A REVIEW OF THE EXPANDED USE OF CO-TRIMOXAZOLE IN HIV-INFECTED AFRICANS

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The HIV/AIDS epidemic affects large numbers of people in sub-Saharan Africa. Most are unaware of their HIV status. Despite two decades of scientific advance, the education of communities and the provision of antiretroviral medication to some, many still succumb to the virus. Can this situation be changed? Antiretroviral (ARV) drugs have been shown to be effective in both poor and rich communities.[1,2] But for the majority, these agents remain unaffordable and difficult to access. After 2 years, the public-sector ARV rollout programme in South Africa remains under-subscribed and under-utilised.[3] Diets, vitamins, micronutrients and herbal concoctions have been advocated.[4] But none has provided the survival benefit, freedom from opportunistic disease, and completeness of recovery, of antiretroviral therapy.[5]

In recent years, research in developing countries has suggested that the daily use of the sulfonamide combination antibiotic, co-trimoxazole (CTX, trimethoprim-sulfamethoxazole, TMP/SMX), enhances the survival of infected adults and children.[6-8] Co-trimoxazole (CTX) use in patients with advanced HIV infection became widespread in the 1980s when efficacy against *Pneumocystis jiroveci* pneumonia (PJP) was demonstrated. In this context, prophylactic CTX was commenced at CD4 levels of 200 cells/µl or less or following an AIDS-defining condition including PJP itself, prolonged and unexplained fever and weight loss. It was discontinued once the CD4 count rose to and remained above 200 cells/µl for at least 6 months.[9]

But the landscape for prophylactic CTX use in Africa appears to be changing. Recent World Health Organization (WHO) Guidelines have recommended expanding CTX use to all HIV-infected persons – where CD4 levels are unknown – with symptomatic WHO stage 2, 3 or 4 disease and where CD4 counts are available, to all with counts below 350 cells/µl. All HIV-infected persons with TB - pulmonary and non-pulmonary – are to be placed on CTX prophylaxis irrespective of their CD4 cell count. A 'universal option' of 'CTX to all' is offered to those who live in regions of high HIV prevalence and inadequate health care support.[10] When is CTX prophylaxis stopped? 'The general view is to continue CTX prophylaxis in adults – in resource-poor settings – indefinitely.'[10]

Much of the data behind these recommendations come from Africa itself: Cote d'Ivoire, Uganda, Malawi, Zambia and South Africa[6-8,11-12] (and see Fig. 1 in Anglaret *et al.*[22]). How ought health workers and planners to interpret these suggestions for their region?

CTX USE AND THE DEVELOPMENT OF ANTIMICROBIAL RESISTANCE

RESISTANCE TO REGIONAL BACTERIAL PATHOGENS AND MALARIA

The sulfonamide antimicrobials were discovered in 1932. A sulfonamide-trimethoprim combination was first registered in 1968.[13] Sadly the general efficacy of CTX has been curtailed by the rapid emergence of drug resistance. Among the enteric bacteriaceae and the pneumococci, resistance to CTX is a global phenomenon.[14-16] Indeed, the level of CTX resistance in many parts of central and southern Africa is high.[11,14,17,18] Resistance is not confined to the bacterial kingdom. Point mutations in the dihydrofolate reductase (*dhfr)* and dihydropteroate synthetase (*dhps*) genes of *Plasmodium falciparum* have rendered the malarial parasite resistant to sulfadoxine-pyrimethamine (Fansidar), a sulfonamide anti-malarial used widely throughout Africa.[19,20]

Sulfonamides prevent the conversion of para-aminobenzoic acid to dihydrofolate via the inhibition of dihydropteroate synthetase, DHPS. Trimethoprim blocks the subsequent formation of tetrahydrofolate through blocking dihydrofolate reductase (*dhfr*). All organisms require folate for their metabolic needs. Mutations in and altered function of these genes form the basis of much of the resistance to these drugs. It has been noted that strains of *Escherichia coli* isolated in the developing world are more often resistant to CTX than are strains in developed regions. Climate, poverty, poor hygiene, a weak health infrastructure, and the indiscriminate use and abuse of antimicrobials have contributed to this.[13] With regard to sub-Saharan Africa, these factors are unlikely to change soon. Drug resistance is transferable between microbial kingdoms. The use of Fansidar in Malawi has been linked to the local increase of CTX resistance in *Streptococcus pneumoniae*.[21]

RESISTANCE TO *P. JIROVECI*

Despite inconsistent reporting, *P. jiroveci* is an important African pathogen.[24-26] Sulfonamide use has been followed by *dhps* gene mutations in this organism. The gradual accumulation of more and more resistant genes is likely to lead to the high-grade sulfonamide resistance that has become 'usual' in other microbes.[27-32]

Despite the high level of background bacterial resistance, the use of CTX in HIV-infected Africans appears to confer survival benefit against regional pathogens, not just at traditional CD4 levels below 200 cells/µl, where it would be expected to prevent *P. jiroveci* pneumonia (PJP), but at levels in excess of 500 cells/µl.[22,23] What then is CTX doing that is benefiting the HIV infected? And were CTX to be used more widely in Africa, what will this mean for further resistance to, and the use of, this antimicrobial in the future?

THE CLINICAL STUDIES

THE COTE D'IVOIRE STUDIES[6,7]

Two randomised placebo-controlled clinical trials evaluated the role of CTX in subjects who either had a WHO stage 2 and 3 HIV diagnosis or presented with smear-positive pulmonary tuberculosis (TB).[6,7] The studies were performed by two groups of investigators based in Abidjan, Cote d'Ivoire. Enrolment ran from 1995/6 to 1998 with a median follow-up of approximately 10 - 12 months. Patients were not provided with ARV therapy. Primary end-points included the occurrence of severe events, particularly death or hospital admission. Secondary outcomes measured morbidity. Both studies were discontinued prematurely in the light of significant benefit in the CTX arms. Few adverse events were recorded in those subjects receiving CTX. In the TB study, benefit was shown to be greater in those whose CD4 level was below 350 but above 100 cells/µl. Anglaret *et al.*[6] noted benefit across all CD4 strata: below 200, between 200 and 499, and above 500 cells/µl. Fewer episodes of pneumonia, isosporiasis, malaria and 'acute unexplained fever' were reported. Subjects enrolled in the TB study and taking CTX experienced fewer enteric and bacteraemic infections. Importantly, the authors note that at that time, many pathogens (including the pneumococci and salmonellae) in the Abidjan area were still sensitive to CTX.

In a subsequent letter to *AIDS*[22] these researchers point out that an Ivoirean consensus statement now recommends the prophylactic use of CTX in all HIV-infected persons with a WHO clinical stage 2, 3 or 4 diagnosis or who have CD4 cell counts below 500 cells/µl. This view is endorsed by 'the Global AIDS Policy Model Investigators', who in addition conclude that prophylactic CTX is cost-effective in a developing world scenario when started at or after WHO stage 2, i.e. at an early stage of HIV infection.[33]

THE UGANDAN DATA[12,23,34,35]

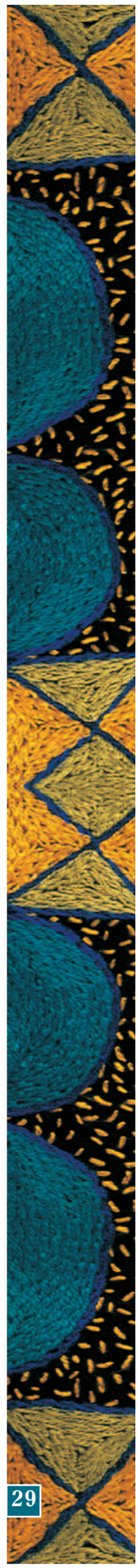
Since 2001, Mermin and co-workers have been researching a stable population of rural HIV-infected Ugandans. Subjects were provided with 'safe' drinking water and CTX. In subsequent follow-up studies ARVs were added, and later still, mosquito-repellant impregnated bed nets. Overall mortality and morbidity rates were examined in addition to rates of occurrence of malaria, diarrhoea illnesses, the numbers of clinic visits and hospital admissions. Participants provided their own 'internal' controls: subjects were monitored for 5 months before commencing CTX therapy.

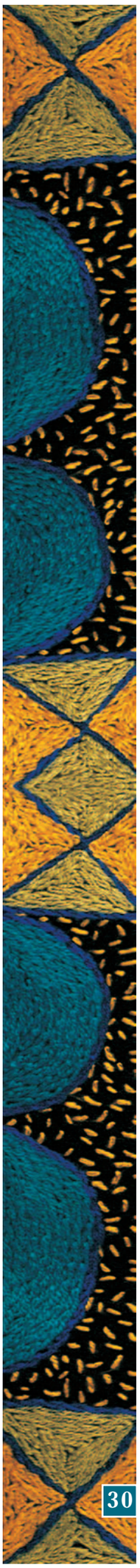
Of bacterial isolates from study subjects 76% were found to be resistant to CTX. Nonetheless, the use of CTX was associated with a reduction in the number of episodes of diarrhoea and fewer deaths. Daily CTX use was associated with a 72% decrease in the rate of malaria among study subjects. Parasite levels were lower. Although the death rate among the HIV-infected was 28 times higher than that of uninfected Ugandans, the use of CTX conferred a 46% reduction in death in the former. Improved survival on CTX was significant only for those whose CD4 levels were below 200 cells/µl or who had a WHO stage 3 or 4 entry-level diagnosis. However, in a further letter to *AIDS*[23] the authors note that in a sub-analysis of their patients with CD4 levels in excess of 500 cells/µl, the annual rate of decline of cells was less than that for the period prior to the start of CTX prophylaxis. They argue for considering commencing CTX prophylaxis at levels above 500 cells/µl.

In a related study the prophylactic use of CTX in HIV-positive household members – usually parents – gave some degree of protection to their uninfected children. Mortality was reduced and there were fewer episodes of malaria or diarrhoea in these children.[34] Before and after CTX-resistance patterns of stool pathogens appeared to remain unchanged and the benefit was attributed to the improved survival of the index case (adult) within the family.

The researchers took their work further with an assessment of the potential benefit of ARV therapy and mosquito-repellant impregnated bed nets in subjects already taking prophylactic CTX. Cumulative benefit was demonstrated with both interventions: fewer episodes of malaria. Benefit for other morbidities such as pneumonia, diarrhoea and bacteraemia was not measured.[35] These later sequential studies lacked contemporaneous control groups and were observational in design. The median duration of actual follow-up while on ART and CTX was short, a mere 126 days. These studies took place in a rural setting in Uganda.

Similar results have been achieved in two peri-urban clinics in Uganda: reduced mortality and fewer episodes of malaria.[36] The latter study was non-randomised, used historical controls and took place between August 1999 and March 2002. The primary benefit seems to have been in malaria control, whereas the incidence of diarrhoea with fever, herpes zoster and oral thrush actually increased on CTX. Worryingly, subjects on CTX had a greater decline in their CD4 cell counts while on CTX. Likewise total white cell counts and mean neutrophil counts were significantly reduced during this period. The fall in CD4 cells and blood counts while on CTX is not discussed in detail.





The Ugandan studies support those from the Ivory Coast and indicate that the role of CTX prophylaxis extends beyond just the prevention of PJP in subjects whose CD4 counts are below 200 cells/µl. Much of this benefit reflects improved malarial control. These clinical trials are not all alike: some have been randomised and placebo-controlled while others have been less tightly structured. The context – urban and rural populations, differing target of primary pathogens and differing resistance patterns – has not been identical. But nonetheless the work has reflected the situation on the ground and has been extremely valuable. The preventive value of CTX appears to vary with regard to respiratory and enteric infections. Its role against malaria is very convincing.

ZAMBIA[11]

Researchers based in Lusaka, Zambia, have evaluated CTX prophylaxis in HIV-infected children. Bacterial pathogens in this region are mostly resistant to CTX. The study was double-blind, randomised and placebo-controlled. It started in 2001 but ended prematurely in 2003 when data confirmed benefit to those children on CTX. The median follow-up was 18.9 months. A small but equal number of children (4 - 5%) in both groups were able to afford ARV therapy (ART). Fewer children in the CTX arm died, and there were fewer hospital admissions in this group. Mortality benefit was independent of age or CD4 category. Pneumonia was strongly associated with mortality and was less frequent in the CTX arm. Somewhat surprisingly, *P. jiroveci* was isolated in only 1 child. An earlier autopsy study based in Lusaka revealed a high prevalence of PJP in this community, 27.5% of children dying with lung disease.[37] South African and Malawian researchers have confirmed a high prevalence of PJP in this part of the subcontinent.[24,25,38] *Pneumocystis* infections are notoriously difficult to nail. The possibility remains that some benefit from CTX may have been accounted for by occult *Pneumocystis* infections. Malaria was not a significant pathogen in this group. Survival benefit appears to be related to the reduction in respiratory disease.

Chintu *et al.*[11] recommend that 'all children with clinical features of HIV-infection should receive cotrimoxazole prophylaxis irrespective of age and CD4 count', 'irrespective of levels of background resistance to the drug'. In a region with cost constraints and where only 5% of children are accessing ART it would seem ill advised to contradict this opinion.

An adult CTX prophylaxis study, the LUCOT trial, has been submitted for publication. The subjects are Zambians with newly treated or previously treated pulmonary tuberculosis. Results demonstrate a 45% reduction in mortality in the CTX arm. The findings are discussed in the WHO Guidelines paper.[10] A further Zambian study noted birth outcomes in pregnant women given CTX prophylaxis. Results indicated reduced chorioamnionitis, prematurity, and neonatal mortality in children born to mothers on the CTX arm. The women had CD4 counts below 200 cells/µl.[39]

SOUTH AFRICA[8]

Grimwade *et al.*[8] looked at the effectiveness of CTX on the mortality of adults with TB in the Hlabisa district of rural KwaZulu-Natal. The study utilised previously diagnosed TB patients (1998 - 2000) from the community as historical controls. HIV seroprevalence in the area – via anonymous testing – had increased from 36% (1993) to 78% in 2001/2. Despite counselling, only 5% of the 1 173 subjects recruited for the study from 2001/2 agreed to be tested for HIV status; 80% tested positive. The researchers found a significant reduction in death at 6 months in those given CTX. Despite continuing with the trial, there was no change in mortality after this period. Adherence to daily CTX had in fact dropped to 43% by 6 months. Malaria was infrequently diagnosed and was felt to be an unlikely reason for CTX-related benefit. The authors admit that there were significant differences in 'type of TB' between the historical cohort and the study subjects. In addition no CD4 levels are given, nor was the degree of immunological suppression of the subjects reflected in any other data provided by the authors. Without this, it is difficult to exclude benefit from *Pneumocystis* prevention as a contributing factor.

CURRENT WHO RECOMMENDATIONS AND USE OF CTX IN HIV-INFECTED PERSONS IN RESOURCE-POOR REGIONS[10]

GENERAL REMARKS

Patients with a history of severe reactions to CTX or other sulfonamides and children with glucose-6-phosphate dehydrogenase (G6PD) deficiency ought not to receive CTX prophylaxis. Dapsone 2 mg/kg once daily orally (100 mg daily in adults) is an alternative. Inhaled pentamidine for *P. jiroveci* prophylaxis is expensive and generally unavailable in resource-constrained areas. Neither dapsone nor inhaled pentamidine possess the breadth of antimicrobial efficacy of CTX.

The continued requirement for CTX prophylaxis in patients who are receiving highly active antiretroviral therapy (HAART) is probably limited to those in whom reconstitution of the immune system is adequate or who experience treatment failure and subsequent regression of CD4 levels. In adults on ART, CTX is stopped after a CD4 count above 200 cells/µl has been achieved and maintained for at least 6 months after starting HAART.[40] This may take some time. The Development of Antiretroviral Therapy for Africa (DART) Study examined the CD4 cell response following the start of HAART in Ugandans. Where subjects initiated ART with CD4 levels above 100 but below 200 cells/µl it took a median of 24 weeks to return levels to above 200. If the initial CD4 level was below 50, the median time to achieve a level above 200 was 72 weeks[10] (as quoted, reference not given). Even on HAART, some patients fail to achieve a level above 200 cells/µl, particularly where initial CD4 levels were extremely low; prudence recommends continuing CTX prophylaxis in such patients.[41]

INFANTS AND CHILDREN

In children aged under 1 year, CTX prophylaxis is provided from 4 - 6 weeks of life and throughout this period, irrespective of CD4 percentage or the use of HAART. Opportunistic infections are common and life-threatening at this age. There are no available data on the value of both

HAART and CTX use in children in resource-poor areas. European and USA guidelines discourage the continuous use of both CTX and HAART in children provided immune reconstitution has taken place[9,42,43] (Table I).

TABLE I. WHO RECOMMENDATIONS WITH REGARD TO THE INITIATION OF CTX PROPHYLAXIS IN INFANTS AND CHILDREN[10]

Situation			
HIV-exposed infants and children	Confirmed HIV infection in infants and children		
	< 1 year	1 – 4 years	> 5 years
CTX prophylaxis is universally indicated, starting at 4 - 6 weeks after birth and continuing until cessation of risk of HIV transmission and the exclusion of HIV infection **[A-III]**	CTX prophylaxis is indicated regardless of CD4% or clinical status of child	WHO stages 2, 3 and 4 regardless of CD4% **OR** any WHO stage with a CD4 < 25% **[A-I]**	Follow adult recommendations

Universal option: Prophylaxis for all infants and children born to confirmed or suspected HIV-infected mothers. This strategy may be considered in settings with high prevalence of HIV, high infant mortality due to infectious disease, and limited health infrastructure. **[C-IV]**

Note that the grading of recommendations as indicated in this WHO Table is based upon the following:

Strength of recommendation:

A. Highly recommended. Should be followed.
B. Consider: Applicable in most situations.
C. Optional.

Level of evidence to support the recommendation:

I. At least one randomised controlled trial with clinical endpoints or several relevant high-quality scientific studies.
II. At least one randomised controlled trial with surrogate markers, at least one high-quality study or several adequate studies.
III. Observational cohort data, one or more case-controlled or analytical studies adequately conducted.
IV. Expert opinion based on evaluation of other evidence.

ADOLESCENTS AND ADULTS

Should the initiation of CTX preventive therapy be restricted to patients with CD4 counts below 200 cells/µl or AIDS-defining diagnoses? Clearly the data that emerge from the studies discussed in this paper suggest not. Mortality and morbidity benefit occurs in those with CD4 counts above 200 cells/µl, possibly even above 500, and/or with early WHO stage 2 and 3 disease.[6,12,23] What is the upper level at which further benefit cannot be demonstrated? Does CTX preventive therapy need to be continued despite successful immune reconstitution on HAART?

Most of the African data reflect a rather specific context: poverty, little or no access to HAART, endemic malaria and/or invasive enteric and respiratory infections. Can these results be applied uniformly to the cities and rural districts of southern Africa?

Poverty exists in these cities, as do other determinants of poor health outcomes: informal settlements, overcrowding, contaminated and insecure water supplies, failing health and hygiene structures, uneducated, displaced and disempowered people. HIV/AIDS is defining the context of life lived in modern Africa. In these ways the cities and rural regions of southern Africa are not too different from the social environment described in the above studies on CTX. However, there are differences too. Malaria is less prevalent in the south of the continent than in central and west Africa. Other organisms that may respond to CTX therapy have variable regional expression in Africa or variable resistance patterns to CTX – *S. pneumoniae*, isosporiasis, non-typhoidal salmonellae, toxoplasmosis and possibly even common enteric pathogens such as *E. coli*. The local prevalence of these conditions may influence the regional effectiveness of CTX prophylaxis.[26]

The rollout of ARVs in South Africa, Botswana and Namibia is generally more widespread than in countries to the north and the influence of immune reconstitution with ARV treatment is likely to significantly diminish the need for CTX prophylaxis in patients on HAART (Table II).

TABLE II. WHO RECOMMENDATIONS WITH REGARD TO THE INITIATION OF CTX PROPHYLAXIS IN ADOLESCENTS AND ADULTS IN RESOURCE-POOR REGIONS[10]

Based on WHO clinical staging criteria alone where the CD4 count is not available	Based on WHO clinical staging and CD4 cell count criteria
WHO stage 3 or 4 **[A-I]**	CD4 below 350 cells/µl **[A-III] OR** WHO stage 3 or 4 and any CD4 level **[A-I]**
WHO stage 2 **[A-III]**	

Universal option: Countries may choose to adopt a Universal CTX for all HIV-infected persons irrespective of CD4 or clinical stage. This strategy may be considered in settings with a high prevalence of HIV and limited health infrastructure **[C-III]**

For grading of recommendations see Table I.

These new WHO Guidelines suggest that where CD4 counts are available, CTX should be given to all with a CD4 count below 350 cells/µl, particularly in resource-poor settings where malaria and invasive bacterial infections are common. Similarly it is suggested that all patients with any form of TB should start CTX prophylaxis irrespective of their CD4 cell count. Long-term adherence to CTX is emphasised and may well be difficult to implement widely, particularly where a 'universal CTX-for-all-and-taken-indefinitely" approach is followed. Women who require CTX prophylaxis and who fall pregnant are urged to remain on CTX. The CTX is continued even while breastfeeding.

Discontinuation of CTX is suggested in the context of immune recovery or toxicity. However, in the absence of CD4 count monitoring 'no consensus was reached' regarding stopping CTX therapy. Discontinuation is suggested after a year on CTX provided the patient is on HAART, has had no symptomatic WHO stage 2, 3 or 4 events and has displayed reliable adherence. CTX is to be restarted when the CD4 count again

falls below the starting value and/or when WHO stage 2, 3 or 4 events occur.[10] In an attempt to avoid confusion as to the cause of hepatic and skin toxicities, it is recommended that CTX prophylaxis be started 2 weeks before commencing HAART where the latter is available. Should the patient develop breakthrough invasive infections, antibiotics other than CTX are advised. This would also apply to the active management of malaria, where a patient currently on CTX would not be expected to derive benefit from sulfadoxine-pyramethamine (Fansidar). Alternative antimalarials must be used.

CONCLUDING REMARKS

The current WHO recommendations for the use of CTX in resource-poor regions focuses on communities where invasive infections are frequent and are accompanied by significant morbidity and mortality in the HIV infected. Southern Africa is a kaleidoscope of developing and developed worlds where some, but not all, access ARV medication and clinical support. The fact that an inexpensive antimicrobial can provide survival advantage must be taken seriously and this benefit should be offered to patients whose CD4 count exceeds 200/µl and who are not on ARVs. However, thorough research into the appropriateness of such a course of action will be required – who to give it to, when to stop, and whether benefit is maintained in the context of the antiretroviral rollout in southern Africa. Invasive bacterial disease is sufficiently important in the southern African context to warrant review in relation to CD4 cell count in much the same decisive way the Cote d'Ivoire investigators have done (see Fig. 1). Answers would inform the issues of whether local patients need to be on CTX when this CD4 count is above 200 cells/µl. Would CTX prophylaxis benefit those on ART? This question requires an answer, which will involve clinical research and evidence-based data. At this time the question in relation to southern Africa remains unanswered.

REFERENCES

1. Ivers LC, Kendrick D, Doucette K. Efficacy of antiretroviral therapy programs in resource-poor settings: A meta-analysis of the published literature. *Clinical Infect Dis* 2005; **41:** 217-224.
2. Fassinou P, Elenga N, Rouet F, *et al.* Highly active antiretroviral therapies among HIV-1 infected children in Abidjan, Cote d'Ivoire. *AIDS* 2004; **18:** 1905-1913.
3. Kapp C. Antiretrovirals give new hope and new life to South Africans. *Lancet* 2004; **363:** 1710.
4. Mills E, Foster BC, van Heeswijk R, *et al.* Impact of African herbal medicines on antiretroviral metabolism. *AIDS* 2005; **19:** 95-97.
5. World Health Organization. *Executive Summary, Consultation on Nutrition and HIV/AIDS in Africa: Evidence, Lessons and Recommendations for Action. Durban, South Africa. 10 -13 April 2005.* Geneva: WHO, .
6. Anglaret X, Chene G, Attia A, *et al.* and the Cotrimo-CI study group. Early chemoprophylaxis with trimethoprim-sulphamethoxazole for HIV-1-infected adults in Abidjan, Cote d'Ivoire: a randomized trial. *Lancet* 1999; **353:** 1463-1468.
7. Wiktor SZ, Sassan-Morokro M, Grant AD, *et al.* Efficacy of trimethoprim-sulphamethoxazole prophylaxis to decrease morbidity and mortality in HIV-1-infected patients with tuberculosis in Abidjan, Cote d'Ivoire: a randomized controlled trial. *Lancet* 1999; **353:** 1469-1475.
8. Grimwade K, Sturm AW, Nunn AJ, Mbatha D, Zungu D, Gilks C. Effectiveness of cotrimoxazole prophylaxis on mortality in adults with tuberculosis in rural South Africa. *AIDS* 2005; **19:** 163-168.
9. Urschel S, Ramos J, Mellado M, *et al.*, and the European PCP-withdrawal Study Group. Withdrawal of *Pneumocystis jirovecii* prophylaxis in HIV-infected children under active antiretroviral therapy. *AIDS* 2005; **19:** 2103-2108.
10. World Health Organization. Guidelines for Cotrimoxazole Prophylaxis for HIV-related Infections in Children, Adolescents and Adults in Resource Limited Settings. Recommendations for a Public Health Approach. Final Draft (2006) World Health Organisation, Geneva, Switzerland. This report is based upon an expert consultation held in May 2005 and available at **http://www. who.int/hiv/pub/meetingreports/ctxprophylaxismeeting.pdf** (accessed June 2006).
11. Chintu C, Bhat GJ, Walker AS, *et al.*, on behalf of the CHAP trial team. Co-trimoxazole as prophylaxis against opportunistic infections in HIV-infected Zambian children (CHAP): a double-blind randomized placebo-controlled trial. *Lancet* 2004; **364:** 1865-1871.
12. Mermin J, Lule J, Ekwaru JP, *et al.* Effect of co-trimoxazole prophylaxis, morbidity, mortality, CD4-cell count, and viral load in HIV infection in rural Uganda. *Lancet* 2004; **364:** 1428-1434.
13. Huovinen P. Resistance to trimethoprim-sulphamethoxazole. *Clinical Infectious Diseases* 2001; **32:** 1608-1614.
14. Feikin DR, Dowell SF, Nwanyanwu OC, *et al.* Increased carriage of trimethoprim-sulphamethoxazole-resistant *Streptococcus pneumoniae* in Malawian children after treatment for malaria with sulfadoxine-pyramethamine. *J Infect Dis* 2000; **181:** 1501-1505.
15. Martin JN, Rose DA, Hadley WK, *et al.* Emergence of trimethoprim-sulphamethoxazole resistance in the AIDS era. *J Infect Dis* 1999; **180:** 1809-1818.
16. Song J-H, Jung S-I, Ki HK, *et al.*, for the Asian Network for Surveillance of Resistant Pathogens Study Group. Clinical outcomes of pneumococcal pneumonia caused by antibiotic-resistant strains in Asian countries: A study by the Asian Network for Surveillance of Resistant Pathogens. *Clin Infect Dis* 2004; **38:** 1570-1578.
17. Scott JAG, Hall AJ, Lowe B, *et al.* Aetiology, outcome, and risk factors for mortality among adults with acute pneumonia in Kenya. *Lancet* 2000; **355:** 1225-1230.
18. Madhi SA, Cutland C, Ismail K, O'Reilly C, Mancha A, Klugman K. Ineffectiveness of trimethoprim-sulphamethoxazole prophylaxis and the importance of bacterial and viral coinfections in African children with *Pneumocystis carinii* pneumonia. *Clin Infect Dis* 2002; **35:** 1120-1126.
19. Roper C, Pearce R, Bredenkamp B, *et al.* Antifolate antimalarials resistance in southeast Africa: a population-based analysis. *Lancet* 2003; **361:** 1174-1181.
20. Baird JK. Effectiveness of antimalarial drugs. *N Engl J Med* 2005; **352:** 1565-1577.
21. Peikin DR, Dowell SF, Nwanyanwu OC, *et al.* Increased carriage of trimethoprim-sulfamethoxazole resistant *Streptococcus pneumoniae* in Malawian children after treatment with sulfadoxine-pyrimethamine. *J Infect Dis* 2000; **181:** 1501-1505.
22. Anglaret X, Toure S, Ouassa T, Dabis F, N'Dri-Yoman T. Thresholds of CD4 cells for initiating trimethoprim-sulfamethoxazole prophylaxis in West Africa. *AIDS* 2000; **14:** 2628-2629.
23. Mermin J, Lule JR, Ekwaru JP, Pitter C. Should cotrimoxaozle prophylaxis be taken by all adults with HIV in Africa? *AIDS* 2005; **19:** 845-846.
24. Ruffini DD, Madhi SA. The high burden of *Pneumocystis carinii* pneumonia in Africa HIV-1 infected children hospitalized for severe pneumonia. *AIDS* 2002; **16:** 105-112.
25. Fisk DT, Mesnick S, Kazazjian PH. *Pneumocystis carinii* pneumonia in patients in the developing world who have acquired immunodeficiency syndrome. *Clinical Infectious Diseases* 2003; **36:** 70-78.
26. Holmes CB, Losina E, Walensky RP, Yazdanpanah Y, Freedberg KA. Review of human immunodeficiency virus type 1-related opportunistic infections in sub-Saharan Africa. *Clin Infect Dis* 2003; **36:** 652-662.
27. Ma L, Borio L, Masur H, Kovacs JA. *Pneumocystis carinii* dihydropteroate synthase but not dihydrofolate reductase gene mutations correlate with prior trimethoprim-sulfamethoxazole or dapsone use. *J Infect Dis* 1999; **180:** 1969-1978.
28. Kazanjian P, Armstrong W, Hossler PA, *et al. Pneumocystis carinii* mutations are associated with duration of sulfa or sulfone prophylaxis exposure in AIDS patients. *J Infect Dis* 2000; **182:** 551-557.
29. Crothers K, Beard CB, Turner J, *et al.* Severity and outcome of HIV-associated *Pneumocystis* pneumonia containing *Pneumocystis jiroveci* dihydropteroate synthase gene mutations. *AIDS* 2005; **19:** 801-805.
30. Helweg-Larsen J, Benfield TL, Eugeg-Olsen J, Lundgren JD, Lundgren B. Effects of mutations in *Pneumocystis carinii* dihydropteroate synthase gene on outcome of AIDS-associated *P. carinii* pneumonia. *Lancet* 1999; **354:** 1347-1351.
31. Navin TR, Beard CB, Huang L, *et al.* Effect of mutations in *Pneumocystis carinii* dihydropteroate synthase gene on outcome of *P. carinii* pneumonia in patients with HIV-1: a prospective study. *Lancet* 2001; **358:** 545-549.
32. Meshnick SR. Drug-resistant *Pneumocystis carinii.* (Editorial.) *Lancet* 1999; **354;** 1318-1319.
33. Yazdanpanah Y, Losina E, Anglaret X, *et al.*, for the Global AIDS Policy Model Investigators. Clinical impact and cost-effectiveness of co-trimoxazole prophylaxis in patients with HIV/AIDS in Cote d'Ivoire: a trial-based analysis. *AIDS* 2005; **19:** 1299-1308.
34. Mermin J, Lule J, Ekwaru JP, *et al.* Cotrimoxazole prophylaxis by HIV-infected persons in Uganda reduces morbidity and mortality among HIV-uninfected family members. *AIDS* 2005; **19:** 1035-1042.
35. Mermin J, Ekwaru JP, Liechty CA, *et al.* Effect of co-trimoxazole, antiretroviral therapy, and insecticide-treated bednets on the frequency of malaria in HIV-1-infected adults in Uganda: a prospective cohort study. *Lancet* 2006; **367:** 1256-1261.
36. Watera C, Todd J, Muwonge R, *et al.* Feasibility and effectiveness of cotrimoxazole prophylaxis for HIV-1-infected adults attending an HIV/AIDS clinic in Uganda. *J Acquir Immune Defic Syndr* 2006; **42:** 373-378.
37. Chintu C, Mudenda V, Lucas S, *et al.*, for the UNZA-UCLMS Project Paediatric Post-mortem Study Group. Lung diseases at necropsy in African children dying from respiratory illnesses: a descriptive necropsy study. *Lancet* 2002; **360:** 985-990.
38. Graham SM, Mtitimila EI, Kamanga HS, Walsh AL, Anthony Hart C, Molyneux M. Clinical presentation and outcome of *Pneumocystis carinii* pneumonia in Malawian children. *Lancet* 2000; **355:** 369-373.
39. Walter J, Mwiya M, Scott N, *et al.* Cotrimoxazole prophylaxis and adverse birth outcomes among HIV-infected women in Lusaka, Zambia. 13th Conference on Retroviruses and Opportunistic Infections, 5 - 8 February 2006, Denver, Colorado. Abstract 126.
40. Kovacs JA, Masur H. Prophylaxis against opportunistic infections in patients with human immunodeficiency virus infection. *N Engl J Med* 2000; **342:** 1416-1429.
41. Duncombe C, Kerr S, Ungsedhapand C, *et al.*, and the HIV-NAT Study Group. Immune recovery and stopping cotrimoxazole prophylaxis in Thai patients treated with NNRTI-based HAART for 216 weeks. 13th Conference on Retroviruses and Opportunistic Infections, 5 - 8 February 2006, Denver, Colorado. Abstract 784.
42. Nachman S, Gona P, Danker W, *et al.* The rate of serious bacterial infections among HIV-infected children with immune reconstitution who have discontinued opportunistic infection prophylaxis. *Pediatrics* 2005; **115:** 488-494.
43. Urschela S, Ramosb J, Melladob M, *et al.*, and the European PCP-withdrawal Study Group. Withdrawal of *Pneumocystis jiroveci* prophylaxis in HIV-infected children under highly active antiretroviral therapy. *AIDS* 2005; **19:** 2103-2108.