

# THE LANCET

## **Supplementary webappendix**

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Walker AS, Ford D, Gilks CF, et al. Daily co-trimoxazole prophylaxis in severely immunosuppressed HIV-infected adults in Africa started on combination antiretroviral therapy: an observational analysis of the DART cohort. *Lancet* 2010; published online March 29. DOI:10.1016/S0140-6736(10)60057-8.

## Webappendix: Statistical Methods

Data to December 2008 were organised into 4-weekly intervals (n=167475, excluding follow-up after randomisation to structured treatment interruptions in a sub-study), corresponding to the nurse visit schedule. Baseline factors were those at cART initiation. Time-dependent variables were the most recent values at/or prior to the start of each interval, allowing laboratory measurements within the first 7 days of the interval only (3236 (1.9%) intervals for current CD4, other laboratory measures such as haemoglobin similar (data not shown)), likely corresponding to late clinic visits. Missing values were imputed by carrying the most recent observation forward (current CD4 >12 weeks before the start of an interval for 3135 (1.9%) of intervals and >24 weeks for 669 (0.4%) of intervals, other laboratory measures similar (data not shown)).

Use of cotrimoxazole for prophylaxis within an interval was defined as at least 7 days use: in 96989 of 97878 (99.1%) intervals it was used for the complete interval. Short (isolated) cotrimoxazole prescriptions for acute events were considered not to be prophylaxis (30 person-years, 0.2% follow-up) since participants being treated for such events were, by definition, already sick and at increased risk of clinical outcomes. Since inaccurate prescription dates could lead to cotrimoxazole prophylaxis initiated following a WHO stage 3/4 event being analysed as use prior to the event, we undertook sensitivity analyses shifting all periods of cotrimoxazole prophylaxis forward by 4 weeks.

Pooled logistic regression models were used to approximate marginal structural Cox models, controlling for time-dependent confounders, using weights based on the inverse probability of a participant's cotrimoxazole prophylaxis use, conditional on prognostic factors (inverse probability treatment weights, IPTW)<sup>1,2,3</sup>. The probability of cotrimoxazole prophylaxis use was modelled to obtain IPTW (treatment model). Then, the association between cotrimoxazole prophylaxis and outcome was estimated in a regression model including baseline variables and time-dependent cotrimoxazole prophylaxis, weighted using IPTW (outcome model).

Baseline factors in treatment and outcome models were randomisation year, age, sex, CD4, haemoglobin, BMI, WHO HIV disease stage, first-line cART regimen and WHO stage 3/4 event in the 4 weeks prior to randomisation. Time since randomisation was adjusted for using natural cubic splines with knots at 12, 48, 96, 144 and 192 weeks.

The probability of cotrimoxazole prophylaxis use in each four week interval,  $A(k)$  where  $A(k)=1$  indicates use of prophylaxis for at least 7 days in interval  $k$ , was estimated using pooled logistic regression models for each centre (n=4, satellite clinic a separate centre) and randomised monitoring group, as follows:

$$\text{logit}(P[A(k) = 1 | \bar{A}(k-1), V, \bar{L}(k)]) = \alpha_0(k) + \alpha'_1 \bar{A}(k-1) + \alpha'_2 V + \alpha'_3 \bar{L}(k)$$

where  $\alpha_0(k)$  is an interval specific intercept (modelled by cubic splines),  $\bar{A}(k-1)$  is cotrimoxazole history up to and including interval  $k-1$ ,  $V$  includes baseline factors described above,  $\bar{L}(k)$  is history of time-dependent confounders up to interval  $k$ . History of cotrimoxazole use was restricted to use on cART, assuming that any residual benefit of use prior to cART on outcomes could be explained by baseline covariates. Cotrimoxazole use in the preceding 6 intervals (24 weeks) predicted current use after 24 weeks on cART, so we fitted separate models for weeks 12-24 and >24 weeks on cART, including cotrimoxazole prophylaxis in the previous 3 ( $k-1, \dots, k-3$ ) and 6 intervals ( $k-1, \dots, k-6$ ) respectively as predictors in our treatment models. Time-dependent variables included in  $\bar{L}(k)$  were current CD4 and haemoglobin (most recent prior to interval  $k$  or in the first 7 days of interval  $k$ ) and BMI (most recent prior to interval  $k$ ), a WHO stage 3/4 event in the previous 4 weeks (interval  $k-1$ ), an earlier WHO stage 3/4 event since randomisation (in interval  $k-2, \dots, 1$ ) and randomisation in the STI study (never, to CT) prior to interval  $k$ , including interactions between these time-dependent variables and cotrimoxazole prophylaxis (on/off) in the previous four week period (interval  $k-1$ ). To estimate the effects of cotrimoxazole on neutrophils (or platelets), baseline and time-dependent neutrophils (platelets) were also included in the treatment model. All factors were checked for positivity and CD4 count was grouped (<50, 50-100, 100-200, 200-350, >350) after 24 weeks on cART as small numbers of participants started cotrimoxazole prophylaxis at high CD4 counts<sup>4</sup>. Sensitivity analyses additionally included last-but-one CD4, haemoglobin (usually 12 weeks prior to current value) and BMI (4 weeks prior to current value) and interactions with calendar period (results similar). Baseline covariates were

assumed to explain cotrimoxazole prophylaxis for the first 12 weeks on cART because there were too few cotrimoxazole changes during the first 12 weeks to model treatment (in total 121 participants started and 12 participants stopped cotrimoxazole prophylaxis), so IPTW weights were fixed as 1 in this period.

To adjust for potential selection bias from loss to follow-up, censoring weights were also estimated<sup>1</sup>. For non-fatal outcomes, censoring from death and from loss to follow-up (or end of follow-up in December 2008) were treated as separate outcomes in multinomial logistic regression models. Treatment and censoring weights were stabilised as standard and the time-dependent product used to weight outcome models, thus weighted regressions estimate the effect of an individual's complete cotrimoxazole history since starting cART on outcomes<sup>1</sup>. We explored the distribution and effect of our stabilised weights by progressive truncation<sup>4</sup>; in presented models weights were truncated at the 1<sup>st</sup> and 99<sup>th</sup> percentiles by centre and randomised monitoring group (in our primary mortality model the overall mean weight was 1.00, minimum 0.04, maximum 8.1; weights had means very close to 1 within centre, monitoring group and by time on cART ( $\leq 72$  weeks,  $>72$  weeks)). Confidence intervals for treatment effects are based on robust variance estimates<sup>1</sup>, confirmed by bootstrapping in main outcome analyses.

Outcome models adjusted for baseline pre-cART factors (as above), centre and randomised monitoring group. Twelve-weekly repeated measures were used for continuous outcomes. Models restricted to outcomes post-12 weeks on cART additionally adjusted for time-dependent variables at 12 weeks and cotrimoxazole prophylaxis use during the first 12 weeks on cART. Heterogeneity across subgroups was assessed using interaction tests.

We excluded Harare participants from analyses of malaria because of low rates of disease. We used history-adjusted marginal structural models<sup>5</sup> to incorporate more than one malaria event per participant. Time was measured from trial entry or 28 days after the previous malaria episode (baseline), and outcome regression models adjusted for time-dependent variables including previous malaria episode and history of cotrimoxazole prophylaxis at this baseline.

We investigated whether the effect of cotrimoxazole prophylaxis differed according to time since cART initiation by fitting interaction terms between current cotrimoxazole use and time on cART modelled using natural cubic splines, representing statistically significant non-linearity using piecewise linear effects and categorisation<sup>6</sup>. We investigated whether the effect of cotrimoxazole prophylaxis differed by current CD4 using history-adjusted marginal structural models, treating each 12-weekly follow-up visit as a new baseline and considering mortality over the following 12 weeks<sup>5</sup>. Time-dependent variables (including current CD4), history of cotrimoxazole prophylaxis and time on cART up to each baseline visit were included as predictors within the outcome regression model with time-dependent variables after baseline adjusted for using IPTW. Results were similar for 48 week follow-up periods or excluding the first 12 weeks on cART.

## References

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