Development of severe anaemia and changes in Haemoglobin (Hb) in a cohort of HIV infected Ugandan Adults receiving Zidovudine, Stavudine and Tenofovir containing antiretroviral regimens

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Abstract

Introduction—Anaemia is a common problem in HIV in sub-Saharan Africa. We describe the contribution of ART regimen on the incidence of anaemia and changes in haemoglobin in Ugandan patients.

Methods—This study was nested in a prevention of cryptococcal disease trial (CRYPTOPRO; ISRCTN7648152). Patients received three different nucleoside reverse transcriptase inhibitor backbones in a non-randomised manner.

Results—Of 852 patients (161 on zidovudine, 628 on stavudine and 63 on tenofovir (all received lamuvidine), the risk of developing grade 4 anaemia was higher (aHR 2.7) for those taking zidovudine compared with stavudine. Those taking stavudine had a greater average increase in haemoglobin than those taking zidovudine (p=0.024) or tenofovir (p=0.014).

Conclusion—In this observational study zidovudine was associated with higher levels of severe anaemia than stavudine or tenofovir; those receiving zidovudine and tenofovir had smaller

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Competing Interests
None of the authors has declared competing interests

Authors’ contributions
RPR: Study design, data collection, data analysis, manuscript preparation
DK: data collection, data analysis, manuscript preparation
JL: Study design, data analysis, manuscript preparation
KW: Data analysis, manuscript preparation
HG: Study design, manuscript preparation
AK: Study design, manuscript preparation
DL: Study design, data analysis, manuscript preparation
haemoglobin rises after ART initiation. We encourage publication of data from African cohorts using tenofovir.

**Keywords**

anaemia ART Africa tenofovir zidovudine Uganda haemoglobin

**Introduction**

Anaemia is a common clinical problem in HIV infected individuals, especially in those with advanced immunosuppression and is an independent risk factor for morbidity and mortality(1, 2). It is more common in Sub-Saharan Africa, where there is often a high background level of anaemia due to non-HIV related factors such as poor nutritional status, TB and malaria(3). In those with advanced immunosuppression, opportunistic infections (OIs) such as pneumonia, candidiasis and HIV wasting syndrome may compound the problem(4–6) and drugs that are commonly used for the treatment of OIs (e.g. ganciclovir, flucytosine, amphotericin, and sulphamides) can also cause anaemia(7).

Overall, the treatment of HIV infection with combination ART reduces the incidence of anaemia and increases haemoglobin (Hb) levels over time (8, 9). However, zidovudine (AZT) has also been clearly demonstrated to cause anaemia(10, 11) and in the USA AZT containing regimens showed a higher rate of new or worsening anaemia in cohort analysis(12, 13). Consequently, WHO and other guidelines advise avoiding AZT in patients with existing anaemia(14). Risk of anaemia is known to be greater in those starting AZT compared with tenofovir (TDF) from the Gilead 934 study(15) but the 903 study of d4T compared with TDF did not report a difference in Hb or anaemia(16). Initially, many ART programmes in Sub-Saharan Africa used stavudine (d4T) as first line treatment, but in most countries this has now been replaced with AZT or tenofovir (TDF), which overall have fewer side effects, and the most recent WHO guidelines highlight TDF (with lamivudine) as best first line NRTI option (17). Unfortunately, there are still challenges in Sub-Saharan Africa as often particular drugs or combinations may not be available despite guidelines being in place. Additionally, unlike in Western settings, where ART combinations are closely tailored to individual patients, this luxury is often not available in Sub Saharan Africa, where patients are treated with a public health approach and often receive whatever regimen is available despite individual relative contra-indications to a particular regimen.

Findings from Sub-Saharan Africa suggest that anaemia in patients receiving ART is more common than in industrialized countries (18). Rates of severe anaemia in the first six to 12 months of ART vary between 3.9 and 9.6/100 patient years at risk (PYAR) (18–20). In the TREAT Asia HIV observational database anaemia occurred in 13% of patients treated with AZT (21). One study has shown a higher incidence of anaemia after switching from d4T to AZT (22) and the need to switch from AZT because of severe anaemia is reported to be up to 15% of patients (23). However, other studies have shown that those on AZT containing therapy have less anaemia than ART naive patients (23, 24) and that starting AZT in those with severe baseline anaemia can be acceptable(25). Direct comparisons of rates of anaemia on different ART regimens in sub-Saharan Africa are rare, but findings from the IeDEA
database (from Sub-Saharan Africa, Asia pacific and South America) recently published have shown a 0.5 g/dL decrease in Hb in the first 3 months of AZT compared with alternatives (mainly d4T, but this was not fully explored)(20).

Between 2004 and 2008, we performed a randomised placebo controlled trial of primary prophylaxis for cryptococcal disease with fluconazole (CRYPTOPRO) (26). Participants received three different ART combinations, providing an opportunity to compare the incidence of anaemia and changes from baseline Hb between three different non-AZT and AZT containing regimens.

Methods

This study was nested in a randomised double blind placebo controlled trial of primary prophylaxis of cryptococcal disease in HIV infected individuals (CRYPTOPRO) [ISRCTN 7648152], which ran between 2004-2008 and full trial details have been previously published. The trial was performed among Ugandan adults in Masaka region, South West Uganda and compared fluconazole 200mg with identical placebo thrice weekly in patients with a CD4 <200 cells/ul. Participants in the CRYPTOPRO who commenced had the potential to be observed for 48 weeks before the end of the trial were eligible for inclusion in the anaemia study analysis. Participants were excluded from the anaemia study analysis if they did not start ART, did not have a pre-ART Hb reading or had grade 4 anaemia at the time of initiating ART. There were seven patients commencing ART with grade 4 anaemia were analysed separately.

ART was provided free of charge by three local service providers: The AIDS Support Organisation (TASO), Kitovu Mobile Home Care and Orphans Programme (Kitovu MAHCOP, and Masaka Ministry of Health (MOH)). These providers obtained their ART supplies through the Uganda Ministry of Health ART roll out programme and / or from donations. Participants received a backbone of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) (stavudine/lamivudine (d4T/3TC), zidovudine/lamivudine (AZT/3TC), or tenofovir/emtricitabine (FTC/TDF), depending on the provider and availability of supply, in combination with either nevirapine or efavirenz (non-nucleoside reverse transcriptase inhibitors (NNRTIs). At the time, the TDF/FTC was only provided at one clinic, and it was the first line NRTI backbone of choice at that site. All patients received cotrimoxazole as per Ugandan MOH guidelines.

Participants were seen every eight weeks, and complete blood count (CBC) plus CD4 count every 16 weeks until the CD4 was >200 cells/µl. Hb was checked if the participant was unwell or admitted to Masaka MOH Regional Referral Hospital. The timing of estimations of Hb measurements was determined by the entry into the trial and not when patients started ART. Patients who required a transfusion under strict Hb criteria were not always able to receive a transfusion due to resource and logistical constraints within the hospital at the time of the study.

Anaemia was graded according to the AIDS clinical trials group (ACTG) criteria namely grade 1: haemoglobin 8.0–<9.5 g/dl, grade 2: 7.0–8.0 g/dl, grade 3: 6.5–<7.0 g/dl, and
grade 4: <6.5 g/dl. Mean corpuscular volume (MCV) was classified as microcytic if <68 fl, normocytic if 80-98 fl and macrocytic if >98 fl, using reference ranges for healthy adults in Eastern and Southern Africa (23). If anaemia was diagnosed at baseline or during the trial, investigations were performed to diagnose and treat the cause of anaemia. All participants received co-trimoxazole prophylaxis as per Ugandan national guidelines.

Statistical Analysis

The statistical analysis aimed to investigate associations within the first 48 weeks of ART between type of ART regimen and a) incident grade 4 anaemia episodes and b) change in haemoglobin after initiation of ART (with the initial ART regimen; classified as d4T based, AZT based or TDF based). A four-week window for tests was allowed, so all Hb readings within 52 weeks of initiating ART were considered.

For the analysis of grade 4 anaemia, we used time to event analysis, with the event being grade 4 anaemia. The incidence rates of grade 4 anaemia were summarized by initial ART regimen, and anaemia incidence illustrated using a Kaplan-Meier curve. Cox proportional hazards regression models were fitted to investigate the effect of AZT-based and TDF-based regimens relative to d4T-based regimens. Potential confounders considered in this analysis were Hb at initiation of ART, baseline CD4 cell count, treatment arm in the CRYPTOPRO trial (fluconazole or placebo) and NNRTI used. Subjects with grade 4 anaemia on initiating ART and subjects with no pre-ART Hb reading were excluded from this analysis. Hb at initiation of ART and baseline CD4 count were treated as continuous variables and fractional polynomials were used to allow for non-linearity in their effect (24). Confounders were included in the final model if they were significant at the 15% level, using a backward elimination approach. A liberal inclusion value was used to ensure adequate adjustment for the confounders.

In order to examine the effect of initial ART regimen on change in Hb, for each subject a change in Hb was calculated as the difference between the post ART Hb closest to week 48, and the pre-ART Hb. The change was summarized by initial ART regimen, and further analysis was carried out by fitting general linear models, adopting an analogous strategy to that used for analysing incident anaemia, with the addition of time between pre-ART Hb and post ART Hb as a potential continuous confounder. All data required for the analysis were extracted from the CRYPTOPRO trial database, and all analyses carried out using Stata release 10.1.

Results

855 patients were eligible for inclusion in the anaemia study; of which 852 were available for analysis, (three patients did not have initial Hb estimations). 161 were receiving AZT/3TC, 628 were receiving D4T/3TC and 63 were receiving TDF/FTC. The baseline characteristics of the study population are shown by initial ART regimen in Table I. For the analysis of the incidence of anaemia, 845 patients contributed: seven patients were excluded because of grade 4 anaemia at ART initiation. For the analysis of change in Hb 842 patients contributed; ten (1.2%) were excluded as they did not have an Hb result at least 28 days after initiating ART (8 on d4T, 1 on AZT and 1 on TDF). Four of the ten died within one month.
of initiating ART, and six withdrew from the trial for other reasons. Figure 1 shows the summary of the patients in the separate analyses.

The age, gender and baseline WHO stage distributions were similar in the three groups, and there were roughly equal numbers in the two CRYPTOPRO treatment arms. However, those who received a TDF based regimen had lower CD4 counts and lower MCV. Patients initiated on AZT were more likely to be given efavirenz as their NNRTI than those in the other two groups. The initial mean Hb was similar in the three groups, although all patients with either grade 3 (n=3) or grade 4 (n=7) anaemia at ART initiation were started on a d4T based regimen.

**Grade 4 anaemia post ART**

Figure 2 shows a Kaplan-Meier plot giving the time to the first post ART grade 4 anaemia event among the 845 patients who did not have grade 4 anaemia at the initiation of ART. Overall 51 subjects out of 845 at risk developed grade 4 anaemia, with an incidence rate of 8.7 per 100 person-years at risk (pyar) (95% confidence limits 6.6 – 11.5 per 100 pyar). Table II shows the incidence rates and the adjusted hazard ratios of grade 4 anaemia by ART regimen. The rates were highest for those on an AZT based regimen (17.1 per 100 pyar), followed by those on a TDF based regimen (10.6 per 100 pyar) and were lowest for those on a d4T-based regimen (6.5 per 100 pyar). Confounders included in the regression model were Hb at ART initiation (as a fractional polynomial with power -2, i.e. inverse quadratic), baseline CD4 cell count (as a linear effect) and gender (with females being at a lower risk after adjusting for other factors).

Patients starting on AZT were at a 2.7 times greater risk (95% CI 1.5 – 5.0; P<0.001) of grade 4 anaemia than those starting on d4T. The point estimate of the TDF effect showed an increased risk of grade 4 anaemia compared to subjects on d4T, but due to the small number of subjects on TDF this did not reach statistical significance (aHR 1.6, 95% CI 0.6 – 4.1; P=0.35).

Developing a new grade 4 anaemia was a risk factor for death, with 19/51 (37.2%) subjects who developed grade 4 anaemia on ART dying compared to 43/794 (5.4%) subjects who did not (P<0.001). In eight subjects (5 on d4T, 2 on AZT and 1 on TDF), anaemia was listed as a cause of death; there was no evidence that the risk of death differed between the three regimens although number are very small (Fisher Exact P=0.74). Overall 30/51 (58.8%) of the patients with grade 4 anaemia were hospitalized within a two week window either side of the anaemia event. There was no difference in the probability of being hospitalized between the three ART regimens (18 were on D4T, 9 on AZT and 3 on TDF; Fisher Exact P=0.65). Only 18 (35.3%) of the patients who developed grade 4 anaemia received a blood transfusion (11 on d4T and 7 on AZT; Fisher Exact P=0.29).

**HB rises after initiation of ART**

The change in haemoglobin after 48 weeks on ART differed significantly between the three groups (unadjusted changes were d4T +1.40 g/dl, AZT +1.0 g/dl and TDF +0.84) (P=0.012 from a one way ANOVA) (Table III). We used the Hb reading nearest to week 48 post ART (IQR= 206 – 327 days), therefore an adjusted analysis for the day on which the reading was
made adjusted for day of Hb measured (using a second order fractional polynomial with terms in day and day X log(day))(27), as well as baseline Hb and gender was performed. The predicted changes in haemoglobin at week 48 obtained from this model are shown in Table III, confirming that while all three regimens result in small increases in haemoglobin, the increase in the d4T group was significantly greater than that in the other two groups.

Observation on those starting ART with baseline anaemia

At initiation of ART there were seven patients with grade 4 anaemia, of whom two patients died (28.5%); three patients with grade 3 anaemia, of whom one (33.3%) subsequently died; 23 with grade 2 anaemia, of whom six (26.1%) subsequently died; 107 with grade 1 anaemia, of whom 16 (15.0%) subsequently died, and 740 without anaemia, of whom 54 (7.3%) subsequently died. This indicates that baseline anaemia was associated with increased mortality (Chi-square=15.57 on 4 d.f., P=0.004).

Of those with grade 1 and 2 anaemia at baseline, 10% starting d4T developed subsequent severe anaemia, 13% on AZT, and none on TDF. Table IV includes the details including Hb changes. All 10 patients with baseline grade 3 or 4 anaemia were initiated on d4T. The median post ART haemoglobin in patients who had grade 4 anaemia at baseline was 11.0 g/dl (IQR 9.2 – 12.8) compared to a median post ART of 12.9 g/dl (IQR 11.8 – 13.8) in patients who did not have grade 4 anaemia when initiating ART.

Discussion

In the sub-Saharan African context it is difficult to make comparisons between ART regimens as the regimens are usually decided at a country level, with limited individual patient variation. It is clear from previous findings that many people eligible for ART will have pre-existing anaemia and in keeping with ART provision in a public health setting, the DART trial suggested targeted laboratory monitoring in a resource poor settings(28). The CRYPTOPRO cohort offers a unique insight into patients starting ART in Uganda as the participants were recruited from three different HIV service providers who independently selected the ART regimens for the participants, and so we have been able to present some comparative findings on the effect on Hb of different regimens in Uganda. At the time of this study in Uganda, the supplies for ART were limited; only one site was able to offer TDF/FTC, (this was the first line NRTI backbone at this site) and d4T was frequently the only NRTI backbone in available at the other sites. The major limitation of this study is the non-randomisation of NRTI backbones, however the method of allocation meant that the risk of channelling bias in the study is somewhat reduced, as clinicians started patients on whatever drug was available to them at each site. As the ART regimens were not randomly allocated, this led to baseline inequalities between treatment groups. Of these the most relevant is the lower median CD4 cells in the TDF arm, as there is an association between nadir CD4 and risk of anaemia(3), therefore we adjusted for this in the logistic regression models. Another limitation is the relatively small number of patients on TDF giving limited statistical power to detect differences between TDF and other regimens. All participants had CD4 counts <200 cells/µL, so the cohort is more immunosuppressed than those currently starting ART with the new WHO recommendations of starting at a CD4 count of 500 cells/µL.
Nevertheless, as many ART eligible patients in Africa still begin ART at low CD4 counts, our study provided valuable insights. There may have been some bias related to ART selection as patients initiated on AZT were more likely to be given efavirenz as their NNRTI than those the other two groups, however, we believe this was simply due to NNRTI availability, but it is difficult to interpret. Despite these limitations, we feel that this study offers some important insights about the development of anaemia between patients within the same cohort receiving regimens with AZT, d4T and TDF backbones.

This study showed that clinically important grade 4 anaemia occurred with all ART regimens at an overall rate of 8.7 /100PYO for the first 48 weeks starting ART. As seen in large US cohorts(4) and as well as in one study in Uganda(18), this was associated with increased mortality. The rate of grade 4 anaemia in our study was comparable with previously recorded rates of 4.0-9.6/100PYO in West Africa (19, 29). In those taking AZT there was an increased risk (aHR2.7) of incident grade 4 anaemia compared to d4T. However, in our study this did not lead to excess mortality.

Overall, ART increased the mean Hb in all three arms, which is in keeping with the other studies of post-ART Hb changes (25, 29). However, in the adjusted analysis, the increase was greater for those taking d4T than for both AZT and TDF, which is different to previous reports in Sub Saharan Africa. Our results differ somewhat from those from a similar study in urban Uganda which found no significant difference in Hb increase in AZT compared with d4T containing regimens in urban Uganda, and the reason for the differences are not entirely clear(25). The baseline data of the two cohorts were similar, although the CRYPTOPRO cohort was slightly more immunosuppressed, as all participants had a CD4 count <200, compared to 85% of those in the urban cohort. This may explain a higher incidence of anaemia (6% over 48 weeks) in our cohort than in the urban cohort (4% at 6 months), which may have made a difference between regimens easier to detect.

We have not been able to find other reports in sub-Saharan Africa on the effect of TDF on Hb compared with other ART regimens. TDF is one of the first line regimens recommended in the 2010 WHO ART guidelines, and along with AZT is now being used in preference to d4T sub-Saharan Africa. These findings suggest that Hb might also need to be monitored in those receiving TDF, as whilst there is no statistical difference in the number of grade 4 anaemia events in this group, the Hb rise was the lowest of all 3 regimens. It will be important to study Hb changes and watch for anaemia in larger cohorts of patients receiving TDF in sub-Saharan Africa over the next few years.

**Conclusion**

In this study population we observed higher levels of anaemia and a higher incidence of severe anaemia among patients receiving AZT than other NRTIs. As suggested by the DART trial (28) we would encourage close clinical surveillance in all patients starting ART in order to avoid the potentially fatal consequences of undiagnosed iatrogenic (and other) anaemia. We would encourage those with larger cohorts of patients now receiving TDF as first line in sub-Saharan Africa to describe their experiences, as more information on development of anaemia in this population is important.
Acknowledgements and funding

The CRYPTOPRO trial was funded by the Medical Research Council UK. We wish to acknowledge all the CRYPTOPRO trial participants. We acknowledge also acknowledge all of the CRYPTOPRO trial staff.

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17. WHO. Consolidated guidelines on general HIV care and the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. 2013


Figure 1. Summary of patients analysed

855 eligible for study (Enrolled >48 weeks before end of trial)

852 with baseline Hb

3 No initial Hb

845 analyzed for Anemia incidence

7 with baseline grade 4 anemias

842 analyzed for Change in Hb

10 with no 2nd Hb
4 died, 10 withdrew from study
Figure 2. Time to first post ART grade 4 anaemia
Table I
Baseline characteristics of study participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>D4T (n=628)</th>
<th>AZT (n=161)</th>
<th>TNF (n=63)</th>
<th>Overall (n=852)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>201 (32.0%)</td>
<td>48 (29.8%)</td>
<td>18 (28.6%)</td>
<td>267 (31.3%)</td>
</tr>
<tr>
<td>Female</td>
<td>427 (68.0%)</td>
<td>113 (70.2%)</td>
<td>45 (71.4%)</td>
<td>585 (68.7%)</td>
</tr>
<tr>
<td>Age – Mean (s.d.)</td>
<td>36.0 (8.5)</td>
<td>35.7 (8.2)</td>
<td>36.1 (8.6)</td>
<td>35.9 (8.5)</td>
</tr>
<tr>
<td>CD4 – median (IQR)</td>
<td>114 (57–162)</td>
<td>108 (40–154)</td>
<td>78 (26–139)</td>
<td>110 (49–160)</td>
</tr>
<tr>
<td>CD4 – grouped</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>142 (22.6%)</td>
<td>49 (30.4%)</td>
<td>23 (36.5%)</td>
<td>214 (25.1%)</td>
</tr>
<tr>
<td>50-99</td>
<td>134 (21.3%)</td>
<td>23 (14.3%)</td>
<td>17 (27.0%)</td>
<td>174 (20.4%)</td>
</tr>
<tr>
<td>100 – 149</td>
<td>150 (23.9%)</td>
<td>44 (27.3%)</td>
<td>10 (15.9%)</td>
<td>204 (23.9%)</td>
</tr>
<tr>
<td>150 - 199</td>
<td>202 (32.2%)</td>
<td>45 (28.0%)</td>
<td>13 (20.6%)</td>
<td>260 (30.5%)</td>
</tr>
<tr>
<td>Hb – mean (s.d.)</td>
<td>11.4 (2.1)</td>
<td>11.4 (1.7)</td>
<td>11.6 (1.7)</td>
<td>11.4 (2.0)</td>
</tr>
<tr>
<td>Hb – grouped</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td>7 (1.1%)</td>
<td>0</td>
<td>0</td>
<td>7 (0.8%)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>3 (0.5%)</td>
<td>0</td>
<td>0</td>
<td>3 (0.4%)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>18 (2.9%)</td>
<td>2 (1.2%)</td>
<td>1 (1.6%)</td>
<td>21 (2.5%)</td>
</tr>
<tr>
<td>Grade 1</td>
<td>76 (12.1%)</td>
<td>19 (11.8%)</td>
<td>6 (9.5%)</td>
<td>101 (11.8%)</td>
</tr>
<tr>
<td>WHO stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15 (2.4%)</td>
<td>4 (2.5%)</td>
<td>1 (1.6%)</td>
<td>20 (2.4%)</td>
</tr>
<tr>
<td>2</td>
<td>137 (21.8%)</td>
<td>31 (19.2%)</td>
<td>20 (31.8%)</td>
<td>188 (22.1%)</td>
</tr>
<tr>
<td>3</td>
<td>433 (69.0%)</td>
<td>117 (72.7%)</td>
<td>39 (61.9%)</td>
<td>589 (69.1%)</td>
</tr>
<tr>
<td>4</td>
<td>43 (6.8%)</td>
<td>9 (5.6%)</td>
<td>3 (4.8%)</td>
<td>55 (6.5%)</td>
</tr>
<tr>
<td>MCV - mean (s.d.)</td>
<td>82.0 (9.4)</td>
<td>82.4 (8.0)</td>
<td>79.2 (13.4)</td>
<td>81.9 (9.5)</td>
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<tr>
<td>MCV grouped</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 68</td>
<td>18 (2.9%)</td>
<td>4 (2.5%)</td>
<td>7 (11.1%)</td>
<td>29 (3.4%)</td>
</tr>
<tr>
<td>68 – 98</td>
<td>600 (95.7%)</td>
<td>156 (96.9%)</td>
<td>56 (88.9%)</td>
<td>812 (95.4%)</td>
</tr>
<tr>
<td>&gt;98</td>
<td>9 (1.4%)</td>
<td>1 (0.6%)</td>
<td>0</td>
<td>10 (1.2%)</td>
</tr>
<tr>
<td>Treatment Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td>311 (49.5%)</td>
<td>84 (52.2%)</td>
<td>26 (41.3%)</td>
<td>421 (49.4%)</td>
</tr>
<tr>
<td>NNRTI Nevirapine</td>
<td>564 (90.0%)</td>
<td>102 (63.4%)</td>
<td>60 (96.8%)</td>
<td>726 (85.4%)</td>
</tr>
<tr>
<td>Efavirenz</td>
<td>63 (10.0%)</td>
<td>59 (36.6%)</td>
<td>2 (3.2%)</td>
<td>124 (14.6%)</td>
</tr>
</tbody>
</table>

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### Table II
Comparison of incidence of grade 4 anaemia between initial ART regimens

<table>
<thead>
<tr>
<th>Regimen</th>
<th>No. of events (cumulative incidence)</th>
<th>Person Years of exposure (PYO)</th>
<th>Incidence rate per 100 PYO (95% CI)</th>
<th>Adjusted Hazard Ratio (aHR) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D4T</td>
<td>28 / 621 (4.5%)</td>
<td>433.2</td>
<td>6.5 (4.5 ; 9.4)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>AZT</td>
<td>18 / 161 (11.2%)</td>
<td>105.4</td>
<td>17.1 (10.8 ; 27.1)</td>
<td>2.74 (1.51 ; 4.99)</td>
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<tr>
<td>TNF</td>
<td>5 / 63 (7.9%)</td>
<td>47.2</td>
<td>10.6 (4.4 ; 25.5)</td>
<td>1.58 (0.61 ; 4.13)</td>
</tr>
</tbody>
</table>

^Adjusted for baseline CD4 count, baseline Hb, gender, CRYPTOPRO treatment arm, and NNRTI
### Table III

Predicted change in Hb over 48 weeks of treatment*

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Unadjusted change in Hb (g/dL)</th>
<th>Predicted increase in Hb (g/dL)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>D4T</td>
<td>1.40</td>
<td>1.57</td>
<td>(1.38 ; 1.76)</td>
</tr>
<tr>
<td>AZT</td>
<td>1.00</td>
<td>1.22</td>
<td>(0.91 ; 1.52)</td>
</tr>
<tr>
<td>TNF</td>
<td>0.84</td>
<td>0.98</td>
<td>(0.51 ; 1.45)</td>
</tr>
</tbody>
</table>

*Adjusted for days to Hb reading, baseline Hb and gender.

1 Difference between D4T and AZT statistically significant (P=0.024)

2 Difference between D4T and TDF statistically significant (P=0.014)
### Table IV

Summary of patients with Grade 1 and 2 anaemia at baseline

<table>
<thead>
<tr>
<th>ART regimen</th>
<th>No</th>
<th>Developing Grade 4 anaemia</th>
<th>Received Transfusion</th>
<th>Died</th>
<th>Median Hb at 48 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>d4T</td>
<td>94</td>
<td>10 (11%)</td>
<td>4</td>
<td>12 (13%)</td>
<td>11.9 (IQR 10.3-12.9)</td>
</tr>
<tr>
<td>AZT</td>
<td>21</td>
<td>3 (14%)</td>
<td>0</td>
<td>4 (19%)</td>
<td>11.0 (IQR 9.9-12.2)</td>
</tr>
<tr>
<td>TDF</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>1 (14%)</td>
<td>11.9 (IQR 10.3-12.5)</td>
</tr>
</tbody>
</table>

Due to the small numbers involved, none of the differences between the three treatment regimens approached statistical significance.