schedule of visits and procedures.

Procedure	Assessment time							
	Screening (weeks −2 to −4)	Enrolment (week 0)	Week 4	Week 8	Week 12	Week 24	Every 3 months after	Every 6 months after
Consent for screening	x							
Consent for plasma storage		x						
Consent for enrolment		x						
History & physical examination[a]	x	x	x	x	x	x	x	
CD4 count[b]	x	x			x	x		x
Full blood count	x				x	x	x	
Pregnancy test[c]	x							
Malaria slide		x	x	x	x	x	x	
Adherence assessment		x	x	x	x	x	x	
Study drug prescription/refill		x	x	x	x	x	x	

[a] Doctor assessment. Including a record of all clinical events and any adverse events since previous visit.
[b] CD4 counts at baseline, after first three months, after 6 months and then 6 monthly thereafter.
[c] Pregnancy test in all women of reproductive age at screening. Thereafter, only in event of amenorrhea.

assessed and appropriate management provided. Serious adverse events (SAEs) are reported to the UVRI REC, UNCST and NDA.

2.5. Randomization and blinding

Participants are randomised in a ratio of 1:1 to stop or continue CTX prophylaxis. The randomisation schedule was produced by an independent statistician at the MRC/UVRI Unit using random permuted blocks of variable size with separate randomisations carried out in four strata, defined by the four possible combinations of study site (Entebbe or Masaka) and baseline CD4 count (250–499 cells/mm^3 or ≥500 cells/mm^3). Neither study staff nor the endpoint review committee (ERC) members have access to the randomisation schedule. Eligible participants details are entered by the study clinician on the next available row of the enrolment register in accordance with their CD4 count. The trial number corresponding to that row is used on all trial documents and to identify the prelabelled study medication.

2.6. Unblinding procedures

The randomisation codes are maintained by the independent trial statistician and a copy is held by the trial pharmacist. Unblinding is discouraged during treatment; if however, a trial clinician considers it necessary for a participant's allocated treatment to be unblinded this is first discussed with the chief investigator. If unblinding is considered appropriate the reason for unblinding is recorded in the unblinding register and an unblinding form is completed and sent to the independent statistician, or in his absence to the pharmacist. The unblinding information is disclosed to the attending clinician and is kept confidential to other study staff and is entered on the database.

2.7. Outcomes

There are two co-primary outcome measures: time to the first CTX preventable event (either one of the WHO staging events in Table 1 or else a death adjudicated by the ERC to be CTX preventable), and time to the first grade 3 or 4 haematological adverse event. Secondary outcome measures include the following: all-cause mortality; incidence of all CTX preventable events, all clinical events and SAEs; incidence, severity and outcome of all malaria episodes (asymptomatic and symptomatic) confirmed by positive parasitaemia on a blood slide; incidence of grade 3 or 4 adverse events; mean change in CD4 and haematologic indices after 12 months; adherence to use of ART, trial drug and ITN.

2.8. Assessment of adherence

Adherence to the use of trial drug, ART and ITN is assessed at every scheduled and unscheduled visit using a standard adherence questionnaire and by returned trial drug and ART pill counts. Adherence counselling is given at every visit. An exit interview questionnaire will be administered to capture the possible ingestion of CTX either from supplies left over prior to enrolment or from sources outside the trial during follow-up.

2.9. Sample size

Sample size calculations used the following assumptions: the rate of CTX preventable events in the control arm would be 10 per 100 PYO, based on an analysis of event rates from the DART trial [9] among participants with confirmed CD4 count above 250 cells/mm^3; loss to follow-up rate would be 4% per year; type I error of 0.05 (one-sided for non-inferiority), the upper limit of the 95% confidence interval for the hazard ratio comparing the intervention arm with the control arm should be at most 1.25 in order to demonstrate non-inferiority; power of 80% assuming equal event rates in the two arms. Under these assumptions a total of 2000 participants would be required, among whom a total of 494 CTX preventable events would be expected. For the co-primary safety end-point a sample size of 1000 per arm would have over 80% power to detect a halving in the rate of grade 3 or 4 haematological events at the 5% level, assuming that at least 10% of those in the arm that continues to receive CTX prophylaxis experience such an event. The sample size was estimated using the formula of Schoenfeld [10].

2.10. Trial oversight

The overall trial oversight is provided by the Trial Steering Committee (TSC). There is an unblinded Independent Data Monitoring Committee (IDMC) that meets six monthly and is responsible for reviewing study recruitment targets, the safety and efficacy endpoints and the available external evidence from other related studies. The IDMC is also responsible for advising the TSC on whether to stop, amend or continue the trial as originally planned. The IDMC can recommend stopping the trial if there is overwhelming evidence (as determined by the Peto–Haybittle rule) [11,12] of a difference in the rate of CTX-preventable events between the two arms.

2.11. Ascertainment of the primary endpoints

All potential primary endpoints are captured by the study clinicians and adjudicated by the ERC. Haematological events (anaemia, neutropenia and thrombocytopenia) are assessed through scheduled laboratory tests carried out in the MRC laboratories. Critical CD4 count measurements (<250 cells/mm^3) are confirmed with a repeat test. The DAIDS toxicity grading tables [13] are utilized to grade the severity of the measured laboratory parameters.

2.12. Data management and quality assurance

A database for the COSTOP trial is custom designed in MS ACCESS. All CRFs are printed in duplicate and the data is double-entered and validated before being uploaded into the database. The data from the Masaka site is transferred and merged with the main trial database in Entebbe every two weeks. The monitoring of the trial to assess adherence to the protocol, respect of participant rights and data quality is done routinely by the MRC/UVRI Uganda Research Unit on AIDS monitors and monitors from the East African Consortium for Clinical Research (EACCR) and these are blinded to the treatment allocation.

2.13. Analysis plan

Two data sets will be used for analysis namely per protocol (PP) and intention to treat (ITT) populations. The PP population will consist of all subjects who were considered to have taken at least 80% of their blinded study medication in each period between scheduled study visits. Study participants will remain in the per protocol population as long as their adherence as defined above remains at 80% or higher, and will be dropped from the PP data set at the visit at which their adherence during that period drops to below 80%. Such patients will not re-enter the PP data set. The ITT data set will consist of all subjects who took at least one dose of blinded study medication and for whom there is at least one follow-up assessment.

For the primary analysis of the first co-primary endpoint, namely time to first CTX preventable event or death, the analysis will test for non-inferiority (NI), hence the main analysis will be a per-protocol (PP) analysis. An ITT analysis will also be done on this population as a form of sensitivity analysis. For the primary analysis of the second co-primary endpoint (time to first haematological grade 3 or 4 adverse event) and for all secondary endpoints, analysis will be carried out on the ITT population.

For all time to event analyses, a subject will be considered to be part of the trial until the subject experiences the event, or the trial ends, the subject leaves the trial (due to withdrawal or loss to follow-up), the subject dies or in the case of the co-primary non-inferiority endpoint, the subject no longer qualifies for the per protocol population.

The comparative incidence of first clinical events in the two study arms will be illustrated graphically using Kaplan Meier plots. The incidence rate in each arm will be estimated together with 95% confidence limits, since it is considered safe to stop CTX-prophylaxis if the event rate in the experimental arm is sufficiently low (upper limit of 95% confidence interval is below 1 per 100 pyar), even if the formal non-inferiority limit is not met. Non-inferiority will be tested by fitting a Cox proportional hazards regression model with terms for centre (Entebbe or Masaka), CD4 stratum (250–499 cells/mm^3 *vs.* 500 or more cells/mm^3) and treatment arm and calculating the one sided 95% confidence limit for the hazard ratio for the experimental arm (stopping CTX) relative to the control arm (continuing CTX). The experimental arm will be deemed to be non-inferior to the control arm if the upper limit of the confidence interval is less than 1.25, that is, no more than a 25% increased risk of an endpoint event on the placebo arm. In investigating whether stopping CTX prophylaxis is superior to continuing CTX prophylaxis with respect to the safety endpoint of time to the first grade 3 or grade 4 haematological adverse events, an intention-to-treat approach will be used.

The frequency of such events will be tabulated by treatment arm, separately for neutropenia, anaemia and thrombocytopenia. Further analyses will be carried out using survival analysis methods. The incidence of grade 3 or grade 4 haematological adverse events will be illustrated graphically using Kaplan Meier plots. The primary analysis will be carried out by means of a log-rank test, stratified by the four randomization strata defined by the combinations of study site and CD4 stratum, to compare the event rates between the two study arms. Further analysis will be carried out by fitting a Cox proportional hazard regression model, with terms for site, CD4 stratum and treatment arm.

3. Discussion

The aim of COSTOP is to assess whether CTX prophylaxis can be safely discontinued among HIV-infected African adults that have achieved sustained immune reconstitution following initiation of ART in resource limited settings. According to the recent WHO guidelines [2], completed studies that have evaluated this concept have provided moderate to low quality evidence and the main reason for this is related in part to their design.

Most clinical trials involving the use of drugs are 'forward sighted', comparing a new drug to a standard, placebo or no treatment at all. In a situation where stopping the standard treatment (intervention) needs to be compared to the standard treatment itself (control), the choice of study design and the methodological considerations to be made can be quite challenging. A clear example in this case is the design and conduct of CTX cessation studies.

In our study a non-inferiority design was required since the goal was to determine whether withdrawing CTX would not disadvantage the participant. The use of a matching placebo in the intervention arm in which CTX is withdrawn is particularly important to avoid possible biases associated with knowledge of whether the patient is receiving prophylaxis. It would be very difficult to maintain cessation of CTX prophylaxis among patients randomised to do so in a setting where CTX is readily and cheaply available and CTX widely believed to be always beneficial. Adherence of participants to their allocated treatment arm is clearly much easier if they are unaware of whether they are receiving CTX or placebo. If either the investigators or the patients feel that those allocated to placebo are disadvantaged, this may lead to differential reporting of events and even premature withdrawal of patients from the trial. This could lead to failure to demonstrate non inferiority when it actually exists (type II error). Without double blinding, both the patients and investigators may perceive this to be true based on the previous evidence that HIV infected patients on ART who stop CTX may be at a higher risk of experiencing HIV-related mortality and morbidity [7] while those continuing on CTX may be at risk of adverse drug reactions.

The dose of CTX is maintained at 960 mg daily as is the current practice since a reduction could result in increased event rates in the control arm making it no longer a true control. Study drug and ART adherence are strictly monitored as differential rates of adherence could lead to erroneous conclusions [14]. The primary outcome is a composite of morbidity and mortality due to all CTX preventable WHO staging events. The criteria developed by WHO for the diagnosis of these events is based on both definitive and presumptive evidence by the clinician. In this study, an Endpoint Review Committee is established to ensure precise ascertainment of endpoints based on the presumptive/definitive WHO criteria [15]. Since some diagnoses based on this criterion involve some degree of subjectivity, in the absence of the ERC, a high proportion of incorrect diagnoses could add "noise" to the trial results and hence diminish the difference in the arms. All these design considerations are necessary for the non-inferiority trial to be conducted with rigour and avoid making false conclusions about the effectiveness of the intervention [16].

We are aware of concerns that patients might have access to unused supplies of CTX or be able to purchase CTX during the study. Ideally, adherence to trial medication would be best assessed by serum level measurements of CTX or its metabolites. However, there is no suitable method that would only detect serum or urine levels of CTX or its metabolites without being affected by other drugs that patients take. In the absence of a suitable test it was decided to conduct end-of-trial interviews by independent researchers in order to identify patients who had possibly taken open label CTX at some time during the trial and had correctly reported this to the trial team. If participants have such access, non-inferiority might be inappropriately demonstrated.

In conclusion, this paper describes the design and methods used in the COSTOP trial highlighting some important aspects in designing CTX cessation studies. It is expected that the design and methodological considerations will significantly contribute to the quality of evidence from this study.

Conflict of interest

No conflict of interests declared by the authors.

Authors' contributions

PM, JL, RK and HG conceived the initial trial idea. ZA, AA, AN and JL prepared the initial draft of this manuscript. RK, AK, HG and PM reviewed and made substantive contributions to subsequent drafts. All authors have read and approved the final manuscript.

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Incidence of malaria by cotrimoxazole use in HIV infected Ugandan adults on antiretroviral therapy: a randomized placebo controlled study

R.Kasirye[1,2], K.Baisley[2], P. Munderi[1], J. Levin[1,3], Z. Anywaine[1], A. Nunn[4], A. Kamali[1], H. Grosskurth[2]

1. MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda. 2. London School of Hygiene and Tropical Medicine, London, UK.
3. School of Public Health, University of Witwatersrand, Johannesburg, South Africa. 4. MRC Clinical Trials Unit at University College London, UK

Background

Previous studies among HIV infected patients on antiretroviral therapy (ART) in Africa have shown that malaria incidence increases when cotrimoxazole (CTX) prophylaxis is stopped[1,2] ; however these trials were not blinded. Further research is needed on whether or not CTX should be continued long-term in settings with high malaria endemicity.

Figure 1: COSTOP trial site in Masaka

Study objective

To investigate the effect of stopping CTX on the incidence and severity of malaria in HIV-infected adults on ART in a placebo-controlled trial (COSTOP-ISRCTN44723643).

Methods

HIV-infected adults on ART and CTX with CD4 ≥250 cells/μl randomized (1:1) to continue CTX prophylaxis or receive matching placebo; randomisation stratified by CD4 count (<500 or 500+) and site (Entebbe & Masaka, Figure 1).

Participants followed monthly for the first 3 months and 3-monthly thereafter for 12-38 months.

Malaria defined as fever and positive blood slide; considered severe if participant had ≥1 clinical or laboratory feature of severity[3], or were admitted to hospital for malaria.

Outcomes: overall rate of malaria, and rate ratios (RR) for the effect of treatment arm, estimated using random effects Poisson regression to account for correlation of repeated episodes within participant.

Results

2180 participants enrolled; median follow-up 2.5 years.;453 malaria episodes recorded among 362 participants (range 1-5 episodes/participant).

Malaria incidence 9.1/100 person-years (pys) (95%CI=8.2-10.1); higher in participants on placebo RR=3.47 (CI=2.74-4.39).

Effect of stopping CTX similar by enrolment CD4 (≥250 to <500 versus ≥500) (P=0.27 for interaction). Malaria incidence in placebo arm reduced over time (from 17.3/100 pys in the first year to 9.0/100 pys after two years, P<0.001) (Fig.2 and Table). Effect of stopping CTX reduced slightly with time (P=0.097 for interaction). Effect of stopping CTX greater in Entebbe compared to Masaka (P<0.001).

15 participants (13 on placebo) experienced severe malaria (<1%).;overall incidence of severe malaria 0.33/100 pys (CI=0.20-0.55). One malaria related death (CTX arm).

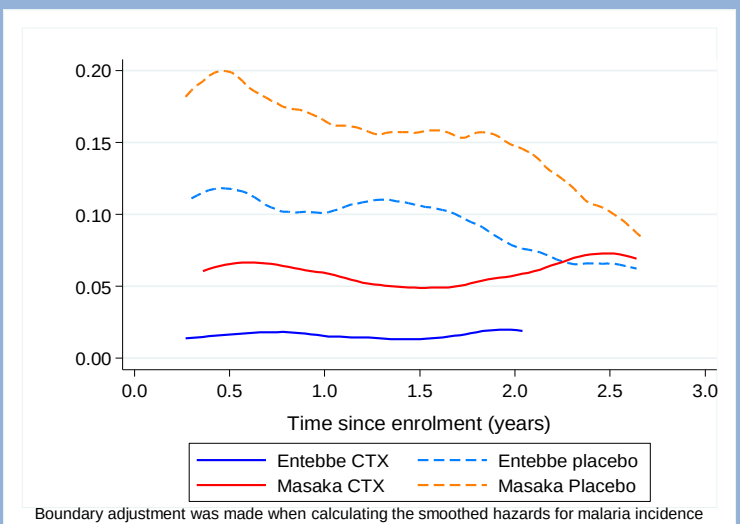

Figure 2: Malaria incidence during follow up by trial arm and site

Table: Malaria incidence by CD4 stratum, follow up time and site

	Trial arm	Episodes	Person-years (pys)	Rate/100 pys	Rate ratio (95% CI)
Malaria incidence	CTX	103	2540	4.1	1
	Placebo	350	2515	14.1	3.5 (2.5-4.4)
Stratum specific					
CD4 count at enrolment					
<500	CTX	46	1322	3.5	1
	Placebo	178	1304	13.9	4.0 (2.8-5.6)
≥500	CTX	57	1218	4.7	1
	Placebo	172	1210	14.3	3.1 (2.2-4.2)
Follow up time					
1ST year	CTX	51	1065	4.8	1
	Placebo	183	1062	17.3	3.6 (2.6-5.0)
2nd year	CTX	29	939	3.1	1
	Placebo	120	924	13.1	4.2 (2.8-6.4)
After 2rd year	CTX	23	536	4.3	1
	Placebo	47	528	9.0	2.1 (1.3-3.5)
Site					
Entebbe	CTX	19	1215	1.6	1
	Placebo	127	1209	10.6	6.8 (4.1-11.1)
Masaka	CTX	84	1325	6.3	1
	Placebo	223	1306	17.2	2.7 (2.1-3.6)

Discussion

Participants on ART who stopped CTX had malaria 3.5 times more frequently than those who continued. The higher frequency is expected because of the antimalarial properties of CTX but has been significantly lower in this trial than in previous studies[1,2].

The difference in malaria incidence between sites is probably due to higher exposure in Masaka where more rural residents were recruited than in Entebbe. The difference between sites in the effect of stopping CTX requires further investigation. Only 1.2% of participants on placebo had severe malaria implying a need to review the necessity of continuous CTX prophylaxis to prevent malaria in ART treated individuals.

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Appendix 5

Form 1

Screening questionnaire (part 1)

COSTOP

Centre: Entebbe ❍ Masaka ❍

Date of screening D D M M M 2 0 Y Y Clinic/ hosp. number

Date of birth D D M M M Y Y Y Y Patient initials Male ❍ Female ❍

Has the patient been given an information sheet and has screening consent been obtained? Yes ❑ No ❑
If not please do so.

LABORATORY EVIDENCE OF HIV INFECTION

Date of positive HIV test D D M M M Y Y Y Y

If the patient does not have a positive HIV test on file take a serum sample for testing.

ANTIRETROVIRAL HISTORY

1. Date of starting ART D D M M M Y Y Y Y

2. Regimen / /

3. Substitutions or switches

	Drug	Dose	Action	Date of action	Reason for action (see codes below)
Example	*Zidovudine*	*0*	*Stopped*	*15.01.10*	*Anaemia(08)*
1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					

1= New or recurrent clinical WHO stage 3 or 4 event
2= CD4 count concerns
3= Intercurrent illness
4= Start of 2nd line regimen
5= Starting of salvage therapy
6= Restarting previous regimen after interruption
7= Pregnancy
8=Adverse event: *name the event*
9=Patient unable to attend ART clinic
10=Voluntary patient decision (*give details do not include adverse events*)
11=Other *(give details in table)*

4. Current ART regimen: / /

5. Has the patient been on ART for at least 6 months? Yes ❑ No ❑
(If no, patient will not be eligible for enrolment)

6. Where is the patient getting ART from?

Entebbe hospital ❑ Masaka Hospital ❑ TASO centre ❑ Uganda Cares ❑
Kitovu mobile ❑ Other ❑ please specify .....

7. Is the patient enrolled into any other antiretroviral therapy study? Yes ❑ No ❑
(If yes, patient will not be eligible for enrolment)

COTRIMOXAZOLE USE

1. Does the patient have history of hypersensitivity to cotrimoxazole? Yes ❑ No ❑
(If yes, patient will not be eligible for enrolment into the study)

2. Has patient been on cotrimoxazole for at least 6 months? Yes ❑ No ❑
(If not, patient will not be eligible for enrolment)

If yes, date started D D M M M 2 0 Y Y

CD4 COUNT HISTORY

1. What was the patient's CD4 count at ART initiation? ---------- *cells/mm³* *not done* ❑

2. Has the patient had CD4 count monitoring of their antiretroviral therapy? Yes ❑ No ❑
If yes, fill the table below

	Date of most recent CD4 count	Laboratory	CD4 count
1.	D D M M M 2 0 Y Y		*cells/mm³*
2.	D D M M M 2 0 Y Y		*cells/mm³*

Please note: *To be eligible for enrolment participant should have at least 2 CD4 counts > 250 cells/mm³.*

1st CD4 count should not be more than 6 months prior to screening

2nd (confirmatory) CD4 count must be done in MRC laboratory not > 4 weeks prior to enrolment.

CONCURRENT ILLNESSES /CONDITIONS

1. Does the patient have any other illnesses / conditions..... Yes ❑ No ❑
If yes give summary below. (Details should be in the clinic notes)

Condition / illness	Date of diagnosis (where available)	Treatment
1.		
2.		
3.		
4.		

(Patients with acute illness is ineligible for enrolment but may be considered after resolution of the illness)

2. Women only

a. LNMP D D M M M 2 0 Y Y

b. Is the patient pregnant? Yes ❑ No ❑ post – menopausal ❑

c. Is the patient using any contraceptive method? Yes ❑ No ❑ (if the patient is pregnant mark no)

(Pregnant women in the 1st trimester are ineligible but may be re-evaluated after the 1st trimester)

Doctor's signature	Print name	Date

Form 1
Screening questionnaire (part 2)

Date of screening D D M M M 2 0 Y Y Clinic/ hosp. number

Date of birth D D M M M Y Y Y Y Patient initials Male ❍ Female ❍

CLINICAL STAGING

1. WHO clinical staging (tick the events ever experienced by the patient)

Stage 1
- ❑ Persistent generalised lymphadenopathy (PGL)
- ❑ Performance scale 1 (last month)

Stage 2
- ❑ Weight loss, **less than 10%** of body weight
- ❑ Minor mucocutaneous manifestations (seborrheic dermatitis, papular pruritic eruption)
- ❑ Fungal nail infections, recurrent oral ulcerations, angular cheilitis
- ❑ Herpes Zoster, within the last 5 years
- ❑ Recurrent upper respiratory tract infections (e.g. bacterial sinusitis)
- ❑ Performance scale 2 (last month)

Stage 3
- ❑ Weight loss**, greater than 10%** of body weight
- ❑ Unexplained chronic diarrhoea, >1 month
- ❑ Unexplained prolonged fever (intermittent or constant), > 1 month
- ❑ Oral candidiasis (thrush)
- ❑ Oral hairy leukoplakia
- ❑ Pulmonary tuberculosis, within the past year
- ❑ Severe bacterial infections (e.g. pneumonia, pyomyositis)
- ❑ Unexplained anaemia (<8g/dl), neutropenia (<0.5 x 10^9 / l) or chronic (> 1 month thrombocytopenia (<50 x 10^9/l)
- ❑ Performance scale 3 (last month)

Stage 4
- ❑ HIV wasting syndrome
- ❑ Pneumocystis carinii pneumonia
- ❑ Recurrent bacterial pneumonia (this episode plus one or more episodes in last 6 months)
- ❑ Toxoplasmosis of the brain
- ❑ Cryptosporidiosis with diarrhoea, >1 month
- ❑ Chronic isosporiasis
- ❑ Cryptococcus, extra pulmonary
- ❑ Cytomegalovirus disease of an organ other than liver, spleen or lymph nodes
- ❑ Herpes Simplex Virus (HSV) infection, mucocutaneous >1 month, or visceral any duration
- ❑ Progressive multifocal leukoencephalopathy (PML)
- ❑ Any disseminated endemic mycosis (e.g. histoplasmosis, coccidioidomycosis)
- ❑ Candidiasis of the oesophagus, trachea, bronchi or lungs
- ❑ Atypical mycobacteriosis, disseminated
- ❑ Non-typhoid Salmonella septicaemia
- ❑ Extra Pulmonary tuberculosis
- ❑ Invasive cervical carcinoma
- ❑ Lymphoma
- ❑ Kaposi's sarcoma (KS)
- ❑ HIV encephalopathy
- ❑ HIV- associated nephropathy
- ❑ HIV- associated cardiomyopathy
- ❑ Performance scale 4 (last month)

Tick clinical stage as the maximum stage of events experienced by the patient:

Clinical stage 1 ❑ 2 ❑ 3 ❑ 4 ❑

OTHER INCLUSION CRITERIA AT SCREENING
(For a patient to be eligible for enrolment, all answers must be in the shaded area)

a) Age 18 years and above..... Yes ❑ No ❑

b) Able to attend 3-monthly clinic follow-up visits..... Yes ❑ No ❑

c) Grade 3 or 4 anaemia, neutropenia or thrombocytopenia ..... Yes ❑ No ❑

ELIGIBILTY ASSESMENT

Is the patient potentially eligible for enrolment? Yes ❑ No ❑

If yes, proceed with lab investigations and ask patient to return after 2 weeks

If no, inform the patient that they will not be eligible for enrolment at this time. Do not proceed with lab investigations.

LABORATORY INVESTIGATIONS

At this visit, a full blood count (hematology), CD4 count and if applicable a pregnancy test should be done. Also do any other tests required for detection and management of HIV related conditions.

Trial scheduled investigations

Hematology ..... Yes ❑ No ❑

CD4 count ..... Yes ❑ No ❑

Urine pregnancy test ..... Yes ❑ No ❑ (For women)

Other investigations

Blood slide for malaria ..... Yes ❑ No ❑

Biochemistry ..... Yes ❑ No ❑

Blood culture ..... Yes ❑ No ❑

Sputum analysis ..... Yes ❑ No ❑

Stool analysis ..... Yes ❑ No ❑

Other microbiology ..... Yes ❑ No ❑ (if yes, specify .....)

Chest X-ray ..... Yes ❑ No ❑

Other tests (please specify) .....

.....

.....

Doctor's signature	Print name	Date

Form 3
Enrolment (nurse) questionnaire

COSTOP

COSTOP trial number | | | | | | |

Date of enrolment D D M M M 2 0 Y Y Clinic/ hosp. number | | | | | | | | | | |

Date of birth D D M M M Y Y Y Y Patient initials | | | Male ❍ Female ❍

Has the patient been given an information sheet and has enrolment consent been obtained? Yes ❑ No ❑

If not, please do so

SOCIO ECONOMIC STATUS

1. What is your religion?
   Protestant ❑ Catholic ❑ Muslim ❑ Seventh day Adventist ❑
   Pentecostal ❑ Traditional ❑ No religion ❑ Other ❑

2. What work do you usually spend most of your time doing?
   Farming ❑ Casual labour ❑ House work ❑ Fishing ❑ Office job ❑
   Petty trading ❑ Shop keeping ❑ Student ❑
   Other ❑ specify……

3. What is your typical monthly income from all sources (in Ugandan shillings)?
   | | | | | | | | |

4. Who contributes most for the regular house hold expenditures
   Self ❑ Spouse ❑ Parent ❑ Children ❑ Brother / sister ❑ Other ❑

5. How many dependants do you support (include children and elderly)? | | |

6. Which of these items do you own or are found in your household?
   (The item should be in working order)
   House ❑ Car ❑ TV ❑ Radio ❑ Cooker ❑ Fridge ❑
   Bicycle❑ Motorcycle ❑ Phone ❑ Boat ❑ None of the above ❑

7. What is your house roof made of?
   Thatch ❑ Old iron sheets ❑ New iron sheets ❑ Tiles ❑

8. What is your highest level of education?
   None ❑ Primary ❑ Secondary ❑ University ❑ Technical / vocational institution ❑

9. What is your marital status?
   Married monogamous ❑ Married polygamous ❑ Widowed ❑ Divorced ❑ Separated ❑
   Cohabiting ❑ Single (never married) ❑ Other ❑ specify……

10. Do you have a casual or regular sexual partner? Yes ❑ No ❑
    (If patient has a spouse or sexual partner, fill the table below, if not go to 11)

Partner number	Partner type 1= Spouse 2= Regular 3= Casual	Number of sexual encounters in the last month 1=None 2=4 or less 3=5 or more	Was a condom used at the last sexual encounter? 1=Yes 2=No	Overall condom use with partner 1 = Always 2 = Most times 3 = Sometimes 4 = Never	HIV status disclosure to partner 1 = yes 2 = No	HIV status disclosure by partner 1 = yes 2 = No

11. Have you disclosed your HIV status to anyone else other than spouse / sexual partner (where applicable)? Yes ❑ No ❑
    If yes, to whom? (Mark all that apply)
    Brother/Sister/Cousins ❑ Parents ❑ Aunt/Uncle ❑ Own children ❑ Employer ❑
    Friend / neighbor ❑ Other; specify……

12. How many biological children do you have? | |

13. Do you want to have more children? Yes ❑ No ❑

14. Are you using any family planning method? Yes ❑ No ❑
    If yes, please specify (all methods you are using now). If no go to 15
    Condom ❑ Pill ❑ IUD ❑ Safe days ❑ Injection ❑
    Implant ❑ Vasectomy ❑ BTL ❑ Other ❑ please specify……

15. Do you sleep under a mosquito net? Yes ❑ No ❑
    (If yes stop here)

16. If no, are you willing to sleep under an insecticide treated mosquito net once enrolled into the study and provided with one? Yes ❑ No ❑
    (If yes stop here)

17. If the patient is not willing to sleep under an insecticide treated mosquito net, what is the reason? ……
    ……
    ……
    ……
    ……

Nurse / Counselor's signature	Print name	Date

Form 4
Enrolment (doctor)

COSTOP

COSTOP trial number | | | | | | |

Date of enrolment D D M M M 2 0 Y Y Clinic/ hosp. number

Date of birth D D M M M Y Y Y Y Patient initials Male ❍ Female ❍

HISTORY AND EXAMINATION

Weight . Kgs **Height** . cms

Blood pressure / mmhg **Temperature** . ^{0}C

1. Performance status today (tick one box only)
   - 1 = asymptomatic, normal activity ❑
   - 2 = symptomatic, normal activity ❑
   - 3 = bedridden, <50% of the day during the last month ❑
   - 4 = bedridden, >50% of the day during the last month ❑

2. Does the patient have any clinical symptoms? Yes ❑ No ❑

(if yes please fill the table below)

Symptom	Yes	No	Symptom	Yes	No
Eye itching / pain / discharge	❑	❑	Cough	❑	❑
Ear pain / discharge	❑	❑	Difficulty in breathing	❑	❑
Fatigue	❑	❑	Muscle aches	❑	❑
Fever or sweats	❑	❑	Bone / joint aches	❑	❑
Confusion	❑	❑	Localized weakness of arms, legs or face	❑	❑
Convulsions	❑	❑	Numbness or tingling of hands and feet	❑	❑
Severe headache	❑	❑	Skin itching / new skin rash	❑	❑
Nausea / vomiting	❑	❑	Urinary symptoms	❑	❑
Mouth sores / ulcers	❑	❑	Genital itching / ulcers discharge	❑	❑
Difficulty / pain on swallowing	❑	❑	Possible pregnancy	❑	❑
Diarrhea	❑	❑	Other (specify below)	❑	❑
Weight loss	❑	❑	.....		

3. Please examine the patient and record any findings in the table below

Any abnormal findings	Yes	No	If yes, give details
Eyes	❑	❑	
Oral cavity	❑	❑	
Ear, nose, throat	❑	❑	
Skin, hair, nails	❑	❑	
Clinical anaemia	❑	❑	
Lymph nodes	❑	❑	
Respiratory system	❑	❑	
Cardiovascular system	❑	❑	
Liver	❑	❑	
Spleen	❑	❑	
Rest of abdomen	❑	❑	
Nervous system	❑	❑	
Genital examination (if indicated)	❑	❑	
Other	❑	❑	

4. Is the patient on any long term medication? Yes ❑ No ❑
   If so please record date started and indication (Should include primary and secondary prophylaxis and contraception).

Medication / drugs	Date started	Indication / reason
i.	D D M M M Y Y Y Y	
ii.	D D M M M Y Y Y Y	
iii.	D D M M M Y Y Y Y	
ix.	D D M M M Y Y Y Y	
x.	D D M M M Y Y Y Y	

5. What was the patients WHO stage at screening? WHO stage (1 ❑ 2 ❑ 3 ❑ 4❑)

6. Does the patient have a new WHO clinical staging event at this visit? Yes ❑ No ❑
   If so, give the name and stage of the event
   Event name .....stage (1 ❑ 2 ❑ 3 ❑ 4❑)

7. Current clinical stage 1 ❑, 2 ❑, 3 ❑ or 4 ❑ (tick clinical stage as maximum stage patient has had)

8. Has any medication been prescribed today? If so give name and reason.

Medication / Drug	Reason Prescribed	Date started
Trial drug	*Enrolled onto study*	D D M M M 2 0 Y Y
		D D M M M 2 0 Y Y
		D D M M M 2 0 Y Y
		D D M M M 2 0 Y Y
		D D M M M 2 0 Y Y

LABORATORY INVESTIGATIONS (mark as appropriate)

(At this visit remember to take a blood sample for plasma storage – **unless patient not consented)**

Trial scheduled investigation

Plasma storage ..... Yes ❑ No ❑
Blood slide for Malaria ..... Yes ❑ No ❑

Other investigations

Haematology ..... Yes ❑ No ❑
Biochemistry ..... Yes ❑ No ❑
CD4 count ..... Yes ❑ No ❑
Stool analysis ..... Yes ❑ No ❑
Sputum analysis ..... Yes ❑ No ❑
Blood culture ..... Yes ❑ No ❑
Other microbiology ..... Yes ❑ No ❑
Urine pregnancy test ..... Yes ❑ No ❑
X-ray ..... Yes ❑ No ❑
Other test (please specify)

Doctor's signature	Print name	Date

COSTOP

Enrolment consent

Centre: Entebbe ❍ Masaka ❍

Date of consent D D M M M 2 0 Y Y

Clinic/ hosp. number

Date of birth D D M M M Y Y Y Y

Patient initials

Male ❍ Female ❍

Please initial box if you agree

I have read/ been read the information sheet concerning the COSTOP Study and understand what will be required of me if I take part in the study. ☐

My questions concerning this study have been answered by:

.....

I understand that at any time, I may withdraw from this study without giving a reason and without affecting my normal care and management. ☐

I understand that I will have to continue taking my antiretroviral drugs plus either cotrimoxazole (septrin) or placebo during the study. ☐

I am willing to allow access to my medical notes to check that the trial is being carried out correctly but understand that strict confidentialitiy will be maintained. ☐

I voluntarily agree to take part in the study. ☐

Patient's signature/ thumb print	Name	Date

Witness' signature	Name	Date

Doctor's signature	Name	Date

N.B: 3 copies: 1 for patient, 1 for researcher, 1 to be kept with clinic notes

UGANDA VIRUS RESEARCH INSTITUTE ENTEBBE
SCIENCE & ETHICS COMMITTEE
22 NOV 2010
P.O. BOX 49 ENTEBBE (U)

Form 5
Randomization

COSTOP

Date of randomization: D D | M M M | 2 0 Y Y

Clinic/ hosp. number:

Date of birth: D D | M M M | Y Y Y Y

Patient initials:

Male ❍ Female ❍

ELIGIBILITY AND CONSENT
(The enrolment consent and enrolment nurse / doctor questionnaires should already have been filled)

Inclusion criteria

a) HIV-infected patient taking cotrimoxazole for at least 6 months..... Yes ❑ No ❑

b) Age 18 years and above..... Yes ❑ No ❑

c) Documented intake of ART for at least 6 months..... Yes ❑ No ❑

d) Clinically asymptomatic..... Yes ❑ No ❑

e) 2 CD4 counts (not more than 6 months apart) ≥250 cells/mm^3 the most recent no more than 4 weeks prior to enrolment ..... Yes ❑ No ❑

(If so, specify the test dates and CD4 counts below)

Date of most recent CD4 test	CD4 count (cells/mm^3)
D D M M M 2 0 Y Y	
D D M M M 2 0 Y Y	

Exclusion criteria

a) Acute illness (opportunistic infection or other co- morbidity). Patients will be considered for inclusion into the trial after resolution of the illness..... Yes ❑ No ❑

b) Enrolled in other ART trial..... Yes ❑ No ❑

c) First trimester pregnancy (when pregnant women reach their second trimester of pregnancy, they could then be re-evaluated for inclusion into the trial)..... Yes ❑ No ❑

d) Unable to attend 3-monthly clinic follow-up visits..... Yes ❑ No ❑

e) Hypersensitivity to Cotrimoxazole..... Yes ❑ No ❑

f) Grade 3 or 4 anemia, neutropenia or thrombocytopenia..... Yes ❑ No ❑

If any answer is in the shaded area, patient is not eligible for enrolment.

Is the patient eligible for randomization? Yes ❑ No ❑

If patient is eligible, obtain the COSTOP trial number by entering the patient's name on the next line of the appropriate enrolment register

RANDOMIZATION

COSTOP trial number

Randomized by (signature)	Print name	Date

Appendix 10
Form 6
Adherence

COSTOP

COSTOP trial number						

Assessment date D D M M M 2 0 Y Y Clinic/ hosp. number

Date of birth D D M M M Y Y Y Y Patient initials Male ❍ Female ❍

This week's visit number

Visit type: Scheduled (on time) ❑ scheduled (early / late) ❑ Sick ❑ Missed ❑ Unannounced home visit ❑

TRIAL DRUG ADHERENCE
(If patient is missing pills or has taken an overdose inform the doctor)

1. How many pills of trial drug has the patient returned?

2. Has the patient taken trial drug more than once a day? Yes ❑ No ❑
   (If no, go to 3)
   If yes, what was the reason .....
   Fill table below

Number of days excess pills were taken		Excess number of pills taken	

3. Has the patient missed any of the trial drug doses since the last scheduled visit? Yes ❑ No ❑

If yes, fill the table below

Reason for missing trial drug	Yes	No	How many days
a) Was away from home?	❑	❑	
b) Was too busy with other things?	❑	❑	
c) Simply forgot?	❑	❑	
d) Had too many pills to take?	❑	❑	
e) Wanted to avoid side-effects?	❑	❑	
f) Did not want other people to notice you taking pills?	❑	❑	
g) Was following the doctor's advice?	❑	❑	
h) Felt sick or ill?	❑	❑	
i) Ran out of pills?	❑	❑	
j) Felt good?	❑	❑	
k) Other, specify.....			
Total number of days missed			

ART ADHERENCE

1. Since the last scheduled visit has the patient missed any of their Antiretroviral drug doses? Yes ❑ No ❑

   If yes fill table below

Drug	Number of days missed	Reason (see codes below)

Reasons for missing ART:
1=Forgot, 2=Was ill, 3=Doctors advice, 4=Was tired of taking pills, 5= Did not want other people to notice, 6=Was away from home, 7=Ran out of pills, 8=Drugs out of stock, 9= Feeling well, 10=Other

BEDNET USE

1. Since the last visit has the patient been sleeping under an insecticide treated mosquito net (ITN)? Yes ❑ No ❑
   If not, fill the table below

Reason for not using ITN	Yes	No	How many days
a) Was away from home?	❑	❑	
b) ITN got damaged / stolen	❑	❑	
c) It was uncomfortable to sleep under the ITN	❑	❑	
d) Gave the ITN to child / spouse / other	❑	❑	
e) Partner was opposed to using ITN	❑	❑	
f) Was using other net (non treated)	❑	❑	
g) Have no bed where to put it	❑	❑	
h) Fears side effects of impregnated medication	❑	❑	
i) Other, specify.....			

Nurse / counselor's signature	Print name	Date

COSTOP

Form 7
Nurse follow-up form

COSTOP Trial number						

Assessment date: D D | M M M | 2 0 Y Y

Clinic/ hosp. number:

Date of birth: D D | M M M | Y Y Y Y

Patient initials:

Male ❍ Female ❍

This week's visit number:

Visit type: Scheduled (on time) ❑ Schedules (early / late) ❑ Sick ❑ Missed ❑

HISTORY AT THIS VISIT

1. Has the patient changed contact details since the last nurse visit? Yes ❑ No ❑
   If yes, please update the contact form.

2. Has the adherence questionnaire been filled? Yes ❑ No ❑
   If not please do so.

SYMPTOM CHECK LIST, HISTORY AND EXAMINATION

Weight(kgs) . Temp.(^{0}c) . BP (mmhg) /

1. Is the patient ill today?..... Yes ❑ No ❑
   If yes what are the main symptoms

Symptom	Duration(days)
(i)	
(ii)	
(iii)	
(iv)	

2.(a) Has the patient taken any new medication since the last visit? Yes ❑ No ❑
If yes, give reason below

Medication	Code	Reason	Code	Dose (mg) / day	Duration (days)
(i)					
(ii)					
(iii)					
(iv)					

(b) Has the patient taken any open label cotrimoxazole (Septrin)? Yes ❑ No ❑
If yes, check that this was documented in 2(a) above.

3. Has the patient been admitted to hospital since the last nurse visit? Yes ❑ No ❑
If yes notify the doctor

Nurse's signature	Print name	Date

Form 8
Doctor follow-up (part 1)

COSTOP

COSTOP trial number | | | | | | |

Assessment date D D M M M 2 0 Y Y Clinic/ hosp. number

Date of birth D D M M M Y Y Y Y Patient initials Male ❍ Female ❍

This week's visit number

Visit type: Scheduled (on time) ❑ scheduled (early/ Late) ❑ Sick ❑ Missed ❑

SYMPTOMS AND SIGNS

1. Performance status today (tick one box only)
   1 = asymptomatic, normal activity ❑
   2 = symptomatic, normal activity ❑
   3 = bedridden, <50% of the day during the last month ❑
   4 = bedridden, >50% of the day during the last month ❑

2. Are there any new symptoms and signs since the last doctor visit? Yes ❑ No ❑
   If so, please fill the table below:

Symptom	Yes	No	Symptom	Yes	No
Eye itching / pain / discharge	❑	❑	Cough	❑	❑
Ear pain / discharge	❑	❑	Difficulty in breathing	❑	❑
Fatigue	❑	❑	Muscle aches	❑	❑
Fever	❑	❑	Bone / joint aches	❑	❑
Confusion	❑	❑	Localized weakness of arms, legs or face	❑	❑
Convulsions	❑	❑	Numbness or tingling of hands and feet	❑	❑
Headache	❑	❑	Skin itching / new skin rash	❑	❑
Nausea / vomiting	❑	❑	Urinary symptoms	❑	❑
Mouth sores / ulcers	❑	❑	Genital itching / ulcers / discharge	❑	❑
Difficulty / pain on swallowing	❑	❑	Possible pregnancy	❑	❑
Abdominal pain	❑	❑	Other (specify below)	❑	❑
Diarrhea	❑	❑	.....		
Weight loss	❑	❑			
Sweats	❑	❑			

3. Please examine the patient and record any findings below

Any abnormal findings	Yes	No	If yes, give details
Eyes	❑	❑	
Oral cavity	❑	❑	
Ear, nose, throat	❑	❑	
Skin, hair, nails	❑	❑	
Clinical anaemia	❑	❑	
Lymph nodes	❑	❑	
Respiratory system	❑	❑	
Cardiovascular system	❑	❑	
Liver	❑	❑	
Spleen	❑	❑	
Rest of abdomen	❑	❑	
Nervous system	❑	❑	
Genital examination (if indicated)	❑	❑	
Other	❑	❑	

4. Does the patient have a fever or is the patient suspected to have malaria? Yes ❑ No ❑
   (If yes please do a blood slide and any other necessary investigation)

5. Does the patient have diarrhea today? Yes ❑ No ❑
   (If yes obtain a stool sample)

6. Does the patient have a cough today? Yes ❑ No ❑
   (If so, take a sputum sample)

DIAGNOSES

Have any diagnoses been made at this visit? Yes ❑ No ❑

If yes, record diagnosis and the respective code below.

Diagnosis	Event code
i)	
ii)	
iii)	
iv)	
v)	

TREATMENT AT THIS VISIT

TREATMENT (Other than ART / Trial drug)

Has any treatment other than ART / Trial drug been prescribed at this visit? Yes ❑ No ❑

If yes, record the treatment below.

Treatment (non ART / Trial drug)	Drug code	Duration (days)
i)		
ii)		
iii)		
iv)		
v)		
vi)		

TRIAL DRUG

Has trial drug been stopped or restarted today? Yes ❑ No ❑

If yes, fill the log below

	Action (stopped/restarted)	Date	Reason / comment
Trial drug		D D / M M M / Y Y Y Y	
1=Adverse event: *name the event* (record on Form 11 if serious or on Form 8 if grade 3/4)	2=Patient is being switched to open label cotrimoxazole.	3=Voluntary patient decision (*give details, do not include adverse events*)	4=Other *(give details in table)* 5=Restarting trial drug after interruption

Doctor's signature	Print name	Date

Form 8

Doctor follow-up (part 2)

COSTOP trial number						

Assessment date D D M M M 2 0 Y Y Clinic/ hosp. number

ANTIRETROVIRAL THERAPY

Has there been a change in antiretroviral therapy since last visit? Yes ❑ No ❑

If yes, please fill the log below.

Drug name	Action (started or stopped)	Date of action	Reason / comment

1=New or recurrent clinical WHO stage 3 or 4 event
2 = CD4 count concerns
3= Intercurrent illness
4=Start of 2nd line regimen
5=Starting of salvage therapy
6=Restarting previous regimen after interruption
7 = Pregnancy
8=Adverse event: *name the event* (record on Form 8 if grade 3/4 or form 11 if serious)
9=Patient unable to attend ART clinic should be on a new line
10=Voluntary patient decision (*give details do not include adverse events*)
11=Other *(give details in table)*

INVESTIGATIONS

Are there any investigations to be done at this visit? Yes ❑ No ❑

If so, please indicate those to be done in the table below;

Scheduled investigations		
1. Hematology	Yes ❑	No ❑
2. CD4 count	Yes ❑	No ❑
3. Blood slide for malaria	Yes ❑	No ❑
4. Plasma storage	Yes ❑	No ❑
Non scheduled investigations		
1. Biochemistry	Yes ❑	No ❑
2. Viral load	Yes ❑	No ❑
3. Blood culture	Yes ❑	No ❑
4. Sputum	Yes ❑	No ❑
5. Stool analysis	Yes ❑	No ❑
6. Other microbiology	Yes ❑	No ❑
7. Urine pregnancy	Yes ❑	No ❑
8. X-ray	Yes ❑	No ❑
9. Other investigation (please specify)		

CLINICAL EVENTS / MALARIA / ADVERSE EVENTS / HOSPITALISATION

1. Has the patient had a new WHO staging event since the last visit? Yes ❑ No ❑
   If yes, fill a WHO staging form (form 10)
2. Does the patient have malaria or has a previous malaria episode resolved? Yes ❑ No ❑
   If yes, fill a malaria form (form 9)
3. Does the patient have a new serious adverse event (SAE) or has a previously recorded adverse event resolved since the last visit? Yes ❑ No ❑
   If yes, fill an SAE form (form 11)
4. Has the patient experienced a new adverse (AE) event or has a previously recorded adverse event resolved since the last visit? Yes ❑ No ❑
   If yes, fill the table below.

Event	Grade	Date of onset	Resolved (y/n)	Date resolved

In the table below list all the drugs including trial drug and zidovine that the patient was on at the time of the event and state the relationship of the adverse event to the drugs.

Drug	Date drug started									Relationship of adverse event to drug				
	D	D	M	M	M	Y	E	A	R	Unrelated	Unlikely	Possible	Probable	Definitely
										❑	❑	❑	❑	❑
										❑	❑	❑	❑	❑
										❑	❑	❑	❑	❑
										❑	❑	❑	❑	❑
										❑	❑	❑	❑	❑
										❑	❑	❑	❑	❑
										❑	❑	❑	❑	❑
										❑	❑	❑	❑	❑

5. Has the patient been admitted to hospital or discharged since the last doctor visit? Yes ❑ No ❑
   If so, please fill a hospital admission form (form 14) and SAE form
6. Has the patient experienced a febrile / acute illness? Yes ❑ No ❑
   If so, please fill form 24 and any other appropriate forms

Doctor's signature	Print name	Date

Appendix 13
Form 9
Malaria form

COSTOP

COSTOP trial number						

Date of form D D M M M 2 0 Y Y Clinic/ hosp. number

Date of birth D D M M M Y Y Y Y Patient initials Male ❍ Female ❍

Date of malaria diagnosis D D M M M 2 0 Y Y

What type of malaria report is this? Initial ❑ follow up ❑ Resolution ❑

What type of malaria diagnosis was made? Definitive ❑ Presumptive ❑

What was the highest recorded temperature . ^{0}c

SYMPTOMS AND SIGNS

Symptom		
Fever	Yes ❑	No ❑
headache	Yes ❑	No ❑
loss of consciousness	Yes ❑	No ❑
Convulsions	Yes ❑	No ❑
Chills and rigors	Yes ❑	No ❑
Joint pains / muscle aches	Yes ❑	No ❑
Diarrhea	Yes ❑	No ❑
Vomiting	Yes ❑	No ❑
Yellowing of eyes	Yes ❑	No ❑
Whitening of eyes / palms	Yes ❑	No ❑
Other ❑. please specify		
Signs		
Pallor ❑	Jaundice ❑	Hepatomegally ❑
Splenomegally ❑	None ❑	Other ❑ specify.....

LABORATORY INVESTIGATIONS

1. If patient was treated at the study clinic fill the table below, if not go to 2;

Initial malaria blood slide result			
Positive ❑	Negative ❑		
Parasite count	/ 200 wbc's (thick film)		% (thin film)
Species			
P. Falciparum ❑	P. Malariae ❑	P. Vivax ❑	
P. Ovale ❑	Other ❑, please specify		

2. For patients getting treatment outside the study clinic, give the blood slide result?
Positive ❑ Negative ❑ Not done ❑

TREATMENT

1. Source of treatment? Study clinic ❑ Other Health worker ❑ self medication ❑ None ❑

2. Treatment given (tick all that apply)

Drug		Drug	
Chloroquine	❑	Tab quinine	❑
Sulphadoxine / pyrimethamine	❑	I.V quinine	❑
Artemether / lumfantrine	❑	Herbs	❑
Other artemesin derivatives	❑	Other drug ❑, specify	
Amodiaquine (Kamaquin)	❑		

3. Is the patient on any malaria chemoprophylaxis ? Yes ❑ No ❑

If yes, what anti malarial are they on? (fill table below)

Chloroquine ❑	Doxycycline ❑	Fansidar ❑	mefloquine ❑	Primaquin ❑
Proguanil ❑,	Other ❑	please specify		

OUT COME
(Remind the patient to come back for review within 2weeks of a malaria episode)

1. Have the malaria symptoms completely resolved? Yes ❑ No ❑
If yes, give date?

Date of resolution	D	D	M	M	M	2	0	Y	Y

2. Was a repeat slide done after treatment? Yes ❑ No ❑

a) For a patient reviewed in the study clinic fill the table below, if not go to b

Repeat malaria blood slide result			
Positive ❑	Negative ❑		
Parasite count	/ 200 wbc's (thick film)		% thin film
Species			
P. Falciparum ❑	P. Malariae ❑	P. Vivax ❑	
P. Ovale ❑	Other ❑, please specify		

b) For patients reviewed outside the study clinic. Give the blood slide result
Positive ❑ Negative ❑ Not done ❑

Doctor's signature	Print name	Date

COSTOP

Consent withdrawal form

COSTOP study number						

Date of form D D M M M 2 0 Y Y

Clinic/hospital number

Date of birth D D M M M Y Y Y Y

Patient initials

Male ❍ Female ❍

I no longer wish to (or cannot) take trial drugs but I am willing to attend follow up visits.	
Encourage the patient to continue with all, or at least some, scheduled visits.	
I no longer wish to (or cannot) take trial drugs and do not wish to (or cannot) attend further visits. I agree to my medical records being consulted in future to obtain clinical information	
Set in place procedure to follow the patient up through medical records alone and report any trial outcomes on the appropriate form. Inform the patient that s/he may still return for follow-up visits only	
I no longer wish to (or cannot) take trial drugs and do not wish to (or cannot) attend further visits. I do not agree to my medical records being consulted in future to obtain clinical information for the study	
Discontinue all follow up through medical records.	
I no longer wish to (or cannot) take trial drugs and do not wish to (or cannot) attend further visits. I do not agree to my medical records being consulted in future to obtain clinical information and also withdraw consent for information already collected to be used in any analysis	
Discontinue all follow up through medical records. Consult the Trial Centre for advice on removing records from the trial database.	

Patient's signature (or thumbprint)	Print name	Date

Witness's signature	Print name	Date

Study Team Member's signature	Print name	Date

Study Doctor's signature	Print name	Date

One signed copy to be given to patient, One signed copy (original) to be kept by the researcher,
One signed copy to be kept in the patient file.

Form 17
Unblinding form

COSTOP

COSTOP trial number						

Date of form D D M M M 2 0 Y Y

Clinic/ hosp. number

Date of birth D D M M M Y Y Y Y

Patient initials

Male ❍ Female ❍

This week's visit number

Visit type: Scheduled (on time) ❑ scheduled (late) ❑ Sick ❑ Missed ❑

REQUEST TO UNBLIND PATIENTS IN THE COSTOP STUDY

1. Name and phone number of clinician requesting for Unblinding

2. Reason for Unblinding

3. Has the patient experienced a hypersensitivity reaction? Yes ❑ No ❑
   (If, so please fill a hypersensitivity form)

4. Has this been discussed with the principal investigator or clinical deputy Yes ❑ No ❑
   (if not please do so)

As the clinician responsible for this patient, remember that the unblinding information given to you is confidential and should not be shared with anyone else.

Doctor's signature	Name	Date

Unblinding

Date patient unblinded D D M M M 2 0 Y Y

Has the doctor caring for the patient been informed? Yes ❑ No ❑ (if not, please do so)

Statisticians' signature	Name	Date

Form 14
Hospital Admission

COSTOP

COSTOP trial number						

Date of form D D M M M 2 0 Y Y Clinic/ hosp. number

Date of birth D D M M M Y Y Y Y Patient initials Male ❍ Female ❍

Admission summary

Date of admission	D D M M M 2 0 Y Y
Date of discharge	D D M M M 2 0 Y Y
Date of death (if patient died in hospital)	D D M M M 2 0 Y Y

Hospital admitted to

Entebbe ❑ Kisubi ❑ Mulago (referral) ❑
Masaka ❑ Villa Maria ❑ Kitovu ❑

Other ❑, please specify……

Main diagnoses at discharge or death

1.
2.
3.
4.
5.

Admission details

History

Findings on Clinical Examination

Investigations and results

Treatment / procedures

Condition at discharge

Has Malaria, WHO clinical event, Adverse event or Death occurred ? Yes ❑ No ❑
If Yes, please fill relevant COSTOP study event report form

Doctor's signature	Print name	Date

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
United Kingdom
Switchboard: +44 (0)20 7636 8636

www.lshtm.ac.uk

Observational / Interventions Research Ethics Committee

Ronnie Kasirye
Research Degree Student
IDE / EPH
LSHTM

7 January 2014

Dear Dr. Kasirye,

Submission Title: **Effect of cotrimoxazole on malaria in HIV infected patients on antiretroviral therapy**

LSHTM Ethics Ref: **7022**

Thank you for your response of 6 January 2013, responding to the Observational and Interventions Committees' request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval for the amendment having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Information Sheet	Information Sheet.PDF	22/11/2010	1.0
Information Sheet	luganda information sheet.PDF	22/11/2010	1.0
Information Sheet	Screening consent.pdf	22/11/2010	1.0
Information Sheet	Enrolment Consent.pdf	22/11/2010	1.0
Protocol / Proposal	Appendix 2-COSTOP Screening Questionnaire.pdf	15/3/2012	1.2
Protocol / Proposal	Appendix 3-COSTOP Enrolment quest. Nurse.pdf	15/10/2011	1.1
Protocol / Proposal	Appendix 4-COSTOP Enrolment quest. Doc.pdf	15/10/2010	1.0
Protocol / Proposal	Appendix 5-COSTOP Adherence questionnaire.pdf	15/3/2012	1.1
Protocol / Proposal	Appendix 6-COSTOP Nurse fup.pdf	15/10/2011	1.1
Protocol / Proposal	Appendix 7-COSTOP Doctor's fup.pdf	15/3/2012	1.3
Protocol / Proposal	Appendix 8- COSTOP Unblinding form.pdf	15/10/2010	1.0
Protocol / Proposal	Appendix 9-COSTOP Malaria form.pdf	15/7/2012	1.3
Protocol / Proposal	COSTOP Protocol - Version 5 2.doc	15/5/2013	5.2
Protocol / Proposal	Effect of CTX on malaria in pts on ART 0.7.doc	15/6/2013	0.7
Protocol / Proposal	Appendix 10-COSTOP Hospital Admissions.pdf	15/10/2010	1.0

After ethical review

Any subsequent changes to the application must be submitted to the Committee via an Amendment form onthe online application website. The Principal Investigator is reminded that all studies are also required to notify the ethics committee of any serious adverse events which occur during the project via anAdverse Event form on the online application website. An annual report form is required on the anniversary of the approval of the study and should be submitted during the lifetime of the study onthe online application website. At the end of the study, please notify the committee via anEnd of Study form onthe online application website.

Yours sincerely,

Professor John DH Porter
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

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