Prevalence, incidence and predictors of severe anaemia with zidovudine-containing regimens in African adults with HIV infection within the DART trial

Francis Ssali[1], Wolfgang Stöhr[2], Paula Munderi[3], Andrew Reid[4], A Sarah Walker[2], Diana M Gibb[2], Peter Mugyenyi[1], Cissy Kityo[1], Heiner Grosskurth[3], James Hakim[4], Helen Byakwaga[5], Elly Katabira[5], Janet H Darbyshire[2] and Charles F Gilks[6] on behalf of the DART Trial Team*

[1]Joint Clinical Research Centre, Kampala, Uganda
[2]MRC Clinical Trials Unit, London, UK
[3]MRC Programme on AIDS/Uganda Virus Research Institute, Entebbe, Uganda
[4]University of Zimbabwe, Harare, Zimbabwe
[5]Academic Alliance, Mulago Hospital, Uganda
[6]Imperial College, London, UK

*Corresponding author: Tel: +44 20 7670 4726; Fax +44 20 7670 4818; E-mail: sarah.walker@ctu.mrc.ac.uk

Objective: To describe the prevalence, incidence and predictors of severe anaemia in previously untreated symptomatic HIV-infected adults with CD4$^+$ T-cells <200 cells/mm^3 initiating zidovudine-containing regimens in Africa.

Design: DART is a randomized trial comparing two strategies for HIV/AIDS management in Uganda and Zimbabwe.

Methods: We analysed the occurrence of anaemia at weeks 4 and 12, and then every 12 weeks. We also evaluated sex, age, WHO stage, body mass index (BMI), baseline laboratory measurements and first regimen as predictors of developing grade 4 anaemia (<6.5 mg/dl) by week 48 using logistic regression.

Results: To May 2005, 3,314 participants (65% women, 23% at WHO stage 4, median age=37 years, baseline CD4$^+$ T-cell=86 cells/mm^3 and median baseline haemoglobin=11.4 g/dl) had a median 72 weeks follow-up. Prevalence of grade 4 anaemia was 0.7%, 2.0%, 0.5% and <0.5% at weeks 4, 12, 24 and ≥36, respectively. Overall, 219 (6.6%) participants developed grade 4 anaemia by week 48; women and those with lower haemoglobin, CD4$^+$ T-cell count and BMI at baseline were at significantly higher risk (P<0.05), but not those with lower neutrophils or receiving cotrimoxazole at baseline.

Conclusions: We observed a higher incidence of grade 4 anaemia than in studies from industrialized countries, which is likely to be due in part to population characteristics and in part to a higher rate of concurrent HIV-related clinical events. Clinical vigilance and haemoglobin measurements 4, 8 and 12 weeks after starting zidovudine could help to manage serious anaemia.

Introduction

Anaemia is common in patients with HIV infection, particularly those with advanced disease. In patients not receiving effective antiretroviral therapy (ART), anaemia is associated with increased mortality, independently of CD4$^+$ T-cell count and viral load [1–7]. In addition, symptoms such as fatigue substantially impair quality of life [8,9].

Causes of HIV-related anaemia include impaired haematopoiesis due to increased cytokine production, decreased erythropoietin production, blood loss, haemolysis, neoplasms, opportunistic infections with *Mycobacterium avium* complex or parvovirus B-19, and drugs such as zidovudine or cotrimoxazole [10,11]. Risk factors for HIV-related anaemia already identified include low CD4$^+$ T-cell counts, high viral load, female sex and African-American ethnicity [2,12–15]. Estimates of HIV-related anaemia prevalence show considerable variation [16], ranging from 1.3% to 95% depending on the definition and clinical setting.

Much of the data on HIV-related anaemia are from industrialized countries prior to the introduction of triple drug ART. Increased incidence of anaemia after initiation of zidovudine monotherapy was demonstrated in early placebo-controlled trials [17,18]. In contrast, the data available on the impact of effective ART show a significant decrease in anaemia incidence

and increase in haemoglobin during the first 12–18 months [3,14,19,20]. However, HIV-related anaemia may be of particular concern in resource-limited settings where unfavourable combinations of risk factors predominate and resources for laboratory investigations are limited [21]. Therefore, we describe the prevalence, incidence and timing of severe anaemia and associated risk factors in the Development of Antiretroviral Therapy (DART) trial in Uganda and Zimbabwe.

Patients and methods

Trial design and participants

DART is a randomized controlled trial evaluating two ART management strategies suitable for the resource-limited context of sub-Saharan Africa. The trial enrolled 3,314 symptomatic (WHO stage 2, 3 or 4) HIV-infected adults (≥18 years) with $CD4^+$ T-cell counts <200 cells/mm^3, and without laboratory contraindications to ART or acute infections initiating ART in three clinical sites in Uganda (2 sites) and Zimbabwe (1 site). All participants had not previously received ART other than to prevent mother-to-child HIV transmission. The first randomization compares clinical monitoring only (CMO) with laboratory plus clinical monitoring (LCM). A second randomization compares repeated structured treatment interruptions (STIs: 12 weeks on, 12 weeks off ART) with continuous ART in those achieving $CD4^+$ T-cell counts ≥300 cells/mm^3 at 48 or 72 weeks on continuous therapy. Individual informed consent was obtained from every participant, and the trial received ethics approval in Uganda, Zimbabwe and the UK.

The principle objective of DART is to evaluate different ART management strategies, it is not primarily investigating different drug regimens. Therefore, all participants initiated triple combination ART with co-formulated zidovudine and lamivudine (as Combivir®) plus tenofovir, nevirapine or abacavir in line with WHO guidelines at the time [22], with only 600 participants being randomized between drug regimens (to either abacavir or nevirapine in a double-blind 24 week substudy in Uganda). The other participants received tenofovir or open-label nevirapine, based on drug availability at the time and concomitant medications (for example, for tuberculosis).

Laboratory measurements

All DART participants attend the study clinic every 4 weeks when a nurse administers a symptom checklist and adherence questionnaire, and dispenses a repeat ART prescription, referring the participant to a doctor if there is cause for concern. Participants are also asked to return to the clinic for extra visits if they feel unwell. All participants routinely see a doctor and have a full blood count, tests of liver and kidney function, and lymphocyte subsets measured at weeks 4 and 12, and then every 12 weeks. Laboratory results for LCM participants are returned to the treating clinicians, but those for CMO participants are not returned unless there is a grade 4 toxicity or requested for a clinical reason (authorized by the site Project Leader). Laboratory tests may also be requested outside the scheduled timepoints for clinical reasons. New or recurrent WHO stage 3 and 4 HIV events [23] are reported at the time of diagnosis; all other clinical events are reported at follow-up visits. All WHO stage 4 events are reviewed against criteria for presumptive/definitive diagnosis by an Endpoint Review Committee, which also reviews the cause of death. Data on transfusions before or during the trial are not routinely collected.

Anaemia is graded according to the ACTG criteria [24], 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. Eligibility criteria for enrolment into DART included haemoglobin ≥8.0 g/dl. Mean corpuscular volume (MCV) is classified as microcytic if <80 fl, normocytic if 80-99 fl, and macrocytic if ≥100 fl [25].

Statistical analysis

This analysis considers data to 15 May 2005. Prevalence of anaemia of different grades was estimated at scheduled visits where all participants should have had haemoglobin measured. Where multiple specimens were available, the closest value to each visit week within equally spaced windows was used. Incidence of anaemia was estimated by defining a new episode when the grade deteriorated from baseline (ART initiation), or following resolution of a preceding episode to grade at baseline or better. Here we focussed on grade 4 anaemia episodes, defined as the period between the first grade 4 measurement and resolution, estimating the end of episode as the mid-point between the last measurement before and measurement at resolution. Clinical events and laboratory abnormalities were considered concomitant to the anaemia episode if diagnosed between 14 days (7 days for laboratory abnormalities) before its start and 14 days (7 days for laboratory abnormalities) after its resolution.

As our aim was to describe anaemia after the start of zidovudine-based ART, primary analyses considered the first 48 weeks following ART initiation, which is the period before patients could be randomized to STIs. Differences in continuous variables (for example, MCV) between subgroups at scheduled timepoints were compared by applying the Kruskal–Wallis test. We used univariable and multivariable (backwards elimination; exit probability 0.1) logistic regression

models to identify baseline factors associated with ever developing grade 4 anaemia, investigating sex, age, WHO stage of HIV disease, body mass index (BMI), $CD4^+$ T-cell count, haematological parameters, baseline receipt of cotrimoxazole, and first ART regimen. Goodness of fit of the regression models was assessed by applying the Hosmer–Lemeshow test. Where there was evidence of significant non-linearity in a continuous variable (checked by fractional polynomial regression [26]), we modeled this as two linear associations, choosing the slope changepoint at the maximum of the (profile) likelihood. Finally, time dependent Cox proportional hazard models were used to assess the impact of current cotrimoxazole use on the development of anaemia.

Results

Between January 2003 and October 2004, 3,314 eligible participants were randomized in DART (Table 1). Four hundred and six (12%) participants had anaemia at baseline, including 6 (0.2%) with ≥grade 2 anaemia, among them one grade 4 (5.8 g/dl). The enrolment of these six patients represented a protocol violation. The median follow-up after ART initiation to 15 May 2005 was 72 weeks (interquartile range [IQR]=48–84; range: 1–120).

Prevalence of anaemia over time

Median haemoglobin was lowest at week 4, but then increased by a median 0.6 g/dl 24 weeks after ART initiation, and continued rising thereafter (Figure 1). Prevalence of grade 4 anaemia was 0.7%, 2.0%, 0.5% and <0.5% at weeks 4, 12, 24, ≥36, respectively. As expected, following the initiation of zidovudine, MCV increased by week 24 (to median 101 fl compared with 86 fl at baseline) with no change thereafter (Figure 1) with 6% and 54% of patients having macrocytic MCV at baseline and week 24, respectively. However, patients with grade 4 anaemia at weeks 4 and 12 had significantly lower concurrent MCV than those without anaemia at these timepoints (both $P<0.001$; Figure 1).

Characteristics and timing of anaemia

Overall, 852 (25.7%) participants developed a new episode of anaemia of ≥grade 1 after starting ART (789 [23.8%] by 48 weeks), among them 242 (7.3%) with a new grade 4 anaemia (219 [6.6%] by 48 weeks; Table 2). Sixteen participants had more than one grade 4 episode (13 participants with two episodes and three with three episodes). The first grade 4 episode was detected a median of 12 weeks after ART initiation (IQR=9–24; range=2–96) and had a median duration of 35 days (IQR=13–49). Only 23/242 participants had their first grade 4 anaemia >48 weeks after initiating ART. All 23 had measurements as scheduled before 48 weeks; the greatest previous anaemia grade was 3 in one participant, 2 in two participants, and no anaemia or grade 1 in the remaining participants.

Subsequent analyses were restricted to the first 48 weeks after initiating zidovudine-containing ART. The majority of the 219 participants with new grade 4 anaemia within this period had haemoglobin within normal range at baseline (168/219; 77%). One hundred and thirty-five participants (62%) presented grade 4 anaemia at a scheduled DART doctor/nurse visit (every 4 weeks), and 84 (38%) at an unscheduled visit. As expected, patients who developed grade 1–3 anaemia were more likely to

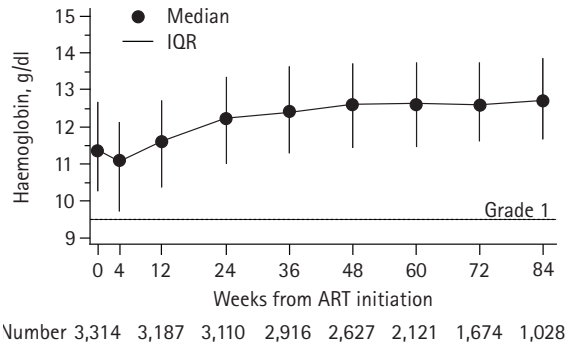
Table 1. Baseline characteristics of DART trial participants (n=3,314)

Country	
Uganda	2,315 (70%)
Zimbabwe	999 (30%)
Female	2,154 (65%)
Age, years	36.7 (31.9–42.2)
WHO stage	
2	670 (20%)
3	1,865 (56%)
4	778 (23%)
$CD4^+$ T –cell count, cells/mm^3	86 (31–139)
0–49	1,107 (33%)
50–99	785 (24%)
100–149	759 (23%)
150–199	663 (20%)
BMI, kg/m^2	21.1 (19.1–23.6)
Haemoglobin, g/dl	11.4 (10.3–12.7)
>9.5	2,908 (88%)
8.0–<9.5 (grade 1)	400 (12%)
7.0–<8.0 (grade 2)	3* (0.1%)
6.5–<7.0 (grade 3)	2* (0.1%)
<6.5 (grade 4)	1* (0.0%)
MCV, fl	86 (80–91)
Neutrophils, $\times 10^9$/l	1.5 (1.1–2.1)
Platelets, $\times 10^9$/l	204 (157–260)
Cotrimoxazole use	
No	1,241 (37%)
Started before randomization	1,654 (50%)
Started at randomization	419 (13%)
Initiated ART with	
Combivir/tenofovir	2,467 (74%)
Combivir/abacavir	300 (9%)
Combivir/nevirapine	547 (17%)

*Minor protocol violation: eligibility criteria included haemoglobin 8.0 g/dl
Values are n (%) or median (interquartile range) unless otherwise specified.
ART, antiretroviral therapy; BMI, body mass index; MCV, mean corpuscular volume; WHO, World Health Organization.

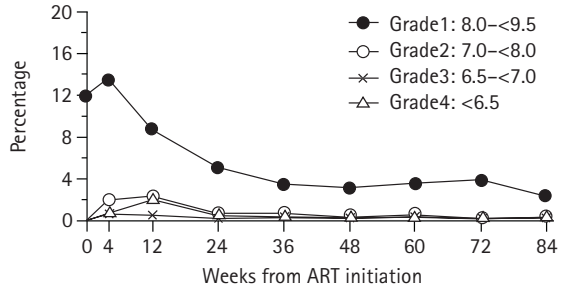
Figure 1. Haemoglobin **(A)**, prevalence of anaemia **(B)** and mean corpuscular volume **(C)** at routine trial visits

A



Number 3,314 3,187 3,110 2,916 2,627 2,121 1,674 1,028

B



C

Anaemia grade	0	4	12	24	36	48	60	72	84
0	86 (2,904)	88 (2,645)	97 (2,748)	101 (2,902)	100 (2,776)	100 (2,521)	100 (2,022)	100 (1,602)	100 (995)
1–3	83 (405)	85 (509)	94 (369)	96 (187)	91 (127)	87 (96)	89 (90)	91 (69)	87 (30)
4		83 (19)	90 (61)	103 (15)	104 (10)	87 (6)	100 (8)	91 (3)	100 (3)

Median corpuscular volume measured per fl, the actual numbers of participants are in parenthesis. ART, antiretroviral therapy; IQR, interquartile range.

subsequently develop grade 4 anaemia – 2.3% of participants without anaemia at week 4, but 11.5%, 15.3% and 18.8% of participants with anaemia grade 1, 2, and 3, respectively, had grade 4 anaemia by week 12 ($P<0.001$). Overall, as relatively few patients developed anaemia of any grade, most of the patients with grade 4 anaemia had previously not had anaemia – 50% of participants with grade 4 anaemia at week 12 had no anaemia at week 4, and 40% had anaemia grade 1 at week 4 (similar results were seen comparing weeks 12 and 24).

Table 2. Incidence of anaemia up to 48 weeks after initiation of zidovudine-containing ART

Most severe episode	*n* (% of 3,314)	Cumulative *n* (% of 3,314)
Grade 4 (<6.5 g/dl)	219 (6.6%)	219 (6.6%)
Grade 3 (6.5–<7.0 g/dl)	40 (1.2%)	259 (7.8%)
Grade 2 (7.0–<8.0 g/dl)	134 (4.0%)	393 (11.8%)
Grade 1 (8.0–<9.5 g/dl)	396 (12.0%)	789 (23.8%)

Description of the most severe episode experienced by each participant up to and including 48 weeks, based on all haemoglobin measurements at scheduled and non-scheduled visits.

Management of anaemia

After the first episode of grade 4 anaemia, stavudine was immediately substituted for zidovudine in 100/219 (46%) participants. In a further 82 (37%) participants, all ART was stopped: ART was restarted in 80/82 participants after a median of 9 days with stavudine substituted for zidovudine in 73 participants; the remaining 7 restarted with zidovudine. Sixteen (7%) participants died before stopping or substituting zidovudine, and for 21 (10%) no substitution or interruption was recorded before anaemia resolution. Overall, 353 of 3,314 (11%) trial participants substituted stavudine for zidovudine. Anaemia (of any grade with or without neutropenia) accounted for 225 (64%) of these substitutions, neutropenia accounted for the rest.

Concomitant clinical and laboratory events

During the first grade 4 anaemia episode, 56/219 (26%) participants had one or more of the following: concurrent bacterial infection (6%), malaria (11%) or a WHO stage 4 HIV event (12%). These events occurred more frequently in participants presenting at non-scheduled visits (39% versus 17%, $P<0.001$). A further 89 (41%) participants had either concomitant neutropenia grade 3/4 (31%), thrombocytopenia grade 3/4 (2%) or both neutropenia and thrombocytopenia grade 3/4 (8%). One hundred and one (46%) participants had none of the events above concurrent with their first episode of grade 4 anaemia.

Mortality

Forty-eight of 219 participants with grade 4 anaemia before 48 weeks subsequently died a median 17 weeks after randomization (26 before and 22 after complete resolution of the anaemia), compared with 154 of those without grade 4 anaemia ($P<0.001$). All 26 who died before complete anaemia resolution had other HIV-related illnesses considered among the

main causes of death by the Endpoint Review Committee (ERC). These included sepsis/pneumonia/bacterial meningitis (*n*=15, 3 with pancytopenia), tuberculosis (*n*=2), cryptococcal meningitis/fungaemia (*n*=2), and one case each of HIV-related cerebral disease, cryptosporidial diarrhoea, hepatocellular carcinoma (hepatitis B), Kaposi's sarcoma, malaria and transverse myelitis. One further patient died from unknown causes after refusing transfusion on religious grounds. In two of these 26 patients, severe anaemia related to zidovudine treatment was considered to have contributed significantly to their death by the ERC. Both patients also had neutropenia and probable sepsis, and died 4–8 weeks after starting ART. Eleven of the 26 who died before complete anaemia resolution had concomitant grade 4 neutropenia, with four participants also having pancytopenia (three in association with sepsis). Of the 22 participants who died after resolution of grade 4 anaemia, nine deaths were considered primarily HIV-related and three primarily drug-related (of note, two were due to lactic acidosis after substituting stavudine for zidovudine). For the remaining 10 participants, we were uncertain whether their deaths were primarily HIV- or drug-related.

Baseline predictors of anaemia

Women and participants with lower haemoglobin, $CD4^+$ T-cell count and BMI at baseline were all significantly more likely to develop grade 4 anaemia after initiation of zidovudine-containing ART (Table 3). Baseline age, neutrophil count, WHO HIV stage and taking cotrimoxazole at trial entry were not additional predictors. However, the risk of developing new grade 4 anaemia did not increase linearly over the range of baseline BMI, with little change in risk in those with higher BMI (P=0.006 for difference in risk above and below 22.5 kg/m^2 – chosen by profile likelihood, see Patients and methods). Furthermore, whereas there was no significant linear association over the whole range of baseline MCV, risk of anaemia appeared to increase substantially with increasing values of baseline MCV >92.5 fl (P=0.001 for difference in risk above and below 92.5 fl; odds ratio [OR]=1.98, 95% confidence interval [CI]=1.35–2.90, per 10 fl increase over 92.5 fl).

In an attempt to disentangle anaemia relating to potential drug toxicity from underlying HIV disease, we also considered predictors of grade 4 anaemia that occurred without any concomitant clinical event (*n*=163), or grade 4 anaemia without any concomitant clinical event and detected only at a routine visit

Table 3. Baseline predictors of developing grade 4 anaemia by 48 weeks

	Univariable model			Multivariable model		
Factor	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Uganda versus Zimbabwe	1.28	0.93–1.74	0.13	–	–	–
Female	1.70	1.24–2.33	0.001	1.58	1.13–2.21	0.007
Age (per 10 years)	0.95	0.79–1.13	0.54	–	–	–
WHO stage						
2	0.72	0.48–1.08	–	–	–	–
3	1.00	–	0.02	–	–	–
4	1.34	0.98–1.83	–	–	–	–
$CD4^+$ T-cell count, per 100 cells/mm^3	0.58	0.45–0.74	<0.001	0.62	0.49–0.80	<0.001
BMI, per unit	0.91	0.87–0.95	<0.001	0.93	0.90–0.98	0.002
Haemoglobin, per g/dl	0.74	0.68–0.81	<0.001	0.79	0.72–0.87	<0.001
MCV, per fl	1.00	0.98–1.01	0.63	–	–	–
Neutrophils, per 10^3 cells/mm^3	1.02	0.89–1.17	0.79	–	–	–
Platelets, per 10^6 cells/mm^3	4.51	1.03–19.83	0.046	–	–	–
Cotrimoxazole use						
No	1.00	–	0.21	–	–	–
Started before randomization	0.87	0.64–1.17	–	–	–	–
Started at randomization	1.24	0.82–1.87	–	–	–	–
Initiated ART with						
Combivir/tenofovir	1.00	–	0.98	–	–	–
Combivir/nevirapine	0.96	0.66–1.40	–	–	–	–
Combivir/abacavir	1.00	0.62–1.62	–	–	–	–

Final multivariable model shown is based on backwards selection (P<0.1) from all factors in the Table. There were no significant interactions. Hosmer–Lemeshow goodness-of-fit test: P=0.49. ART, antiviral therapy; BMI, body mass index; CI, confidence interval; MCV, mean corpuscular volume; WHO, World Health Organization.

(*n*=112) via sensitivity analyses. Predictors were mainly unchanged, but there was an additional effect of age at ART initiation, showing an increasing risk with older age (in both models: OR=1.3 per 10 years, *P*=0.03). However, whereas the influence of baseline CD4+ T-cell count remained nearly unchanged in the first sensitivity analysis, it was attenuated in the second (OR=0.72 per 100 cells/mm^3, 95% CI=0.52–1.02; *P*=0.06). Of note, in the second sensitivity analysis there was also a trend towards higher risk of anaemia associated with initiating cotrimoxazole at randomization (OR=1.91, 95% CI=1.09–3.36; *P*=0.03). In addition, grade 3/4 neutropenia occurred in 46% of grade 4 anaemia with concomitant clinical event versus 38% of anaemia without clinical event and detected at a scheduled visit (probably drug related).

In a final sensitivity analysis, we examined predictors of developing anaemia of any grade (*n*=789). The only differences in risk factors were a stronger effect for baseline haemoglobin (OR=0.63, 95% CI=0.60–0.68, *P*<0.001), a smaller effect of baseline CD4+ T-cell count (OR=0.71, 95% CI=0.61–0.83, *P*<0.001), and additional small influences of age (OR=1.19 per 10 years, 95% CI=1.06–1.33, *P*=0.004) and baseline neutrophil count (OR=1.14 per 10^9 cells/l, 95% CI=1.05–1.24, *P*=0.002).

Lastly, current use of cotrimoxazole was not associated with increased risk of grade 4 anaemia, overall (OR=0.82, 95% CI=0.63–1.08, *P*=0.16, effect of other predictors virtually unchanged) or for the other subgroups defined above.

Discussion

In this large cohort of HIV-infected adults with symptomatic disease and low CD4+ T-cell count commencing zidovudine-containing triple-drug ART in Africa, we observed a substantial overall increase in haemoglobin maintained beyond 48 weeks, which is similar to studies from industrialized countries [14,19,20]. However, in spite of this we observed a higher rate of anaemia compared with studies from industrialized countries. Whereas effective ART is likely to decrease HIV-related anaemia, zidovudine increases the risk of anaemia because of its myelosuppressive potential [17,18]. A recent meta-analysis of data from randomized trials confirms that anaemia is more common with zidovudine- than stavudine-based triple-drug ART [27]. Nevertheless, those studies from industrialized countries, which included non-randomized control groups not receiving triple-drug ART, found higher anaemia prevalence than in those taking effective ART.

Another meta-analysis of clinical trials, which included 6,797 ART-naive patients predominantly from US and Europe, recently reported a cumulative incidence of grade 2–4 anaemia (<8 g/dl) after ≥24 months of triple-drug ART for 1.8% of individuals taking zidovudine as a separate drug, 1.2% of those taking zidovudine within a coformulation (Combivir®/Trizivir®) and 1.1% of those taking ART without zidovudine [28]. Baseline haemoglobin and being from geographical areas outside Europe or the Americas were additional risk factors, as was male gender (although after allowing for normal differences in haemoglobin between men and women, there was no difference in risk between an average man and woman, in contrast to our findings). Other studies in industrialized countries have also identified advanced HIV disease, African origin, female sex, low BMI, and older age as risk factors for anaemia [11]. As our population had low CD4+ T-cell counts and was mostly female, it is not surprizing that, despite initial exclusion of patients with anaemia, a substantially higher incidence of 11.8% grade 2–4 anaemia (<8 g/dl) was observed by 48 weeks in our study compared to this meta-analysis and other studies. It is likely that lower normal ranges in Africa than in Western populations [29] could also contribute to the higher incidence, as well as low BMI, general malnutrition and the occurrence of other diseases, such as malaria and helminthiasis. The highest prevalence of anaemia in DART was 4–12 weeks after commencing ART, thus coinciding with the time when zidovudine-related anaemia has been reported to be most common. However, interestingly, over a quarter of patients with grade 4 anaemia in our study had new concomitant HIV-related illness at the time of anaemia, suggesting that the incident anaemia is likely to be related not just to drug-related toxicity, but also to non-drug related clinical events. The same may be true for neutropenia; however, we have not considered neutropenia in more detail here as there are difficulties in defining normal ranges for African populations [29] and there is the additional confounder of cotrimoxazole prophylaxis. Of the 26 patients who died before grade 4 anaemia was resolved, all had acute illnesses, particularly sepsis, although zidovudine-related anaemia was considered to have contributed to death in at least two participants. Compared with the analysis of all grade 4 anaemia, sensitivity analyses suggested a smaller effect of baseline CD4+ T-cell count in predicting patients at higher risk of likely drug-induced toxicity (defined as anaemia identified without concomitant clinical events at a routine blood test) and also suggested that older participants were more likely to develop such drug-related anaemia.

Although several studies have described toxicity of zidovudine-containing as well as non-zidovudine-

containing ART in resource-limited settings, detailed accounts of the development of grade 4 anaemia are rare. Moh *et al*. reported a 2% incidence of grade 4 anaemia (<6.5 g/dl) by 6 months among 498 patients in West Africa starting zidovudine-containing triple-drug ART, which is lower than in our study [21]. However, HIV disease was considerably less advanced in their population with two-thirds having baseline $CD4^+$ T-cell counts >200 cells/mm^3 compared with none of the DART participants, although in other respects their patient population was similar (low baseline haemoglobin, median 11.3 g/dl; 72% women). Furthermore, since pre-enrolment transfusion data were not routinely collected, it is theoretically possible that enrolment of some recently transfused patients may have led to an overestimation of incidence in our study. Similarly to our findings, Moh *et al*. found low baseline haemoglobin to be the key risk factor for developing anaemia. However, in contrast to our study they observed no association between baseline $CD4^+$ T-cell counts or sex, and development of anaemia – possible explanations include different variation in risk of grade 4 anaemia at $CD4^+$ T-cell counts >200 cells/mm^3 or lower power with only 500 patients. Eighty percent of patients in the study of Moh *et al*. had already received cotrimoxazole at baseline and most of the remainder started cotrimoxazole within the first 2 months of ART, so this study was not able to assess any additional effect of cotrimoxazole. In contrast, fewer patients were receiving cotrimoxazole at trial entry in our study (depending on when they had been diagnosed). Although a non-randomized comparison, so selection bias cannot be excluded, we did not find evidence that concurrent use of cotrimoxazole increased the overall risk of anaemia, nor that risk was different in patients who started cotrimoxazole before randomization, at randomization or after randomization (or never). However, in one of the sensitivity analyses there was a trend towards an increased risk of anaemia not likely to be related to clinical events in participants who had initiated cotrimoxazole with ART.

Since haemoglobin <8.0 g/dl at screening was an exclusion criterion for DART (although at screening, a few patients were noted to have received recent blood transfusions and a few with haemoglobin <8.0 g/dl were enrolled in error), we are not able to draw any conclusion about the risks of zidovudine-based therapy in a population with haemoglobin <8.0 g/dl (or with other laboratory contraindications to ART or acute infections). Nevertheless, many of these people may urgently require treatment and are from areas where non-zidovudine based therapy may not be available.

The highest prevalence of anaemia occurred at week 12, which was also the median time for detection of grade 4 anaemia. According to the trial protocol, we performed routine haemoglobin tests at 4 and 12 weeks after starting ART, but not at week 8. All grade 4 anaemia results were returned to clinicians, whether the patient was randomized to CMO or LCM, and additional haemoglobin tests could be performed if clinically indicated. In fact, 38% of those developing grade 4 anaemia were identified through an additional haemoglobin test at a non-scheduled timepoint, about a third of which were between weeks 4 and 12. As many patients had advanced HIV disease before starting ART, differentiating symptoms of anaemia developing after ART from symptoms persisting from HIV-related illnesses present prior to starting ART was difficult for both patients and physicians. Thus, patients may not have presented very promptly with symptoms of anaemia. In view of the fact that previous post-baseline haemoglobin measurements were not able to identify the majority of those with grade 4 anaemia, this study suggests that even in resource-limited settings, an additional measurement of haemoglobin 8 weeks after starting zidovudine-based ART could help to manage serious anaemia, as well as at 4 and 12 weeks – particularly as these can be done at low cost without involving advanced technology (using the WHO fingerprick card) [30].

In conclusion, we observed overall increases in haemoglobin following initiation of zidovudine-based ART in this population of adults with advanced HIV disease and low $CD4^+$ T-cell counts. However, against this background of overall haemoglobin increase, we also observed a higher incidence of grade 4 anaemia than in studies from industrialized countries, likely due in part to population characteristics, and in part to a higher rate of concurrent HIV-related clinical events. Nevertheless, anaemia is a clinically detectable and reversible toxicity of an otherwise successful ART regimen. Clinicians initiating participants on zidovudine-containing regimens in resource-limited settings need to be alert to clinical signs of anaemia because a non-negligible minority of participants are likely to develop it. Education and counselling of participants who are used to feeling unwell about the symptoms of anaemia and clinical vigilance by healthcare workers is vital. In addition, if possible, we recommend that a haemoglobin test should be performed at 4, 8, and 12 weeks in patients initiating zidovudine-containing ART in resource-limited settings. Further, the risks of anaemia and of other toxicities after starting non-zidovudine-containing ART in advanced HIV disease, particular those which may be non-reversible, need to be better quantified in future studies.

Acknowledgements

The authors would like to thank all the patients and staff from all the centres participating in the DART trial (see additional file).

The DART trial is funded by the UK Medical Research Council, the UK Department for International Development, and the Rockefeller Foundation. First-line drugs are provided by GlaxoSmithKline, Gilead and Boehringer Ingelheim.

Additional file

The additional file 'The full list of DART trial members' can be accessed via the Volume 11 Issue 6 contents page for *Antiviral Therapy*, which can be found at www.intmedpress.com (by clicking on 'Antiviral Therapy' then 'Journal PDFs').

References

1. Moore RD, Creagh-Kirk T, Keruly J, *et al.* Long-term safety and efficacy of zidovudine in patients with advanced human immunodeficiency virus disease. Zidovudine Epidemiology Study Group. *Arch Intern Med* 1991; **151:**981–986.
2. Sullivan PS, Hanson DL, Chu SY, Jones JL, Ward JW. Epidemiology of anemia in human immunodeficiency virus (HIV)-infected persons: results from the multistate adult and adolescent spectrum of HIV disease surveillance project. *Blood* 1998; **91:**301–308.
3. Mocroft A, Kirk O, Barton SE, *et al.* Anaemia is an independent predictive marker for clinical prognosis in HIV-infected patients from across Europe. EuroSIDA study group. *AIDS* 1999 **13:**943–950.
4. Graham NM, Piantadosi S, Park LP, Phair JP, Rinaldo CR, Fahey JL. CD4+ lymphocyte response to zidovudine as a predictor of AIDS-free time and survival time. *J Acquir Immune Defic Syndr* 1993; **6:**1258–1266.
5. Saah AJ, Hoover DR, He Y, Kingsley LA, Phair JP. Factors influencing survival after AIDS: report from the Multicenter AIDS Cohort Study (MACS). *J Acquir Immune Defic Syndr* 1994; **7:**287–295.
6. Moore RD, Keruly JC, Chaisson RE. Anemia and survival in HIV infection. *J Acquir Immune Defic Syndr Hum Retrovirol* 1998; **19:**29–33.
7. Creagh-Kirk T, Doi P, Andrews E, *et al.* Survival experience among patients with AIDS receiving zidovudine. Follow-up of patients in a compassionate plea program. *JAMA* 1988; **260:**3009–3015.
8. Sullivan PS, Dworkin MS. Prevalence and correlates of fatigue among persons with HIV infection. *J Pain Symptom Manage* 2003; **25:**329–333.
9. Ludwig H, Strasser K. Symptomatology of anemia. *Semin Oncol* 2001; **28:**7–14.
10. Moyle G. Anaemia in persons with HIV infection: prognostic marker and contributor to morbidity. *AIDS Rev* 2002; **4:**13–20.
11. Volberding PA, Levine AM, Dieterich D, Mildvan D, Mitsuyasu R, Saag M. Anemia in HIV infection: clinical impact and evidence-based management strategies. *Clin Infect Dis* 2004; **38:**1454–1463.
12. Wills TS, Nadler JP, Somboonwit C, *et al.* Anemia prevalence and associated risk factors in a single-center ambulatory HIV clinical cohort. *AIDS Read* 2004; **14:**313–315.
13. Semba RD, Shah N, Klein RS, Mayer KH, Schuman P, Vlahov D. Prevalence and cumulative incidence of and risk factors for anemia in a multicenter cohort study of human immunodeficiency virus-infected and -uninfected women. *Clin Infect Dis* 2002; **34:**260–266.
14. Moore RD, Forney D. Anemia in HIV-infected patients receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2002; **29:**54–57.
15. Levine AM, Berhane K, Masri-Lavine L, *et al.* Prevalence and correlates of anemia in a large cohort of HIV-infected women: Women's Interagency HIV Study. *J Acquir Immune Defic Syndr* 2001; **26:**28–35.
16. Belperio PS, Rhew DC. Prevalence and outcomes of anemia in individuals with human immunodeficiency virus: a systematic review of the literature. *Am J Med* 2004; **116 Suppl 7A:**27S–43S.
17. Richman DD, Fischl MA, Grieco MH, *et al.* The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. *N Engl J Med* 198; **317:**192–197.
18. Volberding PA, Lagakos SW, Koch MA, *et al.* Zidovudine in asymptomatic human immunodeficiency virus infection. A controlled trial in persons with fewer than 500 CD4-positive cells per cubic millimeter. The AIDS Clinical Trials Group of the National Institute of Allergy and Infectious Diseases. *N Engl J Med* 1990; **322:**941–949.
19. Semba RD, Shah N, Klein RS, *et al.* Highly active antiretroviral therapy associated with improved anemia among HIV-infected women. *AIDS Patient Care STDS* 2001; **15:**473–480.
20. Semba RD, Shah N, Vlahov D. Improvement of anemia among HIV-infected injection drug users receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2001; **26:**315–319.
21. Moh R, Danel C, Sorho S, *et al.* Haematological changes in adults receiving a zidovudine-containing HAART regimen in combination with cotrimoxazole in Cote d'Ivoire. *Antivir Ther* 2005; **10:**615–624.
22. World Health Organization. Scaling Up Antiviral Therapy in Resource-Limited Settings: Guidelines for a Public Health Approach. Geneva, Switzerland: WHO; 2002 [cited 4 Sept 2006]. Available from: http://www.who.int/hiv/pub/ prev_care/ScalingUp_E.pdf.
23. World Health Organization. Acquired immune deficiency syndrome (AIDS): interim proposal for a WHO staging system for HIV-1 infection and disease. *Wkly Epidemiol Rec* 1990; **65:**221–228.
24. Regulatory Compliance Center, National Institute of Allergy and Infectious Diseases. Division of AIDS table for grading severity of adult adverse experiences. Aug 1992 [cited 4 Sept 2006]. Available from: http://rcc.tech-res.com/ tox_tables.htm.
25. Lee G. Anemia: A diagnostic strategy. In *Wintrobe's Clinical Hematology*, 10th edition, 1999; pp. 908–940. Edited by Lee G, Paraskevas F, Foerster J, Lukens J. Philadelphia: Lippincott Williams and Wilkins.
26. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. *Int J Epidemiol* 1999; **28:**964-974.
27. Moyle G, Sawyer W, Law M, Amin J, Hill A. Changes in hematologic parameters and efficacy of thymidine analogue-based, highly active antiretroviral therapy: a

meta-analysis of six prospective, randomized, comparative studies. *Clin Ther* 2004; **26:**92–97.

28. Edwards M, Burkle W, Cutrell A, Liao Q, Brothers C, Hernandez J. Characterization of anemia in HIV-infected (HIV+) subjects treated with antiretroviral therapy (ART) with and without zidovudine (+/-ZDV) in 54 clinical trials. *IAS Conference on HIV pathogenesis and treatment*. 2005, Rio de Janeiro, Brazil. Abstract TuFo0106.
29. Lugada ES, Mermin J, Kaharuza F, *et al*. Population-based hematologic and immunologic reference values for a healthy Ugandan population. *Clin Diagn Lab Immunol* 2004; **11:**29–34.
30. World Health Organization. Haemoglobin Colour Scale: Operational Research Agenda and Study Design (WHO/EHT/04.15). Geneva, Switzerland: WHO; 2004 [cited 4 Sept 2006]. Available from: http://www.who.int/medical_devices/publications/en/Res_Agenda.pdf.

Accepted for publication 15 May 2006