Response to Antiretroviral Therapy in Adults and Children in Resource-limited Settings

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for

MASTER OF PHILOSOPHY

DECLARATION

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

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I would like to thank Sarah Walker and Debbie Ford for their fantastic support and guidance throughout this work.

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ABSTRACT

The main aim of this thesis was to address outstanding questions in the treatment of adults and children in low/middle-income settings using data from the DART and ARROW trials. DART and ARROW both investigated whether delivery of ART in Africa with or without routine monitoring of CD4 counts led to similar outcomes in terms of efficacy. In addition ARROW investigated different numbers of drugs at ART initiation. Despite work already carried out on these data, many important clinical and epidemiological questions remained.

I have investigated the patterns of mortality in DART and ARROW using flexible parametric models to assess the change in hazard of death over time, overall and for specific causes of death. Deaths were most commonly related to HIV disease both during the first year after ART initiation and subsequently. HIV-related deaths after 1 year on ART were also the main driver of long-term differences between CD4 vs. no-CD4 monitoring strategies. The contribution of HIV-related-malignancies, likely triggered by pre-cancerous events occurring before ART initiation, and longer-term ART mortality highlights the importance of earlier HIV diagnosis and access to care. Low but increasing risks of deaths from trauma/suicide in adults highlight the importance of long-term psychosocial support and empowering patients to manage their own treatment.

Analysis comparing the DART STI/CT (structured treatment interruption/continuous therapy) randomised groups showed that interrupting ART is associated with lower CD4 counts and poorer clinical outcomes over the long-term even after returning to ART. These findings suggest that substantially greater efforts should be devoted to reducing the risk of any treatment interruption, including strengthening supply chain management, early contact of patients not attending drug refill visits and making short supplies of ART available for patients who travel.

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Chapter 1: Introduction

1.1. Human immunodeficiency virus

1.1.1. Background to HIV

HIV (Human immunodeficiency virus) is a member of the retrovirus family, specifically a lentivirus. Unlike most viruses that store their genetic material on long double strands of DNA (Deoxyribonucleic acid), the genes of a retrovirus are made up of a single strand of RNA (Ribonucleic Acid). This small difference means that HIV replication is more complicated than that of many other viruses and HIV can only replicate inside a human cell. The life cycle of HIV (Figure 1.1.1) [1] starts when a virus particle attaches itself to a human cell via a special protein on the outside of the cell called CD4; the viral envelope which covers the protein shell of a virus then fuses with the cell membrane and the HIV genetic code is released into the human cell where it is transcribed from RNA to DNA. It then travels to the cell's nucleus and integrates with the human DNA. The cell is then 'programmed' to make new HIV genetic material and proteins. The new proteins are cut up by the HIV protease (a protein cutting enzyme) to make functional new HIV particles.

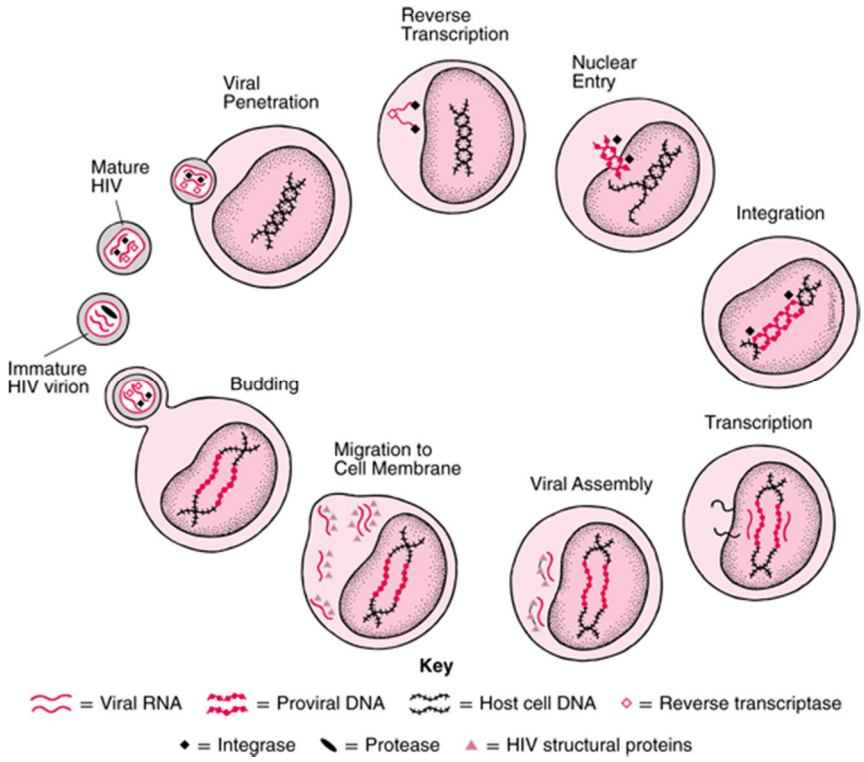


Figure 1.1.1 The HIV Life Cycle (reproduced from [1])

These cells with CD4 proteins on their surface are white blood cells known as T helper cells. They are part of the human immune system helping the body to fight infection by moving throughout the body, identifying and destroying germs such as bacteria and viruses. This aspect of immune system health can be monitored by counting the number of CD4 cells in a microlitre

of blood. A normal CD4 count is from 500 to 1500 cells/μL of blood; however, it is more important to pay attention to the pattern of results than to any one test result, because counts can vary substantially around acute illness. Someone infected with HIV will have a slow decline in CD4 count. As CD4 counts decline the immune system gets weaker, is less able to fight off infections, and this is when people are most likely to become unwell. Although CD4 counts indicate the risk of developing illness, some people can have high CD4 counts and still get infections that healthy people would fight off. Others can have low CD4 counts and have few complications, at least for a while [2].

HIV infection leads to low levels of CD4 through three main mechanisms 1) direct viral killing of infected cells, 2) increased rates of apoptosis (programmed cell death) in infected cells and bystander cells (cells in the nearby environment that may not themselves be directly infected), and 3) the killing of infected CD4 cells by CD8 cytotoxic lymphocytes that recognise infected cells. When CD4 counts decline to below a critical level (<350 cells/μL) [3] the body's immunity declines and people become more susceptible to opportunistic infections (OIs), infections that would not usually occur in someone with a healthy immune system. These OIs and particular cancers which are more common in HIV-infected people form the definition of "AIDS" (acquired immune deficiency syndrome), indicating severe disease. "AIDS" is a definition from the US Centers for Disease Control (CDC). WHO have defined equivalent clinical criteria to diagnose the progression of HIV disease; stage 1 is asymptomatic disease and stage 4 is equivalent to a clinical diagnosis of AIDS [4]. AIDS/WHO stage 4 is the final stage of HIV disease at which point the immune system is so severely damaged that the person can no longer fight infection. If left untreated, HIV/AIDS leads to progressively greater immune system failure, life-threatening OIs, cancers and eventually death.

1.1.2. Transmission of HIV

HIV is only transmitted via bodily fluids such as semen, vaginal secretions, breast milk and blood (including menstrual blood). Blood contains the highest concentration of the virus followed by semen, vaginal secretions and then breast milk. HIV can be transferred from a HIV-infected mother to the baby during pregnancy, birth or breast-feeding; this is known as mother to child transmission (MTCT). The following 'bodily fluids' are not infectious; saliva, tears, sweat, faeces and urine [5]. Activities that allow HIV transmission include unprotected sexual activity and direct blood contact, including injecting drug needles, blood transfusions, accidents in health care settings, and treatments with certain blood products, including factor VIII products (clotting agents commonly used to treat haemophiliacs). From the late 1970s to the mid-1980s, over 4500 people with haemophilia became infected with HIV through receiving infected blood products. Many of these people have developed AIDS and died; of the 1,243 people in the UK with haemophilia who were known to become infected with HIV, less than 250 are still alive in 2016 [6].

Sexual transmission via heterosexual activity accounts for the majority of newly infected individuals in low/middle income countries (LMIC). People who inject drugs suffer disproportionately, particularly in Eastern Europe. In the absence of any intervention, 15-45% of

infants will be infected through MTCT, with most infections occurring during birth; however this rate can be reduced to 5% or less with effective treatment given during pregnancy, delivery and breastfeeding. In 2016, 24% of pregnant women did not have access to treatment to prevent transmission to their children and in that same year around 160,000 children became infected with HIV [7]. Despite this, breastfeeding now accounts for the majority of MTCT [8].

1.1.3. Prevalence

HIV/AIDS was first clinically diagnosed in the USA in 1981; how many people were already infected at this time or how long the virus had been in humans is not clear as there are other rare diseases with similar symptoms to HIV/AIDS. The number of adults (aged 15-49) and children in the world living with HIV has risen from 8 million in 1990 to 37 million by the end of 2016 [9]. It is estimated that 0.8% of adults aged 15-49 years are living with HIV worldwide, with the largest population (25.5 million) being in Africa; East and Southern Africa account for over 50% of all adults living with HIV in the world. [10, 11] (Figure 1.1.2). Overall, prevalence in adults (age 15-49) is higher among men who have sex with men (MSM) than those who have sex with women (MSW) and it is estimated than on average the prevalence among MSM in capital cities is 13 times higher than in the general population [12]. Certain groups such as sex workers also have significantly higher HIV prevalence rates. For example, in 2016 in Lesotho, the reported HIV prevalence among the general population was 25%, the second highest in the world, and prevalence was even higher among sex workers at 72% and men who have sex with men at 33% [13].

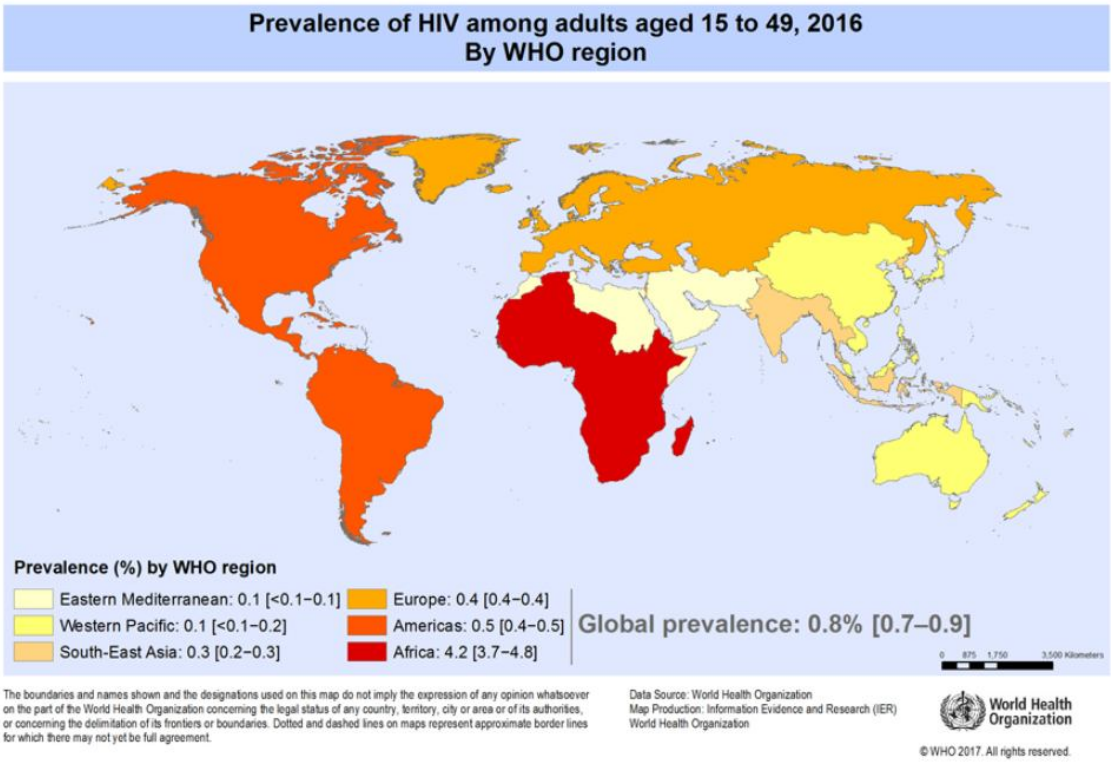
In the heterosexual population, HIV continues to mainly affect women across all regions, likely because of younger age at sexual debut, older partners and socio-economic dependence on male partners. Other factors related to a larger proportion of women having HIV is violence towards women, their first sexual experience being forced, marriage before 18 years of age, the inability to protect themselves from HIV infection and having little knowledge about HIV. In 2015, globally, 51% of people living with HIV were women [14].

At the end of 2003, there were an estimated 2.6 million children under 15 years of age living with HIV infection worldwide; 2.2 million in sub-Saharan Africa. Worldwide this had increased to around 3.3 million in 2011 [15], but has since decreased to just 2.1 million at the end of 2016; the majority of these being in sub-Saharan Africa [9].

People infected with HIV have an increased risk of tuberculosis (TB) due to their vulnerable immune system; without treatment they have a 20-30 times higher lifetime risk of developing active TB, compared with people without HIV [11]. In 2016 there were 10.4 million new cases of TB worldwide, with 10% of these estimated to have been in people with HIV. There were 374,000 deaths from TB in those infected with HIV, 86% of these being in Africa [16]. Although treatable, HIV and TB each speed the other's clinical progression. Because they share modes of transmission, hepatitis B (HBV) and hepatitis C (HCV) are also common among persons who are at risk of or living with HIV infection. For example, in the US a third of people infected with

HIV are co-infected with HBV or HCV [17]. In west and south Africa, 36% of HIV infected people are co-infected with HBV; HCV is less common with an estimated 3.6% of HIV infected individuals being infected with HCV in sub-Saharan Africa [18].

Figure 1.1.2 Prevalence of HIV among adults in 2016 (reproduced from [19])



1.1.4. Incidence

In 2016 there were 1.8 million newly HIV-infected individuals worldwide (16% decline since 2010; 11% decline in adults alone) [10]. The sharpest decline in new HIV infections was seen in sub-Saharan Africa where there was a 56% decline among children and a 29% decline among adults from 2010-2016 [13]. Despite this there were still 790,000 new HIV infections in sub-Saharan Africa in 2016, accounting for 46% of the adults and children newly diagnosed with HIV in 2016. In sub-Saharan Africa, 56% of new infections among adults were in women aged 15 years and older; 66% of infections in women aged 15 years and older are among women aged 15-24 [14]. In 2014 UNAIDS developed a 90-90-90 treatment target to end AIDS globally, which aims at 90% of people living with HIV knowing their status, 90% of HIV diagnosed receiving treatment and 90% of those on HIV treatment achieving viral suppression [20]. In 2016, in East and Southern Africa, it was estimated that 76% of people with HIV knew their status, of those 79% were on antiretroviral therapy and 83% of those were virally suppressed [21].

1.1.5. Deaths and survival

In 2016 there were 1.1 million deaths worldwide related to HIV. In sub-Saharan Africa this was 790,000; 72% of all deaths in the region. HIV related mortality has fallen significantly since 2004 where it was more than double this in sub-Saharan Africa [21]. The main reason for this decline is likely to be enormous widening of access to effective antiretroviral treatment (ART) in sub-Saharan Africa and worldwide over the last decade [22]. There has also been the introduction of the test and treat strategy in 2015 which means people are tested and then hopefully treated straight away regardless of their CD4 count [23]. This has meant that patients are treated earlier, which has shown to improve clinical outcome [24]. In the absence of treatment, life expectancy is severely reduced; median survival time after infection with HIV is estimated to be 4 to 12 years without treatment, depending on age at infection [25]. The median survival rate after diagnosis of HIV-AIDS in LMIC where treatment is not available ranges between 6 and 19 months [26]. Children in Africa typically present with symptoms in the first year of life and by one year one-third will have died without treatment and by age two at least half [27].

However, with effective treatment, started early enough, survival may be prolonged significantly. In 2010, the UK CHIC study published data regarding life expectancy in the UK [28] showing that life expectancy for a HIV-infected individual on treatment has improved by over 15 years during 1996-2008, but is still 13 years lower than that of the general UK population. They showed that mortality rates have decreased such that, compared with the general population, the risk of death in successfully treated patients is similar to that of people with unhealthy lifestyles (such as heavy smoking, drinking or obesity). The ART Cohort Collaboration reported in 2008 [29] that in high income countries, life expectancy has improved by 81% since 1996, with HIV infected adults aged 20 years expecting to live for another 49 years. However, in LMIC less data are available and few population data are available for comparison. Mills et al [30] reported in July 2011 a crude mortality rate of 3.2 deaths per 100 person years (PYs) in a cohort of 22,315 persons who initiated HIV treatment in Uganda between 2000 and 2009. Life expectancy in the whole cohort was a further 27 years at 20 years of age, and a further 28 years at 35 years of age, with men having a consistently shorter life expectancy than women (a further 19 years for men, but 31 years for women from 20 years of age). Longer life expectancy was associated with increasing baseline CD4 counts at ART initiation [30]. More recently, in 2013 Nakagawa et al. [31] reviewed literature on life expectancy in several countries (UK, US, Uganda and Netherlands) from 2010 onwards and found that with earlier diagnosis, access to ART and good lifelong adherence, people with HIV can expect to have a life expectancy which is nearly the same as that of people who are HIV negative. They also found modelling studies that suggest life expectancy could improve further with an increased uptake of HIV testing, better antiretroviral regimens and treatment strategies, and the adoption of healthier lifestyles by those living with HIV. They found that earlier diagnosis is one of the most important factors associated with better life expectancy. However, as people live longer with HIV, further research is needed on how to treat an ageing HIV population – this is one of the new challenges of treating those with HIV.

1.1.6. Treatment of HIV

At this present time there is no cure for HIV; as discussed above, with treatment HIV may be managed as a chronic condition. The standard treatment for HIV is combination antiretroviral therapy (ART), one of the most important advances in the history of HIV/AIDS. The first antiretroviral (ARV) drug was approved in 1986, zidovudine (ZDV), and was taken as monotherapy (single ARV drug) which was only partly efficacious. In 1991 several more drugs of a similar class (nucleoside reverse transcriptase inhibitors, NRTIs) became available, and from 1991-1995 HIV-infected people were treated with dual therapy which again was only partially efficacious. Unless HIV viral load (VL) is suppressed to very low levels, HIV can become resistant to a drug. Resistance to one drug can also lead to resistance to another (cross-resistance). Mono and dual therapy did not suppress VL enough, so resistance developed and the drugs became ineffective. In 1995-6, two new classes of antiretrovirals (non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs)) became available and people moved to 3-drug combination therapy (2NRTI+PI or 2NRTI+NNRTI). This combination treatment suppresses VL so much that development of resistance to the drugs is reduced, and in some cases viral replication may be stopped completely. This new combination therapy was also known as highly active antiretroviral therapy (HAART), and in 1997 became the standard of care.

When I started my MPhil in 2011, the main types of ARVs used in combination ART in first-line therapy in LMIC were:

1) Nucleoside reverse transcriptase inhibitors (NRTIs) which suppress the replication of retroviruses by interfering with the reverse transcriptase enzyme, which is encoded from the genetic material of the virus and transcribes retroviral RNA into DNA (Figure 1.1.1); without this process the viral genome cannot become incorporated in the host cell. The chemical structure of a NRTI consists of a modified version of a natural nucleoside. NRTIs include zidovudine (ZDV), lamivudine (3TC), emtricitabine (FTC) and abacavir (ABC).

2) Nucleotide reverse transcriptase inhibitors (NtRTIs), which act as NRTIs, but have an additional phosphate group. Tenofovir (TDF) is a NtRTI.

3) Non-nucleoside reverse transcriptase inhibitors (NNRTI) – these are similar to NRTIs in the sense that they inhibit the transcription of the viral DNA, however they are unable to incorporate into the viral DNA so they simply attach themselves to the reverse transcriptase and inhibit its movement – nevirapine (NVP) and efavirenz (EFV) are NNRTIs. In LMIC, NNRTIs are recommended for first-line treatment with NRTIs [32].

4) Protease inhibitors (PIs) which block the protease enzyme and prevent the cell from producing new viruses. In LMIC PIs are recommended for second-line treatment [32].

Other classes of ARVs (integrase inhibitors (II) such as raltegravir or dolutegravir, fusion inhibitors such as maraviroc) were not recommended as preferred first- or second-line treatment [27], until the guidelines were updated in 2016.

1.1.7. Guidelines for treatment

In 2002 WHO released their first guidelines for taking a public health approach to the scale up of ART [33], covering treatment initiation criteria and recommending therapies and management of patients on treatment. During the time of the DART and ARROW trials, subsequent updated guidelines were published in 2006 and 2010 [27, 34]; a summary of which can be found in Table 1.1.1. Since then, and since the DART and ARROW trials were closed, the guidelines were updated in 2013, 2015 and 2016 [32]. The 2006 update changed the thresholds for treatment initiation with the aim of having all those who needed ART (based on an ART initiation threshold of CD4 <200 cells/μL) having access by the end of 2010 [34]. In 2010, the threshold was increased to <350 cells/μL, substantially increasing the numbers defined as being in need of ART [27]. In 2013 the guidelines were updated so the threshold for ART initiation was increased to ≤500 cells/μL, but giving priority to those with severe/advanced disease or CD4 ≤350 cells/μL [35]. In 2016 the guidelines removed this threshold and it is now recommended that all those diagnosed with HIV should start treatment regardless of WHO clinical stage and at any CD4 count [32]; this was based on the results of the START trial [36]. In addition (as discussed previously) the aim is for 90% of people with HIV to be aware of their status, 90% of those should be receiving ART and 90% of those should be reaching viral suppression (VL≤50 copies/L) [37].

In summary, the current WHO 2016 guidelines recommend that all HIV-infected children, adolescents and adults, including pregnant women, regardless of their WHO clinical stage or CD4 count should initiate ART. As a priority ART should be initiated in all children ≤2 years of age or children younger than 5 years who are WHO stage 3/4 or CD4 count ≤750 cells/μL or CD4% <25, and any children ≥5 years with WHO stage 3/4 or CD4 count ≤350 cells/μL; this priority is also given to HIV-infected adolescents and adults [32].

Current guidelines recommend [32] that HIV infected patients start on a first-line therapy that should consist of 2NRTI + (NNRTI or II), one of which should be tenofovir (or abacavir for children) plus lamivudine (or emtricitabine); the recommended NNRTI is efavirenz. Alternative first-line regimens can include zidovudine as an NRTI or nevirapine as the NNRTI or the replacement of the NNRTI with the integrase inhibitor dolutegravir. Dolutegravir is not recommended for pregnant or breast feeding women or children. Stavudine should be discontinued in first-line regimens due to its well-known metabolic toxicities. On failure of first-line therapy patients should move onto second-line therapy that consists of a ritonavir-boosted PI+2NRTIs, one of which should be zidovudine or tenofovir, based on their first-line therapy. Current management recommends that all patients should have CD4 cell count testing to optimise pre-ART care and those receiving ART should have access to laboratory monitoring consisting of HIV VL (at 6 months and 12 months after initiating ART and every 12 months

thereafter) and CD4 cell count every 6 months until patient is stable on ART. Management recommendations during the period that DART and ARROW were active are outlined in Table 1.1.2.

Table 1.1.1 Changing recommendations for criteria to initiate ART in LMIC over the period when the DART and ARROW trials were active

	Year of treatment recommendation		
Age*	**2002 [33]**	**2006 [34, 38]**	**2010 [27, 38]**
Adults	Initiate ART if: • WHO stage 4 regardless of CD4 count • WHO stage 1/2/3 with CD4<200 cells/μL • WHO stage 2/3 TLC<1200 cells/μL if CD4 unavailable • WHO stage 4 irrespective of TLC if CD4 unavailable • TB/HIV co-infection – finish TB treatment before initiating ART unless high risk of progression	• HIV+, asymptomatic o Initiate ART if CD4 ≤200 cells/μL irrespective of WHO clinical stage • HIV+, symptomatic o WHO 4 irrespective of CD4 o CD4 200-350 cells/μL consider treatment and initiate before it drops to <200 o WHO stage 2/3 and CD4 ≤200 cells/μL o WHO stage 3 if CD4 not available • HIV+, pregnant o WHO 1/2 and CD4 ≤200 cells/μL (do not treat if CD4 unavailable) o WHO 3 and CD4 ≤350 cells/μL • Co-infected with TB o CD4 ≤350 cells/μL o Can be delayed if CD4 ≥200 cells/μL	• HIV+, asymptomatic o Initiate ART if CD4 ≤350 cells/μL irrespective of WHO clinical stage • HIV+, symptomatic o Initiate ART if WHO clinical stage 3/4 irrespective of CD4 o Initiate ART if WHO clinical stage 2 and CD4 ≤350 cells/μL • Patients co-infected with HIV and TB should start ART as soon as possible after TB treatment irrespective of CD4 cell count • Patients who require treatment for their HBV infection, irrespective of CD4 count
Infants	(<18 months) Initiate ART if HIV infection has been virologically confirmed and: • WHO paediatric stage 3 • WHO stage 1/2 and CD4<20% • When virological confirmation unobtainable then WHO stage 3 and CD4<20% (no stage 4 defined)	(<12 months) Initiate ART if: • WHO 3/4 irrespective of CD4 • WHO 2 and CD4 <25% or <1500 cells/μL or TLC <4000 cells/μL when CD4 not available • WHO 1 and CD4 <25% or <1500 cells/μL or do not treat when CD4 not available	Initiate ART in first year of life irrespective of CD4 count or WHO clinical stage

Table 1.1.1 Changing recommendations for criteria to initiate ART in LMIC over the period when the DART and ARROW trials were active (continued)

Children	2002	2006	2010
12-35 months	(>18 months) Initiate ART if: • WHO stage 3 regardless of CD4 % • WHO 1/2 and CD4<15%	(12-35 months) Initiate ART if: • WHO 3/4 if CD4 not available • WHO 4 irrespective of CD4 • WHO 3 and CD4 <20% or <750 cells/μL if child has TB, LIP **(**lymphocytic interstitial pneumonia), oral hairy leukoplakia or thrombocytopenia	Initiate ART irrespective of CD4 count or WHO clinical stage
36-59 months		(36-59 months) • Initiate ART as 12-35 months but CD4 guided treatment based on <15% or <350 cells/μL; use TLC <2500 cells/μL if CD4 not available	Initiate ART with CD4 count ≤750 cells/μL or CD4% <25, whichever is lower, irrespective of WHO clinical stage
≥5 years		• Initiate ART as 12-35 months but CD4 guided treatment based on <15% or <200 cells/μL, use TLC <2000 cells/μL if CD4 not available (but only up to 8 years)†	As with adults, initiate ART if CD4 ≤350 cells/μL irrespective of WHO clinical stage

TLC – Total lymphocyte count, *categories based on most recent guidelines, †Little evidence for children >8 years

Table 1.1.2 Changing management recommendations over the period when the DART and ARROW trials were active

		2002	2006	2010
Adults	**Tests**	• Minimum lab test is HIV antibody test, haemoglobin and haematocrit • Basic recommended tests include WBC, hepatitis, hepatotoxicity • Desirable tests include bilirubin, serum lipid and CD4 count • Clinical monitoring is essential • Where laboratory monitoring is limited, close clinical monitoring is crucial	• Haemoglobin at ART initiation (for those on ZDV at baseline, weeks 4, 8, and 12) • WBC and differential at initiation (at 4,8, and 12 weeks is optional) • CD4 cell count testing at initiation, every six months (may need to be more often if patient appears to be unwell) • Full chemistry as required (every six months for those receiving second-line drugs) • Viral load as and when required but should not be used for treatment decision making	• All patients should have access to CD4 cell-count testing to optimise pre-ART care and ART management • HIV RNA (viral load) testing is recommended to confirm suspected treatment failure • Drug toxicity monitoring should be symptom directed
	Management	• Countries are encouraged to use CD4 cell counts in ART programmes and consider low cost CD4 methodologies • CD4 % can be used instead of CD4 counts (CD4<15% = CD4 200 cells/μL. TLC ok when CD4 cannot be measured. • Assessment of VL not considered essential	• Patients on ART should be monitored clinically every 2, 4, 8, 12 and 24 weeks after starting ART, then every 6 months thereafter • Contact with healthcare team trained to manage is recommended with referral to physician if needed • Pay attention to any reduction in frequency of infections or increased toxicities	• Laboratory monitoring is not a prerequisite for the initiation of ART • CD4 and viral load (VL) testing are not essential for monitoring patients on ART • Symptom-directed laboratory monitoring for safety and toxicity is recommended for those on ART • If resources permit use VL: o as a targeted approach to confirm suspected treatment failure based on immunological and/or clinical criteria o in a routine approach, every 6 months, with objective to detecting failure earlier than would be the case if using immunological and/or clinical criteria to define failure

Table 1.1.2 The changing management recommendations over the period when the DART and ARROW trials were active (continued)

Infants and children	**Tests**	• As with adults but %CD4 instead of CD4	• As with adults but %CD4 instead of CD4	• As with adults but %CD4 instead of CD4 • Viral load should be used whenever possible to confirm suspected clinical or immunological failure
	Management	• As with adults VL is not a useful test as children have raised HIV RNA levels anyway	• Frequency of clinical monitoring depends on response to ART, at least at weeks 2, 4, 8 and 12 after starting ART, then every 2-3 months after stabilisation on therapy • Assessment of CD4 every 6 months • TLC not suitable • For those on ZDV, haemoglobin (weeks 4, 8, 12) is recommended • For those on nevirapine, liver function tests during the first few months • The inability to provide such monitoring should not prevent children from receiving ART	• Clinical monitoring depends on response to ART o Infants, at weeks 2, 4, 8 then every 4 weeks o Children, at weeks 2,4,8,12, then every 2-3 months • Where clinical monitoring is unavailable monitoring should be targeted to assessment of clinical events

Table 1.1.2 The changing management recommendations over the period when the DART and ARROW trials were active (continued)

		2002	2006	2010
Adults	**Treatment**	• Treatment should be standardised • Single first-line and limited number of second-line regimens: first-line • ZDV/3TC/EFZ or ZDV/3TC/NVP (for pregnant women consider EFZ only in addition to ZDV and 3TC) • ZDV/3TC/ABC • ZDV/3TC/RTV-PI or ZDV/3TC/NFV • Other dual nucleoside combinations can be substituted for ZDV/3TC, including stavudine (d4T)/3TC, d4T/didanosine (ddI) and ZDV/ddI, depending on country-specific preferences. • ZDV and d4T should never be used together because of proven antagonism between these drugs • Dual NRTI combination of d4T/ddI should only be used during pregnancy when no other alternatives exist, because of the potential increased risk of lactic acidosis with this combination in pregnant women.	First-line: • Adults and adolescents o ZDV or TDF + 3TC (or FTC) + EFV or NVP • HIV+, pregnant o ZDV + 3TC + NVP • HIV/TB co-infection o ZDV or TDF + 3TC (or FTC) + EFV • HIV/HBV co-infection o TDF + 3TC (or FTC) + EFV Second-line: • Adults and adolescents o ABC + ddl or TDF + ABC or ddl +3TC or TDF +3TC (±ZDV) + ATV/r or FPV/r or IDV/r or LPV/r or SQV/r • HIV+, pregnant o As adults and adolescents but different boosted PI (LPV/r or NFV or SQV/r) • HIV/TB co-infection o As adults and adolescents but PI is LPV/r or SQV/r with adjusted ritonavir (RTV)* • HIV/HBV co-infection o 3TC and/or TDF containing regimens • ZDV, TDF, ABC or d4T. • Stavudine (d4T) is now recommended at the dose of 30 mg twice daily for all adult and adolescent patients regardless of body weight	First-line: • Adults and adolescents o No change but where d4T is used as principal option for starting ART there should be a plan to move towards ZDV or TDF based regimens • HIV+, pregnant o ZDV + 3TC + NVP (or EFV, but not during first trimester) o See guidelines for women with prior MTCT regimens • HIV/TB co-infection o ZDV or TDF + 3TC (or FTC) + EFV (recommended within 8 weeks of start of TB treatment) • HIV/HBV co-infection o Require regimens that contain both TDF + 3TC (or FTC) + EFV Second-line: • Adults and adolescents o TDF (if ZDV used first-line) or ZDV (if TDF used first-line) + 3TC (or FTC) + ATV/r or LPV/r • HIV+, pregnant o As adult and adolescent • HIV/TB co-infection o If rifabutin available then same as adults, else same NRTI backbone as adults plus LPV/r or SQV/r with adjusted dose of RTV • HIV/HBV co-infection o ZDV+TDF+3TC(or FTC)+ATV/r or LPV/r

Table 1.1.2 The changing management recommendations over the period when the DART and ARROW trials were active (continued)

		2002	2006	2010
Infants/ Children	**Treatment**	• ZDV, 3TC, d4T, DDI and ABC have formulations appropriate for children • Combinations ZDV/3TC and ZDV/3TC/ABC only available in tablet • NVP liquid formulation • EFZ children >3 years	• Syrups and solutions remain necessary for treating infants and very young children • Solid formulations (parts of scored tablets or combination preparations for older children • Regimen of 2 NRTI plus 1 NNRTI: o ZDV + 3TC + NVP/ EFV o d4T + 3TC + NVP /EFV o ABC + 3TC + NVP/ EFV	• Infant or child <24 months not exposed to ARVs: NVP + 2 NRTI • Infant or child <24 months exposed to NNRTI: LPV/r + 2 NRTI • Infant or child <24 months with unknown ARV exposure: NVP + 2 NRTI • Children 24 months to 3 years: NVP + 2 NRTI • Children >3 years: NVP or EFV + 2 NRTI

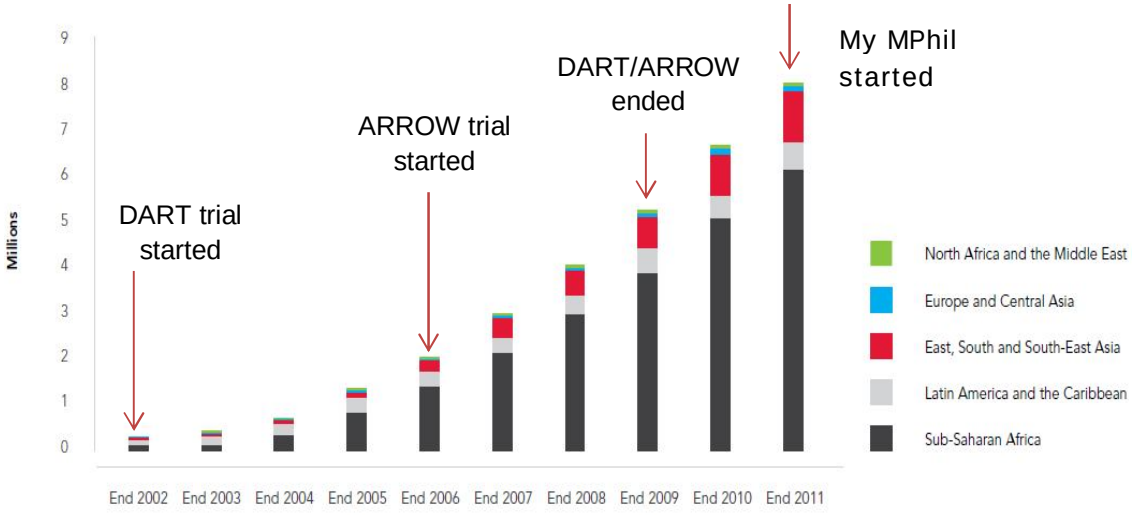
*see guidelines for dosing [27, 33, 34, 38]

Note: ABC=abacavir, ddl=didanosine, d4T=stavudine, TDF=tenofovir, ZDV=zidovudine, 3TC=lamivudine, FTC=emtricitabine, NVP=nevirapine, EFV=efavirenz, ATV/r=ritonavir-boosted atazanair, FPV/r= ritonavir-boosted fosamprenavir, IDV/r= ritonavir-boosted indinavir, LPV/r= ritonavir-boosted lopinavir, NFV=nelfinavir, SQV/r= ritonavir-boosted saquinavir,

1.1.8. Numbers on treatment and coverage

In 2002 it was estimated that there were 6 million people in LMIC who needed ART (based on a CD4 threshold for ART initiation of <200 cells/μL), but only 230,000 had such access (50% of these were in Brazil), with only 50,000 on ART of the 4.1 million in need in sub-Saharan Africa [34, 39]. The major gap between need and access led WHO to set a target of 3 million individuals receiving ART by the end of 2005, known globally as the "3 by 5" plan. As shown in Figure 1.1.4 this target was not met, but nevertheless the numbers on ART in LMIC had still increased to over 1 million by the end of 2005, ~0.8 million in sub-Saharan Africa, and have continued to increase substantially thereafter. Figure 1.1.3 shows the progress in scaling up of ART since 2002 until the end of 2011 where ART had reached 8 million people [15]. In 2016 19.5 million people infected with HIV were receiving ART; 11.7 million in sub-Saharan Africa [40].

Figure 1.1.3 People receiving ART in low- and mid- income countries 2002-2011 (reproduced from [15])



Since the guidance on treatment update in 2016, recommending that all HIV positive individuals start treatment regardless of their CD4 count, the coverage of ART in HIV positive individuals in East and Southern Africa has increased to 61% in adults and 51% in children [21] (Figure 1.1.4).

Figure 1.1.4 Antiretroviral therapy coverage and number of AIDS-related deaths in East and Southern Africa (adults and children) (reproduced from [40])

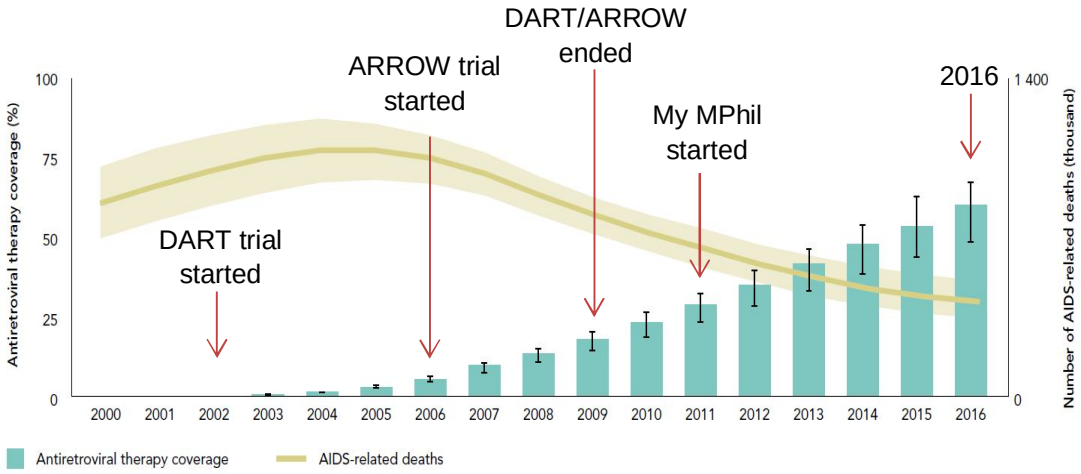


Table 1.1.3 ART coverage in sub-Saharan Africa 2002, 2006, 2011 and 2016

		2002 [39]	2006 [41]	2011 [42]	2016 [11]
Adults	ART coverage	1%	28%	56%	61%
	CD4 treatment threshold	CD4<200 cells/μL	CD4<200 cells/μL	CD4<350 cells/μL	Start ART regardless of CD4 count (priority given to those with CD4 <350 cells/μL)
Infants/ Children	ART coverage	NI*	15%	28%	51%
	CD4 treatment threshold	Infants: CD4<20% Children: CD4<15%	Infants: CD4<25% Children: CD4<20%	Infants & children <2yrs: treat in first year irrespective Children (>2 yrs): CD4<25% (>5yrs) CD4<350 cells/μL	Start ART regardless of CD4 count (priority given to those with CD4 <350 cells/μL)

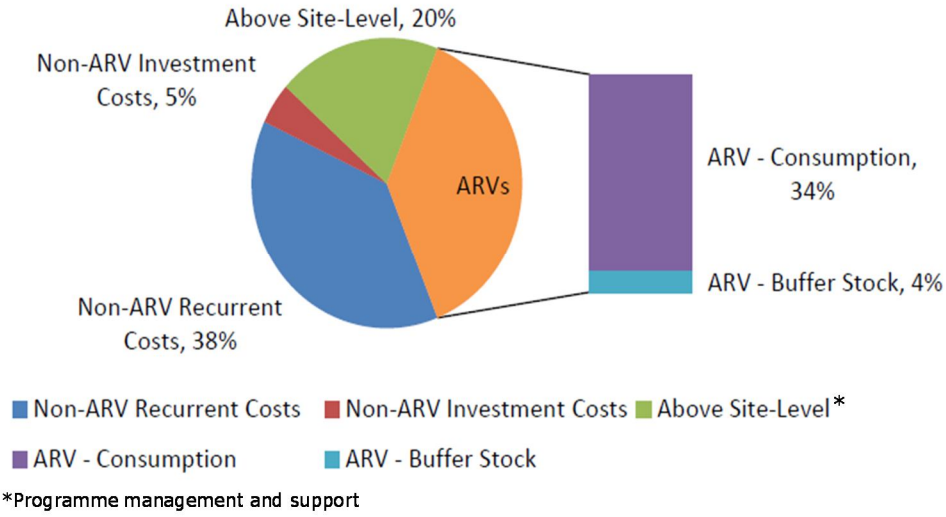
*Not included in the report;

Table 1.1.3 shows the change in coverage in sub-Saharan Africa from 2002-2016. The estimated 11.7 million [10.3 million-12.1 million] people on ART in the region in 2016 represented an almost threefold increase over 2010 numbers. Sustained progress is needed to ensure that 81% of all people living with HIV in the region are accessing treatment by 2020 (the second part of the 90-90-90 target). In 2016, the gap to fully achieving this second 90 from the 90-90-90 target was starting an additional 4.1 million people on antiretroviral therapy. In 2016, worldwide, 54% of adults and 43% of children living with HIV were currently receiving lifelong antiretroviral therapy [11]. Global ART coverage for pregnant and breastfeeding women living with HIV was high at 76%.

One major change that has aided the increase in people needing ART being able to receive it is reductions in the cost of the drugs. Generic drugs have been produced which are identical to the 'branded' drugs, but costing a fraction of what they did before. In 1996 when ART first became available it cost $10,000-15,000 per patient per year. But by 2001 drugs were costing

as little as $295 per person per year [43]. In PEPFAR programmes (one of the major US donors), about 30 to 40 percent of the costs related to treatment are drug-related costs, and the other 60 to 70 percent are systems and delivery costs [44], with 34% ARVs and 4% buffer stock [45] (Figure 1.1.5).

Figure 1.1.5 Costs of ART provision, by major cost component (reproduced from [45])



1.1.9. Practical Challenges of treatment

Effective triple-drug ART has resulted in a major reduction in mortality and morbidity in HIV-infected adults and children in high-income countries and LMIC but there have been many barriers to ART in LMIC. The increase in the number of people needing treatment since the 90-90-90 targets have been introduced has resulted in an increased burden on the already limited resources in LMIC.

In order for the scale up of ART in sub-Saharan Africa to take place, health care systems need to be strengthened. There have been several barriers to scale up that have been cited in the literature recently, including inadequate supply and poor retention of health professionals [46, 47]. In 2008 WHO defined task shifting as a necessary condition for scaling up ART in sub-Saharan Africa [48]. Task shifting is the process by which specific tasks are moved, where appropriate, to less specialised healthcare workers. It has been split into 4 types; Type I – shift from medical doctor to non-physician; Type II – shift from non-physician to nurse; Type III – shift to lay community providers and counsellors; Type IV – shift to people living with HIV themselves. By using lower level health care workers at smaller healthcare facilities, access to HIV care could be easier and will hopefully also reduce the cost of travelling to clinic. By sharing medical tasks with lower level health care workers, the strain on the already overburdened health care staff is reduced and by involving people who have HIV, practical skills needed to manage having HIV can be directly passed on to those people who need it [47]. It has been shown that when full care and patient self-management fail, patients discontinue treatment, and are at risk of drug resistance and treatment failure [49].

The HIV epidemic affects populations in many ways, not just medically but socially and psychologically. An ART programme needs to be well integrated into the public healthcare system and be comprehensive in addressing the social, psychological and economic needs of HIV care, for example social support, socioeconomic status and education. If this can happen then HIV could become more widely accepted like other chronic conditions such as diabetes. In the early 2000s there was limited integration of ART services into the public system [47]. More and more people are now recognising that integration is essential. The main concerns about this are that it may take resources away from other vital health services, leading to general health care suffering.

Loss to follow-up is a major challenge in ART programmes [50], since failure to attend inevitably means running out of drugs, leading to treatment failure or other poor clinical outcome. Recent studies have shown that psychosocial support and regular home visits from community health workers and peer adherence counsellors has increased patient retention [51]. Tracing defaulters has also been highlighted by several studies as one of the standard tasks of community support providers; for example Kabore et al [51] reported that defaulter tracing by community healthcare workers was associated with better ART outcomes in Malawi.

Other challenges are the cost of treatment (although, as described above, this has come down in recent years) and the supply chain, including not only drugs but laboratory supplies and testing kits. Ensuring there are no interruptions in treatment requires a guaranteed supply of ART. This needs careful planning: accurate forecasting is necessary to reduce over-purchasing and therefore extra strain on money and storage space. However, the converse is that stock-outs are common as sites try not to over purchase drugs [52]. As well as the practical challenges to treatment, a number of clinical challenges exist. In particular, there is the challenge of monitoring patients for toxicity and treatment response during time on ART [46], a topic the DART and ARROW trials addressed.

1.2 Data available

1.2.1. The DART Trial

The major driver behind increasing rollout of ART in LMIC was reductions in the cost of drugs. However, the cost and infrastructure required for monitoring patients taking ART, as carried out in high-income countries, was and still is extremely high. In order to carry out toxicity and CD4 monitoring (or now VL monitoring) sites need to buy and maintain machines, purchase reagents and employ suitably trained staff. At the time the DART trial was being designed, in 2002, in Africa patients were already receiving ART without routine laboratory monitoring but no one had formally investigated the effects of this and whether this could be done safely, and there were still concerns regarding the lack of infrastructure to administer ART and to sustain adherence and monitor toxicity in LMIC [53]. Laboratory tests are done 3-monthly in high-income countries because they are available and are de facto the standard of care. Due to the potential barriers in LMIC (as discussed above) there was a need to explore the question of the necessity of these laboratory tests with the introduction of ART in Africa. In 2003 the Medical Research

Council Clinical Trials Unit, in partnership with Imperial College London and three African research groups in Uganda/Zimbabwe, started the large African DART (**D**evelopment of **A**nti**R**etroviral **T**herapy in Africa) trial [54] to evaluate the need for laboratory tests required for monitoring toxicity and efficacy of ART. At this time there had been no clinical trials evaluating such questions. The other major question addressed by DART was regarding structured treatment interruptions (STI), whereby periods on ART are followed by periods off treatment. At the time the trial was being designed there was some evidence to suggest that STIs were safe and could reduce toxicity and costs, and improve adherence while maintaining clinical and immunological well-being.

The DART trial was an open-label non-inferiority randomised trial evaluating two strategic approaches to the management of ART in symptomatic HIV infected adults in Africa. The first strategy compared clinically driven monitoring (CDM) with laboratory plus clinical monitoring (LCM). The second approach compared structured treatment interruptions, 12 weeks on, 12 weeks off with continuous ART (CT) in patients who achieved CD4 cell counts of ≥350 cells/μL after 48/72 weeks on continuous ART. Patients were randomised at three sites in Uganda and one in Zimbabwe to receive either CDM or LCM and a second randomisation took place after 52 weeks (or 76 weeks, depending on CD4 counts measured at 48 or 72 weeks) to either structured treatment interruptions (STI) or continuous therapy (CT).

The hypotheses for the DART trial were that CDM would result in a similar outcome to LCM and that ART administered as structured intermittent therapy would result in similar outcomes to continuous ART. The primary efficacy endpoint was progression of clinical HIV disease (as indicated by a new non-recurrent WHO 4 stage HIV event) and/or death and the primary safety endpoint was Serious Adverse Events (SAEs). Routine full blood tests were carried out at screening, weeks 4 and 12 and then 12 weekly thereafter. All results were returned to clinicians in the LCM arm but in the CDM arm results were only returned if requested for valid clinical reasons or if there was a grade 4 laboratory toxicity (protocol safety criteria). No total lymphocyte counts (TLC) or CD4-cell counts were ever returned for CDM participants. All participants received ART and were reviewed by a nurse every 4 weeks. Any decision to switch to second-line ART was based on clinical criteria (new or recurrent WHO 4 event; or WHO 3 event at clinician discretion) in both groups or laboratory criteria (CD4 <100 cells/μL [<50 cells/μL before July 2006]) in the LCM group. Switching before 48 weeks was discouraged following the 2006 WHO guidelines [33].

Table 1.2.1 DART Baseline characteristics and follow-up

DART	LCM n (%)	CDM n (%)	Total n (%)
Baseline	**1656**	**1660**	**3316**
Women	1092 (66%)	1064 (64%)	2156 (65%)
Age (years)			
Median (IQR)	36 (18-67)	36 (18-73)	36 (18-73)
CD4 cell count at ART initiation (cells/μL)			
Median (IQR)	86 (0-199)	86 (1-199)	86 (0-199)
WHO stage			
2	363 (22%)	310 (9%)	673 (20%)
3	916 (55%)	948 (57%)	1864 (56%)
4	377 (23%)	402 (24%)	779 (24%)
First-line treatment received: ZDV+3TC plus			
TDF	1232 (74%)	1237 (75%)	2469 (74%)
ABC	150 (9%)	150 (9%)	300 (9%)
NVP	150 (9%)	150 (9%)	300 (9%)
Follow-up			
Lost to follow-up	112 (7%)	122 (7%)	234 (7%)
Treatment status at end of trial			
Still on first-line treatment	1295 (78%)	1346 (81%)	2641 (80%)
Drug substitution in first-line	281 (17%)	288 (17%)	569 (17%)
Switched to second-line	361 (22%)	314 (19%)	675 (20%)
Self-reported adherence across all visits			
Missed any pills in last 4 days	3.3%	3.5%	3.4%
Missed any pills in last 28 days	7.8%	8.5%	8.2%
Median follow-up (IQR) [years]	4.9 (4.4-5.3)	4.9 (4.4-5.3)	4.9 (4.4-5.3)
Adverse events: patients with any			
Serious	260 (16%)	283 (17%)	543 (16%)
ART modifying	416 (25%)	422 (25%)	838 (25%)
Grade 4	643 (39%)	683 (41%)	1326 (40%)
Grade 3/4	1151 (70%)	1168 (70%)	1319 (40%)
New WHO 4 event/death	356 (21%)	459 (28%)	815 (25%)
New WHO4/event-free survival at 5 years % (95%CI)	78% (76-80)	76% (70-74)	77% (70-80)
Death	164 (10%)	218 (13%)	382 (12%)
Overall survival at 5 years % (95%CI)	90% (88-91)	87% (85-88)	88% (85-91)
Comparison of CDM vs. LCM [HR (95%CI)] p-value			
Adverse events			
Serious	1.12 (0.94-1.32)		p=0.19
ART modifying	1.01 (0.88-1.16)		p=0.86
Grade 4	1.07 (0.96-1.20)		p=0.19
Grade 3/4	1.03 (0.95-1.11)		p=0.51
Events			
Death	1.35 (1.10-1.65)		p=0.004
New WHO 4 event/death	1.31 (1.14-1.51)		p=0.0001

Table 1.2.1 shows baseline characteristics, treatment received and follow-up data for the main trial for LCM and CDM comparison. 3316 patients aged ≥18 years with CD4 cell count <200 cells/μL, who were ART naïve and had no clinical or laboratory abnormalities were randomised between January 2003 and October 2004, and followed until December 2008. There was a median of 4.9 years of follow-up (14937 person years). As the upper 95% confidence limit for the new WHO 4 event/death outcome lies above the pre-specified non-inferiority margin of 1.18 the team were unable to conclude that in terms of efficacy CDM is not inferior to LCM (that is, non-inferiority was not demonstrated). However, in terms of toxicity there was no difference found between CDM and LCM (HR 1.12 (96%CI 0.94-1.32) p=0.19 for serious adverse events)

suggesting that ART can be delivered safely without routine laboratory monitoring for side-effects. All trial results can be found in DART main publication [54].

These results had major implications for ART programmes in Africa at a time when there was uncertainty about long-term funding and sustainability and when >40% people in need could still not access treatment. The trial showed that routine laboratory monitoring for toxicity had no benefit and ART could be safely delivered with good quality clinical care [54], although small (~3% in absolute terms), real improvements could be gained if routine CD4 monitoring was also used.

In March 2006 the second randomisation between STI and CT was terminated by the Independent Data Monitoring Committee and the Trial Steering Committee after harms associated with STIs emerged in the SMART trial [55]; all patients were moved back to CT. The results were published with a median follow-up of 51 weeks after randomisation to STI vs. CT [56]. Table 1.2.2 outlines the baseline characteristics for the STI/CT comparison along with the results reported.

Table 1.2.2 STI substudy, baseline characteristics and follow-up

DART	**STI n (%)**	**CT n (%)**	**Total n (%)**
Baseline	**408**	**405**	**813**
Women	296 (73%)	299 (74%)	595 (73%)
WHO stage at ART initiation			
2	103 (25%)	93 (23%)	196 (24%)
3	219 (54%)	238 (59%)	457 (56%)
4	86 (21%)	74 (18%)	160 (20%)
Age at STI/CT randomisation (years)			
Median (IQR)	37 (32-42)	37 (32-43)	37 (32-43)
CD4 cell count at STI/CT randomisation (cells/µL)			
Median (IQR)	357 (324-428)	359 (328-415)	358 (325-419)
ART at STI/CT randomisation:ZDV+3TC plus			
TDF	271 (66%)	276 (68%)	547 (67%)
ABC	35 (9%)	47 (12%)	82 (10%)
NVP	102 (25%)	82 (20%)	184 (23%)
Follow-up			
Median follow-up (weeks)	51 (37-65)	51 (37-64)	51 (37-65)
Adverse events			
Serious	9 (2%)	10 (2%)	19 (2%)
ART modifying	2 (<1%)	10 (2%)	12 (1%)
Grade 4	23 (6%)	26 (6%)	49 (6%)
Grade 3/4	80 (20%)	80 (20%)	160 (20%)
New WHO 4 event/death	24 (6%)	9 (2%)	33 (4%)
Death	5 (1%)	4 (<1%)	9 (1%)
Comparison STI vs. CT [HR (95%CI)] p-value			
Adverse events			
Serious	0.88 (0.36-2.17)		p=0.78
ART modifying	0.20 (0.04-0.92)		p=0.02
Grade 4	0.81 (0.46-1.43)		p=0.47
Grade 3/4	0.98 (0.72-1.34)		p=0.90
New WHO 4 event/death	2.73 (1.27-5.88)		p=0.007
Death	1.30 (0.35-4.83)		p=0.70

Between July 2004 and March 2006, 813 patients were randomised to receive either CT or STI. Nine patients died and 4 were lost to follow-up. Although absolute event rates were low, the incidence of new WHO 4 events/death was increased more than two fold in the STI arm. Interrupting ART also resulted in rapid and significant declines in CD4 cell count. These results meant the trial team were unable to recommend 12 week STIs initiated at a CD4 cell count ≥300 cells/μL.

1.2.2. The ARROW trial

As with adults there is a high level of infrastructure required to carry out routine laboratory monitoring in children. Even though the adult DART trial was well underway there had been no trials to investigate the need for routine laboratory monitoring in HIV infected children on treatment. There were good reasons to believe that the results in children could be different to adults, and that adult results may not be generalisable, because of differences in frequency of ART toxicity, predictive value of CD4s and the clinical spectrum of paediatric HIV and its co-morbidities, particularly in Africa [57]. There was also the urgent need to develop better treatment strategies for children in LMIC, since lower rates of virological suppression had been reported in children compared with adults in the same calendar periods in high-income settings. In particular, it was hypothesised that a 4-drug induction strategy might increase efficacy and durability of first-line treatment. Some small observational studies and one larger national UK study had noted better virological response with 4 drugs compared to 3 [37]. But there had been no randomised trials comparing 4 vs. 3 drugs in children.

The ARROW trial (**A**nti**R**etroviral **R**esearch f**O**r **W**atoto) [57] was an open-label parallel group randomised trial designed to investigate the role of routine laboratory monitoring by comparing efficacy and toxicity in children and adolescents receiving standard ART with clinically driven monitoring (CDM) versus laboratory and clinical monitoring (LCM), similar to DART. The trial included a second factorial randomisation, at ART initiation. It compared a continuous first-line ART three-drug two-class regimen, comprising 2NRTIs+NNRTI with an induction with four drugs (two classes, 3NRTI+NNRTI) followed by maintenance with three drugs. Children were randomised 1:1 to receive either CDM or LCM. In addition children were randomised 1:1:1 in a factorial manner to lamivudine + abacavir + NNRTI continuously; induction maintenance with 4 drug lamivudine + abacavir + NNRTI + zidovudine for 36 weeks, then lamivudine + abacavir + NNRTI; or lamivudine + abacavir + NNRTI + zidovudine for 36 weeks, then lamivudine + abacavir + zidovudine.

Routine blood tests were carried out at screening, randomisation to the trial, weeks 4, 8, 12, and then 12 weekly. As in DART, results for LCM participants were returned to clinicians but not for the CDM participants; haematology/biochemistry test results could be requested for a valid clinical reason (CD4/TLC were never returned). All children received ART as syrups or tablets dosed according to the WHO weight-band tables. Children were reviewed 4-6 weekly by a nurse using a standard symptom checklist. Switch to second-line ART was based on clinical criteria in all participants (new/recurrent WHO4 event, or WHO3 events at clinical discretion,

particularly if recurrent/persistent) and laboratory criteria for patient on LCM (confirmed on ART CD4<15% 1-2 years, <10% 3-4 years and <100 cells/μL ≥5 years). Primary endpoints for the monitoring comparison were progression to a new non-recurrent WHO 4 event/death (efficacy) and grade 3/4 AEs not solely HIV related (safety). For the ART strategy comparison primary endpoints were change in CD4 percentage from randomisation to 72 and to 144 weeks (efficacy) and grade 3/4 AEs (safety). Secondary endpoints for both comparisons included mortality, new WHO4 event/death, new WHO 3/4 event/death and grade 3/4 AEs (for further information see Kekitiinwa et al. 2013) [58].

Table 1.2.3 ARROW Baseline characteristics and follow-up (monitoring strategy)

ARROW	**LCM n (%)**	**CDM n (%)**	**Total n (%)**
Baseline	**600**	**606**	**1206**
Female	302 (50%)	308 (51%)	610 (51%)
Age (years)			
Median (IQR)	6 (3-9)	6 (2-9)	6 (2-9)
CD4 at ART initiation			
Cells/μL median (IQR) if >5 yrs	237 (94-366)	244 (114-369)	243 (104-366)
CD4%, median (IQR)	12 (7-17)	13 (8-17)	12 (7-17)
WHO stage			
1	7 (1%)	13 (2%)	20 (2%)
2	174 (29%)	160 (27%)	334 (28%)
3	337 (56%)	346 (57%)	683 (57%)
4	82 (14%)	87 (14%)	169 (14%)
First-line treatment received (ABC+ lamivudine) plus			
NVP	125 (21%)	129 (21%)	254 (21%)
EFV	72 (12%)	71 (12%)	143 (12%)
ZDV + NVP	248 (41%)	256 (42%)	504 (42%)
ZDV + EFV	155 (26%)	150 (25%)	305 (25%)
Follow-up			
Lost to follow-up	13 (2%)	20 (3%)	33 (3%)
Treatment status at end of trial			
Still on first-line treatment	565 (94%)	577 (95%)	1142 (95%)
Switched to second-line	35 (6%)	28 (5%)	63 (5%)
Median follow-up (years)	4.0 (3.7-4.4)	4.0 (3.7-4.4)	4.0 (3.7-4.4)
New WHO 4 event/death	39 (7%)	47 (8%)	86 (7%)
Death	29 (5%)	25 (4%)	54 (4%)
Comparison CDM vs. LCM [HR (95%CI)]			
New WHO 4 event/death	1.13 (0.73-1.73)		p=0.59
Death	0.80 (0.49-1.44)		p=0.45

This cohort consists of 1206 HIV infected children from three centres in Uganda and one in Zimbabwe. Median follow-up was 4 years (total 4685 children-years (CYs)). For new WHO4 events/death the upper 95% confidence interval for the absolute difference (+0.32/100CY [95%CI -0.47,1.12]) lay below the pre-specified non-inferiority margin of +1.6 suggesting that overall non-inferiority has been demonstrated. However, from the second year, there were small but significant clinical event excesses in CDM, with CD4 monitoring providing clinical benefit; nevertheless, the 95% CI for the difference in event rates after one year still lay just within the non-inferiority margin, still demonstrating non-inferiority (+0.99 (95% CI 0.37-1.60)). Event rates were very low (substantially lower than in DART) and long-term survival high, suggesting ART

roll-out should take priority over laboratory monitoring. All trial results can be found in the ARROW trial publication [58].

Table 1.2.4 ARROW Baseline characteristics and follow-up (first-line ART strategy)

ARROW	Arm A	Arm B	Arm C
Baseline	**397**	**404**	**405**
Female	204 (51%)	197 (49%)	209 (52%)
Age (years)			
Median (IQR)	6 (2-9)	6 (3-9)	6 (2-9)
CD4 at ART initiation			
Cells/μL median (IQR)	221 (87-341)	242 (108,369)	262 (114, 373)
CD4%, median (IQR)	12 (7-18)	12 (7-17)	12 (8-17)
WHO stage			
1	4 (1%)	7 (2%)	9 (2%)
2	119 (30%)	116 (29%)	99 (24%)
3	217 (55%)	228 (56%)	238 (59%)
4	57 (14%)	53 (13%)	59 (15%)
First-line treatment received (ABC+ lamivudine) plus			
NVP	253 (64%)	1 (<1%)	0 (0%)
EFV	143 (36%)	0 (0%)	0 (0%)
ZDV + NVP	0 (0%)	154 (38%)	151 (37%)
ZDV + EFV	1 (<1%)	249 (62%)	254 (63%)

There was no significant difference between the three first-line strategy arms for mean CD4 percentage change at week 72 (p=0.33) or 144 (p=0.69). However, at week 36 (when all children moved to 3-drugs), CD4% responses were significantly greater in the 4-drug induction arms (p<0.0001).

1.3. Aims of the thesis

The main aim of this thesis was to address some of the outstanding questions in the treatment of HIV-infected adults and children in LMIC. The DART and ARROW trials as described in sections 1.2.1 and 1.2.2 had just under 15000 person years and 5000 children years of follow-up on individuals starting ART in Africa respectively. Apart from the randomised comparisons in these trials, the epidemiological potential of these datasets is immense given the substantial resources that were employed to ensure high completeness of visits and extensive data collection. Some work has already been done on the DART dataset in particular, however many important clinical and epidemiological questions still remain. I aim to address the following questions in this thesis.

1.3.1. Modelling causes of death

The aim is to investigate further the patterns of on-ART mortality in the DART and ARROW trials, overall and by specific causes of death in relation to time on ART; both across and within each of the CD4 monitoring groups separately. Using different modelling techniques I aim to assess the change in hazard of death over time on ART for specific causes of death. Whilst patterns of mortality in the first year after initiating ART and early causes of death have been previously described in both of these cohorts [38], no analyses of long-term mortality have been

carried out to date. As patients survive longer on ART and the number of patients on ART increases, understanding causes of long-term mortality may provide indications of further interventions that could be used to prevent these deaths in patients receiving ART. Full analysis results can be found in Chapter 2.

1.3.2. Structured and unstructured treatment interruptions

Preliminary simple analysis comparing the DART STI/CT randomised groups long-term suggests that the STI group is likely to remain at increased risk of death many years after undertaking STIs as a consequence of persistent deficits in long-term CD4. I aim to assess the effects of planned and unplanned treatment interruptions on clinical and immunological outcomes using the DART and ARROW data sets. This is relevant for current patients in ART programmes where stockouts are common. Full analysis results can be found in Chapter 3.

Chapter 2: Cause-Specific Mortality in DART and ARROW

(Work for this Chapter was done, and this Chapter was mainly written in 2012-2013. Based on comments I received during my upgrade, the literature search was updated in 2014.)

2.1. Introduction

2.1.1. Background

HIV-related illness is currently the leading cause of death in sub-Saharan Africa [59, 60]. In 2013 there were an estimated 1.2 million AIDS related deaths among adults and children in sub-Saharan Africa [61]. Antiretroviral therapy (ART) has been shown to improve immune function and decrease the risk of opportunistic infections (OI) and death [62, 63] and mortality rates have declined significantly over the last decade as the number of people on ART in sub-Saharan Africa has increased from 50,000 in 2001 to ~1.2 million in 2006 and to ~7.0 million in 2012 [64]. In low/middle-income countries mortality after starting ART is high. Between 8 and 26% of patients receiving ART in sub-Saharan Africa die within the first year of treatment with most deaths occurring in the first few months [65]. Similarly to adults, high early mortality has also been reported in children [66-69].

2.1.2. Literature search

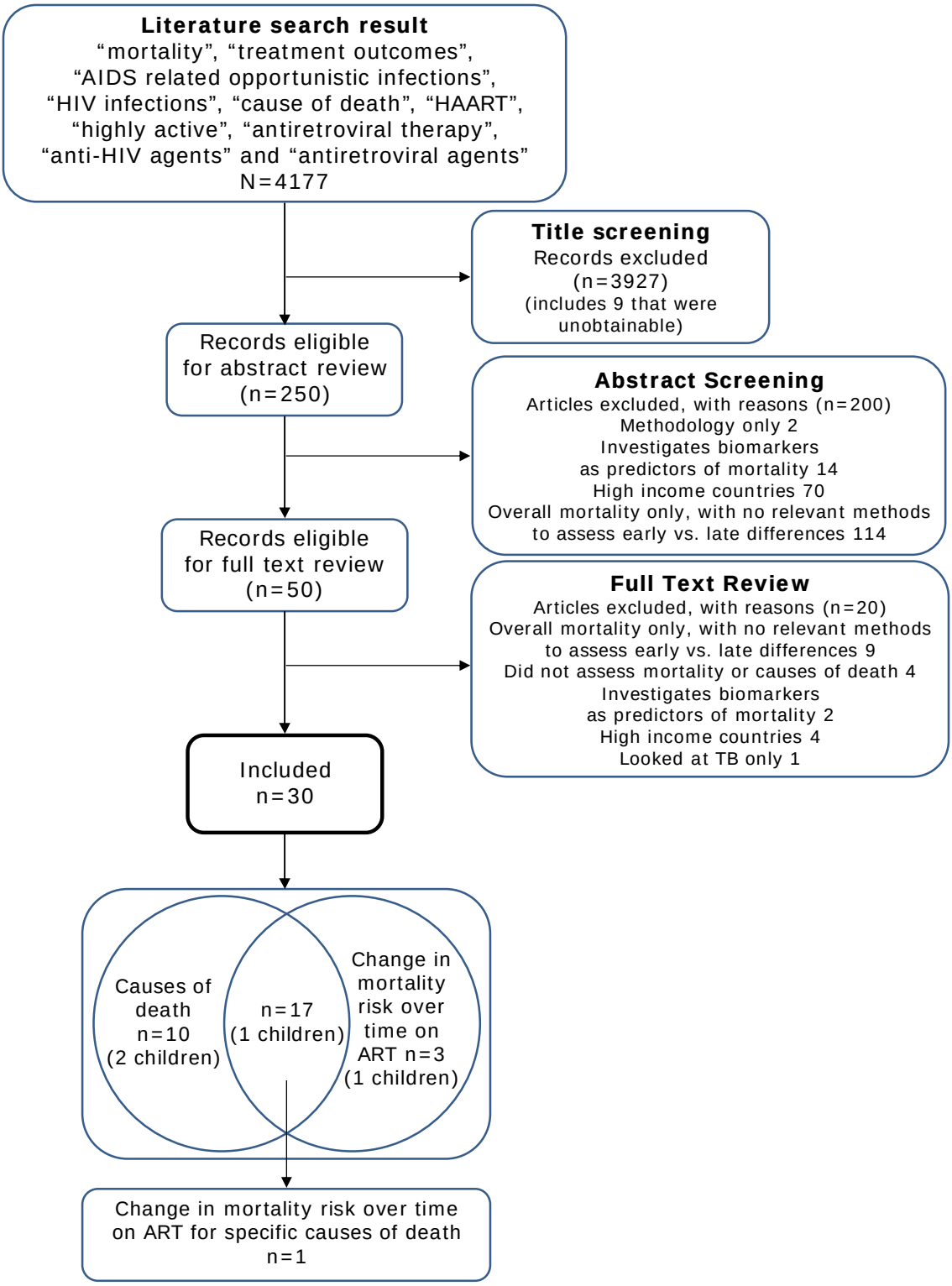
A search of the literature was carried out in Medline for articles published between January 2002 (when ART rollout to low/middle-income countries started) and July 2014. The aim of this literature search was to find articles reporting on the cause of death by time on ART in low/middle-income countries. Keywords used for the search included “mortality”, “treatment outcomes”, “AIDS related opportunistic infections”, “HIV infections”, “cause of death”, “HAART”, “highly active”, “antiretroviral therapy”, “anti-HIV agents” and “antiretroviral agents”. A total of 4177 potentially relevant citations were identified. Titles and abstracts were screened to capture potentially relevant studies (Figure 2.1.1). Studies were selected for full text review if they met the following criteria:

i) included data from low/middle-income countries (including this in the search strategy was too restrictive on the search excluding many relevant papers);

ii) mentioned individual causes of death in the abstract and not just overall mortality.

Fifty studies received full text review, of which 30 met my inclusion criteria (10 included cause of death only (2 paediatric); 3 included change in mortality over time only (1 paediatric) and 17 included both causes of death and change in mortality over time (1 paediatric)) (Figure 2.1.1). Of the 30 articles included, 86% were from sub-Saharan Africa, 10% were multi-regional and 4% were from the Americas (South and Central America).

Figure 2.1.1 Flow diagram of inclusion and exclusion for literature search



Below I summarise the findings of the 30 papers included from my search. I start with those studies describing both causes of death and change in mortality over time (n=17).

Amuron et al. [70] investigated mortality in 1453 patients initiating ART in an AIDS support clinic in Uganda. Overall there were 197 deaths and follow-up was for 42 months. They reported higher mortality rates in the first year (12/100 person-years (PYs)) compared to thereafter (2/100PYs) (154 patients (78% of all deaths) died in the first year). Low CD4 at baseline, being male, WHO stage 3/4 disease, lower weight and not taking cotrimoxazole prophylaxis were all independently associated with increased mortality and the most common causes of death were tuberculosis (TB), diarrhoea and cryptococcal meningitis. They concluded that earlier ART initiation is required to prevent these early deaths that occur in the first year of ART.

Etard et al. [71] evaluated survival and investigated causes of death among 404 HIV infected adults in Senegal. Overall there were 93 deaths and median follow-up time was 46 months. Overall incidence of death was 6/100PYs with a slightly higher mortality rate in the first year of 7/100PYs (47 patients (51% of all deaths) died in the first year), decreasing over time. BMI, haemoglobin level and CD4 at baseline were predictors of survival with the most common cause of death being mycobacterial infections, neurological disorders and septicaemia. Like Amuron et al. they conclude that earlier initiation of ART could reduce early mortality.

Grinsztejn et al. [72] analysed a cohort of data from 915 patients in Brazil (Rio de Janeiro) and 859 patients in the US and made a comparison between the two. Outcome was early mortality (death occurring during the first year after initiating HAART). Mortality rates and causes of death were investigated according to specific periods (first 30 days, 31-90 days, 91-180 days, 181-360 days) and the effect of CD4 count on the risk and cause of death was assessed in a Cox proportional hazards model. They did however only assess the first year. They found higher mortality rates (14/100PYs) in the first 30 days on ART in Rio de Janeiro with decreasing mortality rate up to the end of the first year (64% of deaths occurred in the first 90 days of HAART initiation). This decreasing trend was not seen in the US where rates were low throughout (47% of deaths were from days 180-365 in the US). The most common causes of death in Rio de Janeiro were AIDS-defining illnesses, TB, cryptococcus and Kaposi's sarcoma (KS). In the US the most common cause of death was non-AIDS defining illnesses; there were no deaths from TB in the US. They concluded that in both countries there is a need for earlier initiation of ART to reduce early mortality.

Karstaedt et al. [73] described causes of death in 2943 patients in a single-centre cohort in Soweto, South Africa. Causes of death were described according to time on ART and in particular, for deaths beyond the first year. The most common causes of death were acute sepsis, TB and *Mycobaterium avium* complex (MAC) bacteraemia. Overall there were 305 deaths. Median follow-up was 2.7 years. They showed that the percentage of deaths decreased over time from 165/305 (54%) in the first 3 months on ART to only 6/305 (2%) after 3 years on ART. They found the same causes of death to be common in the first twelve months and thereafter.

Kouanda et al. [74] investigated causes of death and factors associated with mortality in a cohort of 5508 patients in Burkina Faso initiating HAART. They used Kaplan-Meier methods to explore potential predictors of death and two Cox models to estimate hazard ratios (HRs) for death (one for baseline and one for time-dependent covariates). Median duration of follow-up was 23 months and by the end of follow-up 690 (13% of all patients) had died. Overall incidence of death was 6/100PYs which reduced over time with the highest rate reported at 6 months of 18/100PYs. Median time to death was 2.8 months and the most common causes of death were wasting, TB and anaemia.

In 2005, Lawn et al. [62] described rates, risk factors and causes of death among 712 patients accessing community based ART in Cape Town, South Africa. There were 68 deaths during 563 PYs of follow-up and 44 deaths (65% of all deaths) in the first 90 days of ART initiation. They showed decreasing mortality rates over time with a rate of 18/100PYs in the first month after ART initiation decreasing to just 3/100PYs at 6-9 months after ART initiation. Causes of death were wasting, TB, acute bacterial infections, malignancy and immune constitution disease. Similarly to previous studies they concluded that treatment should be started earlier and certainly for patients with WHO stage 3/4 disease.

In 2009, Lawn et al. [75] described mortality rates in the first 4 years of ART in 2423 patients accessing community based ART in Cape Town, South Africa. There were 197 deaths over 3155 PYs of follow-up. Mortality was highest in the first month on ART (27 deaths/100PYs). They also showed that risk was high for months 0-4 (16/100PYs) but then rapidly decreased after that to just 4/100PYs for months 8-12. Death rates were as little as 0.4/100PYs in the 4^{th} year of ART. They also assessed the association between CD4 cell count and mortality and showed decreasing mortality rate with higher current CD4 count. The most common causes of death were TB, acute sepsis and cryptococcal meningitis.

MacPherson et al. [76] considered a retrospective cohort of 1353 patients initiating HAART in a region of South Africa and assessed mortality rates and potential predictors of death. There was 966PYs of follow-up and at 24 months 124 (9% of all patients) had died. Median time to death was 57 days in those who died. The overall mortality rate was 13/100PYs. The mortality rate up to 6 months was 20/100PYs declining to just 3/100PYs between 12 and 24 months. The most common causes of death were TB and diarrhoea.

Marazzi et al. [77] assessed excessive early mortality in the first year of ART in 3456 patients initiating ART in centres in Mozambique, Tanzania and Malawi. Two hundred and sixty deaths were recorded in the first year of initiating ART and the overall mortality rate for the first year of ART was 10/100PYs. The highest mortality rate was seen in the first 3 months of treatment at 16/100PYs (137 (53% of all deaths) occurred in the first 3 months) and the mortality rate declined to just 1/100PYs from 9-12 months. BMI, haemoglobin levels and viral load (VL) were independent predictors of mortality. The main causes of death were malaria, anaemia and TB.

Moh et al. [78] assessed the incidence and determinants of mortality following early ART in 792 patients in West Africa. Median follow-up was 8 months and 9 (1%) patients were lost to follow-up. There were a total of 18 (2%) deaths. The most common cause of death was TB, followed by cerebral toxoplasmosis, KS, bacteraemia, malaria, cardiac insufficiency and trauma. They found the incidence of mortality was greatest in those patient with pre ART CD4<200 cells/μL and found no deaths in those with pre-ART CD4≥350 cells/μL. They suggested their data supported the need for ART to be started before a patients CD4 drops to below 350 cells/μL. Patients with severe morbidity had a higher risk of death with the most frequent morbidity being TB.

Moore et al. [79] examined data from 1132 patients initiating ART in an AIDS care project in Uganda to describe mortality over time and determine clinical predictors of death. Overall there were 112 deaths over a median of 3 years of ART. Mortality was 16/100PYs in the first 3 months after ART initiation and decreased over time to just 0.3/100PYs after 24 months. They reported TB to be the most common cause of death followed by candida disease. They found an association between deaths in the first 3 months and WHO 3/4 disease stage, TB at baseline, BMI and non-TB opportunistic infection (OI); after 3 months there were associations with time-updated CD4<200 cells/μL, adherence <95%, non-TB OI and low time-updated haemoglobin. Early treatment was considered the key to survival along with good adherence.

Mzileni et al. [80] described mortality trends and causes of death among 2605 HIV-infected patients in the HAART era in South Africa between July 2004 and December 2006. They found mortality to be higher in males as well as for those with CD4<200 cells/μL at baseline. Mortality rate was highest in those with CD4<50 cells/μL. There were a total of 205 deaths, 95% of which occurred in the first year of ART. Mortality rates significantly decreased over time to just 2% between 19-30 months. The main causes of death were TB, diarrhoea, cryptococcus meningitis and pneumonia.

Woradria et al. [81] looked at TB-HIV coinfection and its effects on mortality in 302 TB-HIV co-infected patients enrolled in ART programs in Uganda. Total study follow-up was 331PYs. There were 53 deaths in total. Thirty-six (68% of all deaths) were in the first 6 months of ART initiation, 23 (43%) in the first 3 months (of which 12/23 (52%) died before being able to start ART). Overall mortality was 16/100PYs. During the first 3 months the mortality rate was 34/100PYs, reducing to just 6/100PYs after 9 months on ART. The most common causes of death were gastroenteritis, severe anaemia and KS.

Wamalwa et al. [69] conducted a study investigating both causes of death and mortality rates over time in 135 children in Kenya. Median follow-up was 21 months and 11 (8%) children were lost to follow-up. There were a total of 20 deaths, 18 (90%) occurring in the first 4 months after ART initiation. Overall mortality was 8/100 child years (CYs). In the first 4 months the rate was 46/100CYs and dropped to just 1/100CYs from 4 months to two years. Low haemoglobin was an independent risk factor for mortality. The most common cause of death was pneumonia, along with TB, diarrhoea, non-Hodgkin's lymphoma and anaemia. They suggested that children who present with severe wasting and anaemia should be prioritised for ART treatment.

Zachariah et al. [82] assessed risk factors for high early mortality in 1507 patients initiating ART in Malawi. There were a total of 190 deaths on ART. Sixty-one percent (n=116) of deaths occurred in the first 3 months of ART initiation and 79% (n=150) during the first 6 months. They found significant risk factors for mortality to be stage WHO 3/4 disease, pre-ART CD4<50 cells/μL and increasing grades of malnutrition. They concluded that BMI and clinical staging were important in highlighting who are still at very high risk despite ART. The most common causes of death were oral recurrent candida, KS, bacterial pneumonia and TB.

In addition Kowalska et al. [83] assessed changing mortality overtime for specific causes of death in middle-income countries in Europe, Israel and Argentina. They analysed a cohort of 12,069 patients and looked at the crude incidence rate of death due to specific causes. A total of 1297 patients died during a median follow-up time of 5.4 years. They assessed all cause, AIDS-related (884, 68%) and non-AIDS related deaths (413, 32%) by time on ART (<2, 2-3.99, ≥4 years). They also assessed changing mortality over time for the following more specific categories of causes of death: non-AIDS related infection (12, 9%), liver related (LR) (182, 14%), non-AIDS defining malignancies (125, 10%), cardiovascular disease (CVD) (122, 9%) and deaths due to violence (90, 7%). The overall mortality rate was 2/100PYs. They found that the incidence rate of death was highest in the first year on ART (3/100PYs) and decreased with more time on ART for all cause (~1/100PYs) and AIDS related deaths, non-AIDS related infection deaths, LR deaths and violent deaths, however, the rates of non-AIDS related deaths, deaths from non-AIDS defining malignancies and cardiovascular deaths remained fairly constant over time on ART.

The following ten studies described causes of death only, without considering time on ART. Barlow et al [66] assessed the effectiveness of generic adult fixed-dose combination ART (tablet) on 104 children affected with HIV in Uganda. Median follow-up was 96 weeks and no children were lost to follow-up. There were a total of 6/104 (6%) deaths, of which 4/6 (67%) were in the first 4 months of initiating ART. The other two deaths occurred at 44 and 56 weeks respectively. Causes of death included toxoplasmosis, malaria, pneumonia, renal failure and severe diarrhoea with dehydration.

Bourgeois et al. [84] assessed the effectiveness of generic antiretroviral drugs in 109 adult patients from Cameroon. They assessed survival and virological and immunological response and also assessed causes of death. The median follow-up was 16 months; there were 3 patients lost to follow-up and a total of 9 deaths, four (44%) of which occurred in the first 6 months of ART initiation. They found that the most common cause of death was HIV disease followed by TB with other deaths being due to poor general health, hepatitis and pancreatic disease.

Bussmann et al. [85] assessed the long term clinical outcomes of 633 patients receiving ART in Botswana. The median follow-up was 42 months and 126 (20%) patients died. The most common causes of death were wasting syndrome, TB and advanced AIDS.

Chihana et al. [86] assessed mortality and causes of death in 17,373 patients in rural northern Malawi. From September 2004 to August 2009 there were 905 deaths. The most common cause of death was AIDS/HIV, followed by gastrointestinal, cardiovascular disease and cancer.

Coetzee et al. [87] assessed outcomes in 287 patients up to 2 years after ART initiation in Khayelitsha, South Africa. One patient was excluded and one was lost to follow-up. Median follow-up was 14 months and 6 had stopped ART. There were a total of 38 deaths and the most common cause of death was TB followed by KS.

Cox et al. [88] carried out an autopsy study describing causes of death in 46 patients in Uganda. They made comparisons between 35 HIV positive (10 of whom were on ART) and 11 HIV negative patients and found the causes of death to be different; in HIV positive patients the main cause of death was TB and the leading cause of death in HIV negative patients was non-infectious disease which included liver failure, cardiac failure and pulmonary thromboembolism.

Fassinoua et al. [67] assessed the effect of ART in 78 children in the Côte d'Ivoire. Total follow-up for the 78 children was 1927 children-months. There were only 9 deaths (5 (56%) of which occurred in the first 3 months of ART initiation). The most common cause of death was lower respiratory infections.

Karcher et al. [89] evaluated risk factors for treatment denial and loss to follow-up in 159 patients in western Kenya. Median time on treatment was 9 months. They considered death to be a reason for loss to follow-up and reported causes of death in 11 of the total 15 deaths. TB was the most common cause of death followed by cryptococcal meningitis and pneumonia. They reported an overall mortality rate of 19/100PYs.

Laurent et al. [90] compared the tolerability and effectiveness of two major first-line ART regimens in 169 HIV-1 infected patients in Cameroon. Follow-up was for around 24 months. They reported an overall mortality rate of 11/100PYs and there were 19 (11%) deaths. Cause of death was mainly attributed to advanced HIV, followed by poor general health, TB and wasting.

Wester et al. [91] assessed the initial response in 153 ART naïve patients initiating HAART in Botswana. Median follow-up was 96 weeks and there were 24 (16%) deaths. The most common causes of death were advanced HIV and TB, followed by KS, liver disease, wasting and cryptococcal meningitis.

The following three studies assessed change in mortality over time only. Bong et al [68] assessed risk factors for early mortality in 439 children in a hospital in Malawi. There were a total of 49 (11%) deaths. They found that 71% (35/49) of deaths occurred in the first 3 months and 89% (44/49) in the first 6 months of ART. 38 children were lost to follow-up. Children who were stage WHO 3/4 at baseline had severe wasting or CD4 cell count below the threshold for severe immunodeficiency (CD4<200 cells/μL) had a significantly greater risk of early death at 3 or 6 months. They recommended scaling up prevention of mother-to-child transmission (PMTCT) to prevent children becoming infected with HIV.

Braitstein et al. [92] made a comparison between low (N=4810) and high (N=22,217) income countries, of mortality in HIV infected patients in the first year of ART. There were cohorts in Africa, Asia and South America and Europe and North America. There were lower CD4 counts for those starting ART in low-income countries compared to high income countries (N=22217). There was 2236PYs and 20,532PYs of follow-up in low and high income countries respectively; loss to follow up was 15% and 5%. Mortality was higher in low-income countries compared to high income countries, with a total of 124 (3%) and 414 (2%) deaths respectively; 97/124 (78%) and 255/414 (62%) deaths occurred in the first 6 months on ART. The difference between high and low-income countries in terms of mortality changed over time, with the hazard ratio (HR) of low vs. high going from 4.3 (95% CI 1.6-11.8) in the first month to just 1.5 (0.7-3.0) in months 7-12. They suggested that earlier diagnosis and initiation of treatment in low-income countries would help reduce this difference and reduce the high early mortality in both settings.

Hoffmann et al. [93] assessed data from a cohort study of 16,356 patients taking ART in South Africa. They included 15,060 patients with a median follow-up time of 1.8 years. They found that mortality was highest early during ART with a mortality rate of 34/100PYs in the first 3 months after ART initiation. The mortality rate declined to just 3/100PYs in years 2-4 after ART initiation.

Gupta et al. [94] carried out a systematic review of observational studies of early mortality post-ART initiation in low- and middle-income countries in Asia, Africa, and Central and South America. This systematic review includes several studies from my search and in total they included 50 studies (76% from sub-Saharan Africa) where 14 studies reported cause specific mortality. They found the most common causes of death to be TB, wasting, advanced HIV and chronic diarrhoea. Median follow-up time ranged from 3-55 months and sub-Saharan Africa had the highest probability of 12 month mortality of 0.17.

In summary, I found no studies looked at changes in individual causes of death over timescales covering the first few years on ART in low-income countries. Only one study looked at change for specific categories of death (non-AIDS vs AIDS related). Those studies that did investigate change in mortality all found a decrease in mortality over time on ART with the highest mortality rates being in the first 3-6 months on ART. Common causes of death across these studies were TB, other infections (including diarrhoea, gastroenteritis, pneumonia and septicaemia), KS and anaemia.

2.1.3. Data available for analysis

Data are available from the Medical Research Council Clinical Trials Unit (MRC CTU) DART trial [54]. In DART patients with CD4 count <200 cells/μL were randomly assigned to either laboratory and clinical monitoring (LCM) or clinically driven monitoring (CDM). The aim was to investigate whether delivery of ART with or without routine monitoring of CD4 counts led to similar outcomes in terms of efficacy and with or without routine haematology/biochemistry tests led to similar outcomes in terms of toxicity. In all participants 12 weekly CD4 counts (for efficacy) and 12 weekly full blood counts/haematology and biochemistry (for toxicity) were

performed. For LCM participants test results were returned to treating clinicians and for CDM participants all tests were carried out but were not returned. The DART trial showed that CDM was inferior to LCM (HR 1.35 95%CI 1.10-1.65, p=0.004) for mortality, but there was no difference between the groups in any adverse event outcome. Because of this, the aim of this chapter is to investigate further patterns of mortality overall and by specific causes of death in relation to time on ART; both across and within each of the CD4 monitoring groups separately. Using different modelling techniques I aim to assess the change in hazard of death over time for specific causes of death without having to make any arbitrary classifications of time upfront as done in the papers reviewed in section 2.1.2. Whilst patterns of mortality in the first year after initiating ART and early causes of death (COD) have been previously described in this cohort [95], no analyses of long-term morbidity have been undertaken to date. As patients survive longer on ART and the number of patients on ART increases, understanding causes of long-term mortality may provide indications of further interventions that could be used to prevent these early deaths. Therefore in this chapter I investigate the different methods that can be used to model this change in mortality risk.

2.2. Methods

2.2.1. Objectives

The primary outcome of interest in this chapter is mortality, firstly overall mortality, secondly cause-specific mortality. I am specifically interested in the effect of time on ART (antiretroviral therapy) and the effect of monitoring strategy in the DART trial (LCM: Laboratory and clinical monitoring with 12 weekly CD4 counts monitored by physicians, CDM: clinically driven monitoring, CD4 counts are performed 12 weekly but not given to the physicians and are therefore not used for patient management). Time on ART has been calculated as time from randomisation (enrolment and ART initiation) to either death or 31st December 2008 (end of follow-up under the randomised strategy) whichever occurred first. Any patients who died after 31st December 2008 have been censored on this date (i.e. not included as a death).

Deaths were reported using the death case report form. All deaths were reviewed by an Independent Endpoint Review Committee (ERC) to assign cause and relationship to drug. This was done on the basis of a structured clinical narrative written by the clinician managing the patients. The ERC consisted of an Independent Chair, project leaders from each site and two other independent clinicians. No member reviewed deaths (endpoints) from their own site. As well as determining the cause, the ERC classified all deaths by whether they were definitely/probably related, uncertainly related or unlikely to be related to any drug including antiretrovirals and concomitant medications. All reviews were done blind to the CD4 monitoring randomisation and CD4 count. Where cause of death was considered to depend on the CD4 count, the ERC provided two adjudications e.g. “cause=A if CD4<X, otherwise if CD4≥X, cause = B”, and the unblinded trial manager administrating the ERC then input the appropriate adjudication into the database.

2.2.2. Methods

There are several methods that can be used to analyse survival data. Here I will consider just three main approaches. They are Poisson Regression, Cox Regression and Flexible Parametric Models (FPMs). All of these methods estimate rate or hazard ratios (HRs) to measure how much a covariate increases or decreases the mortality risk. These methods are outlined along with their regression models, advantages, disadvantages and what they have been used for in this chapter in Table 2.2.1.

Table 2.2.1 Different methods proposed for modelling the effect of covariates on time-to-event data/hazards

	Poisson [96]	**Cox** [97]	**Flexible parametric models (FPM)** [98]
Regression formula	$h_{ij}(t \mid \mathbf{x}_{ij}) = \lambda_j \exp(\mathbf{x}_{ij}\boldsymbol{\beta})$ where *i* is the subject, *j* is the interval and λ_j is the baseline hazard rate in the interval *j*	$h_i(t \mid \mathbf{x}_i) = h_0(t)\exp(\mathbf{x}_i\boldsymbol{\beta})$ where *i* is the subject $h_0(t)$ = baseline hazard	Weibull hazard function: $h_i(t \mid x_i) = \lambda_i \gamma\, t^{\gamma-1}$ with $\lambda_i = \exp(\alpha + \mathbf{x}_i\boldsymbol{\beta})$ $H(t \mid x_i) = \int_0^t h_i(u \mid x_i)du = \lambda_i t^{\gamma}$ $\ln(H(t \mid x_i)) = \alpha + \mathbf{x}_i\boldsymbol{\beta} + \gamma \ln(t)$ Generalised using spline functions $z_1(.), z_2(.) etc$: $\ln H(t \mid x_i) = s(\ln t;\gamma) = \gamma_0 + \gamma_1 \ln t + \gamma_2 z_1(\ln t) + \gamma_3 z_2(\ln t) + \ldots\ldots + \mathbf{x}_i\boldsymbol{\beta}$
Method	• Uses a piecewise exponential model to split time into several intervals allowing the hazard to change over time, where the hazard follows an exponential model within each time interval	• Full likelihood is decomposed into a term containing only functions of the baseline hazard and a term containing only functions of covariates X_i and ß. • The latter is called the partial likelihood and is maximised to provide a semi-parametric estimate of ß	• Extension of the parametric Weibull model in which log cumulative hazard increases linearly with log time • Uses restricted cubic splines made up of cubic polynomial segments which are joined at values known as knots to allow non-linear variation in log time
Assumptions	• Hazard rate is constant within each chosen time interval, $h_0(t) = \lambda_j$ (where *j* is the interval)	• Hazard rate is not directly estimated but it is treated as a sum of probability elements at the times that events have occurred enabling it to vary freely with the data	• Hazard varies over time but has a parametric form
Advantages	• Allows some flexibility in the shape of the baseline hazard (e.g. compared to simple exponential or Weibull) • Can be a good approximation to the Cox model depending on how much the hazard is changing • Allows rates to be estimated and easily compared over time and by covariates	• Considered standard method for analysing survival data because it does not make any assumptions about the shape of the underlying hazard	• Does not require the time scale to be split a priori in order to flexibly model the hazard • Introduces flexibility into the survival functions in a way that can be modelled directly (parametric) • Interpretation of parameters in a proportional hazards FPM is exactly that of the Cox model (but can also use log normal or proportional odds) • Enables easier investigation of the hazard function over time and by covariates

	Poisson [96]	**Cox** [97]	**Flexible parametric models (FPM)** [98]
Dis-advantages	• Could miss changes in the hazard if split too few times. • If split too often then there would be too many model parameters • Could be considered biologically implausible as risk does not change abruptly when moving from one interval to the next	• Does not provide smoothly varying estimate of the hazard and so it is difficult to visualise exactly how the risk is changing	• Choosing an appropriate degree of complexity for the time varying model can be arbitrary and is not straight forward • Unable to fit some models due to low event numbers when comparing factors* • Models may be sensitive to exactly when the first events occurred*
Used in this chapter to:	• Compare cause-specific mortality rates between 0-1 and >1 years on ART for LCM and CDM • Calculate cause-specific rates and their 95% CIs	• Compare cause-specific hazard ratios between CD4 monitoring vs. no CD4 monitoring for 0-≤1 year and >1 years on ART • Calculate cause-specific hazard ratios and their 95% CIs	• Predict hazard functions over time for overall mortality and for individual causes of death • Predict hazard differences between CD4 monitoring strategies over time

*Findings discovered whilst using this method with my data

2.2.3. Comparison of randomised groups using cause-specific Poisson regression models

Using a piecewise exponential or Poisson model, the time at risk can be split into several intervals, and the constant underlying baseline hazard λ_{ij} modelled in each interval:

$$h_{ij}(t \mid \mathbf{x}_i) = \lambda_j \exp(\mathbf{x}_{ij}\boldsymbol{\beta})$$

This model can then be fitted using Poisson regression as follows:

$$d_{ij} \sim Poisson(\mu_{ij})$$

$$\ln(\mu_{ij}) = \ln(y_{ij}) + \alpha_j + \mathbf{x}_i\boldsymbol{\beta}$$

Where d_{ij} is the indicator for whether or not the *i*th patient has died in interval *j* and μ_{ij} is the mean of the Poisson distribution. Modelling is now on the log scale so the time at risk for the *ith* subject in interval *j*, y_{ij} is incorporated as an offset $\ln(y_{ij})$ and $\alpha_j = \ln(\lambda_j)$.

Using the Poisson model with split times may be a good approximation to the Cox model, depending on how much the hazard is changing. Splitting the time scale into several intervals allows some flexibility in the shape of the baseline hazard function, but it is still discontinuous and does not vary smoothly. In this analysis I have split time at 1 year, based on the observation that approximately half (47%) of the deaths occurred in the first year on ART and because this is the time point which WHO use to define failure of first-line treatment [99]. I will estimate a constant baseline mortality rate within the first year and a different but constant rate thereafter. I can then directly test whether there is a difference in the hazard in the two time intervals and whether these differences also vary across the different CD4 monitoring strategies. Using the Poisson regression model as above, I fit both CD4 monitoring strategy and

time period along with their interaction; I am then able to estimate the incidence rate ratio (IRR) for early (0-≤1 year) vs. late (>1 years) deaths in the CD4 monitoring group and similarly the IRR in the no CD4 monitoring group.

2.2.4. Flexible parametric models (Royston-Parmar models)

When using exponential piecewise Poisson regression models, it is required that the data are split into time intervals over which the hazard is considered constant. If split too often there would be too many parameters in the model; not only can this make analysis time very long, but including such a large number of dummy variables results in a noisy estimate of the baseline hazard function. If split too few times a change in hazard rate could be missed and therefore a model fitted which poorly reflects the data, which may then impact on the estimates of β. Further, these splitting times are completely arbitrary, as shown by the different thresholds used in the studies identified in my literature review (variably 1 month, 3 months, 6 months or a year). For example, in the above, it may be unrealistic to assume the hazard rate changes abruptly at 1 year. Smooth functions of the hazard using either splines or fractional polynomials (FPs) should better model how the hazard is changing over time. But there is still the limitation of where to put the knots and how to choose the FPs.

One spline based method is that of Royston-Parmar, also known as flexible parametric models (FPMs) [98, 100]. FPMs are a parametric approach to survival analysis, but they introduce far more flexibility into the survival/hazard functions than standard parametric survival models such as Weibull or log-normal. The hazard function is not discontinuous at various points in time as in the piecewise exponential models, and it is possible to obtain continually varying hazard predictions and confidence intervals (CIs); however, one still has to choose specific points in time for the spline knots. Below I use the overall survival data from the DART trial to summarise the theory behind these models and show how they are simply an extension of the parametric Weibull model, which itself is an extension of the exponential model for survival data.

The cumulative hazard function for the Weibull model is as follows:

$$H(t) = \lambda t^{\gamma_1}$$

for some $\gamma_1 > 0$, where γ_1 is a shape parameter. The Weibull hazard function:

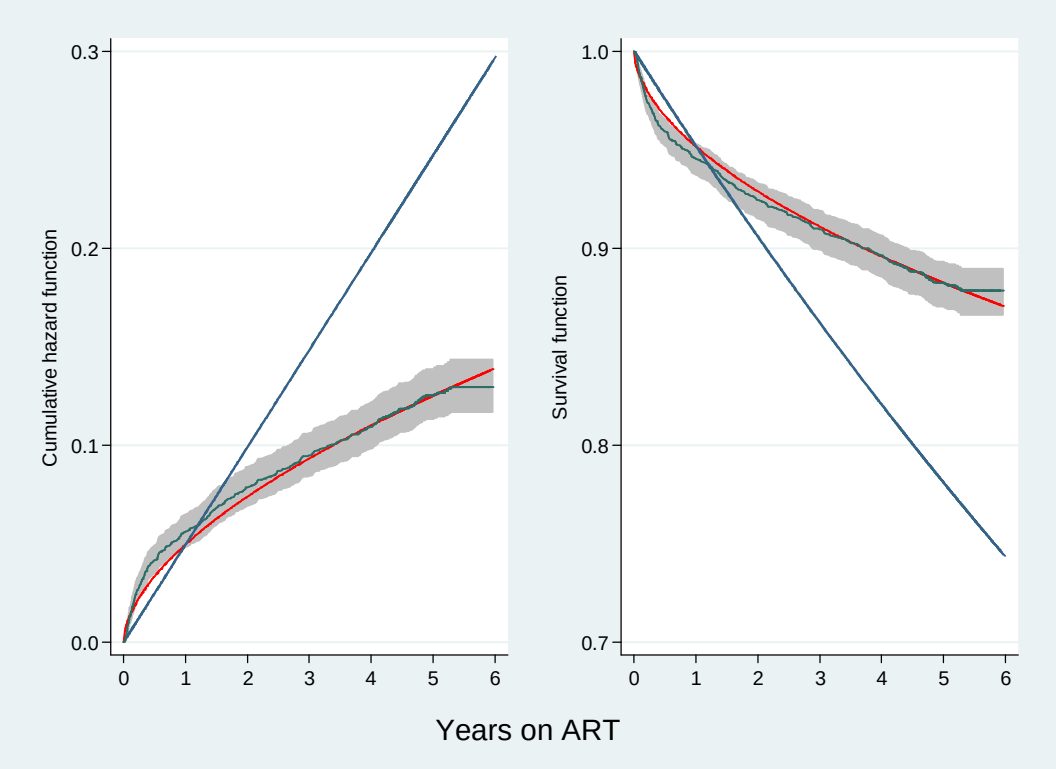
$$h(t) = dH(t)/dt = \lambda\gamma_1 t^{\gamma_1 - 1}$$

is constant when γ_1=1 (simple exponential), monotonic increasing when γ_1>1 and monotonic decreasing when γ_1<1. Effects of co-factors are estimated as $\lambda_i = \exp(\gamma_0 + \mathbf{x}_i\boldsymbol{\beta})$.

In DART, for mortality, γ_1=0.58 (95% CI 0.52-0.64) suggesting strong evidence that the Weibull model may be a better fit than the simple exponential model (γ_1=1). Comparing the Nelson-Aalen (NA) non-parametric estimator of the cumulative hazard function as in Figure 2.2.1 (left panel) and the 95% CI compared with the estimate from the Weibull model over time (red line)

and the exponential model (straight blue line), and a similar comparison for the estimates of the survival function (right panel) shows that the Weibull model is indeed a better fit than the simple exponential model. However, the Weibull parametric model makes very strong assumptions about how the hazard is changing over time. Figure 2.2.1 shows that the Weibull model lies just on the edge of the 95% CI for the NA estimate during the first 2 years suggesting that it is potentially not modelling the early hazard as well as it could. Investigating the hazards for each of the models in Figure 2.2.1 confirms that there are quite large differences in the way these models estimate the change in hazard over time. In particular, the Weibull model estimates an extremely high initial hazard (red line above Nelson-Aalen estimate in Figure 2.2.1 below), which then abruptly drops to a much lower and realistic level shortly after ART initiation.

Figure 2.2.1 Neslon-Aalen estimator of the cumulative hazard function and Kaplan-Meier survival function (green) compared with the Weibull (red) and Exponential (blue) model estimations



The approach of Royston and Parmar starts with the Weibull model in its logarithmic form, excluding covariates

$$\ln H(t) = \ln \lambda + \gamma_1 \ln t = \gamma_0 + \gamma_1 \ln t \qquad (1.0)$$

This shows that $\ln H(t)$ is a sum of two components, a constant and a linear function of log time. Figure 2.2.1 shows that this linear form for $\ln H(t)$ does not fit the cumulative hazard function as well as it could do, therefore a more flexible approach is needed.

Equation (1.0) is extended as follows:

$$\ln H(t) = f(\ln t; \gamma)$$

where $f(\ln t; \gamma)$ represents a general family of non-linear functions of time ln*t*, with a parameter vector γ, and is monotonic increasing in ln*t.* A suitable family of such functions is cubic splines, these are piecewise cubic polynomials with a separate cubic polynomial that is fit in each of a pre-defined number of intervals. The number of intervals is chosen by the user, and the split points are known as knots. The spline is cubic between the knots but is only continuous in its 2nd derivative at the knots, providing more flexibility than a simple cubic polynomial alone. The preferred option here is a restricted cubic spline; this is a special case that forces the function to be linear before the first knot and after the last knot (the first and last knots are known as boundary knots) i.e. it includes a linear term in the tails (to prevent over fitting and model instability) and then additional terms involving the cubic polynomials between the boundary knots. Working on the log scale of time (1.0) is generalised using restricted cubic spline functions as follows for m interior knots:

$$\ln H(t) = s(\ln t; \gamma) = \gamma_0 + \gamma_1 \ln t + \gamma_2 z_1(\ln t) + \gamma_3 z_2(\ln t) + \ldots\ldots + \gamma_{m+1} z_m(\ln t)$$

where $\ln t$, $z_1(\ln t)$, $z_2(\ln t)$ and so on are the pre-defined basis functions of the restricted cubic spline, one for each interior knot. When one or more interior knots are specified, the spline function includes a constant (γ_0), a linear function of $\ln t$ with parameter γ_1, and a basis function of $\ln t$ for each interior knot *i*, which also has a regression parameter γ_{i+1} (*i=1…m)*. The no interior knot case is the simple Weibull model ($\gamma_0 + \gamma_1 \ln t$) (df=1 for one function of ln*t* ($\gamma_1 \ln t$) in the model). The number of knots to be used has to be pre-specified, or equivalently the degrees of freedom (df) for the model. The df are one more than the number of interior knots, so a model with df(3) has 2 interior knots and 2 boundary knots and a total of 3 (=df) functions of ln*t* in the model.

Whilst it is also possible to choose the location of the knots to improve the 'fit' of the model, this runs the risk of over-fitting to the specific dataset. It is not recommended and therefore is not done here. Previous investigation [100] has shown that optimal knot positioning does not appear to be critical for a good fit and may even cause problems when the fitted curve follows small scale features of the data too closely. Royston & Parmar [98] suggested knot positions based on empirical centiles of the distribution of the uncensored log survival times, as given in Table 2.2.2, with boundary knots at the smallest and largest uncensored log-survival times. These knot positions provide considerable flexibility without over-fitting.

Table 2.2.2 Positions of internal knots for modelling the baseline distribution function in FPM models. Knots are positions on the distribution of uncensored log-survival times

Interior Knots	df	Centiles of the interior knots
0	1	None (Weibull)
1	2	50
2	3	33, 67
3	4	25, 50, 75
4	5	20, 40, 60, 80
5	6	17, 33, 50, 67, 83

In addition, a decision must be made as to which type of model to use, the proportional hazards, proportional odds or probit-scaled FPM to give the 'best' fit. A proportional hazards FPM models log cumulative hazard as a spline in ln*t* as discussed above; a proportional odds FPM models log cumulative odds as a spline in ln*t* and the probit-scaled FPM models on the normal equivalent deviate scale as a spline in ln*t.*

The simple log-logistic (proportional odds) model is:

$$\text{logit}\left\{1-S\left(t;\mathbf{x_i}\right)\right\} = \text{logit}\left\{1-S_0\left(t\right)\right\}+\mathbf{x}_i\boldsymbol{\beta}$$

where logit(x)=ln{x/(1-x)}

As for the Weibull model where flexibility is increased by extending the model term in ln*t* using spline functions, the log-logistic model can be extended in a similar way:

$$\ln\left(\frac{1-S(t)}{S(t)}\right)=\gamma_0+\gamma_1\ln t+\mathbf{x}\boldsymbol{\beta}=s(\ln t:\gamma)+\mathbf{x}\boldsymbol{\beta}$$

And similarly for the probit scaled FPM the probit (log normal) model is generalised as follows:

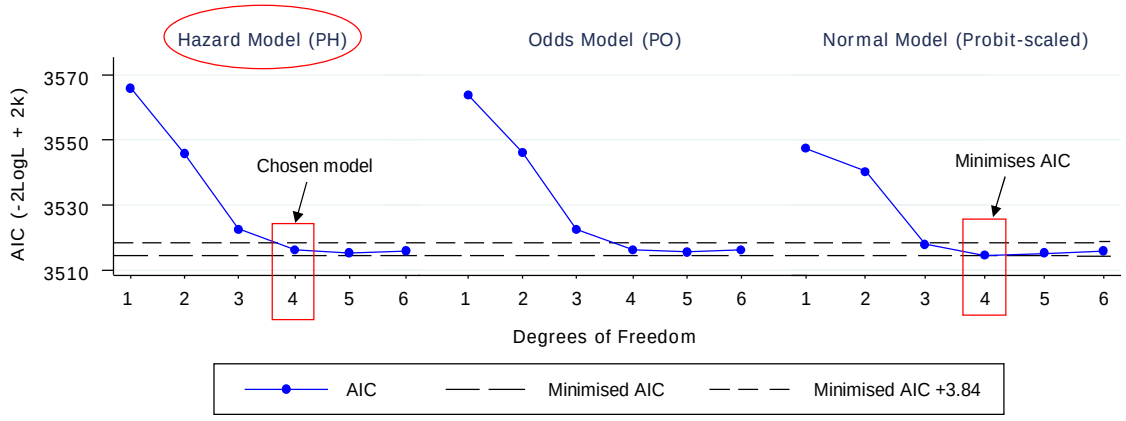
$$S(t)=\Phi\left(\frac{\ln t-\beta_0}{\sigma}\right)\Rightarrow\Phi^{-1}\left\{S(t)\right\}=\gamma_0+\gamma_1\ln t=s(\ln t;\gamma)$$

where $\gamma_1=1/\sigma$ *and* $\gamma_0=-\beta_0/\sigma$

using natural cubic spline for $s(\ln t;\gamma)$

The FPMs described here are the natural cubic spline generalisation of the Weibull, log-logistic and lognormal models. In order to choose which model to use and the number of dfs, each model is fitted to the data and the AIC (Akaike information criterion) calculated, where $AIC=-2\ln(L)+2k$, *k* is the number of parameters in the statistical model, and *L* is the maximized value of the likelihood function for the estimated model. The 'best' model is the model that minimises the AIC, thus trading off improvements in model fit (smaller *-2ln(L)*) against model complexity required to achieve this (number of parameters *+2k*).

Figure 2.2.2 AICs for PH, PO and probit-scaled models with various dfs for overall mortality in DART



The model that minimises the AIC is the normal model with df(4) (an extension of the probit model) (Figure 2.2.2). However, for df(4) and higher, the difference in AICs between model types (i.e. hazard, normal, or odds) is statistically indistinguishable (<3.84, p>0.05 for χ^2 on 1 df). Therefore, instead of the normal model which produces acceleration factors from the log normal model for covariate effects that are hard to interpret (particularly for the wider clinical audience), I have chosen to use the hazard model, with df(4) (an extension of the Weibull model) as this provides standard HRs which are easier to interpret. This choice of model is further supported by Figure 2.2.3 where for the DART data the predicted hazard for overall mortality is very similar whether I use a proportional hazards (PH), proportional odds or probit-scaled FPM with 4 df. This figure also demonstrates the poorness of fit for the simple Weibull model (df=1) (red dashed line).

Figure 2.2.3 Predicted hazard rate (per 100 PY) for all-cause mortality

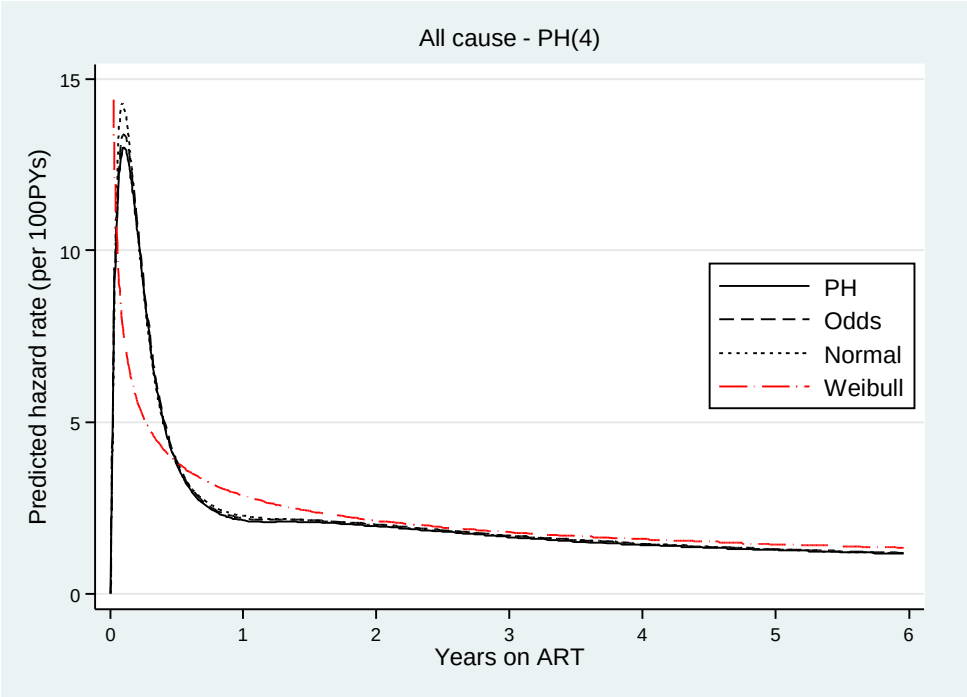


Figure 2.2.4 Assessing the fit of PH(4) – all-cause mortality

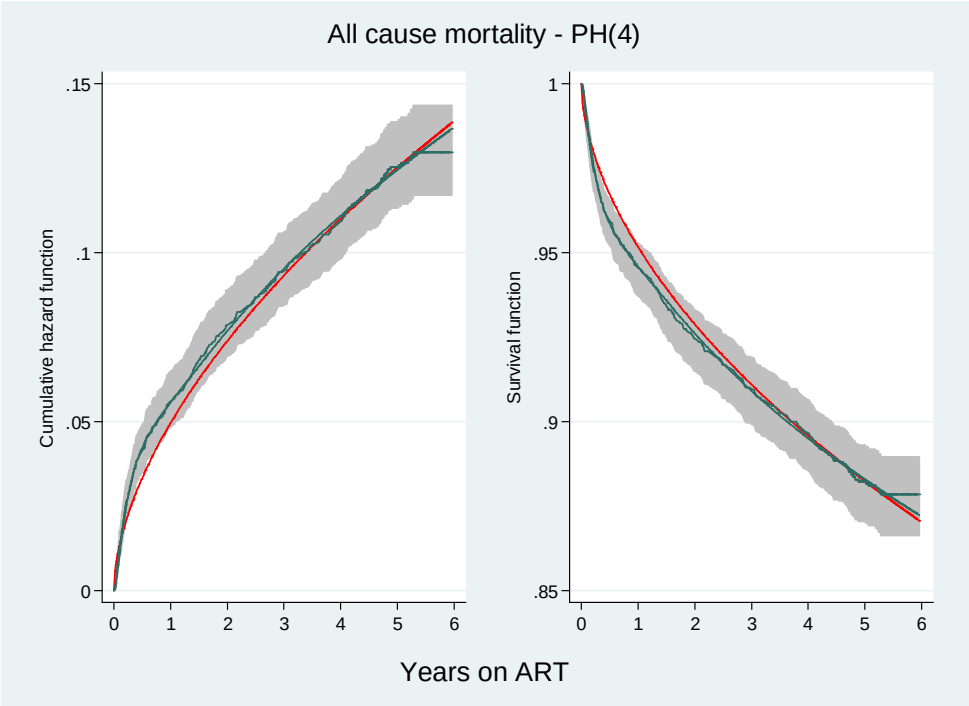


Figure 2.2.4 shows that a PH(4) FPM (green) is a very good fit to the data and is indistinguishable from the Kaplan Meier (blue), in contrast to the Weibull model shown in red (AIC 3522.23 vs. 3572.15 for the basic Weibull model).

2.2.5. Comparing the CD4 monitoring strategies using flexible parametric models

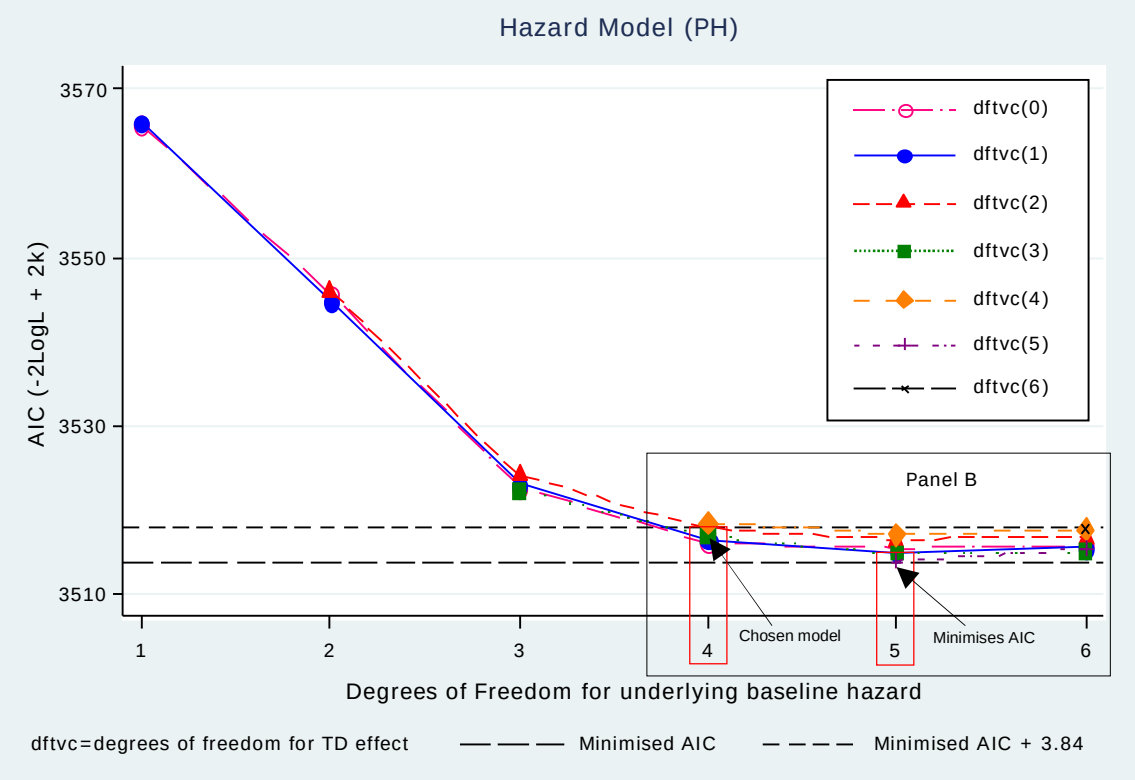
Covariates can be included in the model either by (i) replacing γ_0 with $\gamma_0 + x_i\boldsymbol{\beta}$ which estimates an average (proportional) effect of *x* on the hazards in the PH model (or equivalent for the proportional odds or probit-scaled models) or (ii) by fitting an interaction between covariate x_i and one or more of the $\ln(t)$ terms (including the spline as defined in section 2.2.4 p49). The latter is effectively an interaction model, also equivalent to stratifying the hazard by the covariate (fitting a separate hazard over time in each group). For the DART data, I wish to investigate how the hazard between the monitoring strategies changes over time, so (ii) is likely to be more informative.

Royston and Parmar [98] discussed how time-dependent effects could be fit by including interactions between a covariate and the spline variables in $S\{ln(t)|y, k_0\}$. In an old version of the STATA command stpm, the dfs for the time-dependent effect had to be the same as for the baseline hazard; this tended to lead to over-fitting of the models and certainly of the time-dependent effects. However, it is now possible with the updated stpm2 command to have a different number of df for the baseline hazard and the time-dependent effect (using the dftvc option); of note the optimal number of df for the time-dependent effect is, in nearly all situations, lower than that for the baseline hazard (dftvc<df). To enable the different number of df for the underlying baseline hazard and the time-dependent effect, different sets of spline variables are

calculated, one for the baseline hazard rate and one for each of the time-dependent effects which tends to produce a smoother model. Default knot locations for time-dependent effects are the same as for the baseline hazard knots shown in Table 2.2.2. As before, the model which minimises the AIC is preferred. I therefore chose the dftvc for the CD4 monitoring strategy in DART and the df for the underlying mortality hazard which jointly minimise the AIC. However, I chose to favour parsimony by selecting a less complicated model if the AIC was <3.84 greater than the 'best' model. I have demonstrated selecting the 'best' model when including monitoring strategy as a time-dependent effect in Figure 2.2.5 using the PH model.

Figure 2.2.5 AICs for the PH model with varying df and dftvc

a: All df



b: df(4), df(5), df(6)

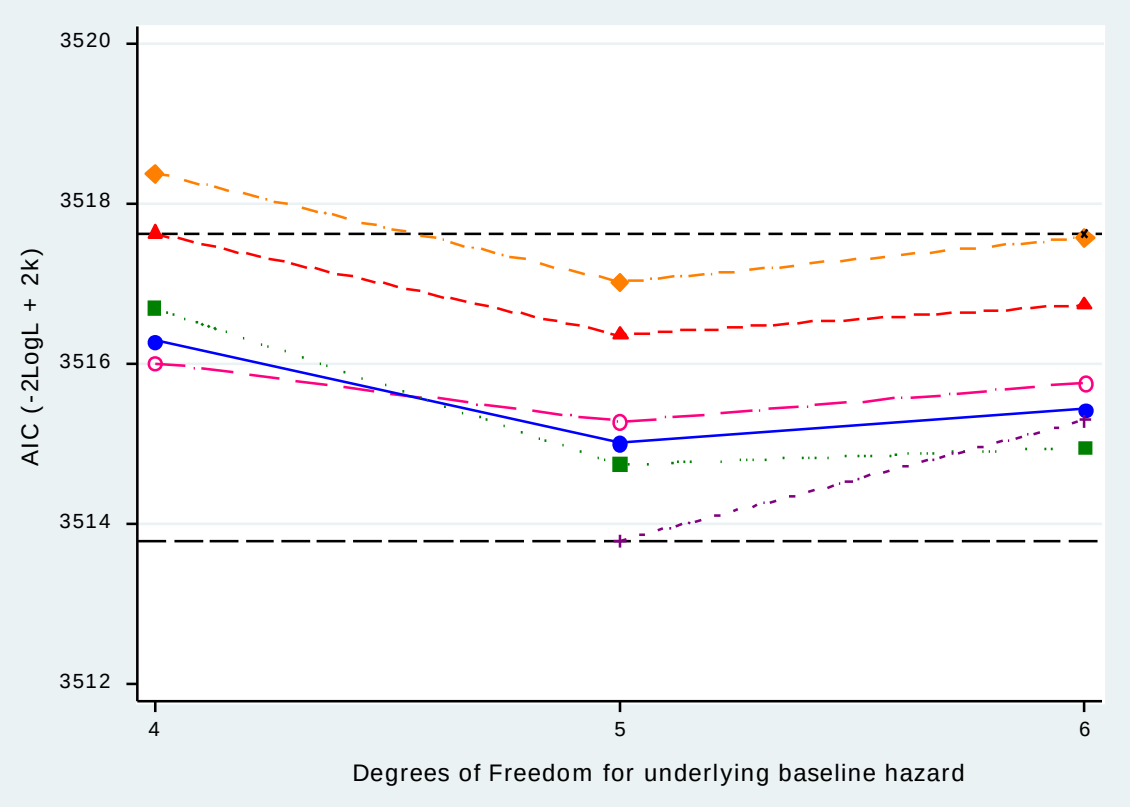
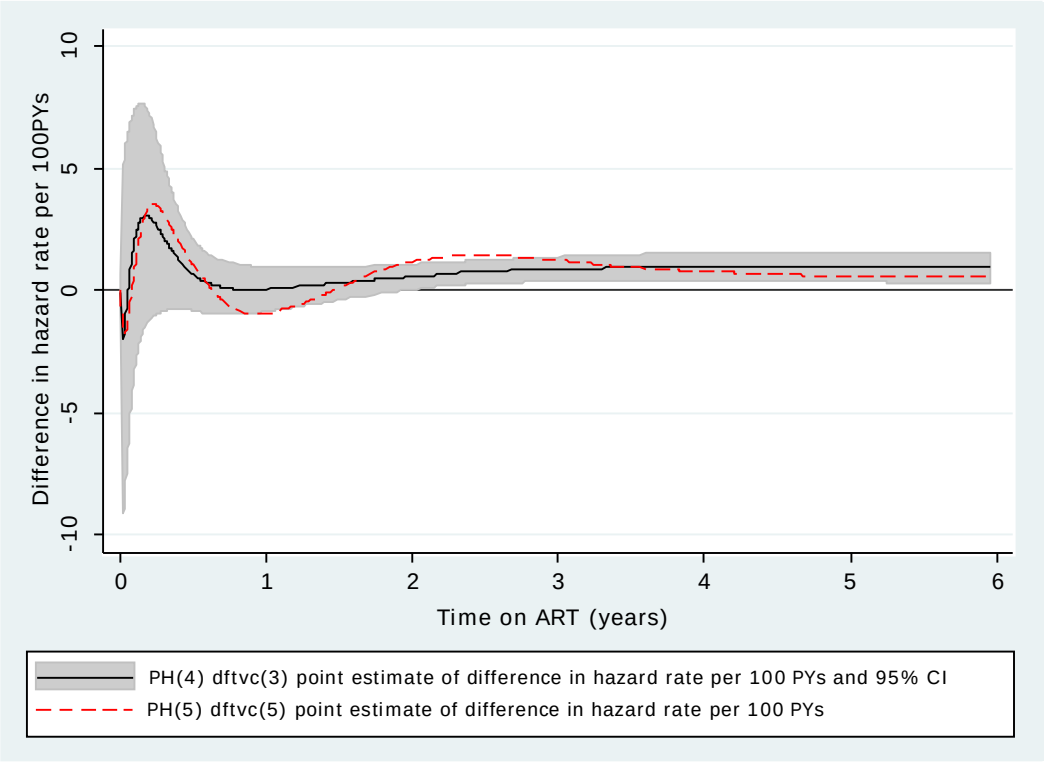


Figure 2.2.5 shows that the model that minimises the AIC has df(5) for the baseline hazard and dftvc(5) for the time-dependent effect of monitoring strategy (AIC=3513.787). However, for baseline df=4, 5, 6, all but 1 of the different combinations of dftvc are within 3.84 of this minimum AIC, meaning that they are statistically similar (Figure 2.2.5b). In addition it has been shown previously that, without including monitoring strategy in the model as a covariate, the 'best' df for the baseline hazard is 4df. With 4df, dftvc(0) (i.e. no time-varying effect, proportional hazards), dftvc(1), dftvc(2) and dftvc(3) have AIC within 3.84 of the minimum AIC (at 3516.005, 3516.287, 3517.627 and 3516.693 respectively). Because I want to model the hazards flexibly, but not introduce too much noise into estimates I have chosen df(4) with dftvc(3) as this is the model with the third lowest AIC for df=4 and is <AIC + 3.84. Although dftvc(0) and dftvc(1) have the lowest AICs for df=4, these either assume proportionality or adds an extra linear ln*t* interaction term in the no CD4 monitoring group which are both fairly strong assumptions. The closeness of the AIC illustrates the challenges of the FPM model selection. From this model the hazard ratio and its 95% CI are calculated for no CD4 vs. CD4 monitoring. Figure 2.2.6 shows the difference in hazards between the two monitoring strategies (black line) along with the 95% CI, compared to the lowest df=5 AIC model in red.

Figure 2.2.6 Difference in mortality hazard rate (no CD4 monitoring-CD4 monitoring) per 100PY



2.2.6. Competing risks

The above sections have focused on a single time to event outcome such as overall mortality. However, each cause of death can be considered an outcome in its own right, with a patient dying from only one of these causes. Thus each of these causes of death are 'competing' to be the one cause of death, that is, these causes can therefore be considered as 'competing risks' in the statistical sense.

A competing risk is an event that happens prior to the event of interest, and means that one can never observe the event of interest. For mortality the different causes of death are the competing risks; if a patient died of septicaemia, then they can never die of say TB (or some other cause). If T is time to the event and R is the cause of failure, with 1 to K specific causes, competing risk analysis is simply finding out about the joint distribution of T and R. There are two important measures in competing risks, these are the cause-specific hazard rate and the cumulative incidence function (CIF). The cause-specific hazard for cause k, $h_k(t)$ gives the hazard rate for the immediate risk of death from cause k at time t conditional on not having already died of cause k or of any other K-1 causes of death. It is estimated by treating events due to competing causes as censored observations. All regression models described in Table 2.2.1 above can be used to model cause-specific hazards, similarly to overall hazards.

The CIF is the probability, as a function of time, that a subject dies of that cause in the presence of all other possible competing causes. The CIF is defined as:

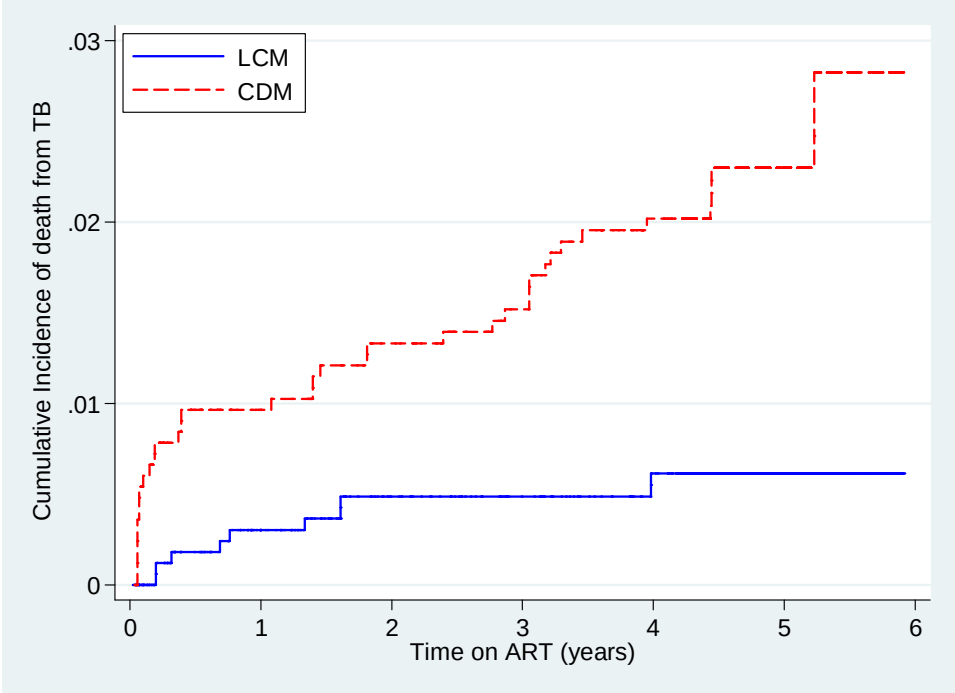
$$\begin{aligned} CIF_k(t) &= P\left(T \le t \mid fail\ \ from\, cause = k\right) \\ &= \sum_{i=1}^{I} \left\{\lambda_k(t_i) S(t_i^-)\right\}^{\Delta_{ik}} \end{aligned}$$

where *k* is the cause, *i* is the subject, $\lambda_k(t_i)$ is the cause-specific hazard at the time the *i*th subject died or was censored, $S(t_i)$ is the overall probability of survival to time t_i and Δ_{ik}=1 if subject *i* died from cause *k*. Therefore the CIF is a function of both the cause-specific hazards and the overall survival probability. Further, since $\sum \lambda_k(t_i) = \lambda(t_i)$, the sum of $CIF_k(t)$ for k=1…..K is the overall survivor function, as estimated from Kaplan-Meier. As $t \to \infty$, the $CIF \to$ the probability of observing the event of interest as the first event of all the competing risks (e.g. *P(die from septicaemia)*): as this is <1, the distribution is improper and has a 'sub-hazard'. Fine and Gray [101] specify a model for the hazard of the sub-distribution which thinks of the hazard as that which generates failure events of interest while keeping individuals who experience competing events as "at risk" so that they can be adequately counted as not having any chance of failing from this specific cause.

As an example I have estimated the CIF for TB in the presence of all other causes of death using competing risks in Figure 2.2.7. However, as overall mortality and cause-specific death rates are low in DART (only 11% died by trial closure), there is relatively little difference

between the sub-distribution hazard and the cause-specific hazard in this specific example. Whilst this cause-specific hazard can be estimated by artificially censoring deaths/events from other causes (as above), one cannot construct a Kaplan-Meier curve as the basis of this cause-specific hazard [102]. I have chosen to model the cause-specific hazard for each type of death separately and all further analyses will be carried out using the cause-specific hazards.

Figure 2.2.7 Modelling CIF for TB in the presence of other causes



2.2.7. Choosing the best model for the individual causes of death

The methods described in sections 2.2.4 to 2.2.5 can be applied to a cause-specific hazard regression model as well as an overall hazard regression model. As with all-cause mortality I could choose the model that minimises the AIC (Table 2.2.3). However, for most individual causes there are small numbers of deaths, meaning power to detect important variation in hazards is low; that is, even when the fit is very good the AIC may be penalised by the larger number of parameters. For causes with ≤13 deaths (and TB) the Weibull (PH model with df=1) is the 'best' fit to the data according to AIC; however, where there are more events, models with greater flexibility are typically chosen, supporting low power being the major determinant behind choice of df=1.

The overall mortality analyses showed that the overall hazard of death is highly non-linear, therefore in this chapter where AIC is minimised in the Weibull model I will use instead FPMs with either df=3 or df=2 provided the difference in AIC is <3.84 compared with the Weibull model (Table 2.2.3).

Table 2.2.3 AIC for FPMs (no covariates) for cause-specific mortality with df(1), df(2), df(3) and df(4)

Cause of death	Number of deaths	AIC			
		Weibull	df=2	df=3	df=4
All-cause mortality	382	3572.152	3551.908	3528.771	**3522.23**
Septicaemia/Neutropenia	44	636.267	618.904	**613.992**	Cannot compute an improvement
Neurological	38	550.480	538.534	**537.826**	539.801
Cryptococcus	32	482.704	477.337	473.033	**472.434**
TB	31	**443.425**	445.205	**444.842**	445.116
HIV related malignancy*	26	362.196	**361.528**	363.385	364.37
Respiratory event	24	366.136	366.597	**365.834**	366.579
GI event	15	235.253	**234.719**	235.927	Cannot compute an improvement
Trauma/Suicide	13	**201.045**	202.955	**201.602**	203.185
Wasting/diarrhoea	11	**186.067**	**187.032**	188.626	188.869
Renal event	11	**168.939**	**170.137**	172.114	174.057
Cardiovascular disease	10	**151.835**	**153.251**	155.098	156.992
Other†	48	638.531	637.755	639.580	**634.032**
Unknown	80	**979.391**	**979.512**	979.747	986.758

*Includes cervical cancer, kaposi's sarcoma, non-hodgkin's lymphoma, other B cell lymphoma and central nervous (CNS) lymphoma

†Includes cryptosporidia, liver, anaemia, other cancer, lactic acidosis, herpes simplex virus (HSV) or cytomegalovirus (CMV), malaria/cholera, stevens-johnson syndrom (SJS)

Shading shows models which minimise the AIC: if this is the Weibull model, df=1 (0 knots), second lowest also shaded and used in results.

The chosen models are as follows: PH(4) for cryptococcus and other as cause of death (COD); PH(3) for septicaemia/neutropenia, neurological, TB, respiratory and trauma/suicide; and PH(2) for HIV related malignancy, GI, wasting/diarrhoea, renal and CVD as COD. As with the overall mortality I wish to compare the monitoring strategies for each individual COD. I have tried to apply the same methods as for the overall mortality, i.e. including monitoring strategy as a time-dependent covariate, however, due to low event rates in certain causes of death this was not possible. For this reason I will return to the Poisson models which still allow some flexibility in the model for mortality from individual causes of death to be fitted, but also allow formal comparisons between the monitoring strategies to be made, despite the low event rates.

2.2.8. Additional work on the difference in CD4 monitoring strategies

In order to estimate how much difference in risk by monitoring strategy could be explained by CD4, I have fitted standard time-updated Cox models. I have used Cox rather than FPM to avoid problems with instability in the baseline hazards for specific causes. I also used Cox over Poisson to allow a more flexible baseline hazard than piecewise exponential and because, although CD4 readings were scheduled 12 weekly, small variations in when the CD4 counts were taken are easier to accommodate in the Cox model.

2.3. Results

2.3.1. Causes of death in DART

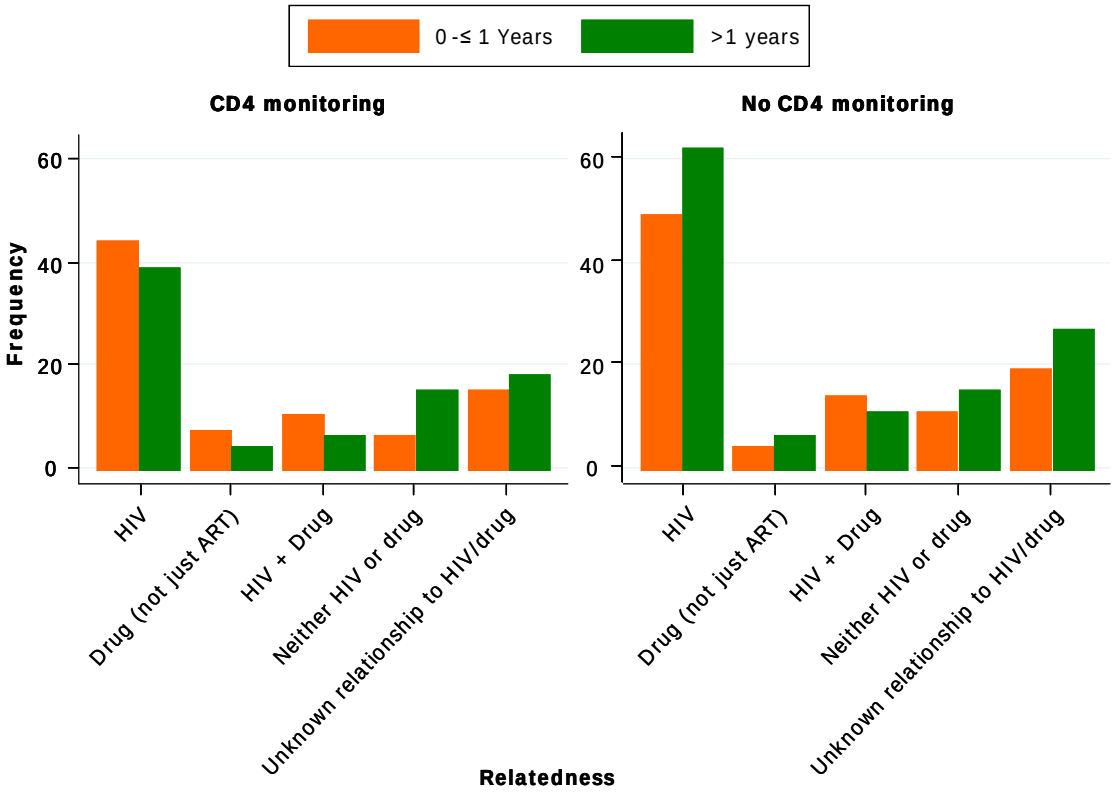
In the DART trial there were 14,937 person years (PY) of follow-up between January 2003 and December 2008 (end of follow-up under CD4 monitoring strategy) with a median of 4.9 years follow-up per patient. A total of 382 patients died on or before 31st December 2008. Of these 303 (79%) had an ascertainable cause of death and 179 (47%) died in the first year on ART. Overall mortality was 179/3316 (5%) in the first year on ART and 203/3094 (6%) subsequently. This equates to 5.6/100PY in the first year of ART and 1.7/100PY thereafter. So, as might be expected, given the severe immunodeficiency at ART initiation in the trial (median CD4 86 cells/µL), overall mortality was substantially higher in the first year on ART. This was also clear in both of the two CD4 monitoring strategies; with 82 deaths (5.2/100PY) in the CD4 monitoring group (LCM) and 97 (6.1/100PY) in the no CD4 monitoring group (CDM) in year 1; and 82 (1.4/100PY) LCM and 121 (2.1/100PY) CDM deaths subsequently ($p<0.001$ for year 0-≤1 vs. year >1 in both LCM and CDM (Table 2.3.1)).

Table 2.3.1 Deaths by time on ART and monitoring strategy

	LCM (n=1656)		CDM (n=1660)		Total	
	0-≤1 yrs	>1 yrs	0-≤1 yrs	>1 yrs	LCM	CDM
Total patients	1656	1552	1660	1542	1656	1660
Deaths All	82 (5%)	82 (5%)	97 (6%)	121 (7%)	164 (10%)	218 (13%)
Causes						
HIV	44	39	49	62	83	111
Drug (not just ART)	7	4	4	6	11	10
HIV and drug	10	6	14	11	16	25
Neither HIV or drug	6	15	11	15	21	26
Unknown relationship to HIV/drug	15	18	19	27	33	46

Regardless of time on ART (exact p=0.3) and regardless of CD4 monitoring strategy (exact p=0.9), most deaths were judged primarily HIV-related (93/179 (52%) early vs. 101/203 (50%) late) or were related to both HIV and drugs (24 (13%) early vs. 17 (8%) late), with few deaths judged primarily drug-related (ART/concomitant medication) (11 (6%) early vs. 10 (5%) late). Deaths where cause could not be ascertained also occurred similarly in both periods (34 (19%) early vs. 45 (22%) late) (Figure 2.3.1).

Figure 2.3.1 Deaths by time on ART and monitoring strategy



There were a large number of individual causes of death. For patients with more than one cause of death, the ERC adjudicated the primary cause (disease/condition directly leading to death) separately from the other secondary causes (other disease/conditions leading to the primary cause of death). This enabled me to group the deaths into the categories outlined in Table 2.3.1. The primary diagnosis was used to determine the cause of death category for analysis. Neurological events excluded cryptococcal meningitis as this is presented separately under cryptococcal disease. Patients were only classified as dying from septicaemia/neutropenia if they had an infection or evidence of a fever in the presence of their neutropenia; else they were included with anaemia/thrombocytopenia/pancytopenia/ neutropenia. Of note is that most septicaemia/pneumonia/meningitis infections causing death had little definitive support for a specific infecting micro-organism (bacteria, virus or fungi) either in the text given for the primary cause of death, the structured narrative, any organism data associated with concurrent WHO stage 3/4 events or from the microbiology form. This was predominantly because microbiological facilities were limited or not available.

Table 2.3.2 Detailed cause of death by treatment and time on ART

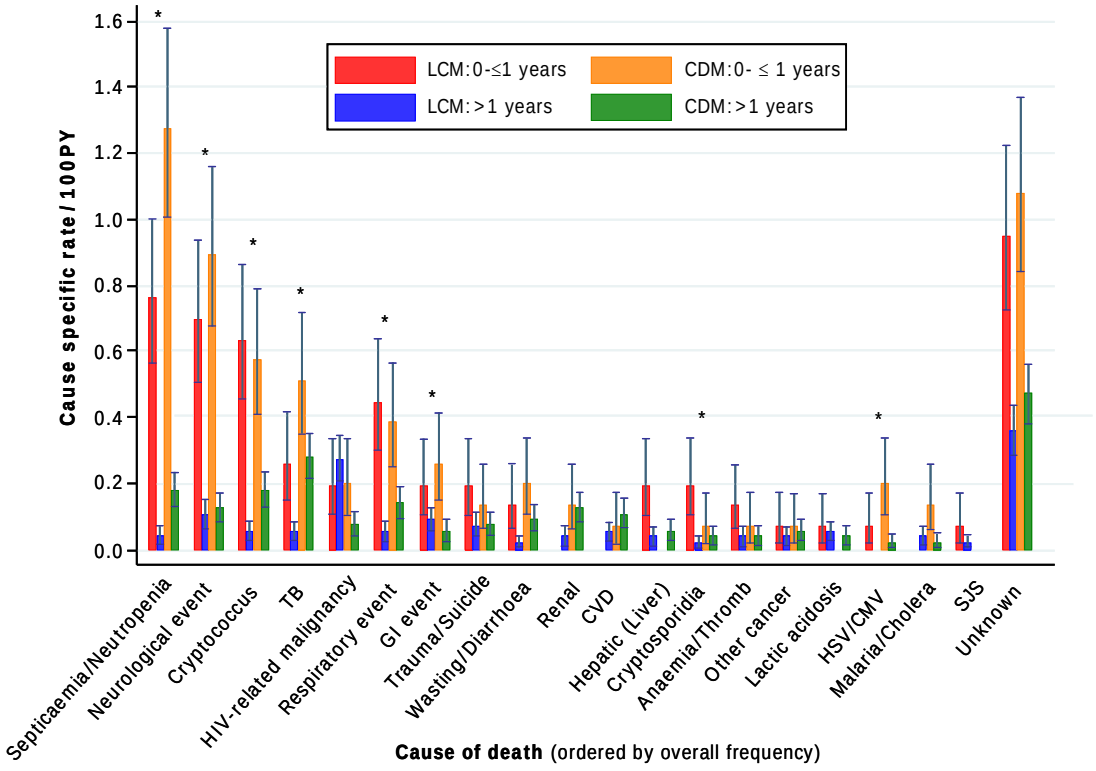
Cause of death*	LCM (CD4 monitoring)		CDM (no CD4 monitoring)		Total	
	0-≤1 yr	>1 yr	0-≤1 yr	>1 yr	0-≤1 yr	>1 yr
Septicaemia/Neutropenia	12 (15%)	2 (2%)	20 (21%)	10 (8%)	32 (18%)	12 (6%)
Neurological event	11 (13%)	6 (7%)	14 (14%)	7 (6%)	25 (14%)	13 (6%)
Cryptococcus	10 (12%)	3 (4%)	9 (9%)	10 (8%)	19 (11%)	13 (6%)
Tuberculosis (TB)	4 (5%)	3 (4%)	8 (8%)	16 (13%)	12 (7%)	19 (9%)
HIV-related malignancy	3 (4%)	16 (20%)	3 (3%)	4 (3%)	6 (3%)	20 (10%)
Respiratory event	7 (9%)	3 (4%)	6 (6%)	8 (7%)	13 (7%)	11 (5%)
Gastro-intestinal (GI) event	3 (4%)	5 (6%)	4 (4%)	3 (3%)	7 (4%)	8 (4%)
Trauma/Suicide	3 (4%)	4 (5%)	2 (2%)	4 (3%)	5 (3%)	8 (4%)
Wasting/Diarrhoea	2 (2%)	1 (1%)	3 (3%)	5 (4%)	5 (3%)	6 (3%)
Renal	0 (0%)	2 (2%)	2 (2%)	7 (6%)	2 (1%)	9 (4%)
Cardio-vascular disease (CVD)	0 (0%)	3 (4%)	1 (1%)	6 (5%)	1 (1%)	9 (4%)
Hepatic	4 (5%)	2 (2%)	0 (0%)	3 (3%)	4 (2%)	5 (3%)
Cryptosporidia	3 (4%)	1 (1%)	1 (1%)	2 (2%)	3 (2%)	4 (2%)
Anae/Pan/Thromb/Neut†	2 (2%)	2 (2%)	1 (1%)	2 (2%)	4 (2%)	3 (2%)
Other cancer	1 (1%)	2 (2%)	1 (1%)	3 (3%)	2 (1%)	5 (3%)
Lactic acidosis	1 (1%)	3 (4%)	0 (0%)	2 (2%)	1 (1%)	5 (3%)
HSV/CMV‡	1 (1%)	0 (0%)	3 (3%)	1 (1%)	4 (2%)	1 (1%)
Malaria/Cholera	0 (0%)	2 (2%)	2 (2%)	1 (1%)	2 (1%)	3 (2%)
Stevens-Johnson-Syndrome (SJS)	1 (1%)	1 (1%)	0 (0%)	0 (0%)	1 (1%)	1 (1%)
Unknown primary cause	14 (17%)	21 (26%)	17 (18%)	27 (22%)	31 (17%)	48 (24%)
Total	**82**	**82**	**97**	**121**	**179**	**203**

*Ordered by total number of deaths
†Anaemia/Pancytopenia/Thrombocytopenia/Neutropenia with no sign of fever
‡ Herpes simplex virus (HSV)/Cytomegalovirus (CMV)

Of the 179 deaths in the first year on ART, the most common causes were septicaemia/neutropenia 32 (18%), non-cryptococcal neurological events 25 (14%), cryptococcus 19 (11%), respiratory events 13 (7%), and TB 12 (7%). Whilst these causes of death remained amongst the most common after >1 year on ART, accounting for 12 (6%), 13 (6%), 13 (6%), 11 (5%) and 19 (9%) deaths respectively; their cause-specific incidence (i.e. absolute risk of dying from this specific cause) dropped significantly from 0-≤1 vs. >1 years on ART (septicaemia/neutropenia, non-cryptococcal neurological events, cryptococcus all p<0.0001; respiratory events p=0.001 and TB p=0.02) (Figure 2.3.2). Mortality also dropped significantly for cryptosporidia (p=0.04), GI events and HSV/CMV (both p=0.02). There was some evidence to suggest rates of death from wasting/diarrhoea also declined (p=0.06); and for hepatic deaths, trauma/suicide and malaria/cholera and anaemia/pancytopenia/ thrombocytopenia/neutropenia with no sign of fever there was marginal evidence of a decline (p=0.11, p=0.14, p=0.14 and p=0.18 respectively). There was no evidence of a decline in cause-specific mortality for lactic acidosis (p=0.79), renal deaths (p=0.80), SJS (p=0.35), CVD (p=0.40), and other cancer (p=0.64). Of note, HIV-related malignancy was the most common

cause of death after >1 year on ART, responsible for 20 (10%) deaths, and rates were similar from 0-≤1 year to >1 year (p=0.9) (Figure 2.3.2). Although 17% of deaths 0-≤1 year and 24% of deaths >1 year could not have the primary cause of death assigned, rates of death from an unknown primary cause also dropped albeit with only marginal significance (p=0.14).

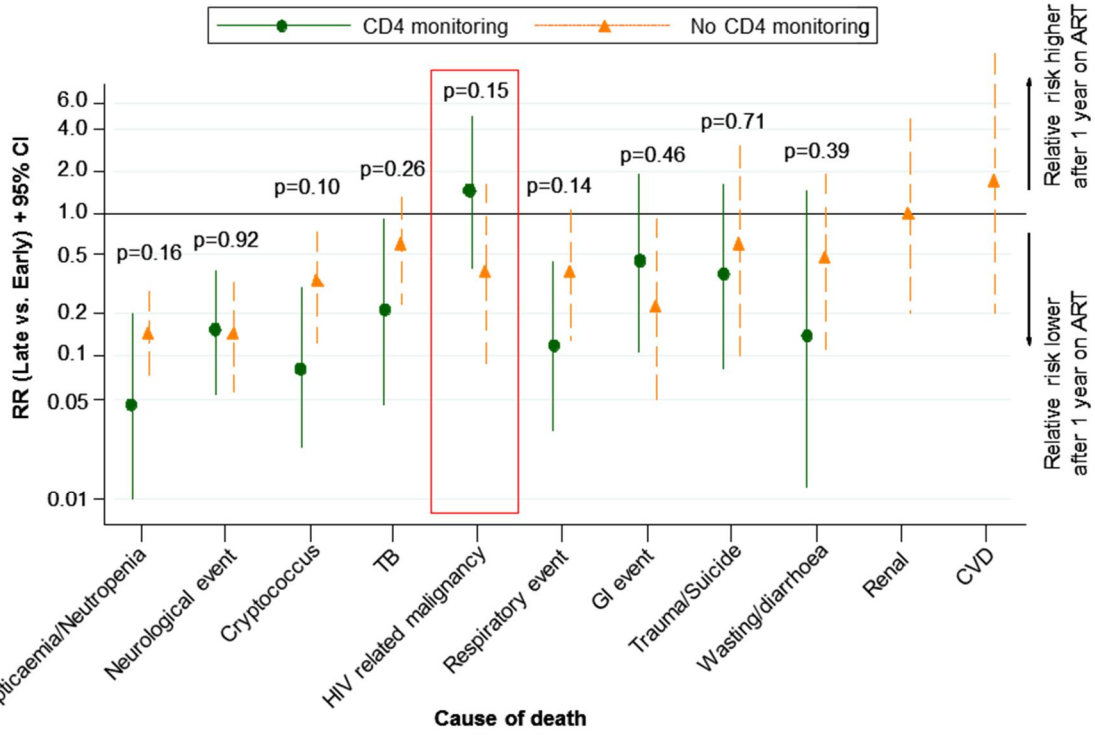
Figure 2.3.2 Cause-specific death rates by CD4 monitoring strategy and time on ART



*p<0.05 for decline in rate between 0-≤1 and >1 years on ART when pooling CD4 monitoring groups

Due to there being a large number of causes of death and with some causes only having a few events I have selected the causes of death with ≥10 events to investigate further. There were just eleven causes included in these further analyses (septicaemia/neutropenia, neurological event, cryptococcus, TB, HIV-related malignancy, respiratory event, GI event, trauma/suicide, wasting/diarrhoea, renal and CVD). By using Poisson models I have been able to assess the change in risk of death from 0-≤1 year to >1 year and to assess whether this differs between CD4 monitoring strategies for this subset of causes (Figure 2.3.3).

Figure 2.3.3 Risk ratios (RR) for late vs. early deaths by CD4 monitoring strategy



Note: p-values show the heterogeneity tests for whether the change in risk of dying from each specific cause >1 vs. 0-≤1 on ART is the same (or different) according to CD4 monitoring strategy
Renal and CVD events are only included for CDM as there were no events in the LCM group for 0-≤1year, so the full interaction model cannot be fitted.

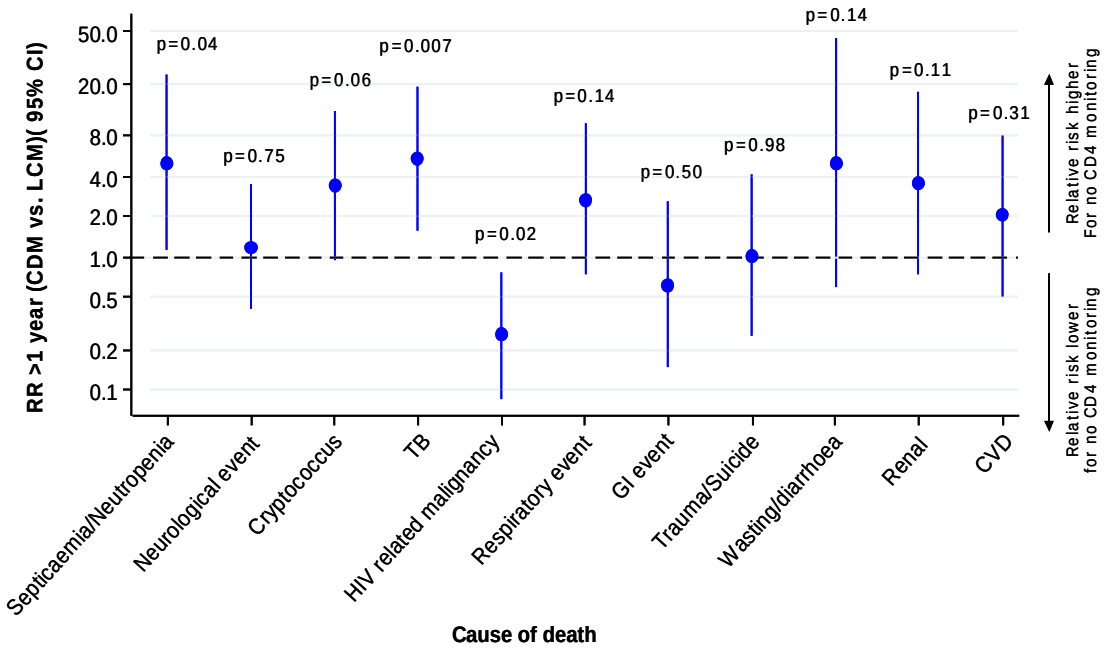
There was marginal evidence that the declines in cause-specific death rates from 0-≤1 to >1 years on ART varied between CD4 vs. no-CD4 monitoring strategies (Figure 2.3.3) for cryptococcus (p=0.10), respiratory events (p=0.14), HIV-related-malignancies (p=0.15) and septicaemia/neutropenia (p=0.16). For cryptococcus, respiratory events and septicaemia/neutropenia, declines in cause-specific mortality appeared greater in those receiving CD4 monitoring.

Of note, the relative risk (RR) of death from HIV-related malignancies was estimated to slightly increase after the first year on ART for the CD4 monitoring group, compared to a small decrease in the no CD4 monitoring group, although this observed increase in LCM was not statistically significant (p=0.57). This trend of a smaller reduction in risk in the CD4 monitoring group vs. no CD4 monitoring group was in the opposite direction to that observed for all other causes except GI events which also did not reach statistical significance (p=0.46). HIV-related-malignancies leading to death after 1 year on ART were cervical cancer (n=9), lymphoma (n=5) and Kaposi's sarcoma (n=6) in the CD4 monitoring group; and lymphoma (n=3) and Kaposi's sarcoma (n=3) for the no CD4 monitoring group.

Most follow-up (11,623PY) was >1 year from ART initiation: given the overall mortality (see methods section 2.2.4) and the fact that CD4 monitoring might be expected to have little effect during the first year on ART (when WHO guidelines recommend not switching for CD4 failure) I

also compared long-term impact of CD4 vs. no CD4 monitoring restricting to those who survived 1 year (Figure 2.3.4).

Figure 2.3.4 Cause-specific RR (+ 95% CI) for no CD4 monitoring vs. CD4 monitoring after 1 year on ART



There is strong evidence to suggest that after 1 year of ART, CD4 monitoring reduced the risk of death for TB (p=0.007), with a pronounced almost 6 fold (RR (95% CI) 5.45 (1.59-18.7)) increase in the risk of death from TB for those patients not receiving CD4 monitoring. Overall after 1 year on ART there were 3 vs. 16 TB-related deaths in those with CD4 vs. no CD4 monitoring respectively; and interestingly, after 2 years on ART there were 1 vs. 12 TB-related deaths. There is also good evidence that the absolute risk of dying from septicaemia/neutropenia was higher in the no CD4 monitoring group than in the CD4 monitoring group (p=0.04) after 1 year on ART, and there is a similar but weaker (p=0.06) effect for cryptococcus. There was marginal evidence that after 1 year on ART, the risk of death from wasting/diarrhoea, respiratory events (both p=0.14) and renal events (p=0.11) was higher in the no CD4 monitoring group. However, for HIV related malignancies the no CD4 monitoring group had a lower risk of death after 1 year on ART, reflecting the results above (p=0.02). For all other events there was no evidence of a difference in risk by CD4 monitoring strategy after 1 year on ART.

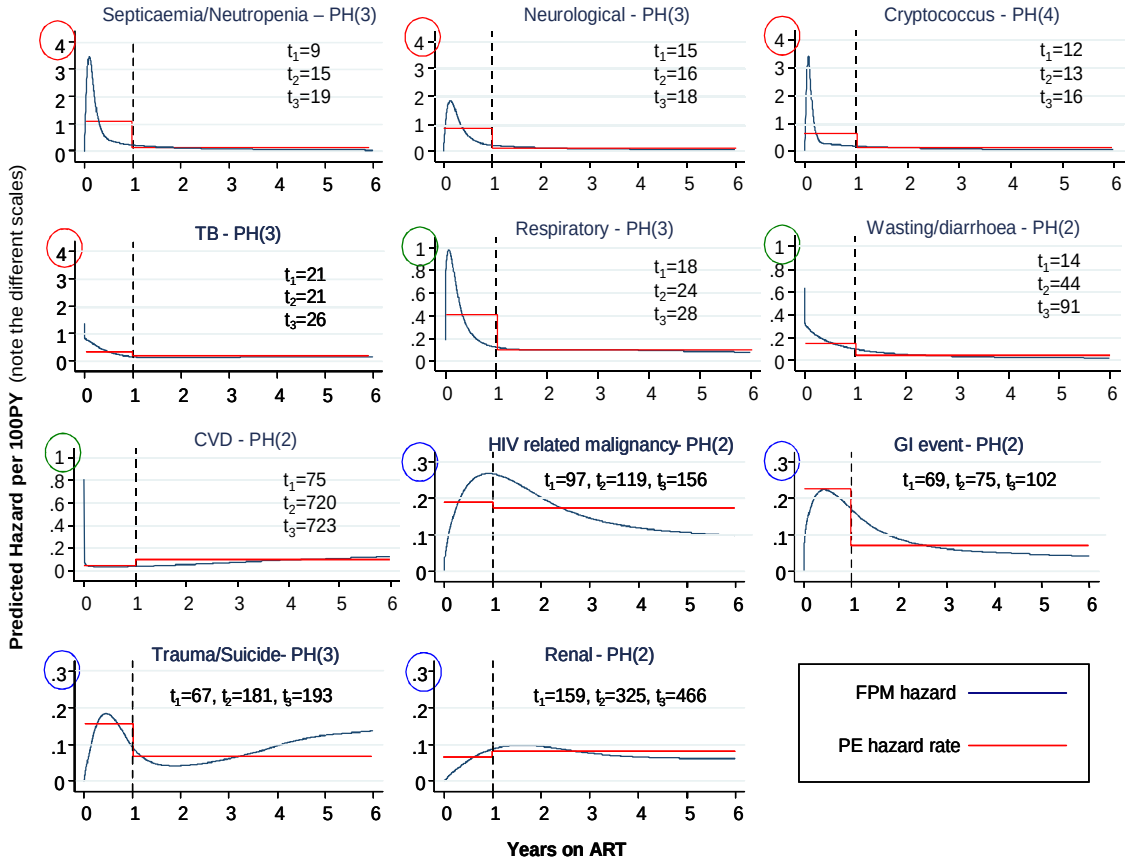
2.3.2. Cause-specific hazards of death in DART

Although simple piecewise exponential (PE) models fitted above give some insight into cause-specific mortality risks (represented as red lines on Figure 2.3.5), and also by monitoring strategy, clearly an abrupt change in risk exactly 1 year after ART initiation is biologically implausible.

The Cox model can only compare CDM vs. LCM, it cannot compare late vs. early hazards directly as these are not directly estimated. Therefore cause-specific hazards of death for

individual causes with ≥10 deaths have been estimated using flexible parametric models as described in section 2.2.4.

Figure 2.3.5 Cause-specific hazards of death over years on ART (CDM and LCM combined)



t_1, t_2, t_3 =time to first, second and third death (days after ART initiation)

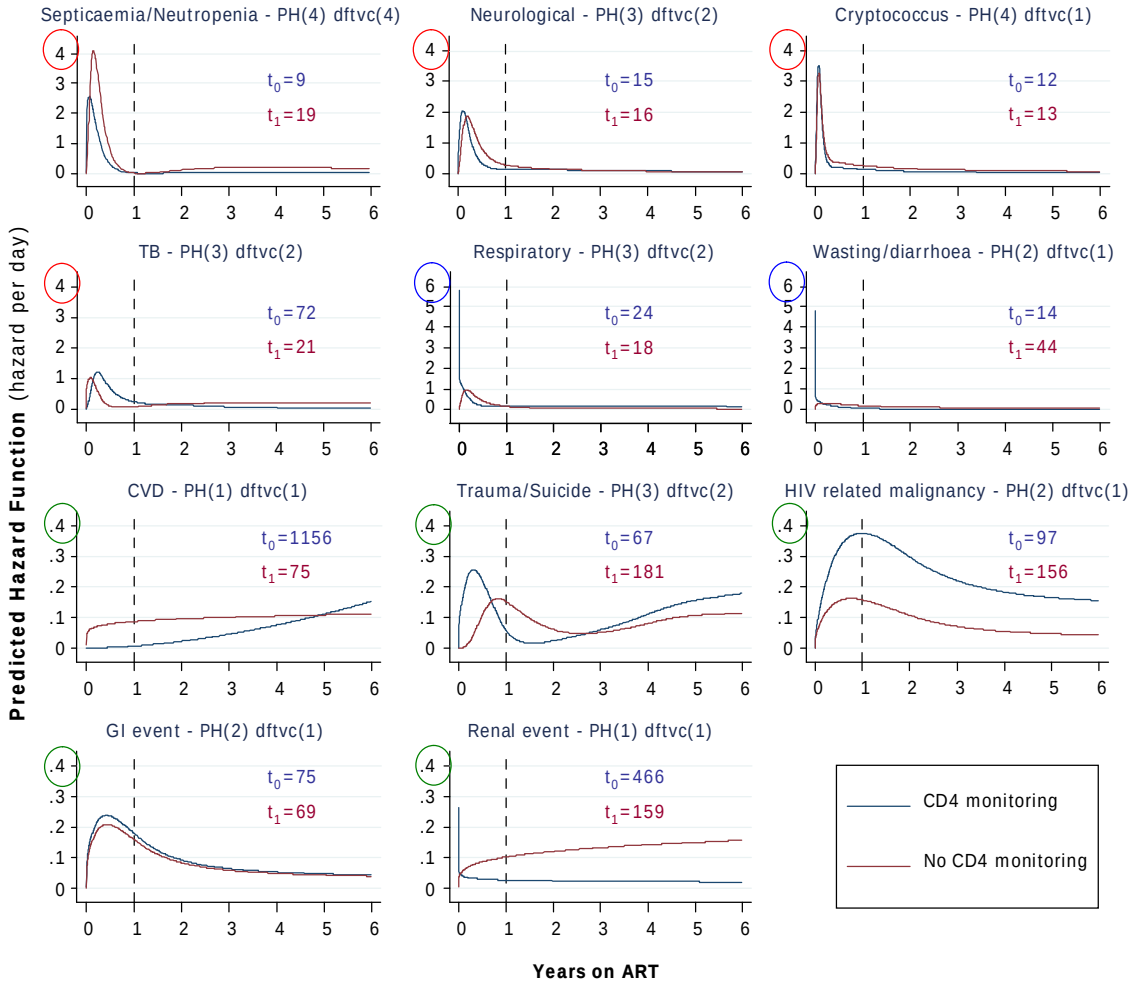
Most causes of death had their highest hazard of death in the first 12 months after ART initiation except CVD (ignoring the implausible early time given that the first death was at 75d) and renal events (Figure 2.3.5). For septicaemia/neutropenia, neurological, cryptococcal and respiratory causes, cause-specific hazards increased from 0 at time t=0 to a maximum at days 25-37, probably because acutely sick patients were ineligible and all enrolled participants had to give consent at day 0 (i.e. could not be moribund). For HIV related malignancies the hazard increased more gradually from 0 at t=0 to a maximum hazard at 11 months, similarly for GI events at 5 months and for trauma/suicide at 6 months. In contrast the risk of dying from TB or wasting/diarrhoea was greatest at ART initiation. These initial changes in hazard appear to be very influenced by the timing of early events (the times of the first 3 events are displayed on Figure 2.3.5). Looking at the most common causes of death, those with earlier deaths (e.g. septicaemia/neutropenia at 9 days) show an increase in hazard from t=0 – whereas only slightly later deaths lead to a hazard greatest at t=0 then declining.

The risk of death from these causes with the highest early risks then declined rapidly to 1 year and stayed low. In contrast, the hazard of death from HIV-related malignancies and GI events declined more steadily out to 6 years. Trauma/suicide had a decline in hazard to 2 years, but then there was a steady increase in hazard which appeared to still be increasing at 6 years.

The risk of dying from renal events increased steadily for the first year on ART and continued to increase until 18 months when it then remained approximately constant. The risk of dying from CVD was very low during the first year and then appeared to increase throughout follow-up; although as seen in Figure 2.3.2 the cause-specific event rate for CVD deaths was very low.

I investigated the individual cause-specific hazards by monitoring strategy (Figure 2.3.6) but estimates were unreliable due to small numbers, particularly with respect to initial hazard trajectories, which appear to depend strongly on timing of first events as above.

Figure 2.3.6 Cause-specific hazards of death during time on ART by CD4 monitoring strategy



In general the trajectories are similar between CD4 and no CD4 monitoring strategies for all causes of death with a higher hazard in the first year than subsequently (apart from CVD and renal). Because of the nature of the interventions I should note that any estimated early differences in cause-specific hazards are unlikely to reflect true differences between the arms; there was no difference in CD4 monitoring received before 12 weeks, and the main purpose of CD4 monitoring is to identify first-line failure and trigger a switch to second-line, which is not recommended by WHO until after 1 year on ART. Thus instability in the hazard estimates close to ART initiation is very likely due to the small number of events and their timing.

Results broadly followed the crude piecewise exponential analysis shown in Figures 2.3.3 and 2.3.4. The hazard of death from septicaemia/neutropenia, cryptococcus and TB appeared to be higher after 1 year on ART for the no CD4 monitoring group, but absolute differences were small. The hazard of death from HIV-related malignancy also appeared greater in the CD4 monitoring group throughout, although absolute differences were small (Figure 2.3.2). The hazard of death from CVD appeared to be greater early on in the no CD4 monitoring group, but as with the overall hazard this may be dependent on the very early death seen in this group and the very late deaths seen in the CD4 monitoring group. Trauma/suicide, CVD and renal events were the only causes where the hazard increased in one or both of the groups. In order to assess long term differences formally I tried to fit FPMs for after 1 year on ART, but there were too few events for models to converge. I successfully investigated the same model for all deaths to increase the number of events. The model that minimised the AIC for overall deaths after 1 year on ART had df(3) and dftvc(4) (Figure 2.3.7), but the hazards showed substantial instability and over-fitting. As above, any early differences appear to be due to the models being very sensitive to when the first event occurred, even after 1 year on ART.

Figure 2.3.7 Hazard of death 1 year after ART initiation, LCM vs. CDM for all deaths

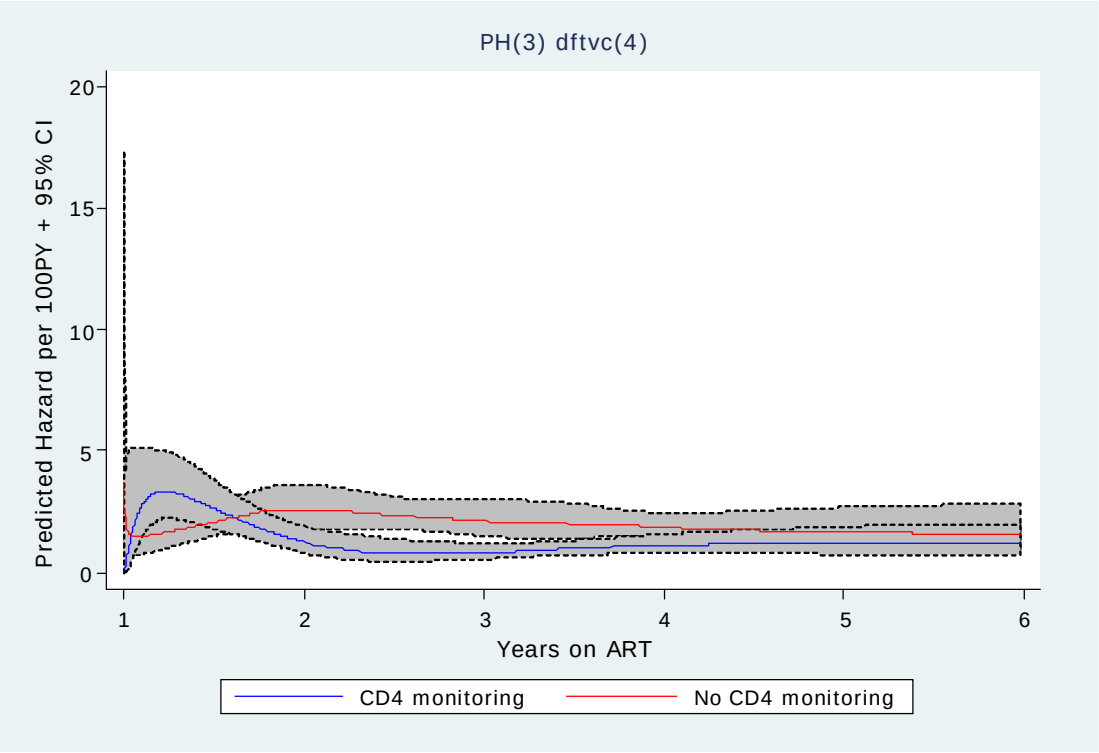
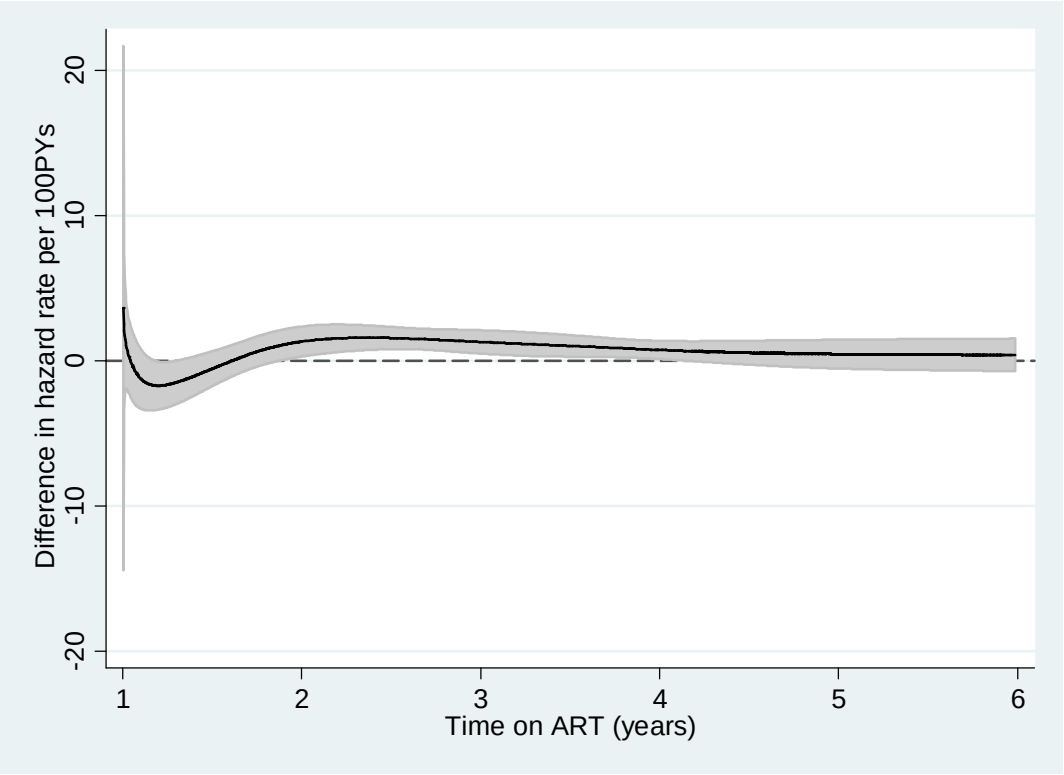


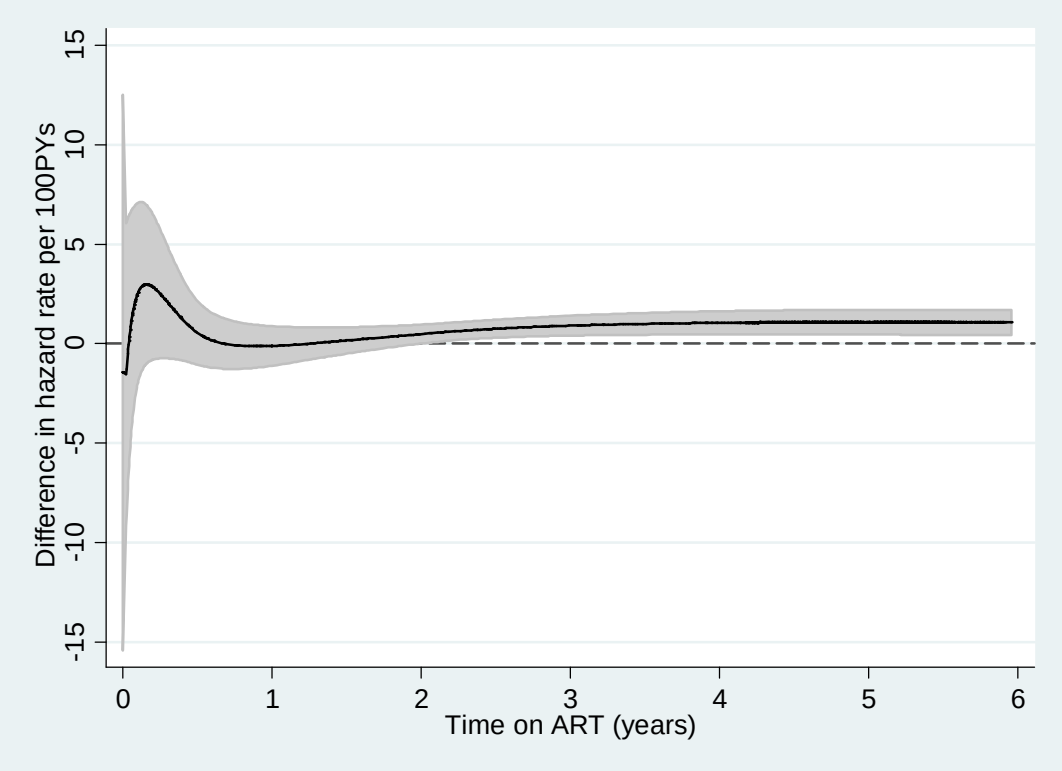
Figure 2.3.8 Difference in hazard rate (CDM-LCM) per 100 PY for overall mortality from 1 year after ART initiation



This model (Figures 2.3.7 and 2.3.8) suggests that the two monitoring strategies appear to be converging as time approaches 6 years, i.e. that there might be no long term difference in mortality under the different CD4 monitoring strategies.

As outlined in section 2.2.5 of the methods, any difference in hazards between the monitoring strategies has been assessed using a FPM for overall mortality including year one. I have re-displayed the difference in the overall mortality hazard rates (CDM-LCM) per 100 PY (including year one) along with the 95% confidence intervals in Figure 2.3.9. As I have seen in some of the individual causes of death, whilst there is a small difference very early on after ART initiation this is not statistically significant and the CIs are very wide. A significant excess in mortality rate in the no CD4 monitoring group appeared from 18-24 months and increased until around 3 years when the excess in mortality rate for the no CD4 monitoring group was between 0.87 and 0.95 deaths per 100PY compared to the CD4 monitoring group. This then appears to remain constant throughout longer term follow-up, in contrast to Figure 2.3.8 above.

Figure 2.3.9 Difference in hazard rate (CDM-LCM) per 100PY overall mortality



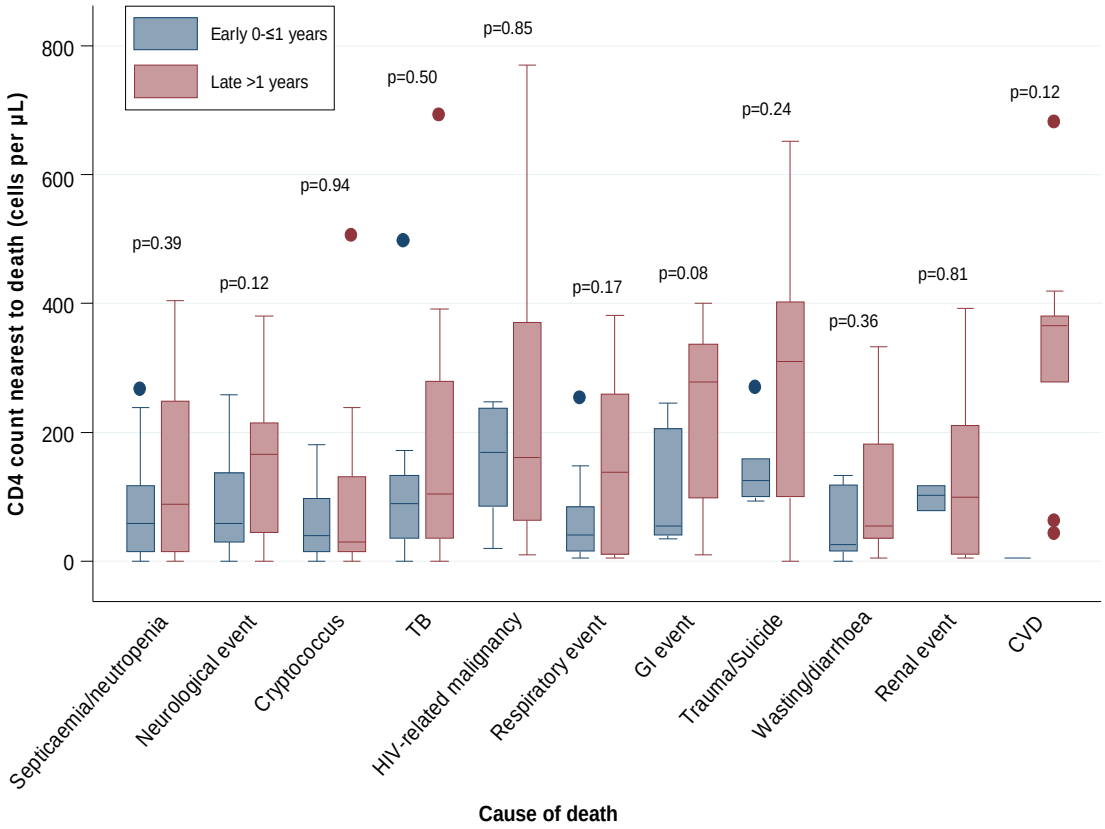
The difference in conclusions drawn about long term effects of CD4 monitoring in Figures 2.3.8 and 2.3.9 is therefore a consequence of the choice of model. In Figure 2.2.6 in methods, df(5) dftvc(5) for overall mortality suggested smaller differences long term than df(4) dftvc(3) in Figure 2.3.9. Thus numbers are too small to reliably refute or support long-term differences; what is clear is that any differences that exist are small.

2.3.3. Role of CD4 count in explaining mortality differences between CD4 monitoring groups

CD4 count is one of the major determinants of mortality risk. In high-income countries, the association between the risk of specific COD and CD4 varies [103, 104], but to date this has not been described in low-income countries. I therefore investigated first whether there was any variation in CD4 count at death by cause and second whether there was any relationship between time-updated CD4 count and mortality (overall and by cause).

Firstly I looked at the latest CD4 count near to the time of death; the median CD4 counts for the 11 individual causes of death can be seen in Figure 2.3.10. Overall, CD4 counts were taken a median of 48 (IQR 26-58, range 0-1085) days before death. There were 70 deaths where the CD4 count was >6 months prior to death and these have been excluded from below.

Figure 2.3.10 Median latest CD4 count at the time of death by cause



Overall, CD4 at death was higher for late vs. early deaths (136 cells/µL, IQR 36-293 vs. 66 cells/µL IQR 19-128, p<0.001). This pattern is the same for many of the individual causes but the power is too low to detect reliably (p≥0.1 in most cases).

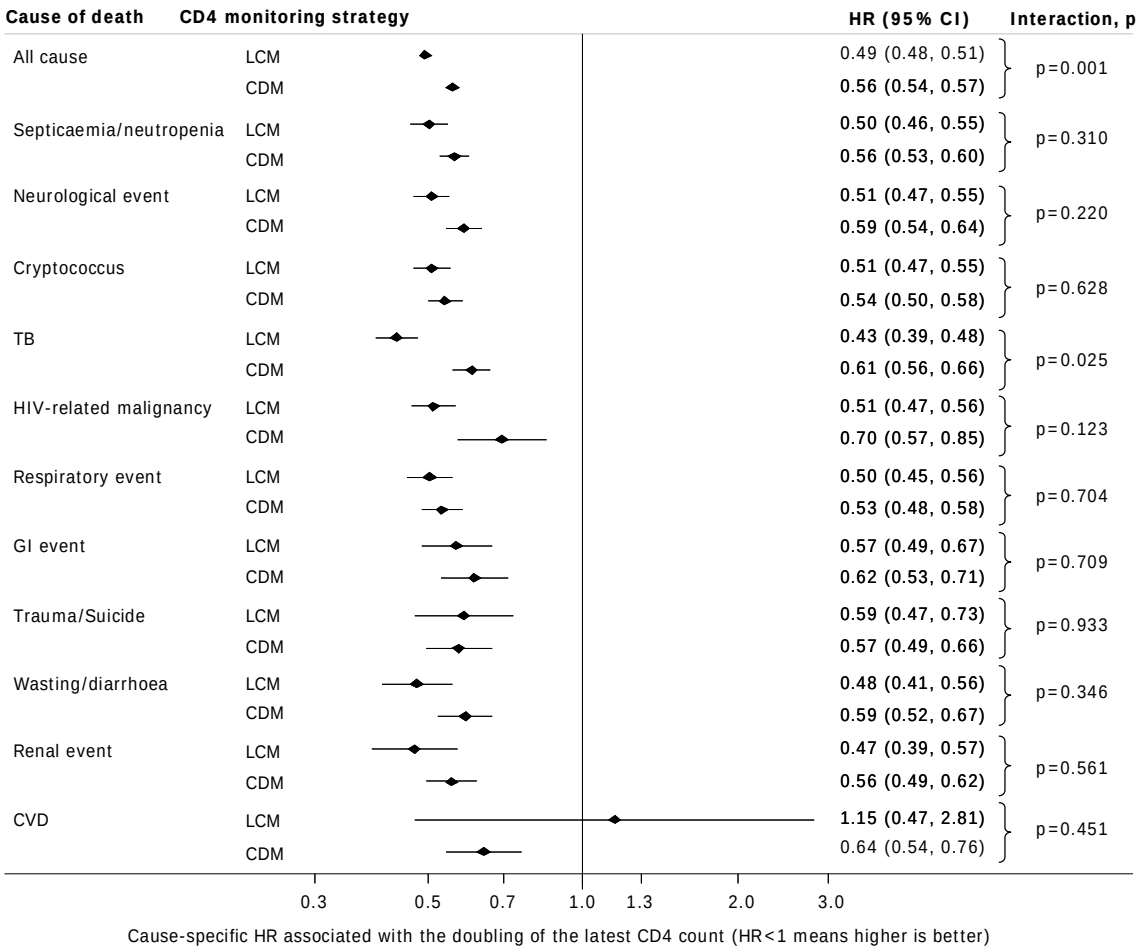
Median CD4 count (cells/µL) was particularly low with very little difference between early vs. late mortality at deaths from septicaemia/neutropenia (0-≤1 years 54; >1 year 85, p=0.39), cryptococcus (0-≤1 year 33; >1 year 26, p=0.94) and wasting/diarrhoea (0-≤1 year 19; >1 year 51, p=0.36). Low CD4 at early deaths for these causes of death might be expected as these are the causes with the earliest peak in cause-specific hazard, so deaths are closer to ART initiation, before CD4 could rise and when patients who are sickest may be overrepresented. However the low median CD4 counts for both early vs. late deaths suggests it is sick and/or failing first-line treatment patients who are dying all the way through the trial and not just at ART initiation. There was also little difference between early vs. late in terms of CD4 count at deaths from TB, HIV-related malignancy and renal events (all p>0.2); however, CD4 counts were slightly higher at HIV-related malignancy deaths than for other causes.

There may be some difference in CD4 counts at deaths from trauma/suicide with a relatively high CD4 count after >1 year on ART. However, this difference is also not significant (p=0.24), possibly due to low numbers of events. For CVD there is a high CD4 count at the time of death for those patients dying >1 year after ART initiation (median 367, IQR 280-380), suggesting this is perhaps not related to immunodeficiency but perhaps a long term effect of ART. As in Table 2.3.2 there is only one death from CVD 0-≤1 years after ART initiation; this patient had a CD4 count of 6 cells/µL at ART initiation and no post baseline counts, so it is difficult to compare the

two treatment periods. Deaths from trauma/suicide and GI events both have median CD4>200 cells/μL >1 year after ART initiation, perhaps suggesting a less strong association with immunodeficiency.

Thus there is some evidence of variation across the different causes in CD4 counts at death. However, this has not assessed the effect of CD4 on those at risk who could, in theory, have had even lower CD4s. To do this I have fitted time-dependent Cox models with interactions between CD4 monitoring group and $\log_2$(CD4). The log transformation has been chosen based on a standard fractional polynomial.

Figure 2.3.11 Cause-specific HRs and 95%CIs for the relationship between specific causes of death and latest CD4



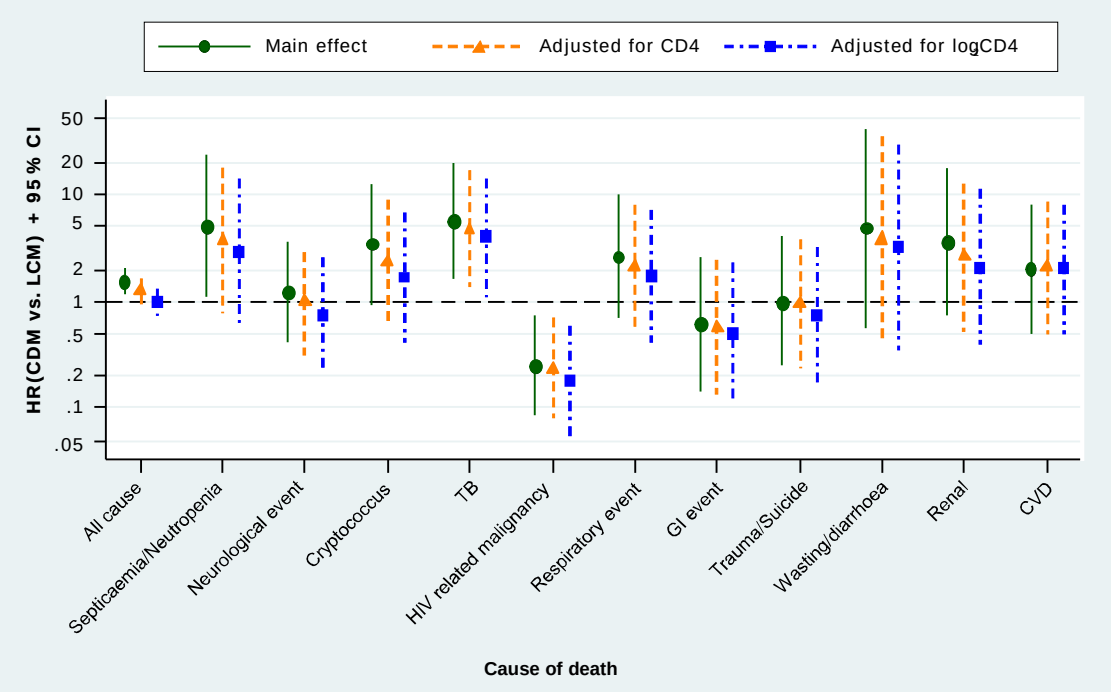
For nearly all causes of death there is a significant decrease in the hazard of death with an increase in CD4 count. Figure 2.3.11 shows that for all-cause mortality a doubling of CD4 count decreases the hazard of death by 51% in the LCM group and 44% in the CDM group. There is strong evidence of an interaction between CD4 and monitoring strategy (p=0.001) in all-cause mortality with slightly more benefit seen from higher CD4 counts in the LCM group. A similar effect was seen for TB where there was good evidence (p=0.025) of an interaction between CD4 and monitoring strategy with greater benefit seen from higher CD4 counts in the LCM group than the CDM group.

As the CD4 monitoring group is the group whose CD4 counts are monitored by the clinicians, this is probably because clinicians modified management to reduce mortality risk on the basis of CD4 counts e.g. by switching to second-line treatment at low CD4 or remaining on first-line at high CD4. This greater benefit of increasing CD4 count in the CD4 vs. no CD4 monitoring group is seen in nearly all other causes of death (other than CVD and trauma/suicide), however due to the small numbers of events in the individual causes of death these differences do not reach statistical significance. There is no evidence of an effect of CD4 on CVD in the LCM group, probably attributed to the fact that there is only 1 death in this group that happens very early on after ART initiation; this is represented by the wide confidence interval.

2.3.4. Time dependent effect of CD4 counts after >1 year on ART in DART

Again as there is no real difference in management between the monitoring strategies up to 1 year after ART initiation, I have looked at the impact of time-dependent CD4 on mortality from 1 year after ART initiation onwards. $\log_2$CD4 has been chosen as the transformation to model as it is a better fit than the linear CD4 model based on AIC. Figure 2.3.12 shows the HRs comparing LCM vs. CDM for first the main effect (as in Figure 2.3.4), then adding CD4 as a time-dependent effect and then finally a transformation of CD4 as time-dependent effect.

Figure 2.3.12 Time-dependent effect of CD4 on CDM vs. LCM from 1 year after ART initiation



After 1 year on ART there is a significant benefit in the LCM group in terms of all-cause mortality (HR (95%CI)=1.50 (1.14-2.00), p=0.004). Adjusting for time-dependent CD4 (using a $\log_2$ transformation) completely removes the treatment effect, HR 0.97 (0.72-1.31). This therefore suggests that the long term differences overall between LCM and CDM are completely explained by differences in the CD4 count. In fact for all causes of death there is a reduction in the benefit of LCM when adjusting for $\log_2$CD4; however for individual causes, these reductions

are small and do not explain much of the overall difference by CD4 monitoring strategies.

2.3.5. ARROW causes of death

Similar analyses have been carried out in the ARROW trial [58]; however due to the low number of deaths seen in the ARROW trial I have only been able to investigate all deaths and not individual causes.

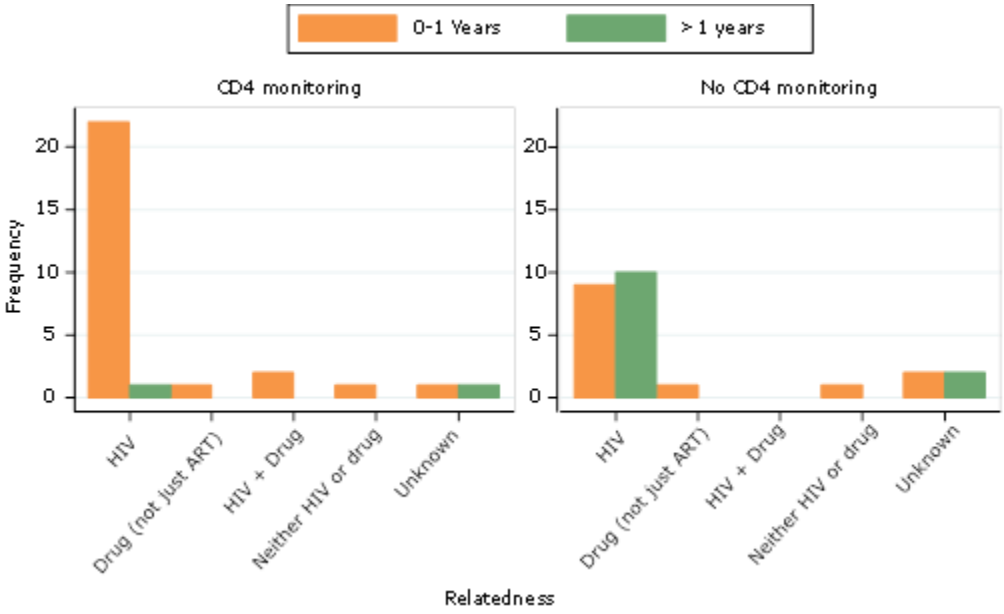
In the ARROW trial there were 4685 child years (CY) of follow-up between 15th March 2007 and 16th March 2012 (planned trial closure) with a median follow-up of 4 years. A total of 54 patients died on or before 16th March 2012. Overall mortality was 40/1206 (3%) in the first year on ART and 14/1157 (1%) subsequently. This equates to 3.4/100CY in the first year of ART and 0.4/100CY thereafter, that is, substantially higher overall mortality in the first year on ART as seen in the DART trial. This was also clear in both the monitoring strategies; with 27 deaths (4.7/100CY) in the CD4 monitoring group (LCM) and 13 (2.2/100CY) in the no CD4 monitoring group (CDM) in year 1; and 2 (0.1/100CY) LCM and 12 (0.7/100CY) CDM deaths subsequently (for year 0-≤1 vs. year>1 p=0.002 in LCM and p=0.003 in CDM respectively) (Table 2.3.3).

Table 2.3.3 Deaths by time on ART and monitoring strategy (ARROW)

	LCM (n=600)		CDM (n=606)		Total	
	0-≤1 yrs	>1 yrs	0-≤1 yrs	>1 yrs	LCM	CDM
Total patients	600	569	606	588	1206	1157
Deaths All	27 (5%)	2 (<1%)	13 (2%)	12 (2%)	29 (2%)	25 (2%)
Cause						
HIV	22	1	9	10	23	19
Drug (not just ART)	1	0	1	0	1	1
HIV and drug	2	0	0	0	2	0
Neither HIV or drug	1	0	1	0	1	1
Unknown relationship to HIV/drug	1	1	2	2	2	4

As in DART, regardless of time on ART (exact p=0.5) and regardless of CD4 monitoring strategy (p=0.7), the majority of deaths were judged primarily HIV related (31/40 (78%) early vs. 11/14 (79%) late). Very few deaths were judged primarily related to drug (2 early vs. 0 late). There were only 6 deaths where cause of death could not be ascertained (Figure 2.3.13).

Figure 2.3.13 Deaths by time on ART and monitoring strategy in ARROW



Due to the low numbers of deaths in the trial overall I have simply looked at the individual causes of death in an exploratory manner. The individual causes of death are outlined in Table 2.3.4. Of the 40 deaths that occurred in the first year the most common cause of death was septicaemia/neutropenia (13 (33%) deaths) followed by respiratory events (11 (28%) deaths). This is slightly different to that of the DART trial where respiratory events were a less common cause at only 7% in the first year, but likely reflects the importance of pneumonia as a key infection in childhood. In ARROW respiratory events became the major cause of death after 1 year on ART (5 deaths, 36%); however wasting/diarrhoea was a more common cause of death than septicaemia/neutropenia after 1 year on ART (3, 21% vs. 1, 7% deaths respectively).

Table 2.3.4 Detailed cause of death by monitoring strategy and time on ART (ARROW)

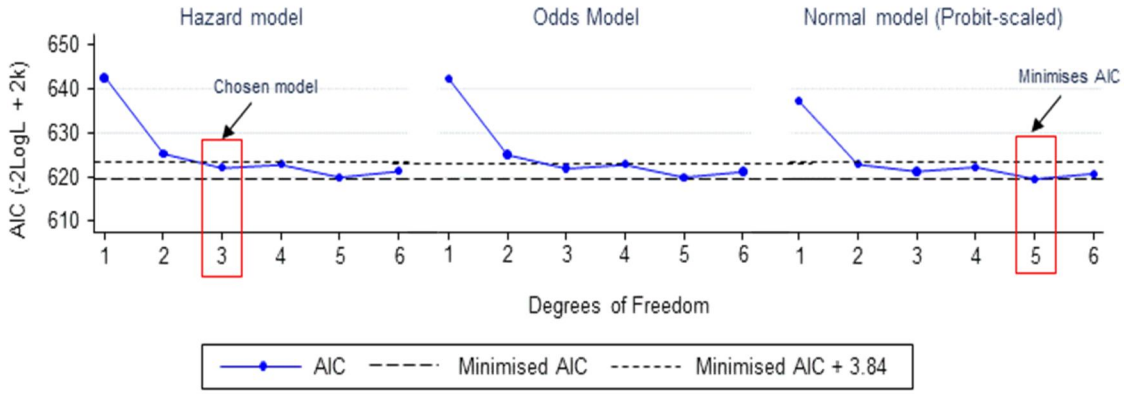
	CDM		LCM		Total	
Cause of death *	**0-≤1 yrs**	**>1 yrs**	**0-≤1 yrs**	**>1 yrs**	**0-≤1 yrs**	**>1 yrs**
Respiratory event	4 (31%)	3 (25%)	7 (26%)	2 (100%)	11 (28%)	5 (36%)
Septicaemia/Neutropenia	4 (31%)	1 (8%)	9 (33%)	0 (0%)	13 (33%)	1 (7%)
Wasting/Diarrhoea	0 (0%)	3 (25%)	3 (11%)	0 (0%)	3 (8%)	3 (21%)
Neurological event	2 (15%)	0 (0)	3 (11%)	0 (0%)	5 (13%)	0 (0%)
Unknown	2 (15%)	1 (8%)	2 (7%)	0 (0%)	4 (10%)	1 (7%)
Tuberculosis (TB)	0 (0%)	2 (17%)	0 (0)	0 (0%)	0 (0%)	2 (14%)
Cardio-vascular disease (CVD)	0 (0%)	1 (8%)	1 (4%)	0 (0%)	1 (3%)	1 (7%)
Anae/Pan/Thromb/Neut†	0 (0%)	0 (0)	1 (4%)	0 (0%)	1 (3%)	0 (0%)
HIV-related malignancy	0 (0%)	1 (8%)	0 (0)	0 (0%)	0 (0%)	1 (7%)
Trauma/Suicide	1 (8%)	0 (0)	0 (0%)	0 (0%)	1 (3%)	0 (0%)
Malaria/Cholera	0 (0%)	0 (0)	1 (4%)	0 (0%)	1 (3%)	0 (0%)
Total	**13**	**12**	**27**	**2**	**40**	**14**

*Ordered by total number of deaths
†Anaemia/Pancytopenia/Thrombocytopenia/Neutropenia with no sign of fever

2.3.6. Modelling the hazard of death in ARROW

As in the DART trial, I used flexible parametric models to estimate the overall mortality risk in the ARROW trial. I have used the same methods as outlined in section 2.2.4 and have started by assessing which model will be the 'best' fit to the data. Figure 2.3.14 shows the AICs for all the different models that could be used.

Figure 2.3.14 AICs for PH, proportional odds and probit-scaled models with various dfs for overall mortality

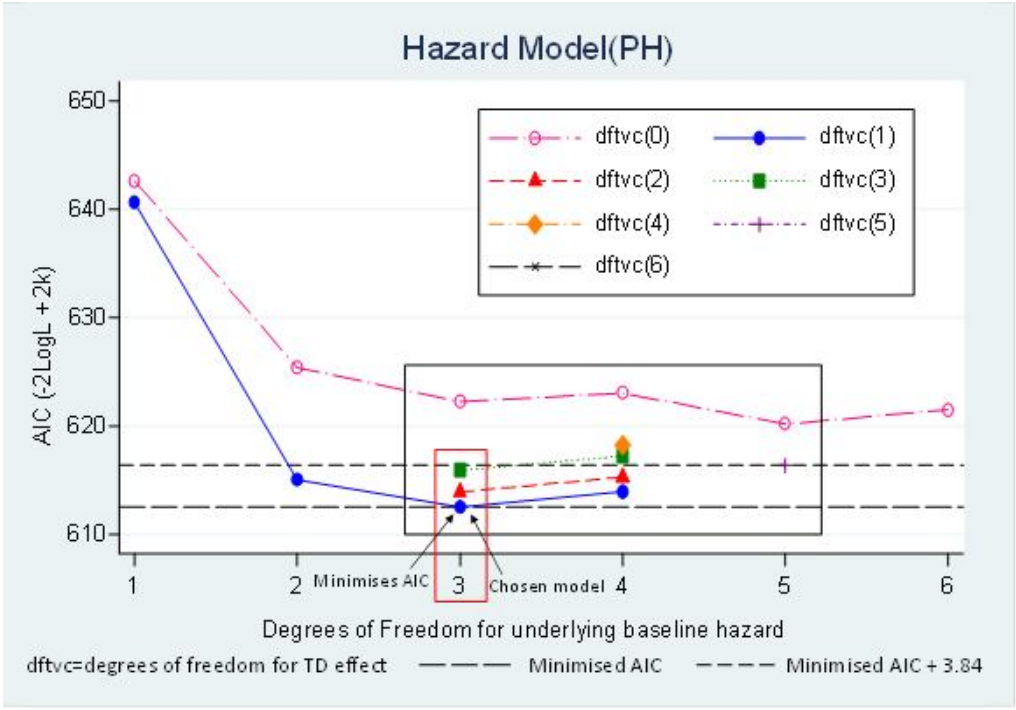


This shows that the best fit to the data (the model that minimises AIC) is the probit scaled model with 5 df; however as in DART I have chosen the hazard model for ease of understanding for clinicians. Selecting the most parsimonious PH model, within 3.84 units of the lowest AIC, the chosen model is therefore the hazard model with 3 df (PH(3)). However, this overall model ignores a major imbalance in year 1 deaths (27 LCM vs. 13 CDM) which can only be due to chance as management was essentially the same during the first year. In contrast, post-one-year there were 12 deaths in CDM vs. 2 deaths in LCM, i.e. substantial crossing of hazards, and so a simple model including only a proportionate effect of CDM vs. LCM in ARROW will average out these two effects and not be meaningful.

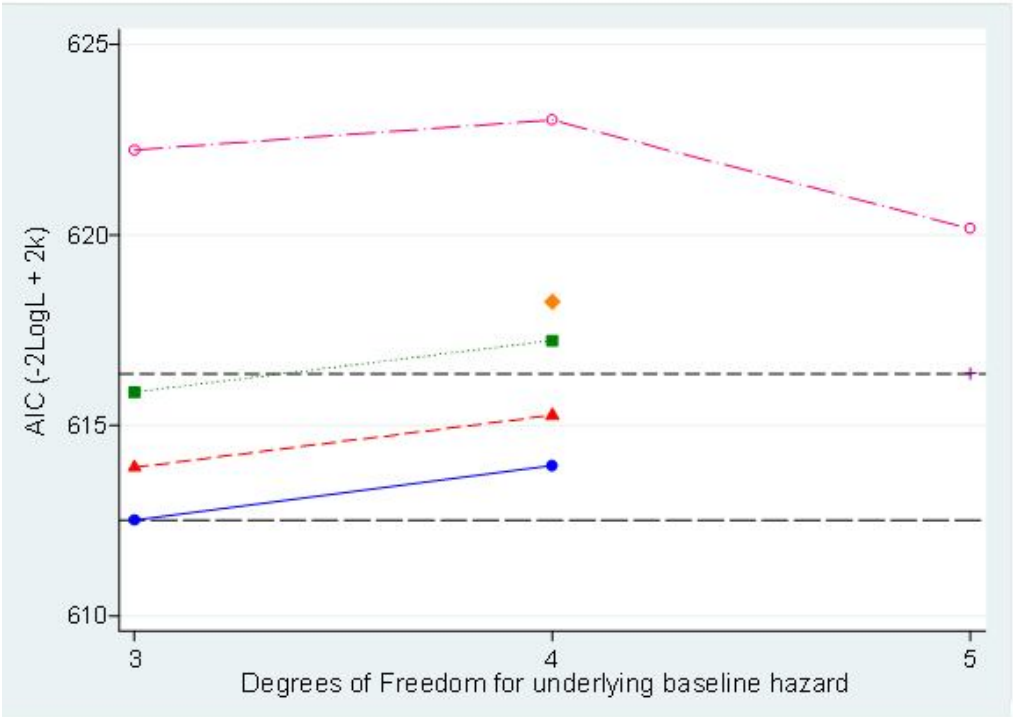
As in DART I therefore need to use the model where I can give a different number of degrees of freedom for the baseline hazard and a time-dependent effect of monitoring strategy. I assessed the best model to fit for the time-dependent effect using stpm2 and have fitted different dftvcs and assessed the fit using the AIC. Figure 2.3.15 shows the AICs for the different models when including monitoring strategy as a time-dependent effect using the PH model.

Figure 2.3.15 AICs for the PH model with varying df and dftvc (ARROW)

a: All df

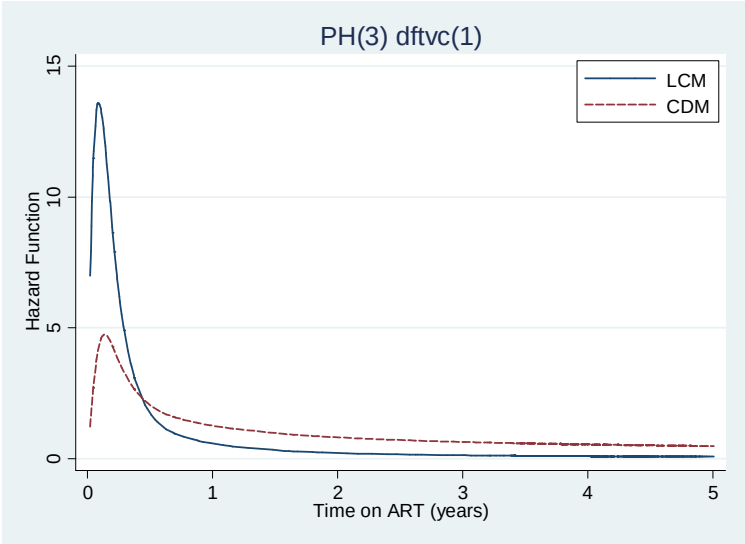


b:df(3), df(4), df(5)



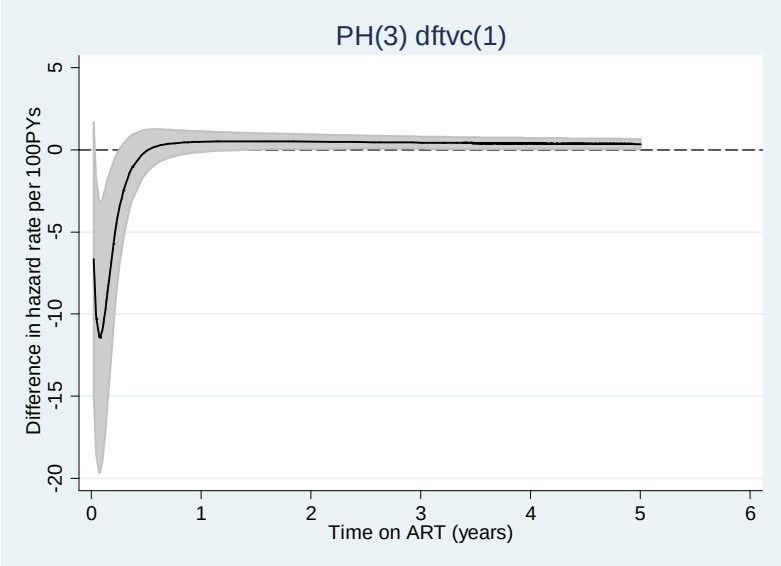
The model that minimises the AIC is with df(3) for the baseline hazard and dftvc(1) for the time-dependent effect of monitoring strategy (AIC=612.5839) and I have chosen to use this model.

Figure 2.3.16 Predicted hazard rate (per 100PY) for all-cause mortality in ARROW by monitoring strategy



As seen in the DART trial, there is a peak in hazard during the first few months of time on ART. As in DART it is likely that the timing of this specific peak is due to the sensitivity of the models to when the first deaths occurred, but that it genuinely reflects initial higher mortality risks shortly after starting ART. This model also shows that this peak in hazard is in fact greater in the LCM arm early on but that the longer term hazard is reduced for LCM. As in DART there is no difference in the monitoring strategies between LCM and CDM before 12 weeks and little difference over the first 12 months as switch to second-line was discouraged before then: therefore this early difference seen in Figure 2.3.16 is likely to be by chance.

Figure 2.3.17 Difference in hazard rate (CDM-LCM) per 100PY for overall mortality in ARROW



The conclusion here is similar to that in DART. Any long term effects of CD4 monitoring that exist are small (estimated difference in hazard rate per 100PY at 5 years in ARROW is 0.35 (95% CI 0.04-0.66) compared to 0.94 (95% CI 0.35-1.53) in DART).

2.4 Discussion

In this analysis I have assessed how the overall hazard of death and the hazard of death for specific causes changes over time from ART initiation. The work in this chapter extends the understanding of changes in mortality risk on ART in low/middle-income countries. A meta-analysis by Gupta et al. [94] found 14 studies (11 in low-income countries which are included in my review above [71, 76, 77, 80, 82, 84, 85, 87, 89-91] and 3 in high-income countries [105-107]) that reported cause-specific mortality up to one year after ART. They found that TB (5-44%), wasting (5-53%), advanced HIV (20-37%) and chronic diarrhoea (10-25%) were the most common causes of death with sub-Saharan Africa reporting the highest all-cause mortality at 12 months. However, this meta-analysis did not look at deaths beyond one year so did not look for changes in mortality risk over time. Similar to the studies in this meta-analysis, I also found TB to be one of the most common causes of death in the first year after initiating ART, however wasting and diarrhoea were not so common causes of death in DART. In contrast to this, Grinsztejn et al. [72] found that in Baltimore (a high income country) the most common causes of death were non-AIDS defining illnesses and no death occurred because of TB.

Most studies that have investigated rates of death over time have looked in specific time intervals, using Poisson regression [83, 104] and have not considered causes of death. A few studies have investigated the change in hazard over time in overall mortality using smoothing non-parametric hazard estimates from Cox regression [71, 72, 74, 93, 94] or Weibull piecewise models [79, 92, 108]. In contrast to the relatively strong assumptions of e.g. parametric Weibull models, the method I have used here, FPMs, is a much more flexible approach which allows for the continuous changing hazard without over fitting change points to the specific data.

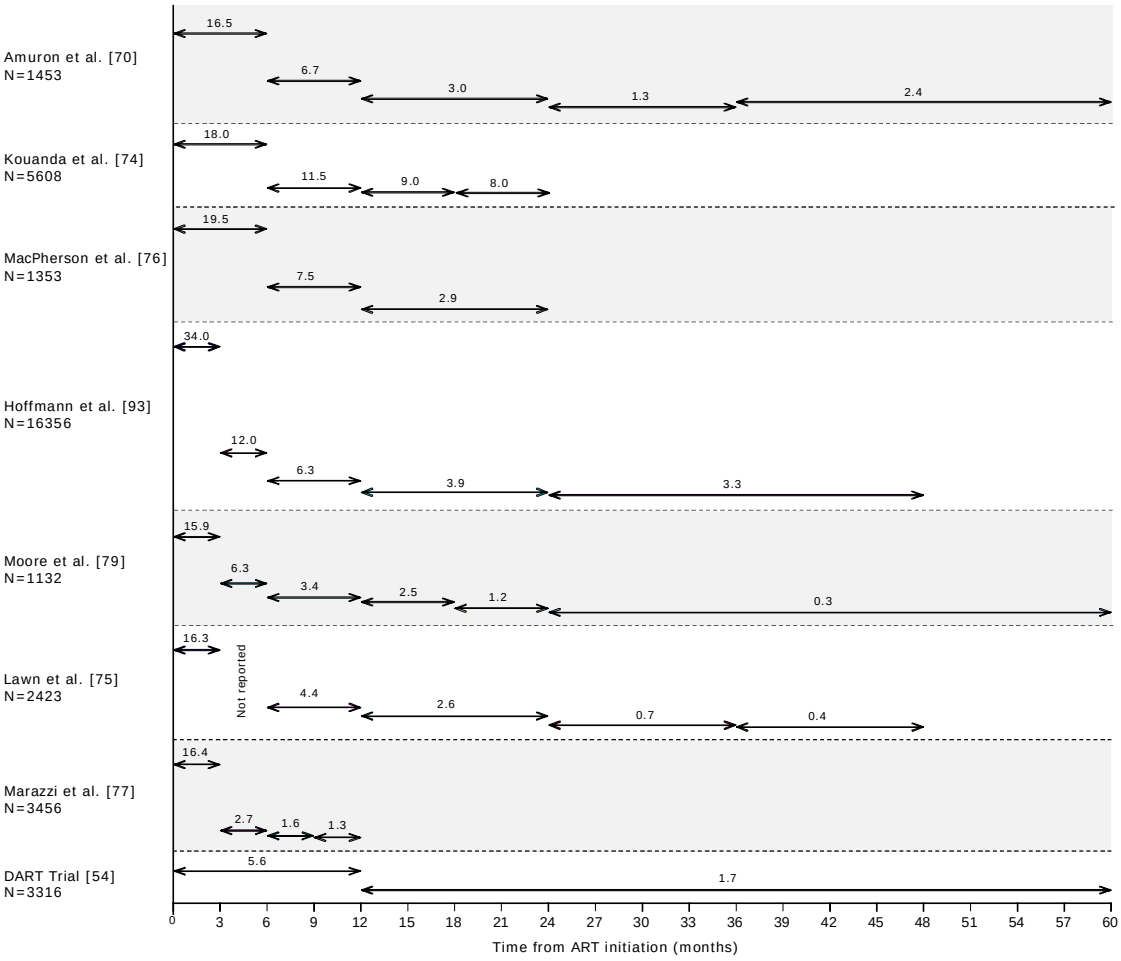
Mortality rates

I started by looking at the simple distribution of deaths over time on ART and from different causes. In DART about half (47%) of the deaths occurred in the first year after ART initiation similar to the studies identified in my literature review. For example, Etard et al. [71] showed that 51% of all deaths occurred in the first 12 months after ART initiation in a Senegalese cohort. Mzileni et al. [80] reported that 95% of deaths occurred in the first year which is considerably higher than that seen in DART or by Etard et al. This could be attributed to the fact that 91% of patients in this study had a CD4 count <200 cells/μL at baseline: but then so did DART (all were <200 cells/μL) and the median CD4 at baseline for Etard was 128 cells/μL. This difference is more likely because the follow-up for the Mzileni study was only 30 months, therefore proportionately more deaths will be in the first year. Most of the studies in my literature search reported the percentage of deaths in the first 3 months rather than the first year, all of which were higher than DART at around 65-80% of deaths occurring in the first 3 months. It is difficult to compare proportions of deaths which occurred in different time intervals since these will depend on the duration of follow-up which varied considerably across studies.

As outlined in the introduction to this chapter, Braitstein et al. [92] showed that mortality rates within the first months following ART initiation were higher in sub-Saharan Africa than in industrialised countries (6.4% vs. 1.8% at year 1). As seen in DART they also showed that the mortality rates in low-income countries fell within the first few months and also found that by around 4-6 months, mortality rates approached those seen in high income countries. They suggested that despite the more advanced immunodeficiency seen in low-income countries, the immunological response to ART is similar in both low and high income countries. Grinsztejn et al. [72] compared the early mortality pattern and causes of death among patients starting HAART in Brazil and the US; they showed that the decreasing trend in mortality rates that occurs in low-income countries was not present to the same degree in high-income countries, although there was still a decrease in mortality. It is likely that the lower mortality rates seen in higher income countries are due to the higher baseline CD4 counts and how accessible antiretroviral treatment is. Any lower mortality rates seen in low-income countries are likely due to those low-income countries that take part in trials (where treatment and care are more accessible), as generally, lower income countries have higher mortality rates, as noted by Moh et al. [78].

Figure 2.4.1 shows that overall mortality changes in DART were consistent with 7 studies of a similar sample size identified by my literature search. Long term rates differed across all studies although they were still declining over time and were lower after 1 year of ART as in DART. Early and long term rates were highest in Hoffman [93], but early (0-3 month) mortality rates were high and relatively similar for all other studies (Amuron [70], MacPherson [76], Kouanda [74], Moore [79], Lawn [75] and Marazzi [77]). Rates were lower for DART (11.0/100PYs) which may be due to the trial setting. In Uganda, Amuron et al. had baseline CD4 counts of 108 cells/µL and in South Africa MacPherson had baseline CD4 counts more similar to that in DART of just 93 cells/µL so I would expect mortality rates to be similar to those in DART if not better. In Burkina Faso, Kouanda et al had a median baseline CD4 of 124 cells/µL which is higher than in DART, as are the baseline CD4 counts in South Africa for Hoffman et al. who had a median baseline CD4 of 127 cells/µL. You might expect mortality rates to be lower in DART than in these examples because of the lower baseline CD4 counts in DART, however I think due to the trial setting this is not the case.

Figure 2.4.1 Changing mortality rates per 100PY during time on ART in low/middle-income limited countries (including DART for comparison)*



*Example of 7 studies shown for illustration and DART for comparison. These studies were chosen as they gave actual details of individual rates over time and had a similar sample size to that of the DART trial.

Mortality rates in Amuron et al. [70] and MacPherson et al. [76] may be higher than some of the other studies as there was active tracing of those lost to follow-up in these studies resulting in better ascertainment of deaths. Similarly, in Hoffman et al [93], the higher rates might be due to better ascertainment of deaths through the linkage to the vital statistics registry. However in DART, mortality ascertainment was near complete so this seems unlikely to explain their higher death rates compared to DART despite similar or higher pre-ART CD4s. Loss to follow-up without confirming death could inflate survival estimates, and reduce mortality estimates in cohorts compared to a trial, the opposite effect to that seen here. Assuming all those lost to follow-up were deaths could potentially make mortality look higher than it actually is. However, this approach puts a maximum band on the greatest possible mortality.

Differences in monitoring could be another explanation for differences in absolute mortality rates between these studies; however, in most studies, monitoring seemed to be regular and included CD4 and viral loads rather than just clinical monitoring so this is an unlikely reason for higher mortality rates compared to DART. One possible question is whether the monitoring was regular enough? Hoffman et al [93] monitored patients using CD4 and HIV RNA at baseline, after 6 weeks on ART and then 6 monthly thereafter. In DART patients were monitored every 12 weeks and were also reviewed by a nurse every 4 weeks – this more regular clinical monitoring might have reduced the mortality rate in the trial. In DART clinical monitoring without CD4s was inferior to CD4 monitoring in terms of mortality so less regular monitoring could potentially increase mortality rates, although this would be expected to have a greater impact after 1 year on ART, not early after starting treatment. Some recent work by the DART team [109] suggests that 12-weekly and 24-weekly CD4 monitoring have similar mortality outcomes, so probably the different frequency of measurement is also not a major driver for mortality differences between these other studies and DART.

In contrast other studies such as Moh et al [78] had very low mortality rates likely due to the higher baseline CD4 counts of 252 cells/μL, and may have underestimated mortality due to there being no active search for patients lost to follow-up. However, their long term rates are very similar to those in DART.

Another question is whether baseline factors other than CD4 differed enough to lead to differences in mortality between DART and these other studies. Hoffman et al. showed associations between pre-ART characteristics and long-term mortality for higher WHO stage, lower CD4 count, higher HIV RNA and TB symptoms. However, baseline characteristics would be expected to provide less information the further from baseline deaths occur. Supporting this lesser impact of baseline cofactors on long-term risk, in rural Uganda Moore et al. [79] found that baseline CD4 cell counts and WHO clinical staging were less predictive of late mortality adjusting for time-updated CD4.

One other important aspect may be that diagnostic tests, second-line ART, hospitalisations and concomitant medications were all available and provided free of charge in DART, whereas patients typically bear the cost of these themselves in other studies. Free access may therefore have lowered mortality rates in DART compared to other studies.

Mortality rates in children appear to be similar to those in adults with there being high early mortality in ARROW, which has also been seen in paediatric studies that took place in hospitals in Malawi [68] and Nairobi [69]. As in adults the highest mortality was seen in the first 0-4 months and is likely due to the low CD4 counts at ART initiation. The key interest in paediatrics is preventing these early deaths and making sure children get the treatment they need as soon as possible, and preventing children getting HIV in the first place by increasing access to antiretroviral therapy to prevent mother to child transmission.

Modelling the changing hazards

Figure 2.4.1 also illustrates the arbitrary timepoints considered in mortality analyses to date. Whilst all these studies show that risk is changing mostly in the first year after ART initiation, there is considerable variation in estimates of when this is happening, because of different categorisations used which assume that there is an abrupt change in the hazard of death at specific timepoints which does not make sense clinically. Etard and Hoffman [71, 93] also presented smoothed estimates of instantaneous hazard of death from time on ART; this appears to show change points in hazard of death at 9 months in Etard and in Hoffman at 9 months and 3 years (not stated explicitly: estimated from curve). Using a different method of piecewise exponential modelling, Moore et al. found evidence for two change points in mortality risk, one at 3 months and one at 10 months. In addition to the DART results shown in Figure 2.4.1, using the flexible parametric modelling I have shown that there is a very rapid decline in hazard around 30-60 days which was not apparent from previous categorisations. The varying estimates found by Etard and Hoffmann show that any modelling strategy that fixes change points on the basis of observed data runs the risk of over fitting, as it does not seem plausible that genuine changes in risk occur at different times in different studies. The different change-points also make comparing results from different studies more difficult. However, even though the FPMs are able to model the hazard continuously and they do not abruptly cut the data they are not without their limitations. As I found when modelling the individual causes of death, the models appeared to be very sensitive to when the first event occurred and the timing of subsequent events. I tried to overcome this by modelling from 1 year after ART initiation; however I still found instability in the boundaries of the models even after 1 year of ART.

Causes of death

Deaths in DART were reasonably consistent with that of the 30 studies in my literature search which found the most common causes of death to be TB, KS and anaemia. Only one study looked at causes of death in the first year separately and they found there to be no difference in the causes of death in the first 12 months compared to thereafter, supporting on-ART deaths being primarily due to HIV from failure of treatment. Of the deaths that occurred during the first year after ART initiation in DART, 52% were considered primarily to be HIV-related, 5% were related primarily to drug and 12% were neither related to drug or HIV (remainder being unknown). By specific cause: septicaemia/neutropenia (18%), neurological events (14%), cryptococcus (11%), TB (7%) and respiratory events (7%) were the most common causes in year one. These results are further supported by data from a prospective cohort study in Uganda which showed TB, cryptococcus and diarrhoea to be the most common causes of death [70]. In the meta-analysis by Gupta et al. [94] it is likely that TB and other opportunistic infections contributed to the substantial proportion of deaths due to wasting.

Over a median 4.9 years follow-up in DART 51% of deaths were primarily HIV-related. Over total follow-up I found that septicaemia/neutropenia was the most common cause of death (12%) followed by neurological events (10%), cryptococcus (8%) and then TB (8%), HIV related malignancy (7%) and respiratory disease (6%). I thus found a higher proportion of HIV-related deaths than the 32% AIDS-related deaths reported for the EUROSIDA cohort by Kowalska et al. [83] over 0-10 years on ART. However, this cohort is from high-middle income countries rather than low-income countries as in DART; and some deaths judged primarily HIV-related in DART were not from AIDS. Further, Kowalska et al. [83] showed that the 68% of all deaths that were non-AIDS-related, were non-AIDS-related infection in 9% (counted as HIV-related in my analysis), liver malignancy 14%, non-AIDS-defining malignancies 10%, CVD 9%, violent death (trauma/suicide) 7% and other 7%, with 12% of all deaths of unknown cause. They also found no increase in the risk of non-AIDS deaths with prolonged exposure to ART. These non-AIDS causes of death occurred rarely in DART, suggesting that long-term outcomes in low/middle-income countries might be quite different. Moore et al. found that in Uganda TB was the most common clinical condition associated with death, followed by candida disease, cryptococcal disease, pneumonia and Kaposi's sarcoma. Zachariah et al. [82] also reported candida to be a common cause of death. Kouanda [74] and Macpherson [76] also found TB to be the most common cause of death. Grinsztejn [14] found that in Brazil AIDS defining illness (62%) was the most common cause of death, then TB 32%, cryptococcus 12%, Kaposi's sarcoma 12%, and HIV related malignancies 18%. The fact that candida was also among the most commonly reported causes of death suggests there may have been issues with ascertainment, since candida is typically non-fatal unless it is an extremely rare bloodstream fungal infection. Ascertainment of death in DART, in theory, is better, as it was a randomised controlled trial with an independent Endpoint Review Committee; however there was still a high proportion of unknown deaths illustrating the challenges of reporting causes of death amongst HIV individuals in low/middle-income countries.

It is difficult to compare results across studies due to different ways of classifying deaths. Differing CD4 at ART initiation may explain some differences but there are other possible explanations. For example patients in Kowalska et al. were from Europe, Israel and Argentina with access to VL monitoring and many more drugs than are available in Africa. As a consequence they would likely switch earlier for failure, possibly repeatedly, and therefore might be more at risk from "serious non-AIDS" type causes of death such as ageing and long-term inflammation-related effects of HIV. In contrast, in low/middle-income countries, switch for first-line failure is typically later, based on immunological/clinical criteria as there are only two regimens available. Thus more deaths may be HIV-related, even in the long term, if patients die on first-line before they are able to switch – or die shortly after switch as a consequence of acute severe illness around switch.

In the DART trial, looking overall and at individual causes of death I have been able to confirm the prolonged benefit of ART with the majority of the most frequent causes showing a significant decline during time on ART. Assessing the causes with lower event rates could lead to definitive conclusions which the data may not really be able to support and so I have not assessed any causes with less than 10 deaths overall. It is however, important to bear in mind the low event rates for some causes within the subgroups for monitoring strategy or years on ART. Two causes of death showed somewhat different patterns and are discussed in more detail here. First, trauma/suicide had a peak in hazard of death at around 6 months after ART initiation and like some of the other causes declined rapidly to around 2 years. However, in contrast it then started to increase steadily, possibly stabilising at 6 years. Evidence suggests that risk for suicidal behaviour increases during the initial weeks following a diagnosis of HIV and then declines as patients adjust to their HIV status. However, as patients' health and quality of life decline, risk of suicide may again increase [110] particularly among middle-aged and older patients, who frequently experience poorer health-related quality of life with disease progression off ART. However, whether this would be the same on longer-term ART is unclear, although given the low rates of mortality might not be expected. These results may suggest the need for long-term psychological support in patients on ART. However, an alternative explanation is that with improving health, patients on long-term ART return to work and experience higher levels of road traffic accidents, falls or other unintentional injuries; in particular road traffic accidents are the 13th most common cause of death in the African region (WHO cause-specific mortality 2008, update from 2011) [64, 111].

I found there to be no overall change in the risk of death from HIV-related malignancies, meaning that post 1 year it was the most common cause of death. Given the timescales these deaths are most likely due to pre-cancerous events that occurred prior to ART initiation. Cervical cancer was the main HIV related malignancy but only led to death (n=8 deaths) in the CD4 monitoring group. Cervical cancer can take a long time to develop. It is a result of a viral infection, human papillomavirus (HPV), which is actually very common in all women and is a virus that most women can get rid of. However, in those with immunodeficiency (e.g. patients in DART) this is not always the case. The risk of malignancy will increase as patients live longer without ART, which is why cervical cancer is an AIDS-defining illness according to the Centre for Disease Control (CDC) definitions. How incidence changes with increasing time on ART is unclear, but it is possible that risks could remain high for first few years on ART if pre-cancerous changes have already occurred before ART initiation. Kaposi's sarcoma (KS) is similarly caused by the human herpes virus 8. Again people with a weak immune system cannot fight this virus and therefore KS is more likely to develop in those off ART; it is a very common malignancy in those with HIV. In DART 5 LCM vs. 1 CDM deaths were from KS. It seems unlikely that these were caused by the CD4 monitoring strategy, but instead are plausibly due to the fact that DART patients had very low CD4 counts pre-ART and were therefore more vulnerable towards these virus-driven cancers which had probably started to manifest within the first few months of treatment before CD4 reconstitution could occur.

The most common causes of death in ARROW were septicaemia/neutropenia in the first year after initiation of ART; thereafter respiratory events became the major cause of death which is consistent with Fassinou et al [67] who also found respiratory events to be the most common cause of death in three out of nine children who died**.**

CD4 count

The vast majority of studies have shown associations between CD4 count and mortality, with both baseline (pre-ART) and time-updated CD4. However, some studies in low/middle-income countries such as Moore et al. [79] and Hoffman et al. [93] have found weaker associations between pre-ART CD4 and early mortality, and instead stronger associations with WHO clinical stage. As pre-ART CD4 is highly associated with WHO stage, this may reflect confounding and over fitting to some specific aspects of these datasets, or to unreliably measured (i.e. inaccurate) CD4 counts.

In high-income countries Achhra et al. [112] found that time-dependent CD4 independently predicted all-cause mortality and non-AIDS deaths in the SMART trial; but not fatal or non-fatal cardiovascular events. The CASCADE collaboration [103] also found an association between cause of death and time-dependent CD4 for all causes except unintentional deaths. In addition data from the D:A:D cohort [104] showed that higher CD4 cell counts have a strong association with a lower risk of mortality from all causes, except, like Achhra, for deaths from CVD which had a marginal association. Marin et al [113] also showed there to be no association between CD4 and CVD deaths. Despite these findings being from high-income countries, they are similar to my results from DART, suggesting the impact of CD4 counts is the same wherever you are if you have HIV and are on ART. These results are further supported by Lawn et al. [75] who showed in 2423 patients on ART in South Africa that the relationship between time-updated CD4 cell counts and mortality rates was extremely strong and that the absolute CD4 cell count at any given time point was the key determinant of mortality risk. My question was slightly different, namely whether the strength of this relationship varied by different causes of death and CD4 monitoring strategy. In DART I have shown that for all-cause mortality and major individual causes of death, including those that are not traditionally thought of as immunodeficiency-related (with the possible exception of CVD deaths), the hazard of death declines similarly and significantly with increasing CD4 count. I have also shown that the associations between CD4 and mortality are similar whether patients were monitored with or without CD4 counts, apart from TB where in the CD4 monitoring group there was a stronger association between cause of death and CD4 than in the no CD4 monitoring group. After assessing the change in risk of TB death as CD4 increases, there was a quicker decline in the risk of TB death in the CD4 monitoring group as CD4 increases above ~50 cells/μL. This could be due to one of two reasons – first clinicians may keep patients in the CD4 monitoring group with TB and with higher CD4s on failing first-line regimen while they treat TB, as this has fewer drug-drug interactions and may allow more successful TB treatment. Second, in the no CD4 monitoring group they do not know the CD4 so may assume it is low and switch the patient to second-line treatment which makes the treatment of TB more complicated.

Strengths and limitations

Most studies I have found have been electronic databases of ART programmes whereas the DART study has the advantage of being a randomised controlled trial with more consistent and complete data collection, particularly regarding death ascertainment and adjudication of cause of death. With over 3000 patients randomised, DART is the largest treatment randomised controlled trial in Sub-Saharan Africa. In addition to this trials tend to have a more consistent drug supply (i.e. no stockouts), they have access to information on concomitant medication and diagnostics and therefore have good 'on ART' estimates of mortality. The DART trial had a loss to follow-up (LTFU) of just 7% whereas Hoffman et al. reported a LTFU of 23% during observation of patients over approximately two years; this was more than 3 times that of the DART trial over 5 years. Moore et al. [79] did not report any LTFU so I am unable to make any comparison between DART and another trial but they did have fewer patients than in DART. With this low LTFU in DART I can be reasonably confident that mortality was ascertained well which is a strength for the study. The presence of an endpoint review committee (ERC) allowed independent assignment of COD, which was also independent of CD4 in DART, this allowed consistent review of deaths across all centres.

One limitation of the work carried out here is that I have not looked at the changing effect of baseline predictors over time which may have given more insight into the changing hazard over time. The other major limitation was the relatively low numbers of deaths from each specific cause of death, despite the size of the DART trial, and even fewer in children in the ARROW trial.

2.5 Conclusions

Deaths were most commonly related to HIV disease both during the first year after ART initiation and subsequently, probably due to advanced immunodeficiency at ART initiation and as a consequence of fairly late detection of failure of first-line therapy respectively. A recent paper from the IeDEA and ART-CC collaborations [114] suggests that ongoing presentation with low CD4 will mean these patients will continue to be a substantial proportion (~25%) of those initiating ART over the next decade. HIV-related deaths after 1 year on ART were also the main driver of long-term differences between CD4 vs. no-CD4 monitoring strategies. The contribution of HIV-related-malignancies, likely triggered by pre-cancerous events occurring before ART initiation to longer-term ART mortality highlights the importance of earlier HIV diagnosis and access to care. Low but increasing risks of deaths from trauma/suicide in adults highlight the importance of long-term psychosocial support and empowering patients to manage their own treatment, as highlighted in Chapter 1.

Chapter 3: Effect of Treatment Interruptions in DART and ARROW

3.1. Introduction

3.1.1. Background

Antiretroviral therapy (ART) has been widely available in low/middle income countries (LMIC) for several years [115] and adherence to treatment in sub-Saharan Africa, as reported by patients, pharmacies, medical records and Medication Event Monitoring, is relatively high compared to high-income countries [116]. However, structural factors such as supply chain interruptions or stockouts, employment migration and conflicts can disrupt adherence in these settings [117, 118] even if the individual has personal intention to adhere. The unstructured nature of such interruptions can make assessing their impact difficult. Several studies have shown that unplanned interruptions like these are common; studies in LMIC [119, 120] and high-income countries [121-125] have shown they contribute to worsening patient outcomes, but most have looked at relatively short-term effects (average follow-up 24 months for references cited).

In the early 2000s structured treatment interruptions (STIs) were widely investigated as a mechanism to reduce toxicity and ART costs. Several randomised trials demonstrated conclusively that such STIs significantly increase the risk of HIV-related events and serious non-AIDS events not traditionally thought to be HIV-related, and they are no longer recommended [126-128]. Long-term follow-up of these randomised trials provides an unbiased mechanism of assessing longer-term effects of treatment interruptions since randomisation ensures balance across different groups, in contrast to possible difficulties with time-dependent confounding in assessing the effect of unplanned treatment interruptions. For example, the SMART trial [129] investigated stopping ART if CD4 was >350 cells/μL and restarting if <250 cells/μL. It showed that mortality risks associated with treatment interruptions reduced after returning to continuous therapy, but still remained elevated 18 months later, compared to those never interrupting. It also showed that despite a hazard ratio of 1.5 for major clinical events, meaning a substantially increased relative risk associated with interruption, the absolute risk between interruption and continuous treatment for major clinical events was small at <2%.

The DART STI sub-study [128], conducted in LMIC, investigated a fixed period 12 weeks on/12 weeks off strategy for patients with CD4 cell count ≥300 cells/μL. Despite absolute rates of WHO stage 4 events/death being low, STIs resulted in a greater than twofold increased relative rate of disease progression compared with continuous therapy (CT) over a median follow-up of 51 weeks. The team therefore concluded that STIs could not be recommended and any further randomisation to the STI arm was terminated. All patients on the STI arm restarted ART with continuous therapy.

The TRIVICAN trial [127], which is the only other trial in sub-Saharan Africa (Côte d'Ivoire) to assess STIs, reported an increase in morbidity both in the CD4 cell count guided arm with interruption and reintroduction thresholds at 350 cells/μL and 250 cells/μL respectively, and the fixed period strategy arm of two months off, four months on. The CD4 guided arm stopped early

due to inferiority compared to the CT arm; patients on the CD4 guided treatment arm had severe morbidity 2.5 fold higher than those on CT, very similar to the results from the DART STI sub-study. The predefined criteria for non-inferiority of -5/100PYs with CD4 <350 cells/μL at 24 months was met by the fixed period strategy with a difference between the arms of -0.87/100PYs. However, the percentage of patients with CD4 <350 cells/μL in the CT arm was lower than anticipated, which made the clinical significance of this non-inferiority uncertain [130].

Other trials in LMIC showed similar results with similar strategies; however all had the same conclusion that STIs could not be recommended in clinical practice. The STACATTO trial [131] investigated a CD4 guided treatment interruption strategy of stopping ART if CD4>350 cells/μL and then restarting CT for at least 12 weeks if two consecutive CD4 counts were <350 cells/μL (higher threshold than in the TRIVICAN and START trials). This trial showed no difference in the primary endpoint of the proportion of patients with viral load (VL)<50 copies/mL. This suggests that using strategies with higher thresholds (and therefore less continuous time off treatment) may minimise the harm associated with STIs.

A review of trials and studies that have investigated STIs, including both CD4 count guided or fixed period interruptions [132], concluded that interrupting treatment results in a variable degree of harm. It concluded that some strategies are less harmful than others, and that interrupting and restarting treatment at higher intervals for CD4 guided treatment, and reducing the time off treatment in each interval could reduce the risks. It also concluded that the benefits of a short period of time off treatment may potentially outweigh the risks. The effect of total duration of time off treatment is something that I will assess in this chapter.

As in adults the introduction of ART has reduced AIDS related mortality and morbidity in children. However, complete HIV suppression requires a high level of adherence to ART to be sustained over a lifetime. There have been 6 randomised trials of various sample sizes assessing the effects of treatment interruptions in children/adolescents, only one of which was in LMIC and 3 of which assessed the effects of planned interruptions. The first was a small pilot trial in South Africa [133] enrolling 30 ART naïve children and randomising them to continuous or intermittent therapy. Children randomised to the STI arm were monitored weekly using VL and CD4 counts. ART was restarted if VL increased by 1 $\log_{10}$ copies/mL. Viral rebounds occurred rapidly within a week of stopping treatment but equally re-suppressed on re-commencing ART. In contrast to adults in the DART STI sub-study, they found that CD4% remained elevated during an interruption and sometimes continued to increase. There have been three randomised controlled trials of planned, CD4 marker-driven, treatment interruption in children with chronic HIV infection. In the PENTA 11 trial [134] HIV-infected children were randomised to CD4-guided STIs or CT. The primary outcome was a composite of a CD4%/cell count outcome (CD4% <15 [age 2-6 years] or CD4% <15 and CD4 cell count<200 cells/μL [7-15 years]) or new CDC stage C diagnosis (i.e. new AIDS) or death. One hundred and nine children were randomised (56 CT and 53 STI); only 1 CT and 4 STI reached a CD4%/cell count outcome. No child died or had a new CDC stage C diagnosis. The CHER trial [135] found that

early limited ART (criteria for re-initiating ART after interruption was CD4%<25% or CD4 count <1000 cells/μL) was superior to deferred ART but did not have a continuous treatment arm for comparison. The other randomised trial based in Botswana, the BANA II study, aimed to evaluate whether structured treatment interruptions could contribute to better long-term treatment outcomes in HIV-infected children [136], results of which are yet to be published.

Short-cycle therapy (5 days on/2 days off) has been evaluated in a number of small studies in adults and young people [137-141] and shows promise; however the concept is somewhat different to the treatment interruption trials since participants have mostly been on efavirenz-based ART (which has a long half-life, 40-55 hours) and the rationale is that there is sufficient drug in the patient's system to continue to suppress the virus for the short treatment break.

Whilst STIs are no longer recommended, unplanned treatment interruptions (UPTIs) are common in both LMIC and high-income countries, but their impact is harder to assess because of confounding. Further research into the role of planned/structured interruptions of ART in both adults and children in low/middle income countries would be useful to estimate short and long term risks of UPTIs, particularly because most trials and studies assessing the effect of treatment interruptions have been based in high-income countries. The populations and patients treated vary greatly between the two settings meaning the results from high-income countries are not necessarily generalisable to LMIC. The work in this chapter assesses the effects of both planned and unplanned treatment interruptions in low/middle-income countries using data from two trials.

3.1.2. Data available for analysis

Data for this chapter are available from the MRC CTU DART and ARROW trials as described in Chapter 2. In particular, data are available from the structured treatment interruption (STI) sub-study within DART. Within the DART trial patients with CD4 cell counts ≥300 cells/μL at 48 or 72 weeks after ART initiation were randomised between either continuous treatment (CT) or a 12 week on/12 week off treatment strategy in order to assess the effect of STIs. Following a review by the Data Monitoring Committee on 16th March 2006 the STI sub-study was terminated due to an increase in the relative rate of disease progression in the STI arm compared to the CT arm. Patients who were in the STI arm of DART when the sub-study was terminated were moved back on to CT. I am therefore able to assess whether or not there is an effect of these STIs on long term outcomes even after returning to CT. In addition data on unplanned treatment interruptions (UPTIs) and reasons for these are available in both DART and the paediatric ARROW trials; there were no STIs by design in ARROW.

The aim of this chapter is to investigate the long-term effects of STIs after patients have returned to continuous therapy using data from the randomised STI sub-study within the main DART trial [128, 142]. I also aim to compare reasons for and outcomes following unplanned treatment interruptions in this and the parallel paediatric trial, ARROW [143] conducted in the same centres in Uganda and Zimbabwe.

3.2. Methods

3.2.1. Introduction

In the DART STI sub-study [128], following a pilot study in 100 patients, patients with CD4 counts ≥300 cells/μL at week 48 or 72 were randomised at either 52 or 76 weeks respectively post ART initiation (if not already randomised) to receive either structured treatment interruptions (STI) or continuous treatment (CT). Patients randomised to the STI group stopped ART immediately; 12 weeks later the same ART was restarted for 12 weeks and the on/off 12 week cycles continued until trial termination or one of the following: a WHO stage 3/4 event or CD4 cell count <50 cells/μL (LCM only) any time after STI randomisation. Either of these events occurring on ART was also considered clinical/immunological failure on first-line ART. Those patients who were taking nevirapine (NVP) had a 2 week staggered stop of NRTIs (NRTIs were continued for 14 days after stopping NVP). CD4 cell counts were recorded at trial enrolment (ART initiation) and then 12 weekly thereafter. Treatment interruptions (TIs) were timed so that the scheduled 12 weekly laboratory tests fell 8 weeks into the STI cycle and 8 weeks after restarting ART. The next STI cycle could be deferred if the patient had received <12 weeks of continuous ART, CD4 cell count was ≤200 cells/μL 4 weeks before the scheduled STI start (LCM only) or the patient was pregnant or breastfeeding.

3.2.2. Objectives

The primary outcomes of interest in this chapter are change in CD4 count, clinical event rates (new WHO3/4 event/death, WHO4 event/death and mortality), switch to second-line treatment and immunological ART failure from 48 weeks after ART initiation (defined in section 3.2.6). I am specifically interested in the effect of STIs on CD4 count over the longer term, the effect of total time off treatment (planned or unplanned) on CD4 count 4 years after ART initiation and the effect of cumulative time off treatment on the above clinical event outcomes.

The main exposure for adults in the DART trial is the number of STI cycles in those randomised to the STI group, which I have considered to be planned interruptions. In addition to these planned interruptions, unplanned interruptions (UPTIs) are also assessed as exposures in both adults and children. An unplanned treatment interruption is defined as stopping all three antiretroviral drugs for ≥4 days, with a reason other than on STI in DART (and not part of the STI pilot) and for any reason in ARROW. The reason for the 4 day threshold is because CRFs prompted clinicians to only record interruptions ≥4 days. I can therefore not be confident that all shorter interruptions were correctly ascertained. In adults I have assessed the effect of the number of STI cycles and the effect of total time off treatment for both planned (STI) and unplanned treatment interruptions (UPTI); in children I have only assessed the effect of UPTIs as there were no STIs by design in ARROW.

Deaths (reporting of which is described in Chapter 2) have been used to calculate overall survival which is defined in adults as time from randomisation into the main DART trial to death and in children as time from randomisation into the ARROW trial to death. As in Chapter 2 for

adults (DART) any patients who died after 31st December 2008 have been censored (i.e. not included as a death). For children (ARROW) this censoring date is 16th March 2012.

For the STI sub-study baseline CD4 values were those nearest to but before and within 120 days of STI/CT randomisation. Subsequent CD4 counts were 8 weeks after starting STI then 12 weekly thereafter. A 6 week window of time either side of each CD4 count was used (apart from at baseline as described above); counts that fell exactly 6 weeks between 2 scheduled timepoints fell in the window of the next time point (e.g. a CD4 count recorded at 26 weeks would be considered a 32 week CD4 count and not a 20 week count). Post STI termination (16th March 2006) baseline values were the closest CD4 count recorded within 6 weeks prior to 16th March 2006 (treating this as the baseline date for the post-STI trial follow-up). Subsequently the closest CD4 count within a window of ±6 weeks every 12 weeks was used.

3.2.3. Change in CD4 counts

I first investigated the change in CD4 count after the termination of the STI sub-study when all patients were receiving CT (Figure 3.2.1). By assessing the difference between those patients who were originally randomised to STI and those who always had CT I can assess the long term effects of these STIs. I have not included any data in Figure 3.2.1 past 144 weeks (~3 years) post 16th March 2006 as <100 patients per arm were at risk after these timepoints. These timepoints are however included in the models used later.

Figure 3.2.1 Absolute CD4 count during and after the STI sub-study

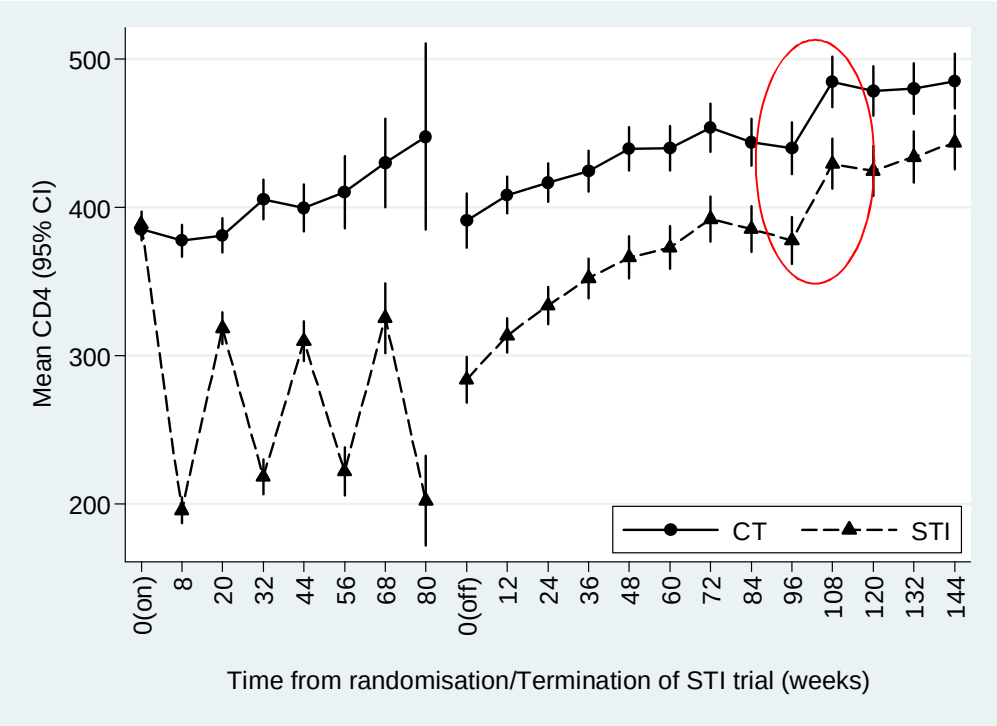
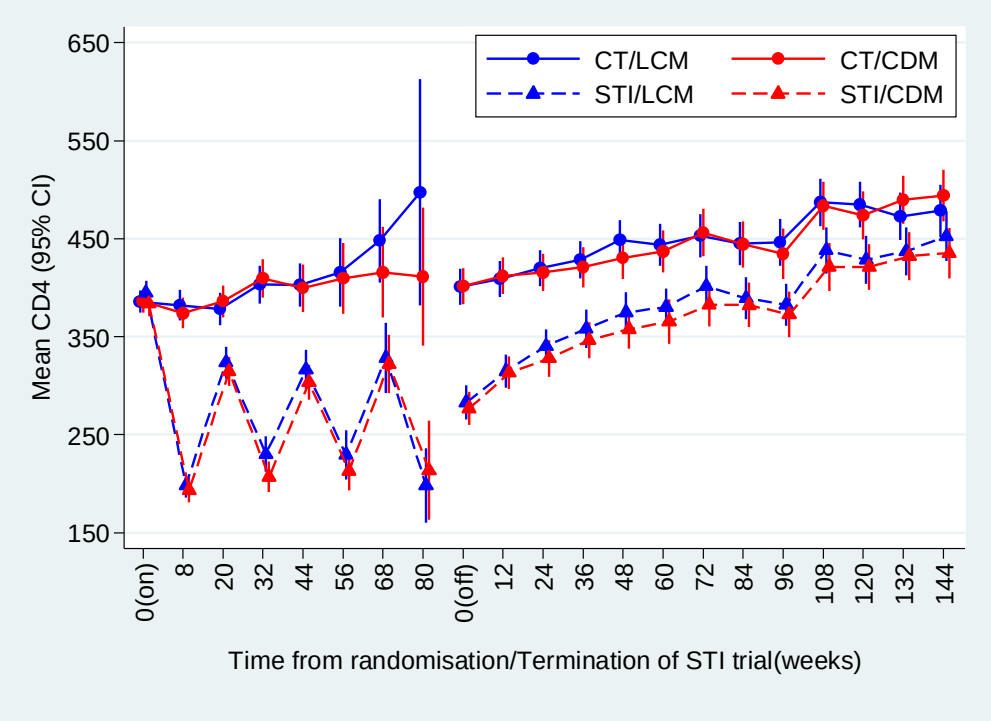


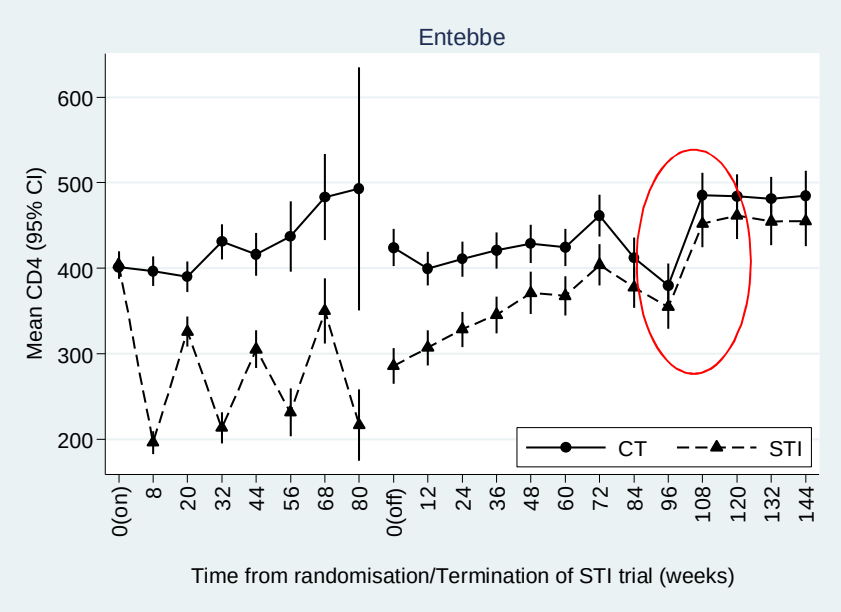
Figure 3.2.1 shows an unexpected rapid increase in CD4 count from 96-108 weeks after 16th March 2006 (overall p<0.001). I first investigated whether this was associated with the randomisation between CD4 (LCM) vs. no CD4 monitoring (CDM) (Figure 3.2.2).

Figure 3.2.2 Absolute CD4 count during and after the STI sub-study by monitoring strategy



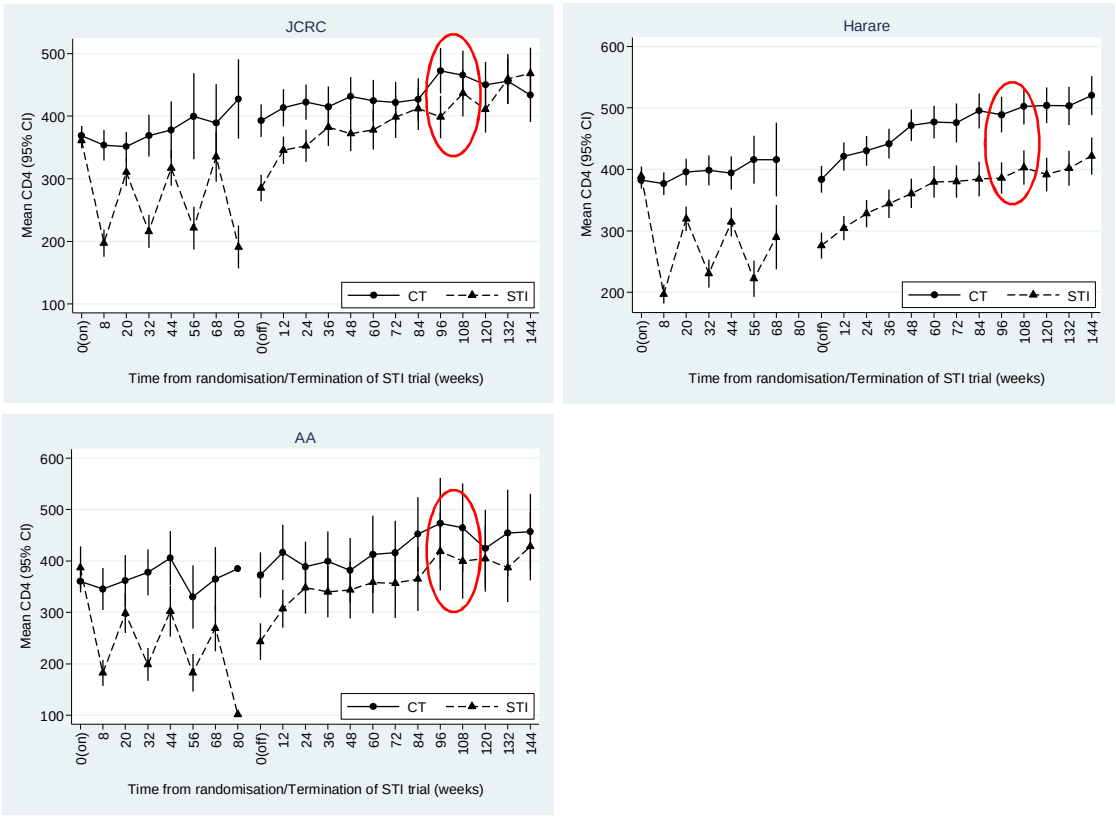
Two points are clear from this plot: Firstly, the mean CD4 count at 80 weeks post randomisation for CT/LCM is larger than might be expected. Investigating this further shows there were 2 outliers with CD4 cell count ≥650 cells/μL at this timepoint and the numbers in this subgroup are small (N=8), so these outliers have a large influence on the mean and its standard error. Secondly, the increase from 96-108 weeks post 16th March 2006 is happening across the board whether STI or CT and whether LCM or CDM (all $p<0.001$). I therefore next looked at results by centre to see if this could be caused by an external factor such as a machine change.

Figure 3.2.3 Entebbe centre (MRC/UVRI)



In the Entebbe centre there is a significant increase (p<0.001) in CD4 in both the CT and STI arms (both p<0.001) from 96-108 weeks preceded by a decrease from 72-84 weeks (Figure 3.2.3). All other centres showed no significant variation between these timepoints (JCRC p=0.41, Harare p=0.32, AA p=0.75) (Figure 3.2.4).

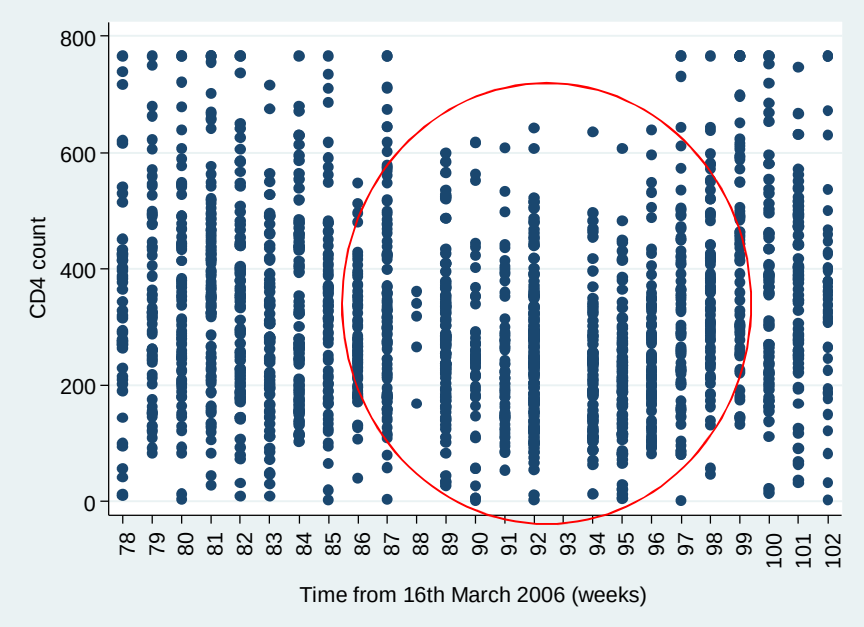
Figure 3.2.4 Absolute CD4 counts at JCRC, HAR and AA centres



The dates that fall around 96 weeks in Entebbe are 6th December 2007 – 27th February 2008, during which time the ARROW trial team (but not the DART team) were informed of a

biochemistry machine change (but not a CD4 machine change). Even though machines are different, it is possible that some change in laboratory practice might be the reason for the change in CD4 counts in the Entebbe centre in DART. Figure 3.2.5 shows absolute CD4 counts in all the Entebbe patients (not just STI/CT patients) by week from 16th March 2006.

Figure 3.2.5 Absolute CD4 counts between 78 and 102 weeks in all Entebbe patients not just the STI sub-study patients



CD4 counts are generally lower between 88-96 weeks [median (IQR) 238 (166-321) cells/µL; in contrast to 78-87 weeks 321 (210-438) and 97-102 weeks 365 (247-474) respectively] and at 93 weeks there are no CD4 counts at all. In fact there were no CD4 counts taken between 21st December 2007 and 2nd January 2008; it is very likely that this is due to the Christmas and New Year period.

Due to the lower CD4 counts at the Entebbe centre from weeks 88-96, from now on I have set all CD4 counts from Entebbe patients between week 88 and week 96 to missing (there is still 80% of all usable data included in the analysis during these timepoints from other centres).

Figure 3.2.6 Absolute CD4 count during and after the STI sub-study by CT/STI randomisation – excluding Entebbe CD4 counts between 19th November 2007 and 11th January 2008

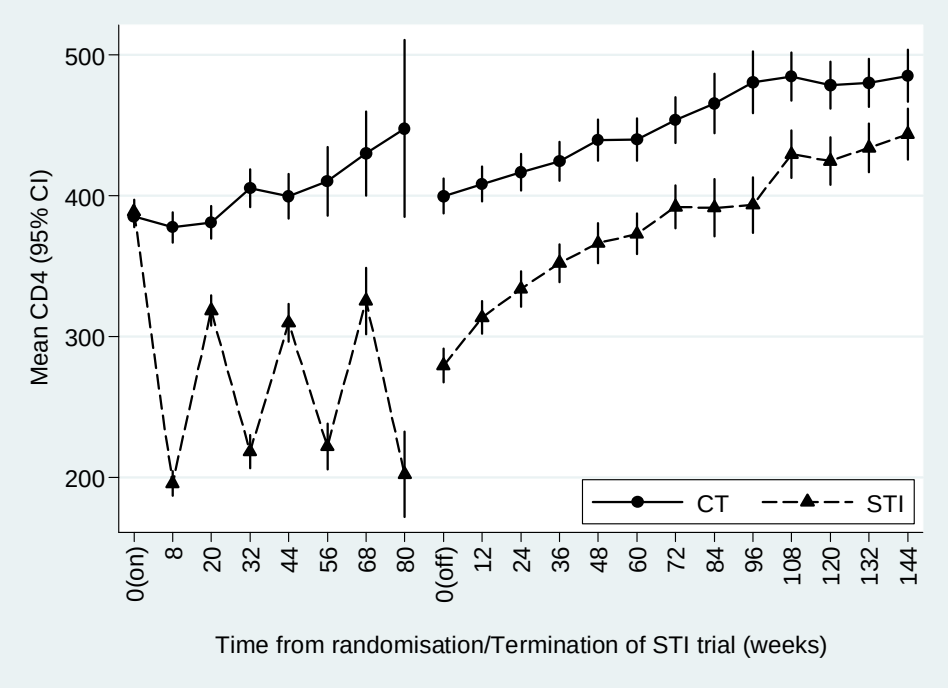


Figure 3.2.6 shows that there is still a slightly larger than expected increase in mean CD4 between 96 and 108 weeks in STI; however this is much less pronounced.

Of note, the problem with the Entebbe CD4 counts had not been identified in the main trial analysis, probably because the specific period of time occurred at very many different timepoints from ART initiation (time zero for the main randomisation).

3.2.4. Mixed modelling for long term trends in CD4 counts

Figure 3.2.6 shows that even up to 2-3 years after returning to CT those patients who received STIs have lower CD4 counts than those on CT. The CD4 count increases more rapidly at re-initiation of ART but from the data, CD4 counts in STI patients never recover to that of the CD4 counts in CT patients (at 144 weeks post 16th March 2006; mean CD4 is 485 cells/µL for CT patients vs. 444 cells/µL for STI patients, p=0.002). One question based on these data, is given more time, would these CD4 counts have recovered?

In DART I assessed the long-term trends in CD4 counts in CT and STI when back on long-term ART using mixed models including fixed and random effects (unstructured covariance matrix) for initial value at 16th March 2006 and a long term slope with heteroskedastic errors depending on STI/CT [144]. Extrapolating using these mixed or random effects models (denoted RE models from now on) allows me to assess whether the CD4 counts for patients in the STI group would ever recover to the same levels as in the CT group even after returning to CT at the end of the STI sub-study (16th March 2006). The reason for using RE models over the standard linear regression is that they are suitable for longitudinal or repeated measures data. Standard linear regression assumes that each observation is independent; using this I would only be able

to assess CD4 as an outcome at a particular point because repeated measurements from one individual are likely to be related to each other. RE models enable me to fit different (random) slopes and intercepts specific to each patient (and depending on the group they were in) during the STI sub-study. Given these individual slopes and intercepts, assuming that the residual error is independent (and normally distributed) is reasonable.

The most basic fixed effects model is

$$cd4_{ij} = \alpha + \beta week_{ij} + \varepsilon_{ij} \quad (3.1)$$

where *i* is the patient and *j* is the week (representing the number of weeks from termination of the STI sub-study on 16th March 2006) and ε_{ij} are the heteroskedastic error terms depending on STI/CT, i.e. $\varepsilon_{ij} \sim N\left(0, \sigma_k^2\right)$ where k indexes STI or CT group membership.

This fits a simple linear regression model to all patients, an intercept (α) followed by a (constant) slope (β) over time (here weeks from termination of the STI trial on 16th March 2006).

The next step is to allow for different intercepts (baseline CD4) and slopes (change in CD4 over time) for each patient (for now ignoring any STI/CT randomisation group effect). These are the random effects.

$$cd4_{ij} = \alpha + \beta week_{ij} + \alpha_i + \beta_i week_{ij} + \varepsilon_{ij}$$

which can also be written as

$$cd4_{ij} = \left(\alpha + \alpha_i\right) + \left(\beta + \beta_i\right) week_{ij} + \varepsilon_{ij} \quad (3.2)$$

Where α_i represents how much an individual patient's intercept varies from the overall population mean intercept, and β_i represents how much an individual patient's slope varies from the overall population mean slope, with $\alpha, \beta \sim MVN\left(\underline{0}\ \Sigma\right)$. If I do not specify a covariance structure for the random effects (α_i and β_i) then the following default independent structure is used:

$$\Sigma = Var\begin{bmatrix}\alpha_i \\ \beta_i\end{bmatrix} = \begin{bmatrix}\sigma_\alpha^2 & 0 \\ 0 & \sigma_\beta^2\end{bmatrix}$$

Instead I have used an unstructured matrix (shown below) which allows the covariance between α_i and β_i to be correlated which is more plausible and supported by a likelihood ratio test (p=0.005).

$$\Sigma = Var\begin{bmatrix}\alpha_i \\ \beta_i\end{bmatrix} = \begin{bmatrix}\sigma_\alpha^2 & \sigma_{\alpha\beta} \\ \sigma_{\beta\alpha} & \sigma_\beta^2\end{bmatrix}$$

Figure 3.2.6 shows that there are different overall intercepts and slopes depending on which STI/CT group a patient is in. In order to include this treatment effect I have fitted:

$$cd4_{ij} = \alpha + \alpha' rxsti_i + \beta week_{ij} + \beta' week_{ij} rxsti_i + \alpha_i + \beta_i week_{ij} + \varepsilon_{ij}$$

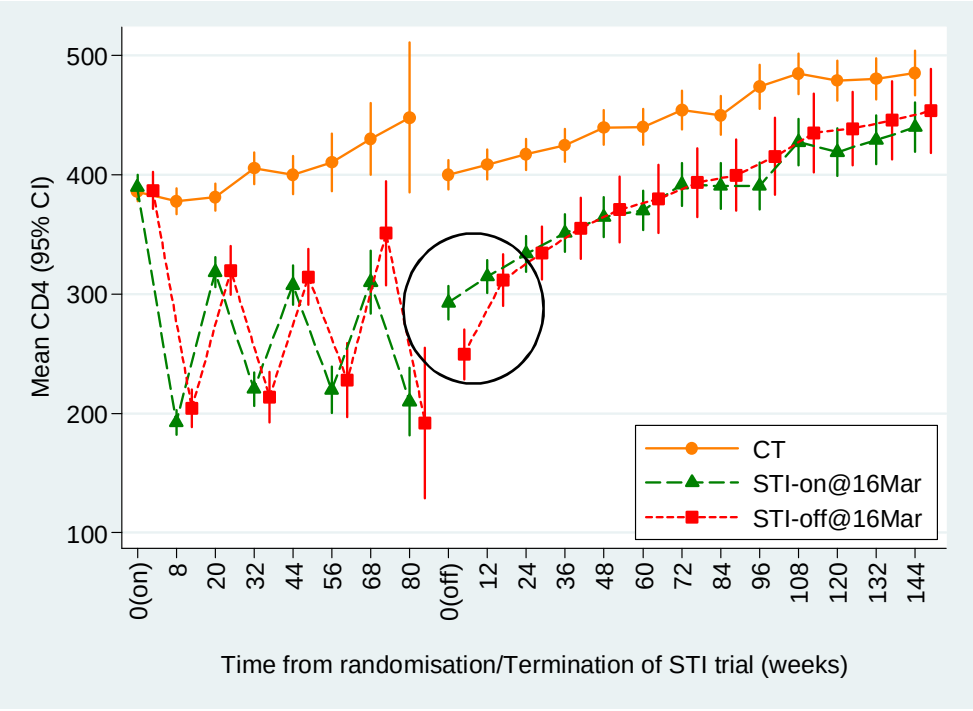
or

$$cd4_{ij} = (\alpha + \alpha' rxsti_i + \alpha_i) + (\beta + \beta' rxsti_i + \beta_i) week_{ij} + \varepsilon_{ij} \quad (3.3)$$

Where *rxsti* is the randomised group allocation variable, α' is the difference in mean baseline CD4 (intercept) between the STI group and the CT group and *β'* is the corresponding difference in change in CD4 over time (slope).

Given the substantial differences in CD4 between on/off ART STI cycles shown in Figure 3.2.6, it is plausible that the CD4 counts in those patients off ART at the time the trial was terminated, i.e. who were in the middle of an STI cycle, may increase differently to those patients who were *on* ART at the time the sub-study was stopped. Allowing different CD4 counts for patients in the CT group, STI patients on ART at the time the sub-study was stopped (STI-on) and STI patients off ART at the time the sub-study was stopped (STI-off) (new variable *newsti*) I found that this is indeed the case and the change in CD4 for STI patients off ART at the time the sub-study was stopped is more rapid (until around 12 weeks) than for those STI patients on ART when the sub-study was stopped (Figure 3.2.7). This suggests I cannot fit a linear slope across the whole follow-up period for patients who are in the STI-off group.

Figure 3.2.7 Change in CD4 count before and after sub-study closure (CT vs. STI-on vs. STI-off)



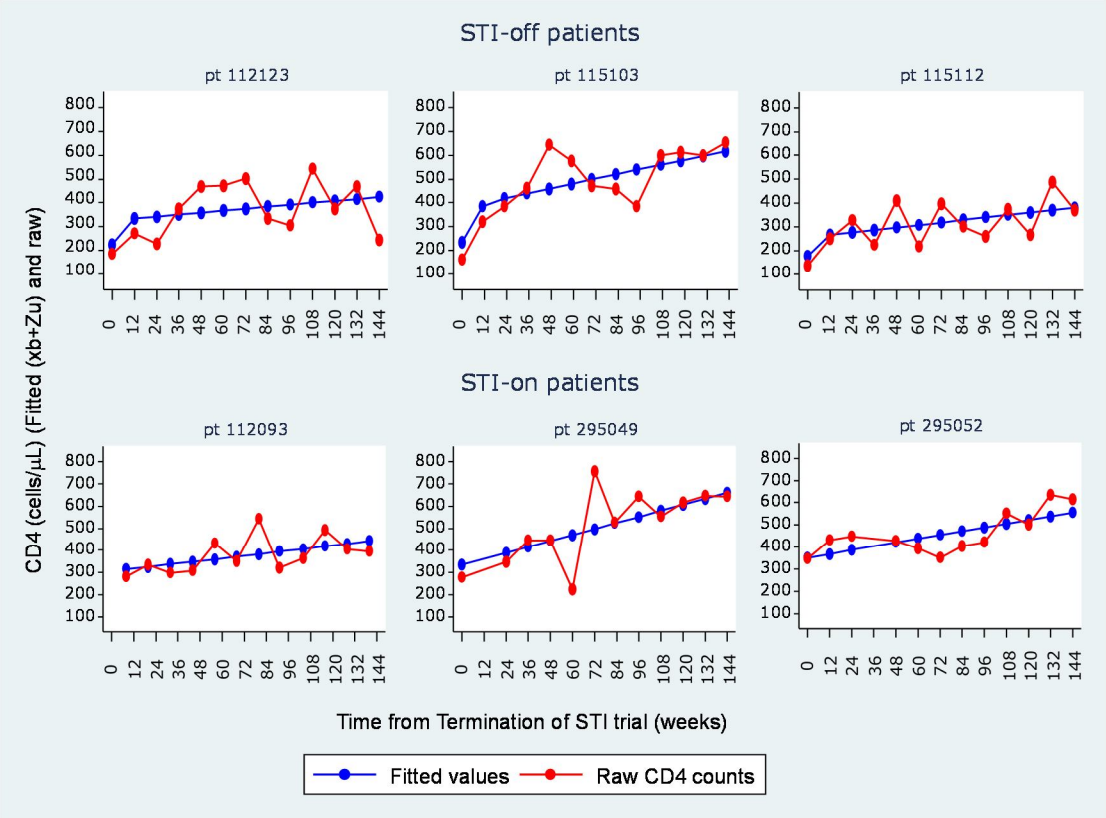
I wish to model this different rate of increase in the initial 12 weeks for STI-off patients. In order to do this I have used piecewise linear functions. I first created a new variable for the first 12 weeks after 16th March 2006 (*initial_week*), this variable will be equal to the week variable until 12 weeks and will then be equal to 12 thereafter. I can then include this variable in the model to allow for the different increase in slope for the STI-off patients. With this new variable my model becomes as follows:

$$cd4_{ij} = (\alpha + \alpha' newsti_i + \alpha_i) + (\beta + \beta' newsti_i + \beta_i) week_{ij} + I(newsti_i = 2)(\gamma + \gamma_i) initial\_week_{ij} + \varepsilon_{ij} \quad (3.4)$$

where CT, STI-on and STI-off are defined as *newsti=0, 1, 2* respectively. The variable *initial_week* only contributes to the model when newsti=2 (STI-off) as I only want to include it for those patients who are off ART when the sub-study was stopped. I define *initial_week* as 0 for all other groups apart from STI-off.

Figure 3.2.8 shows some individual fits for random STI-on and STI-off patients.

Figure 3.2.8 Including the different slope for STI-off patients (individual fits)



I have used model 3.4 to predict whether or not the CD4 counts for the patients in the STI group will ever reach the CD4 counts in the CT group.

3.2.5. Modelling the effect of number of STI cycles on CD4 counts 132 weeks after the sub-study closure

Now I have modelled the effect of STIs on the changing CD4 count over time it is also useful to assess the effect of STIs at a particular timepoint. I have chosen 132 weeks post termination of the STI sub-study because 96% of those randomised to the STI sub-study were still in follow-up and 95% of those had CD4 available at 132 weeks. At 144 weeks this was less; despite 95% of patients still being in FU only 89% had data on CD4.

The effect of number of STI cycles received on the single CD4 count 132 weeks post sub-study closure was assessed using normal linear regression, adjusting for age at ART initiation, sex, centre, randomised monitoring strategy (LCM/CDM), weeks on ART at STI randomisation and CD4 pre-ART and pre STI/CT randomisation.

Linear regression assumes that outcome data are normally distributed and if they are not then the parameter estimates could be misleading. Figure 3.2.9 shows the distribution of CD4 counts at 132 weeks post sub-study closure (note that CD4 count has been truncated at the 99th percentile; 1036 cells/μL, to reduce the impact of outliers on the distribution).

Figure 3.2.9 Distribution of CD4 at 132 weeks post sub-study closure

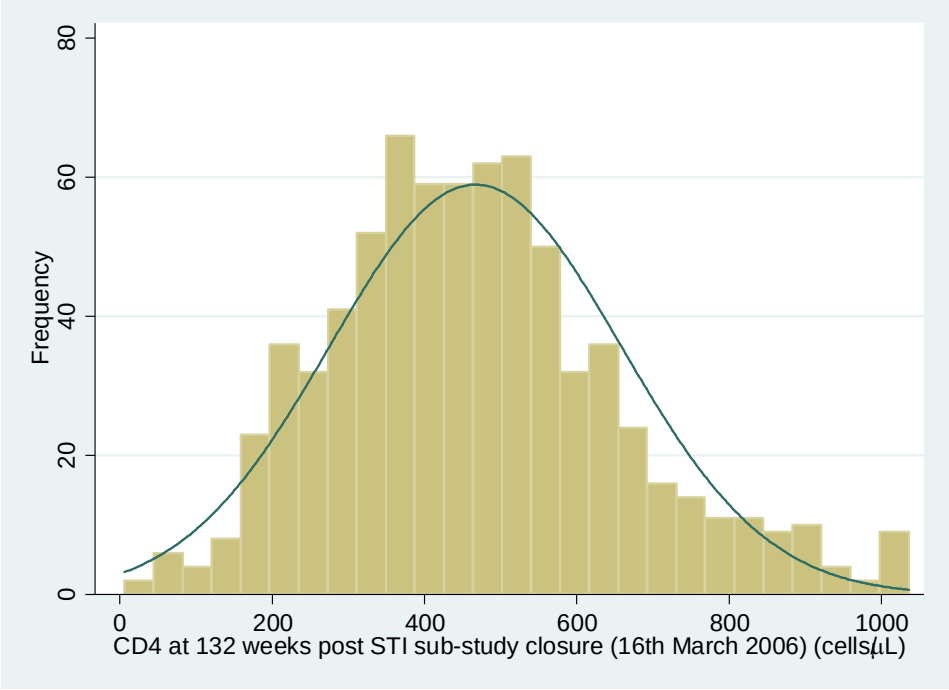


Figure 3.2.9 shows the CD4 counts at 132 weeks post sub-study closure may be slightly skewed. To confirm whether or not this is the case I fitted a Box-Cox regression model to find the maximum likelihood estimates of the parameters of the Box-Cox transform (3.5), that makes these data closest to normality [145-147].

$$\text{transformation, } y^{(\lambda)} = \frac{y^{\lambda} - 1}{\lambda} \qquad (3.5)$$

Here *y* is CD4 at 132 weeks post sub-study closure. The power transform λ has been estimated to be 0.85 (95% CI 0.71-1.00). As the confidence interval just includes 1, this suggests that there the CD4 count at 132 weeks can be modelled in its absolute form, and that departures from normality are not large enough to materially affect results.

3.2.6. Effect of STIs before and after sub-study closure on clinical events

Given the relatively low numbers of events, comparisons between STI and CT in terms of clinical event rates and switch to second-line were made using Poisson regression as described in Chapter 2; section 2.2.3. Clinical events of interest were new WHO 3/4 event/death, WHO 4 event/death and mortality. In addition I assessed the effect of STIs on rates of switch to second-line ART and immunological failure from 48 weeks after ART initiation. The reason for considering immunological failure separately from switch to second-line was because those patients in the LCM group would have been assessed for first-line failure and switch to second-line based on their CD4 counts. But in those patients in the CDM group the CD4 counts would not have been known and so the switch to second-line treatment would have been based on clinical failure alone. Therefore, by following the protocol, switch to second-line was based on different criteria in those monitored with vs. without CD4s. Definitions of immunological failure followed broadly the trial protocols for those monitored with CD4 as follows - adults and children aged ≥5 years: confirmed CD4 50-99 cells/μL or single CD4 <50 cells/μL; children only: aged 3-<5 years confirmed CD4%<10% and aged 1-<3 years confirmed CD4%<15%. As in section 3.2.5, I have adjusted Poisson models for age at ART initiation, sex, centre, randomised monitoring strategy (LCM/CDM), weeks on ART at STI randomisation and CD4 pre-ART and pre STI/CT randomisation.

3.2.7. Effect of total duration off treatment on longer-term CD4 in all trial participants

The analyses above only included participants randomised in the STI sub-study. In both ARROW and DART I investigated the longer-term impact of total time off treatment (planned and unplanned) by modelling CD4% 3 years after ART initiation in all children (ARROW) and absolute CD4 4 years after ART initiation in all adults (DART). I chose these timepoints as the median follow-up in ARROW was 4 years and in DART 5 years and so the majority of all those randomised were still alive and in follow-up one year previously. In both cases I took the closest value within ±12 weeks. I used CD4% rather than absolute CD4 count in children as it varies less with age in uninfected children and is generally preferred when monitoring immune status in children infected with HIV [148]. Models were adjusted for the effect of sex, pre-ART CD4 (CD4% in ARROW), age at randomisation, centre, LCM/CDM and randomised first-line treatment strategy in ARROW. I initially used multivariable fractional polynomials (MFPs) [149] to allow for any non-linearity in the effect of continuous factors.

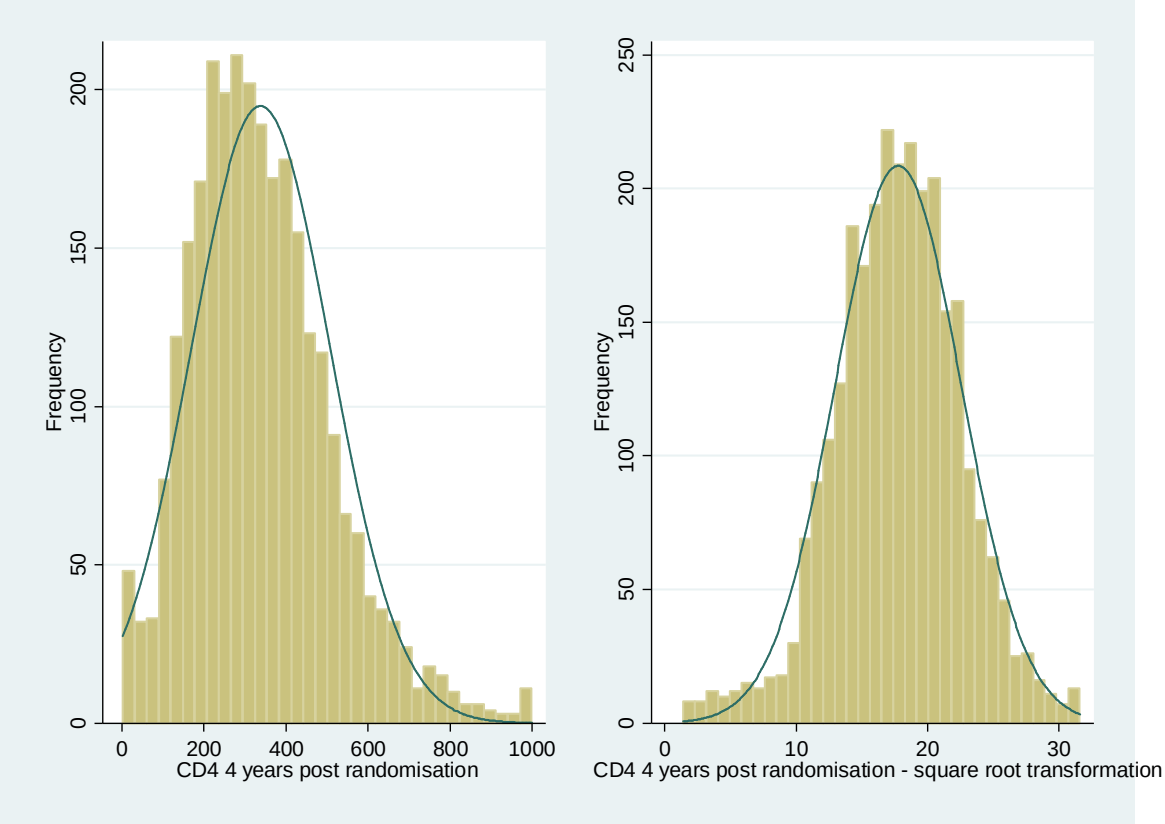
Planned treatment interruptions (STIs) as described in section 3.2.1 are those interruptions that have come from the STI sub-study (DART only) and unplanned treatment interruptions (UPTIs) have come from those interruptions that were not part of the STI sub-study (DART and ARROW). By design there were no STIs in ARROW and so there are only UPTIs. UPTIs were defined as stopping all 3 antiretroviral drugs for ≥4 days (with a reason other than an STI in

DART). As above, I have chosen the cut off of 4 days because the case report forms (CRFs) explicitly asked clinicians not to log treatment interruptions of less than this. While there are some patients who reported a treatment interruption of <4 days, because of the CRF instructions, this will have substantial ascertainment bias and so I have excluded any such interruptions.

a. DART: Finding the best way to model CD4 at 4 years post ART initiation

Before carrying out the analysis described above I investigated how best to model the CD4 count 4 years after ART initiation in the same way as I assessed the best way to model CD4 at 132 weeks after cessation of the STI sub-study in section 3.2.5.

Figure 3.2.10 Distribution of CD4 at 4 years before and after transformation

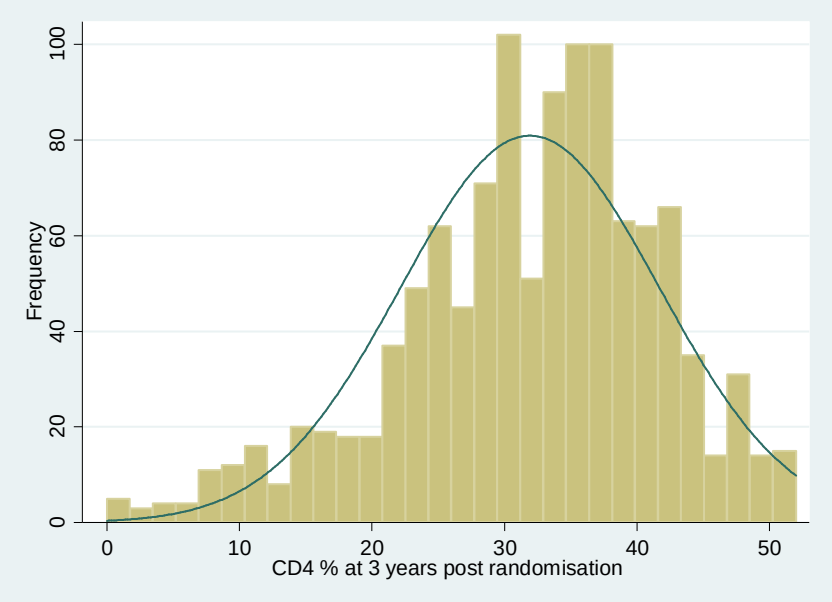


Plotting the distribution of CD4 counts (Figure 3.2.10 left panel) shows that the data are slightly skewed (left panel). Using a Box-Cox transform as described in section 3.2.5 gave $\hat{\lambda}$=0.66 (95% CI 0.62-0.71). As the 95% confidence interval excludes both 1 (linear/no transformation) and 0.5 (square root) there is no intuitively obvious transform to use. Figure 3.2.10 (right panel) shows the square root transformation and the data are only moderately less skewed than the untransformed data. I have therefore decided to model the CD4 at 4 years on the absolute scale, which is easier for interpreting effect estimates. Of note however, I have also truncated the CD4 at 4 years at the 99th percentile (850 cells/μL) to avoid the few outliers potentially influencing the modelling.

b. ARROW: Finding the best way to model CD4% at 3 years post ART initiation

I have also carried out the same check for the CD4% at 3 years after ART initiation in ARROW. Plotting the distribution of CD4% (Figure 3.2.11) shows that the data are slightly skewed, however using a Box-Cox transform as described in section 3.2.5 gave a $\hat{\lambda}$ =1.34 (1.21-1.46). The 95% CI does not contain 1 but the lower confidence limit is reasonably close to 1 and as the data are not highly skewed, for ease of interpretation and consistency with DART I have decided to model the CD4% at 3 years on the linear scale.

Figure 3.2.11 Distribution of CD4% 3 years post ART randomisation



c. DART & ARROW: Finding the best way to model total duration off treatment

The key exposure is the total duration off treatment. Any time off ART that occurred after 4 years (DART) or 3 years (ARROW) has been excluded as occurring before the outcome. Because of the difference in the mean changes in CD4 count shown in Figure 3.2.6 for the STI and CT arms, I have also included a variable to assess the effect of ever/never interrupting ART. Figure 3.2.12 shows the distribution of time off treatment (STIs and UPTIs) in DART to 4 years (excluding those who have not had any time off treatment).

Figure 3.2.12 Distribution of total time off treatment (DART)

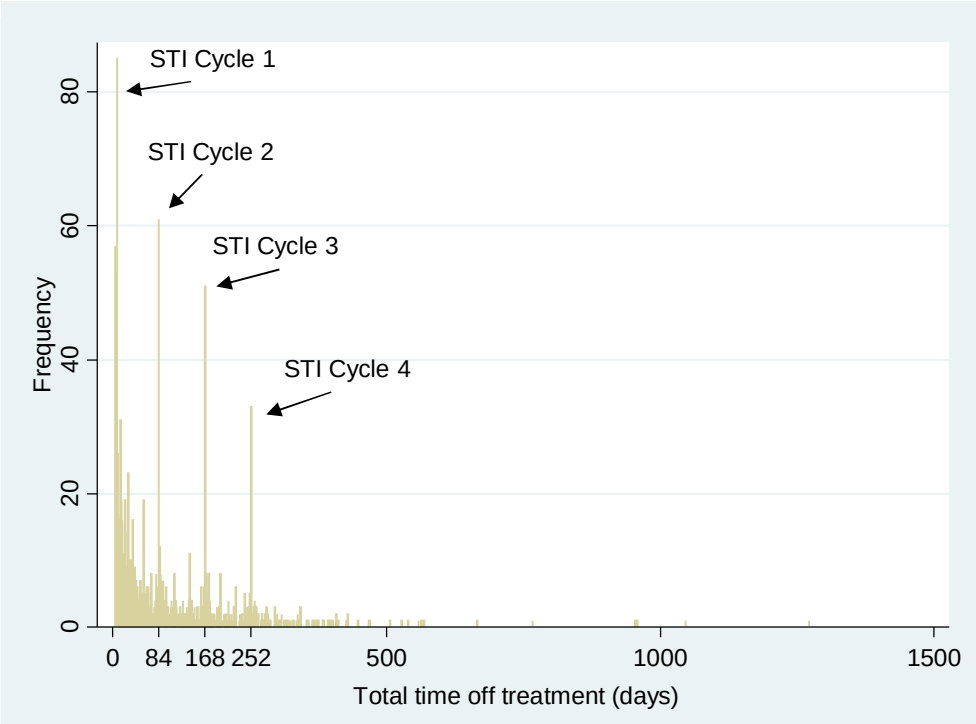
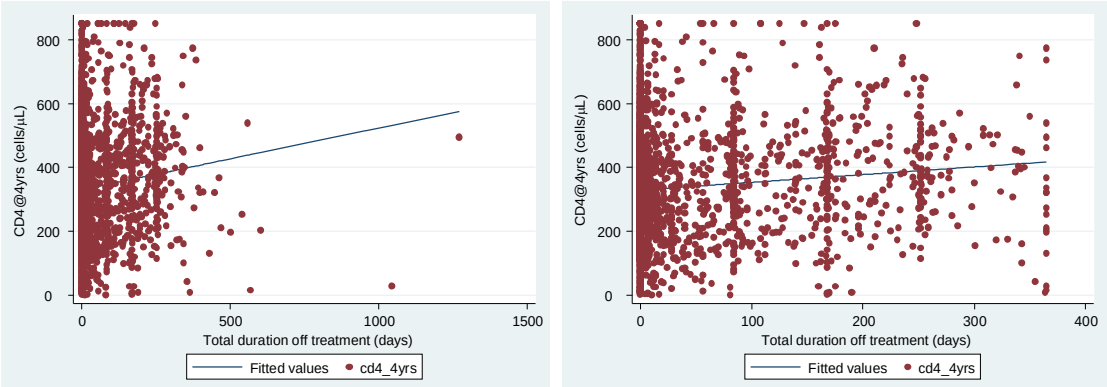


Figure 3.2.12 shows that the distribution of time off treatment is very skewed with almost all patients having a total time off treatment less than 250 days (6 months) with very few patients being off treatment for more than 2 years in total.

I first modelled the effect of total duration off treatment on CD4 at 4 years by using a simple linear regression model.

Figure 3.2.13 Modelling effect of total duration off treatment on CD4 at 4 years using simple linear regression (before and after truncating)



Several outliers appear to have a large influence on the model (Figure 3.2.13 – left panel). As with the CD4 at 4 years (outcome) I have therefore truncated the total time off treatment (exposure) at the 99th percentile (365 days). Further investigation into the distribution of total time off ART, looking at STIs and UPTIs separately, indicates that some very long interruptions were counted as very long STI cycles despite the fact that cycles should only be 12 weeks (84 days) in length (underlined in Table 3.2.1).

Table 3.2.1 Total duration off treatment for STIs by number of STIs

Maximum number of STI cycles	N	Total duration off treatment Median (IQR)	Days off treatment (Min, Max)
0	409	0 (0-0)	(0, 7)
1	85	84 (75, 84)	(17, 262)
2	149	168 (140, 168)	(83, 411)
3	139	247 (212, 252)	(147, 336)
4	31	308 (278, 330)	(254, 342)

This appears to occur where a patient starts an STI as planned, but then fails to re-attend clinic at the end of an STI, leading to a long interruption which was initially for STI but then for failure to attend. Because of this I have capped STI cycles to 91 days (84+7 days); any patient who has had a cycle of STI lasting more than 91 days has the extra time off for that cycle considered as part of a UPTI and added on to the total duration off treatment for any UPTIs for that patient. For example if a patient has a total of 270 days off treatment for just one cycle I have considered this as 91 STI days and 179 UPTI days.

A separate question is whether the linear model is a good representation of the relationship between time off ART and CD4 response (Figure 3.2.13 – right panel). One method to model non-linearity in the relationship between time off ART and CD4 response is to use multivariable fractional polynomials (MFPs) and I have first modelled total duration off treatment using an MFP as described in section 3.2.8 [149]. I have adjusted for pre-ART CD4 (CD4% in ARROW), sex, centre, randomised monitoring strategy and age at ART randomisation. In addition I have adjusted for randomised first-line strategy in ARROW.

3.2.8. Multivariable Fractional Polynomials

Fractional polynomials (FPs) can be used to allow for non-linearity in the effect of a continuous factor in a structured way. The most common FPs are FP1 where the number suffix denotes the degree of FP. FP1 are monotonic [have positive or negative slope], and those with power $p<0$ have an asymptote as $x\to\infty$. FP2 may be monotonic or unimodal [have a maximum or a minimum for some value x]; when p_1 and $p_2<0$ then they have an asymptote as $x\to\infty$. First I distinguish between an FP transformation and an FP function or model. An FP1 transformation of a positive argument $x>0$ with power p is defined as x^p, where p is from $S=\{-2, -1, -0.5, 0, 0.5, 1, 2, 3\}$ (where 0 refers to the natural log of x and not $x^0=1$). An FP1 function or model is defined as $\varphi_1*(x;p)=\beta_0+\beta_1 x^p$. An FP2 transformation of x with powers $\mathbf{p}=(p_1,p_2)$, or for $p_1=p_2$ (p_1, p_2), is the vector x^p with

$$x^{\mathbf{p}} = x^{(p_1,p_2)} = \begin{cases} (x^{p_1}, x^{p_2}), & p_1 \neq p_2 \\ (x^{p_1}, x^{p_2}\log x), & p_1 = p_2 \end{cases}$$

An FP2 function (or model) with parameter vector $\beta=(\beta_1, \beta_2)^T$ and powers $\mathbf{p}$ is $\varphi_2*(x;\mathbf{p})= \beta_0+ \beta_1 x^{p1}+ \beta_2 x^{p2}$.

a. Building an MFP model

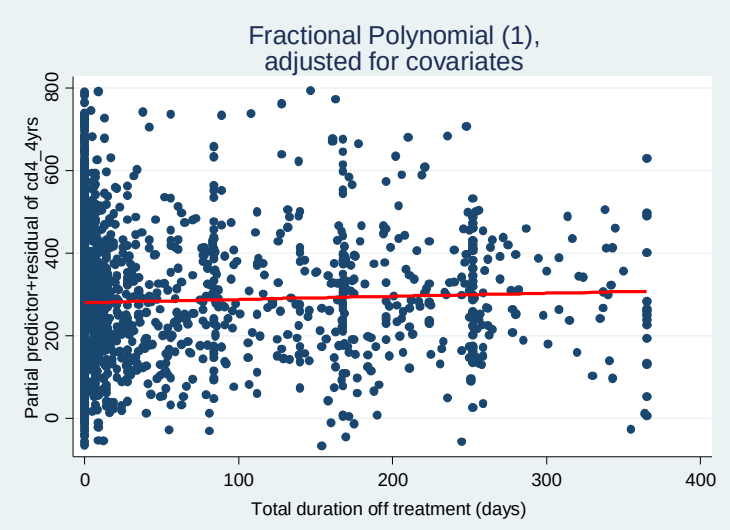
The selection between an FP2 vs. FP1 vs. linear model uses a systematic search for possible non-linearity, provided by a function selection procedure which is added to backwards elimination. When selecting a model there are two significance levels that are needed, α_1 for selecting variables with backwards elimination and α_2 for comparing the fit of the functions within the function selection procedure. Model selection is as follows:

1. Choose α_1 and α_2. Here I have chosen α_1=α_2=0.05. Setting α_1=1 forces no variable selection and α_2 =1 forces no FP selection.
2. Choose maximum permitted degrees of freedom for the FP functions (4df=FP2; 2df=FP1 and 1df=linear function – default is 4df which I have used here for continuous factors, if a binary factor then df=1).
3. The function selection procedure is then as follows:
    i. Inclusion: Test the best FP2 model for *x* (here *x* is the total time off treatment) at the α_1 level against the null model using 4 df. If *x* is significant then continue, if not then drop from the model.
    ii. Non-linearity: Test FP2 for *x* against a straight line at the α_2 level on 3 df. If significant, continue; otherwise, stop, with the chosen model for *x* being a straight line (linear).
    iii. Simplification: Test the best FP2 for *x* against the best FP1 at the α_2 level using 2df. If significant then the final model is FP2, if not then FP1.

When modelling these data using MFPs I have restricted the powers to the set *S={-2, -1, -0.5, 0, 0.5, 1, 2}* to avoid any over fitting of variables that can sometimes occur when using cubic functions. When fitting the model there are several variables that I wish to adjust for regardless of significance and therefore the backwards elimination part of the model selection is not carried out and a full model approach has been used.

Fitting a model as above to my data (i.e. adjusting for pre-ART CD4 (CD4% in ARROW), sex, centre, randomised monitoring strategy and age at ART randomisation and allowing non-linear effects of all covariates), and including the ever/never off treatment variable as described in section 3.2.7c, suggests an FP1 with (1) (i.e. linear) is the best fit to the data (Figure 3.2.14).

Figure 3.2.14 Assessing the fit of an FP1(1) for total duration off treatment



3.2.9. Assessing the effect of cumulative time off treatment on clinical outcomes

Time-dependent Cox regression was used to assess the effect of cumulative time off treatment on clinical outcomes (mortality, WHO4 event/death, WHO3/4 event/death, switch to second-line and immunological failure as defined in 3.2.6) in both DART and ARROW. These models were used because an individual's risk of an event at a particular time depends on their total time off treatment at this timepoint: i.e. depends on a factor which varies across time in each risk set. I therefore expanded my dataset to one record for every risk set a person contributed to and calculated time off ART to each of those timepoints. Given the results from the CD4 outcome analysis (see below), I also assessed the effect of cumulative time off treatment from STIs and UPTIs separately. I used MFP to assess non-linearity in these key exposures.

For most outcomes, there were many events in the first year on ART when risk of events is likely to be high because they have only just started treatment (i.e. not related to interruptions), I therefore also investigated heterogeneity in the effect of time off treatment dividing time on ART at <1 vs. ≥1 years by splitting my data at 1 year as described in section 2.2.3.

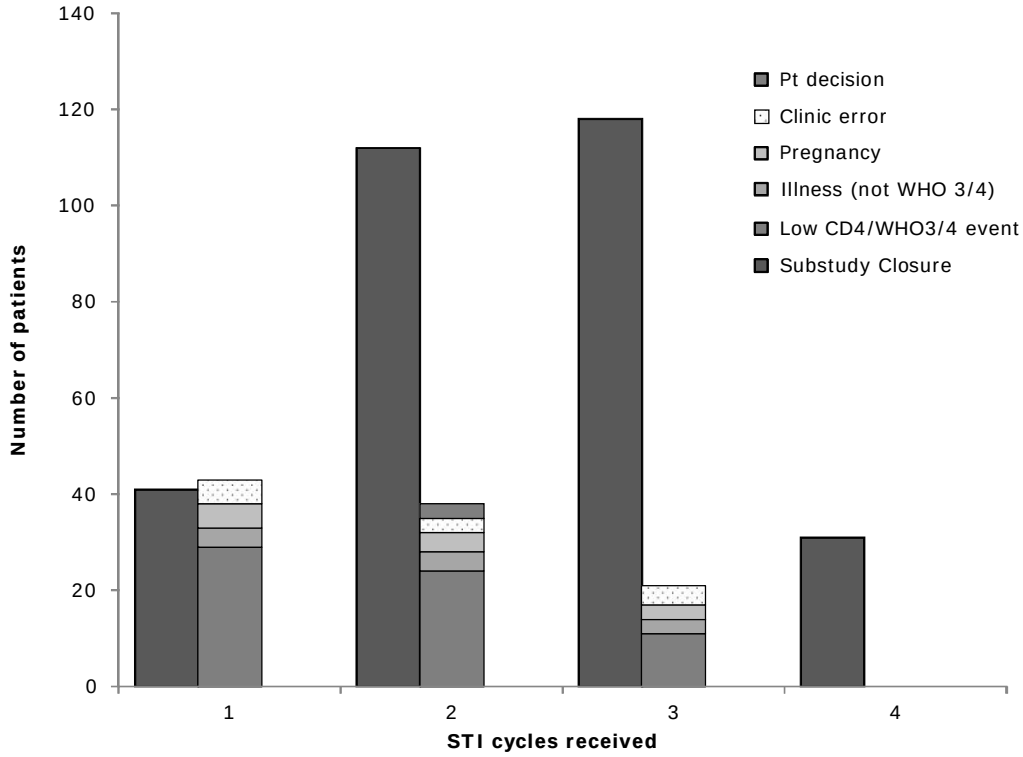
Using the MFP approach I have adjusted for sex, pre-ART CD4, centre, age at ART randomisation and ever/never off treatment. In addition I have adjusted for randomised first-line strategy in ARROW. The MFP model suggests an FP(1) model would be suitable to model the cumulative time off treatment.

The same method has been applied in children using the ARROW data for consistency with the addition of adjusting for the randomised first-line strategy.

3.3. Results

In DART there were 813 participants randomised into the STI sub-study between July 2004 and March 2006 (405 to CT and 408 to STI). A total of 404 participants had at least 1 STI cycle (85, 149, 139 and 31 patients had 1, 2, 3 and 4 STI cycles respectively). There were 4 patients who despite being randomised to the STI arm never actually interrupted ART. Reasons for stopping STIs were sub-study closure (as described in section 3.2.1) in 302 (75%) and clinical/patient reasons in 102 (25%) (Figure 3.3.1). Clinical reasons were mostly due to low CD4 or WHO 3/4 events (64/102, 63%), followed by illness (not WHO 3/4) (12/102, 12%) including malaria, cellulitis, facial palsy and pelvic inflammatory disease, then clinic error (11/102, 11%) including clinician oversight. Patient reasons were mostly due to pregnancy (12/102, 12%) and patient decision (3/102, 3%). As expected those who stopped STIs due to clinical/patient reasons had fewer STIs; 44, 37, 21 and 0 patients stopping STIs for clinical/patient reasons had 1, 2, 3 and 4 STI cycles respectively ($p<0.001$) (Figure 3.3.1).

Figure 3.3.1 Reasons for stopping STIs and cycles received



Another way to assess this is to look at the reasons for stopping STIs by the number of STIs a patient *could* have received before sub-study closure rather than *did* receive. These results are displayed in Figure 3.3.2.

Figure 3.3.2 Reasons for stopping STIs and cycles a patient could have received

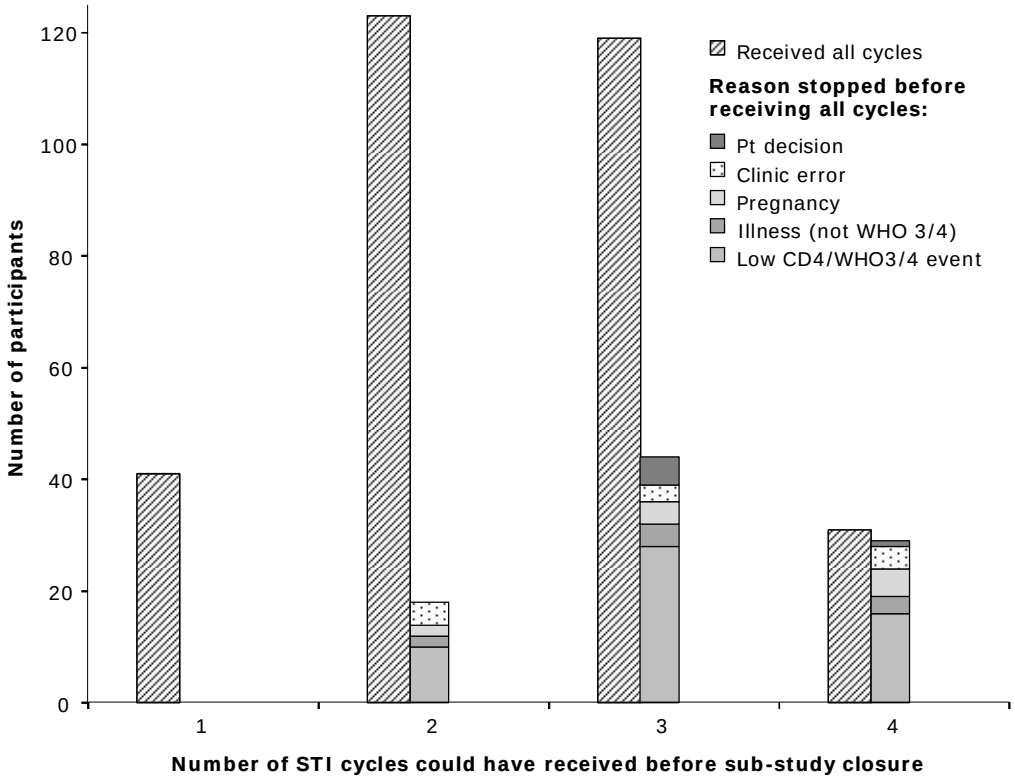


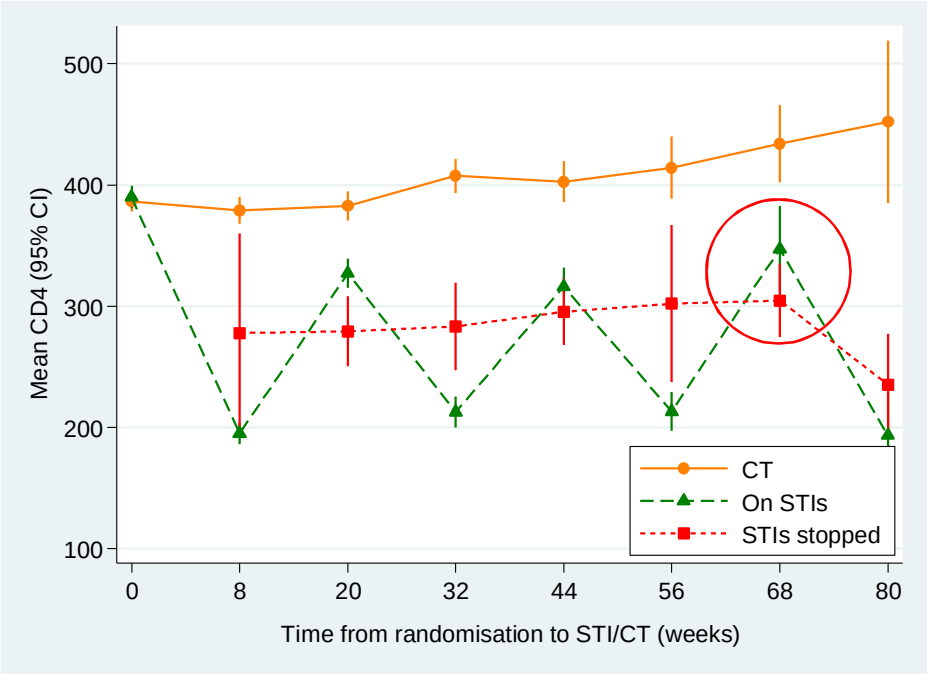
Figure 3.3.2 shows that 100%, 87%, 73% and 52% of patients who could have received 1, 2, 3 or 4 STI cycles before sub-study closure actually received all the cycles they could have done ($p<0.001$). This suggests the more cycles a patient could have received the more likely they were to not receive all their cycles. It also demonstrates that the chance of a patient receiving all 4 cycles they could have received was much lower than receiving all 3, 2 or 1 cycles that they could have received i.e. the population of patients receiving 4 cycles is more highly selected than those receiving fewer cycles. In contrast Figure 3.3.1 shows that of those who only received 1 cycle of STI, sub-study closure accounted for only around 50% of the reasons for stopping, but it does not tell us whether they could have received more. Of those patients who received fewer cycles than they could have done, this was mostly due to low CD4 or a WHO 3/4 event.

3.3.1. Change in CD4 count

Figure 3.3.3 shows the mean CD4 counts during the STI sub-study by sub-study arm (STI/CT); interrupting ART resulted in a rapid and significant drop in CD4 count 8 weeks after each STI and a rapid increase in CD4 count 8 weeks after each restart of ART. It also shows the mean CD4 counts for those in the STI arm who had stopped STIs. What is interesting is that even towards the end of the trial and 8 weeks after the final planned restart of ART (week 68 as highlighted) there was still a net decrease in CD4 compared to at randomisation for those patients on the STI arm; mean change in CD4 for those in the STI arm from STI randomisation to week 68 was -63 (SD 103) cells/μL; in those who had stopped STIs -51 (102) cells/μL and those who were still having STIs -75 (102) cell/μL. In contrast, in the CT arm (as would be

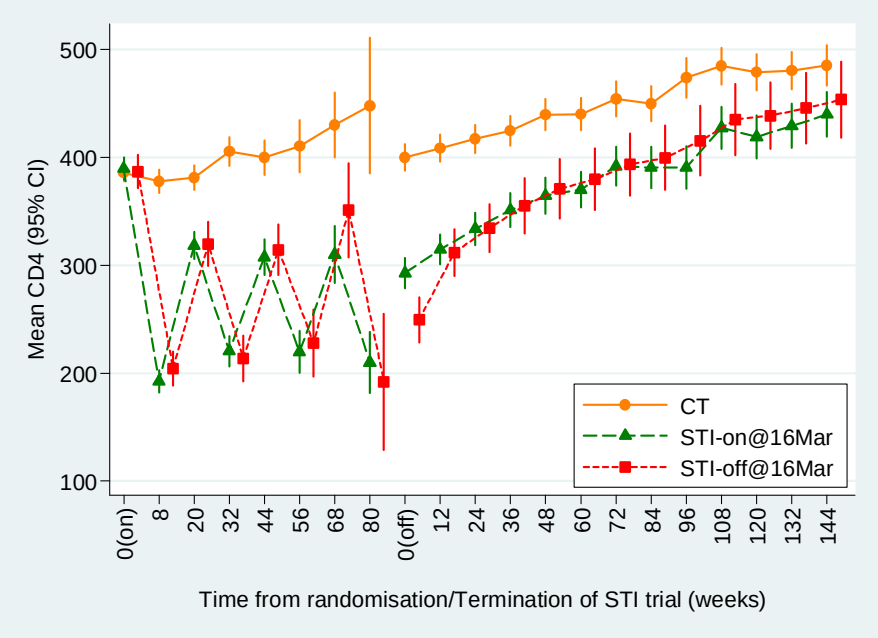
expected) there is generally a steady increase during the course of the STI sub-study (mean change from STI randomisation to week 68 was +40 (SD 121) cell/μL).

Figure 3.3.3 Absolute CD4 count change over STI study



After the sub-study closed on 16th March 2006, STI patients returned to CT and follow up on these patients continued as part of the main DART trial. This allowed me to assess the effects of these STIs on long term CD4 counts. Figure 3.3.4 shows mean CD4 counts before and after sub-study closure in those randomised to CT and STI separately; also split by those who were off ART at the time the trial was terminated (STI-off) and those who were on ART at the time the trial was terminated (STI-on), as described in section 3.2.4.

Figure 3.3.4 Absolute CD4 count during and after the STI sub-study by CT/STI-on/STI-off



STI/CT patients were followed for a median (IQR) 1.9 (1.6-2.2) years post sub-study closure within the DART trial. From the mixed model, patients randomised to CT had a mean CD4 of 402 cells/μL (95% CI 389-414) at sub-study closure and as expected patients randomised to STI had a CD4 that was 93 cells/μL (74-112) lower in those on ART and 169 cells/μL (142-197) lower in those off ART at sub-study closure (both p<0.001). Subsequently CD4 counts in both the STI and CT patients increased. However, CD4 counts in those who had had STIs remained lower than those in the CT group even after 2 years back on CT. I also observed that CD4 increased more rapidly during the first 12 weeks back on CT for those who were off ART at sub-study closure compared to those who were on ART (STI-on or CT) (Figure 3.3.4). From the mixed model, those off ART gained 83 cells/μL (57-108) during the first 12 weeks back on ART, significantly more than those on ART at sub-study closure (p<0.001), who gained on average 9 cells/μL (8-10) during the first 12 weeks (STI-on +11 cells/μL (10-13); CT +7 cells/μL (6-8)). Subsequently both STI groups gained CD4 at similar rates of 48 (41-54) and 49 (40-59) cells/μL/year respectively (heterogeneity p=0.78), significantly faster than the 31 (25-36) cells/μL/year in CT (p<0.001) (Table 3.3.1).

Table 3.3.1 Modelling the effect of STIs on long term CD4 counts

Variable	Estimate and 95%CI	p-value
Fixed effects		
CD4 at sub-study closure (cells/μL)		
CT	402 (389, 414)	-
STI-on ART	-93 (-112, -74)	<0.001
STI-off ART	-169 (-197, -142)	<0.001
Change in CD4 after sub-study closure (cells/μL/week)		
CT	0.59 (0.49, 0.69)	<0.001
STI-on ART	0.92 (0.79, 1.04)	<0.001
STI-off ART	0.95 (0.76, 1.14)	<0.001
Change in CD4 after sub-study closure (cells/μL/week) – initial 12 weeks		
CT	omitted	
STI-on ART	omitted	
STI-off ART	7 (5, 9)	<0.001
Random effects		
sd(CT)	117 (108, 127)	
sd(CTXweek)	0.9 (0.8, 1.0)	
corr(CT, CTXweek)	-0.2 (-0.3, -0.1)	
sd(STI-on)	114 (103, 125)	
sd(STI-onXweek)	0.9 (0.8, 1.0)	
corr(STI-on, STI-onXweek)	-0.2 (-0.4, -0.1)	
sd(STI-off)	88 (71, 110)	
sd(STI-offXweek)	0.9 (0.7, 1.0)	
corr(STI-off, STI-offXweek)	-0.1 (-0.3, 0.2)	
sd(initial_week)	6 (4, 8)	
sd(residual error):		
CT	85 (83-87)	
STI-on	80 (78-82)	
STI-off	79 (76-83)	

Number of observations=9867
Number of groups = 798
Observations per group: Minimum = 1; Average =12.4; Maximum = 19

As the CD4 counts increased at a slower rate for CT and a quicker rate for STI, I asked the question - will these CD4 counts ever recover and be the same as in the CT group? Using the mixed model methods as outlined in section 3.2.4 and assuming our model extrapolates another 1-2 years, by extrapolating from my model the mean CD4 in STI patients on ART at sub-study closure would equal that in CT 5.4 years (95% CI 4.1-7.1 years) after sub-study closure and in STI patients off ART at sub-study closure the mean CD4 would equal that in CT 4.6 (1.5-9.8) years after sub-study closure. As expected from the mean increases shown in Table 3.3.1 above, there is no real difference between STI-on and STI-off in terms of the recovery time of the CD4 counts reflected in the overlapping confidence intervals. For this reason I have also fitted a model with a common STI slope past 12 weeks. Using this updated mixed model the mean CD4 in the STI-on group would equal that in the CT group 5.3 years (95%CI 4.2-6.4 years) after sub-study closure and the STI-off group the mean CD4 would equal that in the CT group 4.9 (1.8-8.0) years after sub-study closure.

3.3.2. Long term effects of STIs

Table 3.3.2 shows results from a multivariable linear regression model investigating the effects of 1, 2, 3 or 4 STI cycles on a patient's CD4 132 weeks after sub-study closure. At 132 weeks post sub-study closure there were 741 (91%) patients with CD4 available; 23 (3%) had died prior to 132 weeks post sub-study closure, 28 (3%) were lost to follow-up and 21 (3%) had no CD4 recorded at 132 weeks.

Table 3.3.2 Long term effects of STIs on CD4 count at 132 weeks post sub-study closure

	N or median (IQR)	CD4 count at 132 weeks post sub-study closure			
		Univariable		Multivariable	
Variable		**Estimate (95% CI)**	**p-value**	**Estimate (95% CI)**	**p-value**
Number of STIs: 0 (CT)	375	0	-	0	-
1	71	-57 (-101, -15)	0.009	-58 (-99, -17)	0.006
2	137	-54 (-87, -20)	0.002	-53 (-84, -22)	0.001
3	127	-33 (-67, 0.76)	0.06	-43 (-76, -11)	0.009
4	31	-5 (-67-57)	0.87	-14 (-73, 45)	0.64
Pre ART CD4 (per 50 cells/mm^3 higher) [Median (IQR)]	133 (83-168)	18 (7, 29)	0.001	10 (-0.6, 21)	0.06
Pre STI/CT CD4 (per 50 cells/mm^{3}higher) [Median (IQR)	358 (325-419)	30 (23, 37)	<0.001	29 (22, 35)	<0.001
Age at ART initiation (per 10 years older) [Median (IQR)]	36 (31-42)	0.20 (-15, 16)	0.98	12 (-3, 27)	0.10
Sex: Male	191	0		0	-
Female	500	74 (46-101)	<0.001	66 (39, 93)	<0.001
Centre:					
Entebbe, MRC/UVRI	302	0	-	0	-
Kampala, JCRC	156	-10 (-43, 23)	0.55	11 (-21, 42)	0.51
University of Zimbabwe, Harare	237	-16 (-45, 13)	0.29	2 (-25 29)	0.88
IDI*, Mulago	46	-46 (-99, 7)	0.09	-27 (-77, 23)	0.28
Monitoring strategy:					
LCM (CD4 monitoring)	380	0	-	0	-
CDM (no CD4 monitoring)	361	6 (-19, 31)	0.63	7 (-16, 30)	0.55
Weeks on ART at STI randomisation:	454	0	-	0	-
52 weeks	287	-34 (-59, -9)	0.008	-16 (-41, 9)	0.20
76 weeks					
Mean CD4 in reference category	-	-	-	439 (406, 472)	<0.001

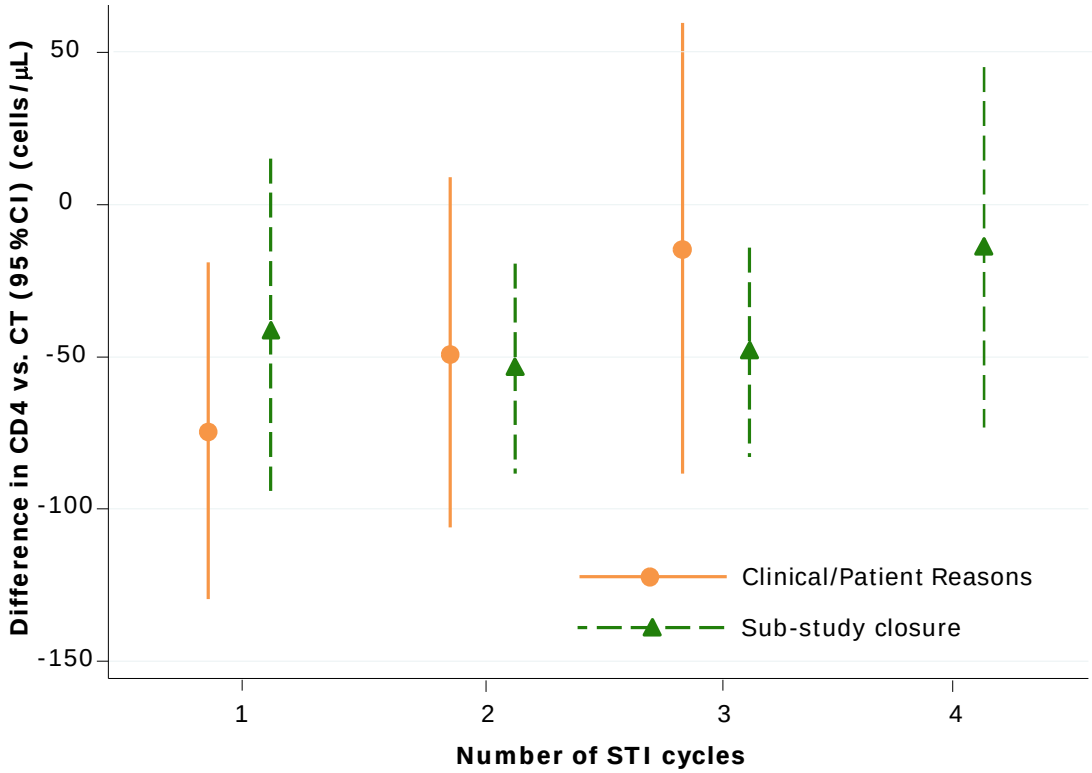
*Infectious Disease Institute
†Reference category: pre ART CD4 =100; pre STI/CT CD4 = 400; Age = 40 years (approximate medians)

Estimated reductions in CD4 132 weeks post sub-study closure associated with one, two, three or 4 cycles of STIs were 58, 53, 43 and 14 cells/μL compared to CT. Overall CD4, 132 weeks after sub-study closure was 51 cells/μL lower in STI vs. CT (95% CI 28-74, adjusted p<0.001) with no evidence of significant variation across number of cycles (heterogeneity p=0.61). Figure 3.3.2 suggests that one reason the point estimate for 4 cycles may be closer to 0 is that the group of patients who undertook 4 cycles is more highly selected (only 52% of those who could

have theoretically had 4 cycles of STIs). As expected there was an effect of earlier CD4 counts on CD4 132 weeks after sub-study closure; with CD4 132 weeks after sub-study closure being marginally higher in those with higher pre-ART CD4 (increase of 10 cells/μL for every 50 cells/μL higher at ART initiation (randomisation into the main trial), p=0.06) and significantly higher in those with higher CD4 at STI/CT randomisation (increase of 29 cells/μL for every 50 cells/μL higher at CT/STI randomisation, p<0.001). I also found a gender effect with females having a higher CD4 at 132 weeks than males (p<0.001). Patient's age at ART initiation had no significant effect, nor did monitoring strategy or weeks on ART at STI randomisation (all p>0.1). There was no evidence that the effect of the number of STI cycles or having undergone any STIs varied by whether patients were monitored using CD4 or not (interaction p=0.19, p=0.42 respectively).

Although patients who stopped STIs for clinical/patient reasons might have been expected to have lower CD4 132 weeks after sub-study closure than those stopping STIs specifically because of sub-study closure, reductions in CD4 were in fact similar in those who stopped their STIs due to clinical/patient reasons, compared with those who stopped due to sub-study closure (heterogeneity p=0.67) (Figure 3.3.5).

Figure 3.3.5 Impact of number of STI cycles and reason for stopping STIs on CD4 132 weeks after sub-study closure (adjusted for other the factors in Table 3.3.2)



Because sub-study closure happened at a fixed date, some patients were restarted on ART in the middle of an STI. Therefore, even though the number of STIs is the same, the actual amount of time off ART varies somewhat. I have therefore also looked at the effect of STIs by considering the effect of total duration off treatment for STIs; results from this analysis support the results above with no evidence of an increasing detrimental effect for longer periods off

(ever/never off ART: CD4 77 cells/μL lower [95%CI -133, -22] at 132 weeks post sub-study closure, p=0.01, and 1.00 cells/μL higher [-0.90, 2.91] per week off treatment at 132 weeks post sub-study closure, p=0.30; methods as described in section 3.2.10 adjusted for the other factors in Table 3.3.2).

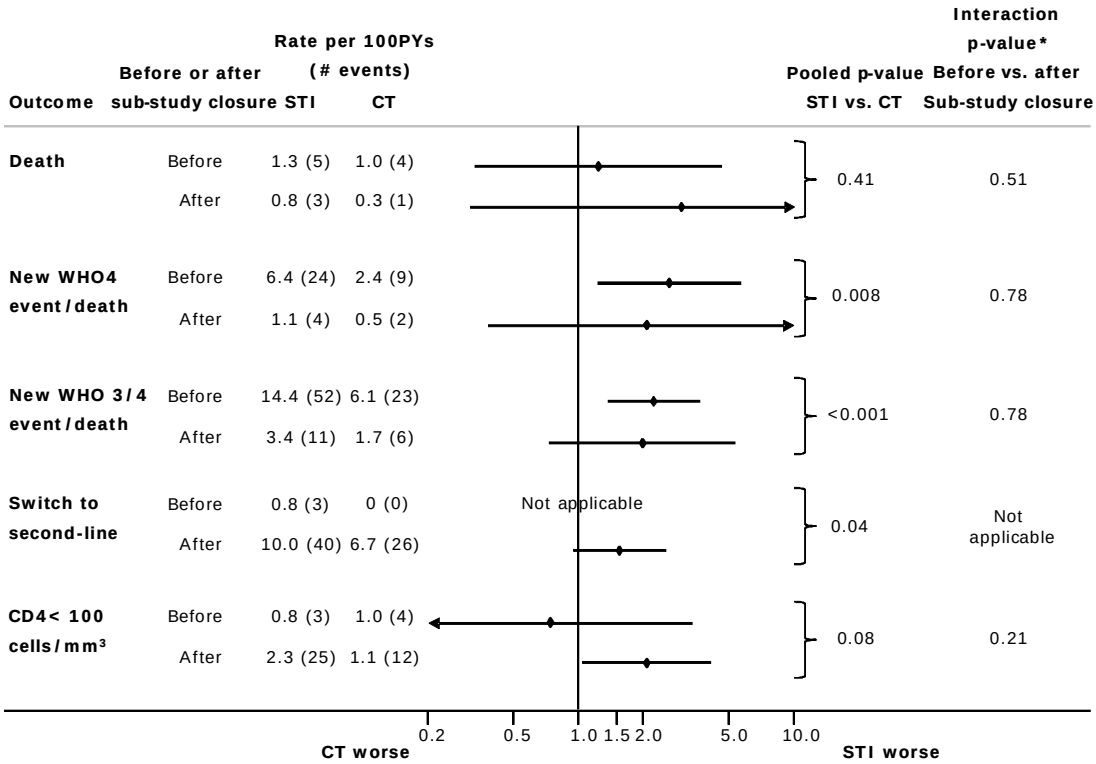
3.3.3. Clinical Outcomes

During the randomised phase there were 773 person years (PYs) at risk and there were 788 PYs at risk subsequently (i.e. after 16th March 2006). During the randomised phase there were higher rates of both new WHO 4 events/death (p=0.008) and new WHO3/4 events/death (p<0.001) in STI vs. CT (Table 3.3.3 & Figure 3.3.6). After sub-study closure, rates of new WHO 4 events/death and WHO 3/4 events/death declined in both groups but remained higher in those having previous STIs. In particular, interaction tests investigating heterogeneity in STI vs. CT effects before and after sub-study closure supported excess risk associated with randomisation to STI persisting post sub-study closure (p>0.7 i.e. no evidence that the STI-associated excess changed). However power was too low to detect any genuine differences between STI and CT in mortality post sub-study closure (Table 3.3.3). After sub-study closure, rates of switch to second-line were greater than during the randomised phase in both groups with a trend towards greater rates in STI vs. CT (p=0.08): there were no switches to second-line in the CT group during the study so I cannot estimate this rate. Rates of immunological failure (CD4<100 cells/μL) were greater after sub-study closure with a significantly higher rate in the STI group (p=0.04) during this period, although there was no evidence of heterogeneity between time periods overall (p=0.21).

Table 3.3.3 Effect of STIs before and after sub-study closure on clinical events, switch to second-line and immunological ART failure

	Before 16th March 2006 Randomised phase	**After 16th March 2006 All patients on continuous therapy**
	STI vs. CT	**STI vs. CT**
Total PYs at risk	773	788
Deaths	5 vs. 4 1.3 vs. 1.0/100PYs RR=1.24 (0.33-4.62), p=0.32	3 vs. 1 0.8 vs. 0.3/100PYs RR=3.02 (0.31-29.0), p=0.34
New WHO4 event/death	24 vs. 9 6.4 vs. 2.4/100PYs RR=2.65 (1.23-5.69) p=0.008	4 vs. 2 1.1 vs. 0.5/100PYs RR=2.08 (0.38-11.38) p=0.40
New WHO3/4 event/death	52 vs. 23 14.4 vs. 6.1/100PYs RR=2.24 (1.37-3.67) p<0.001	11 vs. 6 3.4 vs. 1.7/100PYs RR=1.98 (0.73-5.37) p=0.18
Switch to second-line	3 vs. 0	40 vs. 26 10.0 vs. 6.7/100PYs RR=1.55 (0.95-2.55) p=0.08
CD4<100 cells/μL	3 vs. 4 0.8 vs. 1.0/100PYs RR=0.74 (0.17-3.32) p=0.70	25 vs. 12 2.3 vs 1.1/100PYs RR=2.08 (1.04-4.14) p=0.04

Figure 3.3.6 Effect of STIs on clinical events, switch to second-line and immunological ART failure before and after sub-study closure



*Test whether the difference between STI vs. CT varies during randmonised phase and post sub-study closure (16th March 2006)

3.3.4. Unplanned Treatment interruptions (UPTIs) in DART and ARROW

Planned treatment interruptions (STIs) occurred as a result of the DART STI sub-study. But in addition to these planned interruptions there were patients who had had unplanned treatment interruptions (UPTIs).

In addition to the 404 patients in the DART trial who were part of the STI sub-study and had at least 1 STI cycle, 1055 of 3316 (32%) adults in DART had at least one UPTI lasting ≥4 days recorded on their CRFs; median total UPTI duration per patient was 18 days (IQR 7-49). There were a total of 1955 UPTIs lasting a median of 9 days (IQR 6-24) (Table 3.3.4). Overall 1434 (43%) adults had at least one TI (STI or UPTI) lasting ≥4 days, 1253 (38%) and 1006 (30%) with a TI lasting >7 or >14 days respectively with median total time off treatment of 56 days (IQR 13-163). A similar proportion of patients had UPTIs in both the CD4 and no CD4 monitoring groups (chi-squared p=0.81), therefore I have grouped the two monitoring strategies together below. In DART, of the 1055 patients who had at least 1 unplanned treatment interruption, 90% (n=957) had 3 or fewer interruptions with <1% (n=9) of patients having ≥10 interruptions.

Table 3.3.4 DART Treatment interruptions (planned and unplanned)

Treatment interruptions	Unplanned	Any
Patients with at least one interruption	1055/3316 (32%)	1434/3316 (43%)
Patients with at least one interruption that lasted		
>7days	842/3316 (25%)	1253/3316 (38%)
>14 days	539/3316 (16%)	1006/3316 (30%)
Median length of all time off treatment	18	56
(IQR)	(7-49)	(13-163)
[range]	[4-957]	[4-1272]
Total number of TIs per patient*		
0	2261	1882
1	626 (59%)	618 (43%)
2	243 (23%)	369 (26%)
3	88 (8%)	212 (15%)
4	37 (4%)	125 (9%)
5	24 (2%)	52 (4%)
6	12 (1%)	24 (2%)
7	8 (<1%)	15 (1%)
8	5 (<1%)	6 (<1%)
9	3 (<1%)	3 (<1%)
≥10	9 (<1%)	10 (<1%)
Total number of interruptions	1955	3205
Median length of an interruption	9	21
(IQR)	(6-24)	(7-83)
[range]	[4-957]	[4-1104]

* Percentages are of patients with any interruption

There were no planned treatment interruptions by design in ARROW, but as adults did in DART, children too had unplanned treatment interruptions. 285 of 1206 (24%) children in ARROW had at least one UPTI lasting ≥4 days, with a median total UPTI duration per patient of 13 days (IQR 7-34) (Table 3.3.5), significantly fewer individuals than in DART (32% vs. 24%, p<0.001). In ARROW there were a total of 546 UPTIs lasting a median of 7 days (IQR 6-14). There were 211 (17%) and 115 (10%) UPTIs lasting >7 days and >14 days respectively. Of the 285 children who had at least 1 UPTI, a similar proportion as DART had 3 or fewer interruptions; 252 (88%), with <1% (n=11) having ≥6 UPTIs.

Table 3.3.5 ARROW Treatment interruptions (unplanned)

Treatment interruptions	LCM n=600	CDM n=606	Total N=1206
Patients with at least one interruption	141 (24%)	144 (24%)	285 (24%)
Patients with at least one interruption that lasted			
>7days	108 (18%)	103 (17%)	211 (17%)
>14days	63 (11%)	52 (9%)	115 (10%)
Median length of all time off treatment	16	12	13
(IQR)	(7-36)	(7-32)	(7-34)
[range]	[4-169]	[4-248]	[4-248]
Total number of TIs per patient*			
0	459	462	921
1	77 (55%)*	88 (61%)	165 (58%)
2	31 (22%)	30 (21%)	61 (21%)
3	14 (10%)	12 (8%)	26 (9%)
4	5 (4%)	6 (8%)	11 (4%)
5	7(5%)	4 (3%)	11 (4%)
≥6	7(5%)	4 (3%)	11 (4%)
Total number of interruptions	285	261	546
Median length of an interruption	7	7	7
(IQR)	(6-14)	(5-15)	(6-14)
[range]	[4-88]	[4-248]	[4-248]

*Percentages are of patients with any interruption

In ARROW there was no evidence of a difference in the number of interruptions between randomised monitoring strategies (p=0.35), but there was a slightly higher total time off treatment per patient in the CD4 monitoring group (p=0.06); however as the difference is marginal and the IQRs are similar, monitoring strategies have been grouped together below.

3.3.5. Reasons for unplanned treatment interruptions (DART and ARROW)

In both DART and ARROW the reason for an unplanned treatment interruption (UPTI) was recorded in nearly all patients. A full breakdown of the reasons for UPTIs is shown in Table 3.3.6 (DART) and Table 3.3.7 (ARROW).

In DART the most common reasons for UPTIs were structural (1351, 69%) which mostly consisted of patients being unable to attend clinic (1193, 61%) (Table 3.3.6B); other structural reasons included being in prison/custody, drugs lost or stolen, or running out of drugs (including 1 dispensing error, 1 wrong dose instruction and 1 wrong clinic date). This was followed by adverse events (432, 22%), of which >50% were due to blood disorders. Adverse events included neutropenia, anaemia, hypersensitivity, vomiting (reported as an adverse event), acute hepatic failure; a full list is in Table 3.3.6A. There were 65 (3%) UPTIs that were due to illness including 12 related to psychosis/mental illness which included hearing voices (Table 3.3.6C). There were 102 (5%) UPTIs that were due to voluntary decisions by patient to stop, including 13 related to divine healing/faith. For 38 (37%) of these voluntary decisions there were no further details. In 8 (8%) the patient had forgotten, 3 (3%) were discouraged by relatives and in addition 12 (12%) were due to vomiting, but these had not been reported as adverse events and were simply the reason given by the patient.

Table 3.3.6 Full description of reasons for UPTIs (adults)

A: Adverse events

Adverse event	N	%
Neutropenia	108	25%
Anaemia	104	24%
Lactic acidosis	71	16%
Hepatotoxicity	21	5%
Pancreatitis	12	3%
Renal failure	12	3%
Hypersensitivity	11	3%
Vomiting	11	2%
Raised LFTs	10	2%
Hepatitis	6	1%
Myopathy	5	1%
Congestive heart failure	4	1%
Peripheral neuropathy/paraesthesia	4	1%
Steven Johnson Syndrome	4	1%
Anaemia & neutropenia	3	1%
Diarrhoea	3	1%
Jaundice	3	1%

Rash	3	1%
Abdominal pain	2	0.5%
Intestinal obstruction	2	0.5%
Lipodystrophy	2	0.5%
Lipodystrophy & lactic acidosis	2	0.5%
Liver failure	2	0.5%
Nephritis	2	0.5%
Steatosis	2	0.5%
Upper GI haemorrhage	2	0.5%
Bi-polar affective disorder	1	0.5%
Bone pain	1	0.2%
Bronchospasms	1	0.2%
Cardiomyopathy	1	0.2%
Cardiomyopathy & DVT	1	0.2%
Cirrhosis	1	0.2%
Convulsions	1	0.2%
Hepatic failure (acute)	1	0.2%
Jaundice & liver failure	1	0.2%
Metabolic acidosis	1	0.2%
Muscle toxicity	1	0.2%
Myopathy & raised CPK	1	0.2%
Nausea	1	0.2%
Neutropenia & fatigue	1	0.2%
Non-fatal trauma	1	0.2%
Pancytopenia	1	0.2%
Pruritus	1	0.2%
Raised bilirubin	1	0.2%
Raised creatinine	1	0.2%
Raised LFTs & neutropenia	1	0.2%
SJS & diarrhoea	1	0.2%
Total	**432**	

B: Structural

Structural	**N**	**%**
Unable to attend*	1193	88%
Patient travelled	55	4%
Patient failed to return	43	3%
In prison/custody	19	1%
Drugs stolen	13	1%
Ran out of drugs†	8	1%
Drugs lost	7	1%
Moved away	6	0%
No transport	6	0%
Overdosed	1	0%
Total	**1351**	

* There are no other details for these patients;

† Includes 1 wrong dispense, 1 wrong dose instruction, 1 wrong clinic date

C: Illness

Illness	N	%
Patient felt unwell	13	20%
Psychosis/mental illness	12	18%
Intercurrent illness	10	15%
Admitted to hospital	9	14%
Too ill to take drugs	9	14%
Anti-TB treatment	8	12%
Anorexia/diarrhoea/vomiting	2	3%
Interrupted for palpitations	1	2%
Paralytic ileus, no oral feed	1	2%
Total	**65**	

D: Voluntary decision

Voluntary decision	N	%
No further details given	38	37%
Seeking/has divine healing/faith reasons	13	13%
Vomiting*	12	12%
Patient forgot/misunderstood	8	8%
Discouraged by relatives	3	3%
Pill burden	3	3%
Advised by other doctor	2	2%
Abdominal pain*	2	2%
Felt depressed	2	2%
No longer interested	2	2%
To avoid side effects	2	2%
Domestic problems	2	2%
Diarrhoea*	1	1%
Fear of drugs	1	1%
Fear of hypersensitivity	1	1%
Fear of lipodystrophy	1	1%
Nausea*	1	1%
Felt well	1	1%
Has dental wiring	1	1%
Left without collecting drug	1	1%
Nausea & night terrors	1	1%
Patient was fasting - reason unknown	1	1%
Patient drowsy	1	1%
Refused to take medicine	1	1%
Sweating*	1	1%
Total	**102**	

*Not reported as adverse events but as patient reason

A full breakdown of reasons for unplanned treatment interruptions in ARROW is shown in Table 3.3.7. I have categorised the reasons in ARROW as I did in DART, however voluntary reason is now presented with the subcategories patient and carer as the carer also plays a role in whether or not a child receives treatment. Structural reasons have been split into 'structural patient' and 'structural carer', for the same reason.

As in DART the most common reasons for a treatment interruption in ARROW were structural, here due to the child (335, 61%) which as in DART consisted mostly of being unable to attend clinic (271, 50%); other structural patient reasons included no access to drugs, child travelled or child had moved. This was followed by structural reasons due to the carer (85, 16%) which included loss of drugs by carer, carer travelled or moved or the carer had no transport. The next most common reason was a voluntary (child/carer) decision to stop (child 71, 13%; carer 24, 4%) which mostly consisted of carer or child refusing to give/take drug. Other voluntary reasons for the child included pill burden, social reasons/stigma and forgetting to take drug. As in DART there were some carers who did not administer drugs to the child due to spiritual healing/faith.

Table 3.3.7 Details of reasons for interrupting treatment (children)

A: Adverse event

Adverse event	Total	%
Weakness/dizzy	1	17%
Hepatitis toxicity	1	17%
Hypersensitivity reaction	1	17%
Pre-existing cholesystitis	1	17%
Raised liver enzymes	1	17%
Vomiting	1	17%
Total	**6**	

B: Structural – carer

Structural - carer	N	%
Carer travelled	34	40%
Carer moved/not around	23	27%
Carer did not attend	13	15%
Changed carer	7	8%
Carer lost drugs/syringes/did not take drugs with them	4	5%
Carer no transport	4	5%
Total	**85**	

C: Structural – Patient

Structural - patient	N	%
Unable to attend	271	82%
Patient travelled	22	7%
Patient did not attend	16	5%
Patient moved	6	2%
Patient no transport	6	2%
No access to drugs	4	1%
No food	3	1%
Patient lost drugs	2	1%
In prison†	1	0.3%
Total	**331**	

†Girl aged 13 at randomisation

D: Illness

Illness	N	%
Carer unwell	11	52%
Child too unwell	4	19%
Intercurrent illness	2	10%
Mother developed psychosis	2	10%
Drugs causing child to cough	1	5%
Oral Sores	1	5%
Total	**21**	

E: Voluntary decision – patient

Voluntary decision – patient	N	%
Child refused/decided to stop drugs	29	38%
Child forgot	23	30%
Pill burden	10	14%
Social reasons/stigma	8	11%
Family problems	4	5%
Got married	1	1%
Said pills would prevent pregnancy	1	1%
Total	**76**	

F: Voluntary decision – carer

Voluntary decision – carer	N	%
Carer refused/decided not to give drugs	12	48%
Carer forgot	9	36%
Carer used spiritual healing	3	12%
Not disclosed to spouse	1	4%
Total	**25**	

Table 3.3.8 Reasons for unplanned treatment interruptions

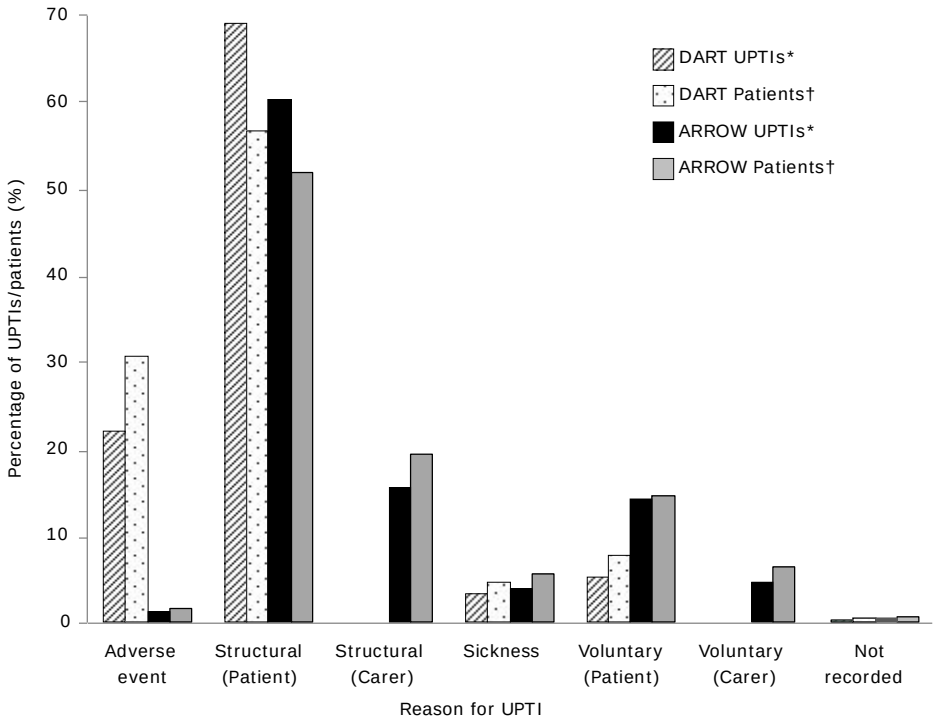
	Adults					Children				
Reason*	**Number of UPTIs**		**Number of patients**		**Duration of UPTI (days)**	**Number of UPTIs**		**Number of patients**		**Duration of UPTI (days)**
	N	**%**	**N**	**%***	**Median (IQR)**	**N**	**%**	**N**	**%***	**Median (IQR)**
Adverse event	432	22%	358	31%	15 (7-35)	6	1%	6	2%	11 (6-39)
Structural										
Patient	1351	69%	660	57%	7 (6-16)	335	61%	190	52%	7 (5-14)
Carer	-	-	-	-	-	85	16%	72	20%	7(5-13)
Illness	65	3%	54	5%	12 (7-23)	21	4%	20	5%	7 (6-13)
Voluntary reason										
Patient	102	5%	89	8%	18 (7-89)	71	14%	53	14%	11 (6-25)
Carer	-	-	-	-	-	24	4%	23	6%	12 (7-22)
Not recorded	5	0.3%	5	0.4%	14 (13-14)	4	0.7%	4	1%	9 (7-12)
Total	**1955**		**1055†**		**9 (6-24)**	**546**		**285†**		**7 (6-14)**

*Percentage of patients reporting any UPTI included for all reasons
†Not equal to column total as patients can occur in more than one category

Table 3.3.8 shows a summary of the main reason categories in adults and children together for ease of comparison. These data are also displayed in Figure 3.3.7. Adverse events were a much less common reason for interrupting ART in children compared to adults. Possible reasons could be greater difficulty in ascertaining AEs in children, which may then get reported as a voluntary reason for not taking drugs. In these specific analyses, adults in DART all took zidovudine long-term as part of their NRTI backbone, whereas only one-third of children in ARROW took zidovudine after 36 weeks. As this drug is known to cause haematological and some gastrointestinal toxicity it may be that rates were genuinely lower in ARROW. However, the main ARROW trial found little difference between randomised ART groups in overall toxicity rates. Thus children may have lower AE rates regardless of the specific drugs as they tend to tolerate drugs well.

In adults the median unplanned total time off treatment per patient per reason differed significantly across the different reasons (p<0.001) with the longest time off being for a voluntary reason (median 18 days IQR [7-89]), followed by adverse events (15 [7-35]) and illness (12 [7-23]). The shortest time off was for structural reasons (7 [6-16]). Results were similar in children with the median unplanned total time off treatment also differing across the different reasons (p=0.01) with the longest time off being for voluntary reasons for not taking drugs (median 11 days IQR [6-25] for patients and 12 [7-22] for carers) and adverse events (11 [6-39]). The shortest unplanned total time off treatment was for non-adverse event illness (7 [6-13]) and structural patient (7[5-14]) and structural carer (7[5-13]) reasons. Generally time off treatment for UPTIs was shorter for children than for adults (p<0.001). Surprisingly, children had less time off for voluntary reasons than adults (median 11 days children vs. 18 days adults, p=0.04) and there was a similar pattern for adverse events (median 11 days children vs. 15 days adults, but non-significant, p=0.73). Time off treatment due to illness was also less in children than adults (median 7 days children vs. 12 days adults, p=0.03). As might be expected, time off for structural reasons was similar with a median of 7 days in both adults and children; the median test however showed that there was a significant difference in time spent off for structural reasons between the two groups based on the data distribution via the sum of ranks (p=0.02).

Figure 3.3.7 Reasons for unplanned treatment interruptions (DART and ARROW)



*The denominator is the total number of UPTIs (DART n=1955; ARROW n=546)
†The denominator is the total number of patients reporting a UPTI included for all reasons (DART n=1166; ARROW n=361)

3.3.6. Effect of total duration off treatment on long term CD4 in DART

Here I am interested in the impact of total duration off treatment on long term CD4 in DART; the outcome of interest is CD4 at 4 years post ART initiation. There were data on CD4 at 4 years for 2826 (85%) adults; 339 (10%) had died prior to 4 years, 139 (4%) were lost to follow-up and 12 (<1%) had no CD4 count available.

As discussed in the methods (section 3.2.7) I assessed the effect of total duration off treatment first using multivariable fractional polynomials (MFPs) excluding any UPTIs that occurred after CD4 at 4 years. All STIs occurred before 4 years. I have adjusted for baseline factors, total duration off treatment and whether a patient ever/never interrupted ART (based on my findings from the STI sub-study analysis) (Table 3.3.9).

Table 3.3.9 Modelling effect of total time off treatment on CD4 at 4 years using MFP

Variable	Estimate** (95%CI)	p-value	Powers
Ever/never had an interruption (any time off treatment)	-3 (-17, 11)	0.70	
Total time off treatment (per week)*	0.5 (-0.11, 1.17)	0.11	1
Sex: Male	0	-	
Female	55 (43, 67)	<0.001	1
Centre:			
Entebbe, MRC/UVRI	0	-	1
Kampala, JCRC	-18 (-32, -4)	0.01	
University of Zimbabwe, Harare	6 (-4, 20)	0.37	
IDI†, Mulago	-16 (-37, 4)	0.12	
Monitoring strategy:			
LCM (CD4 monitoring)	0	-	1
CDM (no CD4 monitoring)	-19 (-30, -8)	-0.001	
Pre ART CD4 (per 50 cells/µL higher)‡	26 (21, 30)	<0.001	1
Age at ART initiation (per 10 years older)‡	-8 (-15, -0.8)	0.03	1

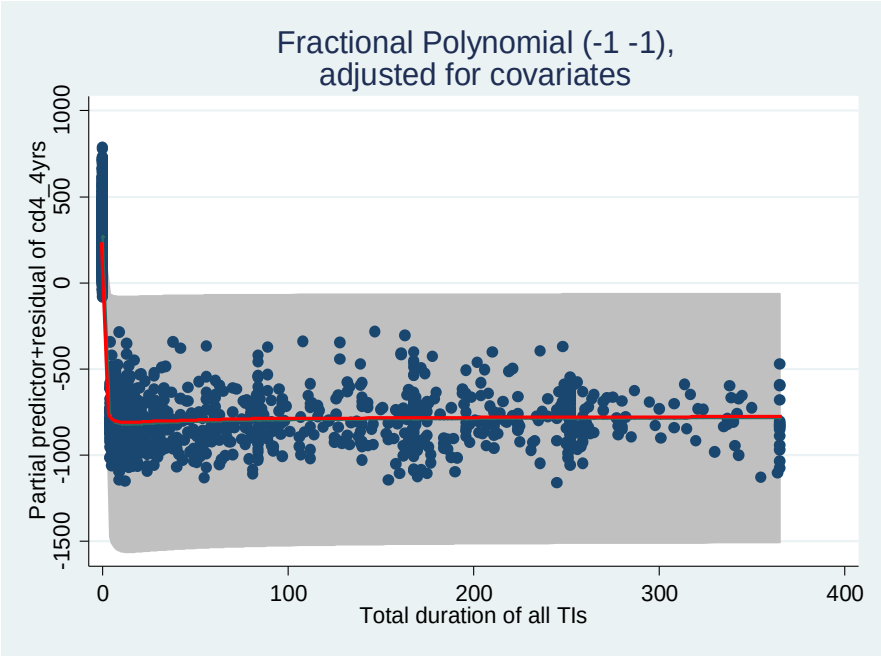
*Both STIs and UPTIs; †Infectious disease Institute; ‡Reference category: pre ART CD4 = 100 cells/µL; age=40 years

**Note there were no transformation of covariates for any covariate

Number of observations = 2826 (number of patients with CD4 at 4 years post ART initiation)

These results suggest that there is no effect of having had any time off ART and the more time off treatment a patient has the higher their CD4 count at 4 years (although not statistically significant). Based on the results shown in Table 3.3.2 this contradicts the randomised comparison of STI vs. CT suggesting there is unadjusted confounding. Even though they are slightly different endpoints, CD4 at 4 years post randomisation should not be too far from CD4 at 132 weeks post sub-study closure. One issue with MFP is that I have chosen a relatively stringent cut off for non-linearity (α=0.05 in the notation of section 3.2.8), hence a linear fit has been chosen in the model for all participants. If I simply allow the model to choose the "best" fractional polynomial for total time off treatment (α=1 in the notation of section 3.2.8) then a FP2 model with the power p_1=-1 and p_2=-1 is chosen; i.e. it has fit total duration off treatment using a FP of order 2 (fitting two FPs) which both have powers of the order of -1. Comparing the AICs for these two models (36121.97 and 36114.29 respectively) would suggest that the FP(-1,-1) model is a modestly better fit to the data. Figure 3.3.8 shows how this model has been fit to the total time off treatment data.

Figure 3.3.8 MFP fit for total time off treatment in all patients (CD4 at 4 years)



The results suggest that there is an initial rapid decrease in CD4 count at 4 years for patients taking any time off ART (Figure 3.3.8; red line) followed by a fairly consistent CD4 for increasing time off treatment. I have investigated this further, first by fitting the same model to the CD4 at 132 weeks post STI sub-study closure endpoint in just the STI/CT sub-study patients to see whether I found a similar result (Table 3.3.10).

Table 3.3.10 Modelling the effect of total time off treatment on CD4 at 132 weeks post sub-study closure using MFP (STI sub-study patients only).

Variable	Estimate** for STIs (95%CI)	p-value	Powers
Ever/never had an interruption (any time off treatment)	-77 (-133, -22)	0.007	1
Total time off treatment (per week)*	1.0 (-0.90, 2.91)	0.30	1
Sex: Male	0	-	1
Female	86 (54, 117)	<0.001	
Centre:			
Entebbe, MRC/UVRI	0	-	1
Kampala, JCRC	-11 (-48, 26)	0.56	
University of Zimbabwe, Harare	-9(-41, 23)	0.08	
IDI†, Mulago	-53 (-112, 6)	0.79	
Monitoring strategy:			
LCM (CD4 monitoring)	0	-	1
CDM (no CD4 monitoring)	-4 (-31, 24)	0.79	
Pre ART CD4 (per 50 cells/μL higher) ‡	20 (7, 32)	0.002	1
Age at ART initiation (per 10 years older)‡	0.1 (-0.1, 0.4)	0.30	1

*For STIs and UPTIs in STI sub-study patients only; †Infectious disease Institute; ‡Reference category: pre ART CD4 = 100 cells/μL; age=40 years

**Note there were no transformations of covariates for any covariate

Number of observations = 741 (number of patients with CD4 at 132 weeks post sub-study closure in the STI/CT patients)

In contrast to the overall model, this model suggests that ever having any time off treatment results in a significant decrease in CD4 and that as total time off increases there is a non-significant increase in CD4 count. The model estimates for the impact of ever having had an interruption are very different.

Tables 3.3.9 and 3.3.10 show that for both CD4 at 4 years and CD4 132 weeks after sub-study closure when including the ever/never off ART variable and allowing the MFP model to fit any transformation to the total time off, the best fit for the total time off is in fact a linear model. However, both models suggest a non-significant increase in CD4 for more time off treatment which is not expected. One possibility is that there is perhaps something different about patients having unplanned treatment interruptions. I fitted several models to investigate the effect of total duration off treatment, considering STIs and UPTIs separately and also the total duration off treatment for all TIs. I used both CD4 132 weeks post STI sub-study closure in STI/CT patients only (Table 3.3.11) and CD4 at 4 years post randomisation in all patients (Table 3.3.12) as endpoints. In all models I have fit an ever/never off ART variable and allowed MFP to choose the “best” model. This does mean that results cannot necessarily be compared across models; however, MFP consistently chose linear functions meaning this was possible.

Table 3.3.11 Effect of duration off treatment on CD4 132 weeks after sub-study closure (STI/CT patients only)

Total duration off treatment		CD4 at 132 weeks after 16th March 2006 n=741				
		Number of STIs (Table 3.3.2)	**Total duration of STIs**	**Total duration of unplanned interruptions‡**	**Total duration of all interruptions**	**Total duration of all interruptions STI and UPTI separately**
Ever/never had an interruption or Number of STI cycles	0 (CT) 1 2 3 4	0 -58 (-107, -9) -55 (-93, -18) -47 (-85, -8) -18 (-88, 52)	-77 (-133, -22)	1 (-38, 39)	-39 (-81, 2)	UPTI: -2 (-40, 37) STI: -77 (-133, -22) **heterogeneity p=0.02**
Total duration off treatment per week (modelled as chosen by MFP*)		n/a	1 (-1, 3)	0 (-4, 5)	-0 (-2, 1)	UPTI: 1 (-4, 5) STI: 1 (-1, 3) **heterogeneity p=0.88**
Sex Male		0	0	0	0	0
Female		86 (54, 118)	86 (54, 118)	86 (54, 119)	85 (53, 117)	86 (54, 118)
Centre Entebbe		0	0	0	0	0
Kampala		-14 (-51, 24)	-11 (-48, 26)	-16 (-53, 22)	-12 (-49, 26)	-11 (-49, 26)
Harare		-9 (-41, 24)	-9 (-41, 23)	-10 (-42, 23)	-10 (-43, 22)	-9 (-41, 23)
IDI†		-53 (-113, 6)	-53 (-112, 6)	-51 (-110, 9)	-49 (-108, 10)	-54 (-113, 5)
Monitoring strategy						
LCM		0	0	0	0	0
CDM		-4 (-31, 23)	-4 (-31, 24)	-4 (-31, 24)	-4 (-31, 23)	-4 (-31, 24)
Baseline CD4 (per 50 cells/μL^3, centred at 100)		20 (7, 32)	20 (7, 32)	19 (7, 32)	21 (8, 33)	20 (7, 32)
Age at baseline (per 10 years, centred at 40)		-0 (-18, 17)	-0 (-18, 18)	1 (-17, 19)	0 (-17, 18)	-0 (-18, 18)
Constant (mean CD4 in reference category)		431 (394, 469)	452 (409, 496)	415 (378, 452)	439 (396, 481)	453 (408, 498)

*MFP has chosen a linear model of order 1 for each model

†Infectious Disease Institute

‡Ignoring time off for STIs

Looking across rows of Table 3.3.11 (STI/CT patients only) I note first that all other effects remain similar to that from my original analysis (Table 3.3.2, repeated in the first column of Table 3.3.11 for reference). However, the impact of treatment interruptions (TIs) varies depending on whether I consider all TIs, planned TIs (STIs) or unplanned TIs (UPTIs). The model where I include STI and UPTI as separate covariates shows different effects for ever/never interrupting ART (test for heterogeneity p=0.02); however they are in the same direction and differ only in magnitude. The effects of total time off treatment are small and there is no evidence of a difference for UPTI and STI (heterogeneity p=0.88). Results are similar in Table 3.3.12 (all patients) where effects of all other factors are similar regardless of whether all TIs, planned TIs or unplanned TIs are modelled but again the effects of STIs and UPTIs appear to be different; this time the effect of STIs is in the opposite direction for the ever/never off depending on whether unplanned or all TIs are modelled, however these effects are small.

Further, even when I have included UPTIs and STIs separately in one model the estimate of effect suggests CD4 at 4 years is 57 cells/μL higher in those who have undertaken STIs. Again, as with the patients receiving all 4 cycles of STIs, those who were randomised into the STI/CT sub-study would be a selected sub population who will have been doing better at STI/CT randomisation compared to those not randomised to the STI sub-study. I therefore next adjusted for whether or not a patient had been randomised to the STI/CT sub-study (5^{th} column of Table 3.3.12). In this final adjusted model those undertaking STIs had lower CD4 counts at 4 years than those not undertaking STIs, in agreement with the main STI/CT analysis; however the effect of UPTIs has remained the same. I note however that power was too low to exclude these differences between STI and UPTIs being compatible with chance.

Table 3.3.12 Effect of duration off treatment on CD4 4 years after ART randomisation (all patients)

Total duration off treatment Continuous variable		CD4 at 4 years				
		Total duration of STI cycles STI/CT pts only (n=782)	Total duration of unplanned interruptions (n=2826)	Total duration of all interruptions (n=2826)	Total duration of all interruptions STI and UPTI separately (n=2826)	Total duration of all interruptions STI and UPTI separately - adjusting for STI/CT randomisation or not (n=2826)
Randomised to STI/CT						
No		-	-	-	-	0
Yes						128 (113, 144)
Ever/never had an interruption		-58 (-102, -14)	-0 (-14, 14)	0 (-0, 0)	UPTI: 1 (-13, 15)) STI: 57 (19, 95) **heterogeneity p=0.006**	UPTI: 2 (-11, 16) STI: -48 (-86, -9) **heterogeneity p=0.02**
Total duration off treatment per week (modelled as chosen by MFP*)		0 (-2, 2)	-2 (-3, -1)	-19 (-117, 79)	UPTI: -2 (-3, -1) STI: -1 (-2, 1) **heterogeneity p=0.11**	UPTI: -2 (-3, -1) STI: -0 (-2, 1) **heterogeneity p=0.11**
Sex	**Male**	0	0	0	0	0
	Female	54 (29, 79)	55 (43, 66)	55 (43, 67)	54 (42, 65)	49 (38, 60)
Centre	**Entebbe**	0	0	0	0	0
	Kampala	21 (-8, 50)	-18 (-31, -4)	-18 (-32, -4)	-15 (-28, -1)	-3 (-16, 11)
	Harare	36 (10, 61)	6 (-8, 19)	6 (-7, 20)	7 (-7, 20)	11 (-2, 24)
	IDI	-11 (-58, 36)	-15 (-36, 5)	-16 (-37, 4)	-12 (-33, 8)	-3 (-23, 17)
Monitoring strategy						
	LCM	0	0	0	0	0
	CDM	-13 (-34, 9)	-18 (-29, -8)	-19 (-29, -8)	-18 (-29, -7)	-18 (-28, -8)
Baseline CD4 (per 50 cells/μL^{-3}, centred at 100)		5 (-5, 15)	27 (22, 31)	26 (21, 30)	24 (19, 29)	15 (11, 20)
Age at baseline (per 10 years, centred at 40)		-3 (-17, 11)	-9 (-16, -2)	-8 (-15, -1)	-9 (-16, -2)	-7 (-13, -0.1)
Constant (mean CD4 in reference category)		370 (336, 405)	281 (268, 296)	282 (268, 297)	272 (257, 287)	248 (234, 263)

*MFP has chosen a linear model of order 1 for each model, †Infectious Disease Institute

As in section 3.3.4, UPTIs differed from STIs in length as well as their unplanned vs planned nature (median [IQR] 18 [7-49] days for UPTIs in DART vs 84 [56-84] days for STIs). In fact 75 (7%) STIs were 84 days or longer and 765 (70%) were 70 days or longer; and the median (IQR) duration of STIs <70 days was only 7 (7-43) days. As described in section 3.2.9, fractional polynomials are better at modelling broad or global non-linearities, whereas other methods, such as natural cubic splines can be better models for local non-linearity. To further try to understand whether there was any evidence for local non-linearity here I tried to estimate the effects of duration of STIs and UPTIs using cubic splines, but they gave no additional insight (data not shown). Unfortunately, none of the additional non-linear spline terms were significant, making it difficult to distinguish signal from noise. There was certainly no evidence to support major non-linearity in effects at low durations of treatment interruptions.

As the STI/CT population is so different and for example Tables 3.3.11 and 3.3.12 suggest that the effect of other factors may differ somewhat between them and patients never randomised to STI/CT, I have therefore decided to fit two separate models, one for patients in the STI/CT sub study and one for all other patients, which I have then combined using meta-analysis techniques to give an overall estimate for UPTIs.

I have first fit my model for the impact of STIs and UPTIs on CD4 4 years after ART initiation in STI/CT patients (Table 3.3.13) adjusting for the other factors as in Table 3.3.9. I have then fitted a model for UPTIs in all other patients who are not part of the STI/CT sub-study (Table 3.3.14), adjusting for the other factors in Table 3.3.9.

Table 3.3.13 CD4 4 years from ART initiation: impact of STIs and UPTIs in STI/CT patients (n=782)

Variable*		Estimate (95%CI)	p-value
Ever/never had an interruption (any time off treatment STIs)		-54 (-98, -10)	0.02
Linear total time off treatment (STIs) (per extra week off ART)		-0.1 (-1.6, 1.4)	0.89
Ever/never had an interruption (any time off treatment UPTIs)		5 (-25, 34)	0.76
Linear total time off treatment (UPTIs) (per extra week off ART)		-0.3 (-3.2, 2.7)	0.86
Sex:	Male	0	-
	Female	54 (29, 79)	<0.001
Centre:			
	Entebbe, MRC/UVRI	0	-
	Kampala, JCRC	20 (-9, 50)	0.17
	University of Zimbabwe, Harare	36 (11, 61)	0.01
	Infectious Diseases Institute, Mulago	-13 (-58, 36)	0.65
Monitoring strategy:			
	LCM (CD4 monitoring)	0	-
	CDM (no CD4 monitoring)	-13 (-34, 9)	0.25
Pre ART CD4 (per 50 cells/µL higher)†		5 (-5, 15)	0.29
Age at ART initiation (per 10 years older)†		-3 (-17, 11)	0.70
Mean CD4 in reference category		367 (332, 404)	<0.001

*Using backwards elimination (alpha=0.05) the following terms are independent predictors for CD4 at 4 years post ART initiation: any time off ART for STIs, sex and centre; †Reference category: pre ART CD4 = 100 cells/µL; age=40 years

Note: there is no interaction between STI/CT randomisation and total time off treatment for UPTIs or having ever had any time off for UPTIs (p=0.124 and p=0.668, respectively)

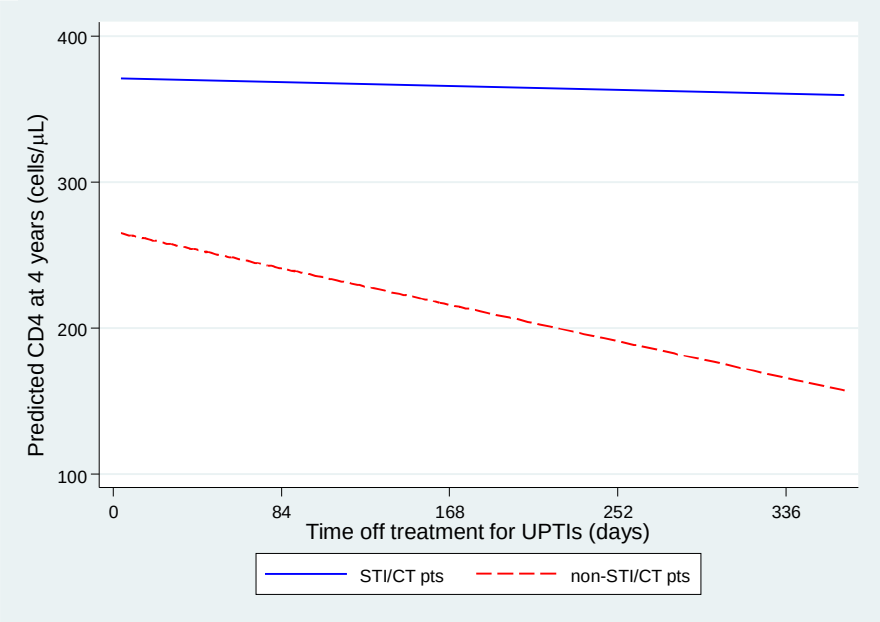
Table 3.3.14 CD4 4 years from ART initiation: impact of UPTIs in non-STI/CT patients (n=2044)

Variable*		Estimate (95%CI)	p-value
Ever/never had an interruption (any time off treatment UPTIs)		0.5 (-14, 15)	0.95
Linear total time off treatment (UPTIs) (per extra week off ART)		-2.1 (-3.5, -0.7)	0.001
Sex:	Male	0	-
	Female	47 (35, 59)	<0.001
Centre:			
	Entebbe, MRC/UVRI	0	-
	Kampala, JCRC	-13 (-28, 1)	0.08
	University of Zimbabwe, Harare	-2 (-17, 13)	0.81
	Infectious Diseases Institute, Mulago	-5 (-26, 16)	0.62
Monitoring strategy:			
	LCM (CD4 monitoring)	0	-
	CDM (no CD4 monitoring)	-19 (-30, -8)	0.001
Pre ART CD4 (per 50 cells/µL higher)†		19 (14, 24)	<0.001
Age at ART initiation (per 10 years older)†		-8 (-16, -1)	0.03
Mean CD4 in reference category		266 (250, 281)	<0.001

*Using backwards elimination (alpha=0.05) the following terms are independent predictors for CD4 at 4 years post ART initiation: total time off for UPTIs, pre-ART CD4, sex and monitoring strategy; †Reference category: pre ART CD4 = 100 cells/µL; age=40 years

Figure 3.3.9 shows the effects of UPTIs in STI/CT patients and non STI/CT patients.

Figure 3.3.9 Effect of UPTIs on CD4 at 4 years post randomisation for STI/CT and non-STI/CT patients



In non-STI/CT patients the more time off treatment for UPTIs a patient has the lower the CD4 count at 4 years. In STI/CT patients there is a similar trend but it is less pronounced. Using meta-analysis techniques (where each population is like a trial in a meta-analysis; STI/CT or non STI/CT patients) I have combined the two estimates for UPTIs to give an overall estimate of -1.8 cells/μL per extra week off ART (95%CI -3.0, -0.5, p=0.006). This suggests that for every cumulative month off ART for a UPTI, CD4 4 years post ART initiation was 8 cells/μL lower (95%CI 2-13, p=0.001). There was no evidence of heterogeneity in this estimate between those who were randomised to the STI/CT sub-study and those who were not (p=0.27). In those patients randomised to STI/CT the model in Table 3.3.13 suggests that CD4 at 4 years was 54 cells/μL (10-98) lower in those who had STIs (p=0.02), however there was no additional effect of longer STIs (p=0.89).

3.3.7. Effect of total duration off treatment on long term CD4 in ARROW

Here I am interested in the impact of total duration off treatment on long term CD4 in ARROW (children); the outcome of interest is CD4 at 3 years post monitoring strategy randomisation. There are two different ways of analysing the CD4 count, either as an absolute count (cells/μL) as I have done for adults or as a percentage of the total lymphocyte count (CD4%). In uninfected children the CD4 count is strongly dependent on age; it is very high (around 4000 cells/μL) when children are born and then drops considerably to around 1000 cells/μL at around 4/5 years of age. The CD4% is much more stable with age. As described in section 3.2.7 I have therefore modelled the CD4% at 3 years.

There are data on 1145 patients for CD4 at 3 years (CD4% and absolute CD4 count); 45 (4%) died prior to 3 years and 16 (1%) were lost to follow-up. For every extra cumulative week off ART (all UPTIs), in a multivariable model CD4% 3 years after ART initiation was 0.4% lower

(0.07-0.6%, p=0.01) (Table 3.3.15) with no additional effect of having any time off treatment (p=0.28), consistent with the UPTI effect in adults.

Table 3.3.15 Modelling the effect of duration off treatment on CD4% 3 years after ART randomisation

Variable		Estimate (95%CI)	p-value
Ever/never had an interruption (UPTIs)		-0.8 (-2.3, 0.7)	0.28
Total time off treatment per week		-0.4 (-0.6, -0.07)	0.01
Sex:	Male	0	-
	Female	2 (1, 3)	<0.001
Centre: Entebbe, MRC/UVRI		0	-
	Kampala, JCRC	2 (1, 4)	0.001
	University of Zimbabwe, Harare	4 (2, 5)	<0.001
	PIDC*	2 (1, 4)	0.008
Monitoring strategy:			
	LCM (CD4 monitoring)	0	-
	CDM (no CD4 monitoring)	-1 (-2, 0.2)	0.11
First-line treatment strategy†:			
	Arm A	0	-
	Arm B	0.4 (-0.8, 2)	0.54
	Arm C	-1 (-2, 0.2)	0.11
Pre ART CD4% (per 5% higher)‡		2.5 (2.0, 2.5)	<0.001
Age at ART initiation (per 5 years older)‡		-4.0 (-4.5, -3.5)	<0.001
Mean CD4% in reference category		29 (27, 31)	<0.001

*Paediatric Infectious Disease Clinic
†Arm A: NNRTI+3TC+ABC -> NNRTI+3TC+ABC, Arm B: NNRTI+3TC+ABC+ZDV -> NNRTI+3TC+ABC, Arm C: NNRTI+3TC+ABC+ZDV -> 3TC+ABC+ZDV
‡Reference category: pre ART CD4%=10, Age=5

There was no evidence of a significant effect of monitoring strategy or first-line treatment strategy (p>0.1) on CD4% three years after ART initiation. As expected there was an effect of baseline CD4% (2.5% higher for every 5% higher at baseline) and also age at ART initiation, with CD4% 4.0% lower for every 5 years older at baseline (both p<0.001). There was also a centre effect with patients in Zimbabwe having the highest CD4% at 3 years after ART initiation (+4% vs. Entebbe, p<0.001), followed by Kampala with a 3% greater CD4% than Entebbe and PIDC with a 2% greater CD4% than Entebbe (both p<0.001).

3.3.8. Effect of cumulative time off treatment on clinical outcomes in adults (DART)

As outlined in the methods (section 3.2.10) I first assessed the effect of cumulative time off treatment on clinical outcomes in adults using the MFP approach; the best power was FP(1). In a multivariable time-dependent Cox model having any time off treatment increased mortality risk (HR=3.12 [95%CI 2.34-4.13], p<0.001) but there was no independent effect of (time-updated) cumulative time off ART on mortality (HR=1.01 per week [0.99-1.03], p=0.17) (Table 3.3.16). As

expected there was a significant effect of pre-ART CD4 with a higher CD4 at baseline leading to a decrease in the risk of death (p<0.001) and an increase in the risk of death for those in the no CD4 monitoring group (p=0.002). There was no overall significant effect of centre (global p=0.14) although there was some evidence to suggest an increase in the hazard of death for patients at the Infectious Diseases Institute compared to those in Entebbe (p=0.02).

Table 3.3.16 Modelling the effect of cumulative time off treatment on mortality in adults

Variable*		**HR (95%CI)**	**p-value**
Any time off ART (ever vs. never)		3.12 (2.35, 4.13)	<0.001
Cumulative time off treatment (weeks)		1.01 (0.99, 1.03)	0.17
Sex:	Male	1.00	
	Female	0.88 (0.71, 1.09)	0.24
Centre: Entebbe, MRC/UVRI		1.00	-
	Kampala, JCRC	1.08 (0.83, 1.41)	0.58
	University of Zimbabwe, Harare	1.16 (0.88, 1.51)	0.29
	Infectious Diseases Institute, Mulago	1.49 (1.07, 2.07)	0.02
Monitoring strategy:			
	LCM (CD4 monitoring)	1.00	-
	CDM (no CD4 monitoring)	1.37 (1.12, 1.68)	0.002
Pre ART CD4 (per 50 cells/μL higher)†		0.67 (0.61, 0.73)	<0.001
Age at ART initiation (per 10 years older)†		1.07 (0.94, 1.22)	0.32

†Reference category: pre ART CD4=100cells/μL, age=40 years
*After model selection using backwards elimination with exit p=0.1 (BE(0.1)) the following variables remain independent predictors of mortality: monitoring strategy (p=0.002), any time off ART and pre ART CD4 (both p<0.001)

Table 3.3.17 Modelling the effect of cumulative time off treatment on mortality in adults (STI and UPTI separately for STI/CT and non-STI/CT patients)

	STI/CT pts only	Non STI/CT pts only	Meta-Analysis combined (All patients)	Tests for heterogeneity, p
STI				
Any time off ART	1.78 (0.46, 6.83)	-	-	-
Total time off ART*	1.00 (0.95, 1.06)	-	-	-
UPTI**				
Any time off ART	1.24 (0.36, 4.35)	3.81 (2.90, 5.00)	3.62 (2.77, 4.72)	p=0.09
Total time off ART*	1.03 (0.91, 1.15)	1.03 (1.01, 1.04)	1.03 (1.01, 1.04)	p=0.98
Heterogeneity, p				
Any time off ART	p=0.70	-	-	-
Total time off ART*	p=0.74	-	-	-

*Effect of cumulative time off ART per week

**There is no interaction between STI/CT randomisation and total time of ART or any time off ART (p=0.62 and p=0.65, respectively)

Note: adjusted for other factors in Table 3.3.16

Assessing the effect of cumulative time off treatment for STIs and UPTIs separately (Table 3.3.17) showed a non-significant trend towards an increased risk in mortality for any time off treatment for STIs in STI/CT patients (HR=1.78 [0.46, 6.83], p=0.40; only 27 events). There was no evidence of an increased risk in mortality for cumulative time off treatment for STIs in STI/CT patients (HR=1.00 per week, 95%CI 0.95, 1.06; p=0.90).

There was a trend towards an increased risk in mortality for any time off treatment for UPTIs in STI/CT patients, this again was non-significant (HR=1.24 [0.36, 4.35], p=0.34). In addition there was no significant evidence of an increased risk in mortality for cumulative time off treatment for UPTIs in STI/CT patients although the point estimate indicated harm (HR=1.03 per week, 95% CI 0.91, 1.15; p=0.43). Tests for heterogeneity suggested there was no difference in the effect of cumulative time off ART for STIs and UPTIs (p=0.74) or for any time off treatment (p=0.70).

In a separate model in non STI/CT patients, there was significant evidence of an increased risk in mortality for any time off treatment for UPTIs (HR=3.81 [2.90, 5.00], p<0.001) and there was significant evidence of an increased risk in mortality for more time off ART for UPTIs (HR=1.03 per week [1.01, 1.04], p<0.001). Using meta-analysis techniques to combine estimates for STI/CT patients and non STI/CT patients, overall there was evidence of an increased risk of mortality for any time off ART for UPTIs (HR=3.62 [2.77, 4.72], p<0.001) and there was significant evidence of an increased risk in mortality for more time off ART for UPTIs in all patients (HR=1.03 per week [1.01, 1.04], p<0.001). Tests for heterogeneity suggested there was no difference in the effect of any time off ART or cumulative time off ART for UPTIs on risk of mortality for STI/CT patients and non-STI/CT patients (p=0.09 and 0.36 respectively).

Table 3.3.18 Modelling the effect of cumulative time off treatment on WHO4 events/death in adults

Variable*		HR (95%CI)	p-value
Any time off ART (ever vs. never)		2.83 (2.33, 3.43)	<0.001
Cumulative time off treatment (per week)		1.01 (0.99, 1.02)	0.09
Sex:	Male	0	-
	Female	0.90 (0.78, 1.04)	0.16
Centre:			
	Entebbe, MRC/UVRI	0	-
	Kampala, JCRC	1.14 (0.95, 1.37)	0.16
	University of Zimbabwe, Harare	1.25 (1.04, 1.50)	0.02
	Infectious Disease Institute, Mulago	1.23 (0.96, 1.58)	0.10
Monitoring strategy:			
	LCM (CD4 monitoring)	0	-
	CDM (no CD4 monitoring)	1.32 (1.15, 1.52)	<0.001
Pre ART CD4 (per 50 cells/µL higher)†		0.66 (0.62, 0.71)	<0.001
Age at ART initiation (per 10 years older)†		1.00 (0.91, 1.09)	0.97

†Reference category: pre ART CD4=100cells/µL, age=40 years
*After model selection using BE(0.1) the following variables remain independent predictors of mortality: monitoring strategy, any time off ART and pre ART CD4 (all p<0.001) and centre (p=0.08)

Results were similar for WHO4 events/death (HR (ever vs. never off)=2.83 95%CI [2.33-3.43], p<0.001; HR 1.01 per extra week off ART [0.99-1.02], p=0.09) (Table 3.3.18). As with mortality there was a significant effect of pre-ART CD4 with a higher CD4 at baseline leading to a decrease in the risk of a WHO4 event/death (p<0.001) and there was an increase in the risk of a WHO4 event/death for those in the no CD4 monitoring group (p<0.001). There was no overall significant effect of centre (global p=0.08) although there was some evidence to suggest an increase in the hazard of a WHO4 event/death for patients at the University of Zimbabwe, Harare compared to those in Entebbe (p=0.02). There was no effect of age or sex (both p>0.1).

Table 3.3.19 Modelling the effect of cumulative time off treatment on WHO4 event/death in adults (STI and UPTI separately for STI/CT and non-STI/CT patients)

	STI/CT pts only	Non STI/CT pts only	Meta-Analysis combined (All patients)	Tests for heterogeneity, p
STI				
Any time off ART	2.69 (1.19, 6.10)	-	-	-
Total time off ART*	0.97 (0.92, 1.02)	-	-	-
UPTI**				
Any time off ART	1.27 (0.55, 2.92)	3.37 (2.78, 4.10)	3.19 (2.65, 3.88)	p=0.03
Total time off ART*	0.99 (0.89, 1.11)	1.03 (1.02, 1.04)	1.03 (1.01, 1.04)	p=0.55
Heterogeneity, p				
Any time off ART	p=0.19	-	-	-
Total time off ART*	p=0.71	-	-	-

*Effect of cumulative time off ART (per week)
**There is no interaction between STI/CT randomisation and total time of ART or any time off ART (p=0.35 and p=0.60, respectively)
Note: adjusted for other factors in Table 3.3.16

Assessing the effect of cumulative time off treatment for STIs and UPTIs separately showed good evidence to suggest an increased risk of a WHO4 event/death for any time off treatment for STIs in STI/CT patients (HR=2.69 [1.19, 6.10], p=0.02) (Table 3.3.19); however there was no evidence of an increased risk of a WHO4 event/death with increasing cumulative time off treatment for STIs in STI/CT patients (HR=0.97 per week [0.92, 1.02]; p=0.22). There was a trend towards an increased risk in mortality for any time off treatment for UPTIs in STI/CT patients, however this was non-significant (HR=1.27 [0.55-2.92], p=0.57) and there was no evidence of an increased risk in mortality for cumulative time off treatment (HR=0.99 per week [0.89-1.11; p=0.90). Tests for heterogeneity suggested that there was no difference in the effect of cumulative time off treatment or anytime off ART on the risk of a WHO4 event/death for STIs and UPTIs (p=0.19 and p=0.71 respectively).

In non STI/CT patients there was an increased risk of a WHO4 event/death with any time off treatment for UPTIs (HR=3.37 [2.78, 4.10], p<0.001) and an increased risk of a WHO 4 event/death with more time off ART for UPTIs (HR=1.03 per week [1.02, 1.04], p<0.001).

Using meta-analysis techniques to combine estimates for STI/CT and non STI/CT patients, there was an increased risk of a WHO4 event/death for any time off treatment for UPTIs in all patients (HR=3.19 [2.65, 3.88]) with this risk being higher in the non STI/CT patients (p=0.03). There was significant evidence of an increased risk of a WHO4 event/death for more time off ART for UPTIs in all patients (HR=1.03 per week [1.01, 1.04]. However, there was no evidence that this differed for STI/CT patients compared to non-STI/CT patients (p=0.55).

Table 3.3.20 Modelling the effect of cumulative time off treatment on CD4<100 cells/µL after 48 weeks in adults

Variable		HR (95%CI)	p-value
Any time off ART (ever vs. never)		1.82 (1.52, 2.19)	<0.001
Cumulative time off treatment (per week)		1.01 (0.99, 1.02)	0.25
Sex:	Male	0	-
	Female	0.68 (0.58, 0.79)	<0.001
Centre:			
	Entebbe, MRC/UVRI	0	-
	Kampala, JCRC	1.15 (0.95, 1.40)	0.15
	University of Zimbabwe, Harare	1.11 (0.91, 1.36)	0.29
	Infectious Disease Institute, Mulago	1.18 (0.91, 1.53)	0.22
Monitoring strategy:			
	LCM (CD4 monitoring)	0	-
	CDM (no CD4 monitoring)	1.00 (0.86, 1.16)	0.98
Pre ART CD4 (per 50 cells/µL higher)†		0.47 (0.43, 0.51)	<0.001
Age at ART initiation (per 10 years older)†		0.90 (0.82, 1.00)	0.05

†Reference category: pre ART CD4=100cells/µL, age=40 years

*After model selection using BE(0.1) the following variables remain independent predictors of mortality: age at ART initiation (p=0.06) and any time off ART, pre ART CD4 and gender (all p<0.001)

The effects of cumulative time off ART were also similar to mortality for CD4<100 cells/µL after 48 weeks, with having any time off treatment associated with an increased risk of CD4<100 cells/µL after 48 weeks (HR=1.82 [1.52, 2.19], p<0.001) but no independent effect of (time-updated) cumulative time off ART (1.01 per week [0.99, 1.02], p=0.25) (Table 3.3.20). Like mortality and WHO4 events/death there was an effect of pre-ART CD4 on CD4<100 cells/µL after 48 weeks with a higher pre-ART CD4 associated with a decreased risk of a CD4 count <100 cells/µL after 48 weeks (p<0.001). Unlike the other clinical endpoints there was a gender effect with a decreased risk of CD4<100 cells/µL after 48 weeks for women (p<0.001) and there was some evidence (p=0.05) to suggest a small decrease in the risk of CD4<100 cells/µL with increasing age. There was no evidence of a monitoring strategy effect or an effect of centre on CD4<100 cells/µL after 48 weeks (all p>0.1).

Table 3.3.21 Modelling the effect of cumulative time off treatment on CD4<100 cells/μL after 48 weeks in adults (STI and UPTI separately for STI/CT and non-STI/CT patients)

	STI/CT pts only	Non STI/CT pts only	Meta-Analysis combined (All patients)	Tests for heterogeneity, p
STI				
Any time off ART	4.72 (2.06, 10.78)	-	-	-
Total time off ART*	0.97 (0.93, 1.02)	-	-	-
UPTI				
Any time off ART	2.17 (1.03, 4.54)	1.87 (1.56, 2.24)	1.89 (1.58, 2.25)	p=0.71
Total time off ART*	0.92 (0.79, 1.07)	1.03 (1.02, 1.04)	1.03 (1.02, 1.04)	p=0.15
Heterogeneity, p				
Any time off ART	p=0.17	-	-	-
Total time off ART*	p=0.49	-	-	-

*Effect of cumulative time off ART (per week)
**There is no interaction between STI/CT randomisation and total time off ART or any time off ART (p=0.78 and p=0.36, respectively)
Note: adjusted for other factors in Table 3.3.16

Assessing the effect of cumulative time off treatment for STIs and UPTIs separately showed there was strong evidence to suggest an increased risk of immunological failure (CD4<100 cells/μL) with any time off ART for STIs in STI/CT patients (HR=4.72 [2.06, 10.78], p<0.001) but no evidence of an increased risk of immunological failure for increasing cumulative time off ART for STIs in STI/CT patients (HR=0.97 per week [0.93, 1.02], p=0.25) (Table 3.3.21). For UPTIs in STI/CT patients there was significant evidence to suggest an increased risk of immunological failure for any time off ART for UPTIs in STI/CT patients (HR=2.17 [1.03-4.54], p=0.04; 63 events) but no evidence to suggest an increased risk of immunological failure for increasing cumulative time off ART (HR=0.92 per week [0.79, 1.07], p=0.28). Tests for heterogeneity suggest that there was no difference in the effect for STIs and UPTIs for any time off ART (p=0.17) nor cumulative time off ART on immunological failure (p=0.49).

In non STI/CT patients there was strong evidence to suggest an increased risk of immunological failure for any time off ART for UPTIs in non STI/CT patients (HR=1.87 [1.56, 2.24], p<0.001) and for increased cumulative time off ART for UPTIs, (HR=1.03 [1.02, 1.04], p<0.001).

Using meta-analysis techniques to combine estimates for STI/CT patients and non STI/CT patients, there was evidence to suggest an increased risk of immunological failure for any time off ART for UPTIs in all patients (HR=1.89 [1.58, 2.25]), which did not differ between STI/CT and non STI/CT patients (p=0.71). There was also evidence to suggest an increased risk of immunological failure for increasing cumulative time off ART for UPTIs in all patients (HR=1.03 [1.02, 1.04]), which again did not differ between STI/CT and non STI/CT patients (p=0.15).

3.3.9. Effect of cumulative time off treatment on clinical outcomes in children (ARROW)

In a multivariate time-dependent Cox model, having ever interrupted ART showed a similar trend towards increased mortality in children (Table 3.3.22) (HR=3.42 [95% CI 1.44, 8.08], p=0.005), similar to adults, with no independent effect of (time-updated) cumulative time off ART (HR=1.05 per week [0.91, 1.22], p=0.51). As in DART there was an effect of pre-ART CD4 with a higher pre-ART CD4% associated with a decrease in the risk of mortality (HR=0.59 per 5% higher [0.47, 0.74], p<0.001). There was no evidence of an effect of gender, age, monitoring strategy, centre or first-line treatment strategy (all p>0.1).

Table 3.3.22 Modelling the effect of cumulative time off treatment on mortality in children

Variable		HR (95%CI)	p-value
Any time off ART (ever vs. never)		3.42 (1.44, 8.08)	0.005
Cumulative time off treatment per week		1.05 (0.91, 1.22)	0.51
Sex:	Male	0	-
	Female	0.91 (0.53, 1.55)	0.72
Centre:			
	Entebbe, MRC/UVRI	0	-
	Kampala, JCRC	0.35 (0.14, 0.89)	0.03
	University of Zimbabwe, Harare	0.80 (0.36, 1.76)	0.58
	Paediatric Infectious Disease Clinic	0.81 (0.36, 1.86)	0.63
Monitoring strategy:			
	LCM (CD4 monitoring)	0	-
	CDM (no CD4 monitoring)	0.86 (0.50, 1.46)	0.57
First-line treatment strategy†:			
	Arm A	0	-
	Arm B	0.65 (0.33, 1.29)	0.22
	Arm C	1.04 (0.56, 1.93)	0.90
Pre ART CD4% (per 5% higher)‡		0.59 (0.47, 0.74)	<0.001
Age at ART initiation (per 5 years older)‡		1.08 (0.74, 1.57)	0.73

†Arm A: NNRTI+3TC+ABC -> NNRTI+3TC+ABC, Arm B: NNRTI+3TC+ABC+ZDV -> NNRTI+3TC+ABC, Arm C: NNRTI+3TC+ABC+ZDV -> 3TC+ABC+ZDV

‡Reference category: pre ART CD4%=10, Age=5 years

*After model selection using BE(0.1) the following variables remain independent predictors of mortality: pre ART CD4% (p<0.001) and any time off ART (p=0.001)

Table 3.3.23 Modelling the effect of cumulative time off treatment on WHO 4 events/death in children

Variable		HR (95%CI)	p-value
Any time off ART (ever vs. never)		1.33 (0.57, 3.12)	0.51
Cumulative time off treatment (per week)		1.08 (0.89, 1.30)	0.43
Sex:	Male	0	-
	Female	0.90 (0.59, 1.38)	0.63
Centre:			
	Entebbe, MRC/UVRI	0	-
	Kampala, JCRC	0.71 (0.35, 1.42)	0.34
	University of Zimbabwe, Harare	0.66 (0.33, 1.30)	0.23
	Paediatric Infectious Disease Clinic	1.12 (0.56, 2.22)	0.75
Monitoring strategy:			
	LCM (CD4 monitoring)	0	-
	CDM (no CD4 monitoring)	1.22 (0.80, 1.87)	0.36
First-line treatment strategy†:			
	Arm A	0	-
	Arm B	0.87(0.52, 1.45)	0.58
	Arm C	0.96 (0.57, 1.61)	0.88
Pre ART CD4% (per 5% higher)‡		0.62 (0.52, 0.74)	<0.001
Age at ART initiation (per 5 years older)‡		1.31 (0.99, 1.75)	0.06

†Arm A: NNRTI+3TC+ABC -> NNRTI+3TC+ABC, Arm B: NNRTI+3TC+ABC+ZDV -> NNRTI+3TC+ABC, Arm C: NNRTI+3TC+ABC+ZDV -> 3TC+ABC+ZDV

‡Reference category: pre ART CD4%=10, Age=5 years

*After model selection using BE(0.1) the following variables remain independent predictors of mortality: pre ART CD4% (p<0.001) and cumulative time off ART (p=0.08)

In children, having ever interrupted ART showed a non-significant trend towards an increased risk of WHO4 events/death (HR=1.33 [0.57, 3.12], p=0.51) with no evidence of an increased risk of WHO4 events/death with increasing time off ART (HR=1.08 per week [0.89, 1.30] p=0.43) (Table 3.3.23). There was a significant effect of pre-ART CD4%, with a decrease in the risk of WHO4 events/death with a higher pre-ART CD4% (HR=0.62 per 5% higher [0.52, 0.74], p<0.001). There was no evidence of an association of centre, monitoring strategy, gender, first-line treatment strategy or age at ART initiation (all p>0.05).

Table 3.3.24 Modelling the effect of cumulative time off treatment on immunological failure[§] in children

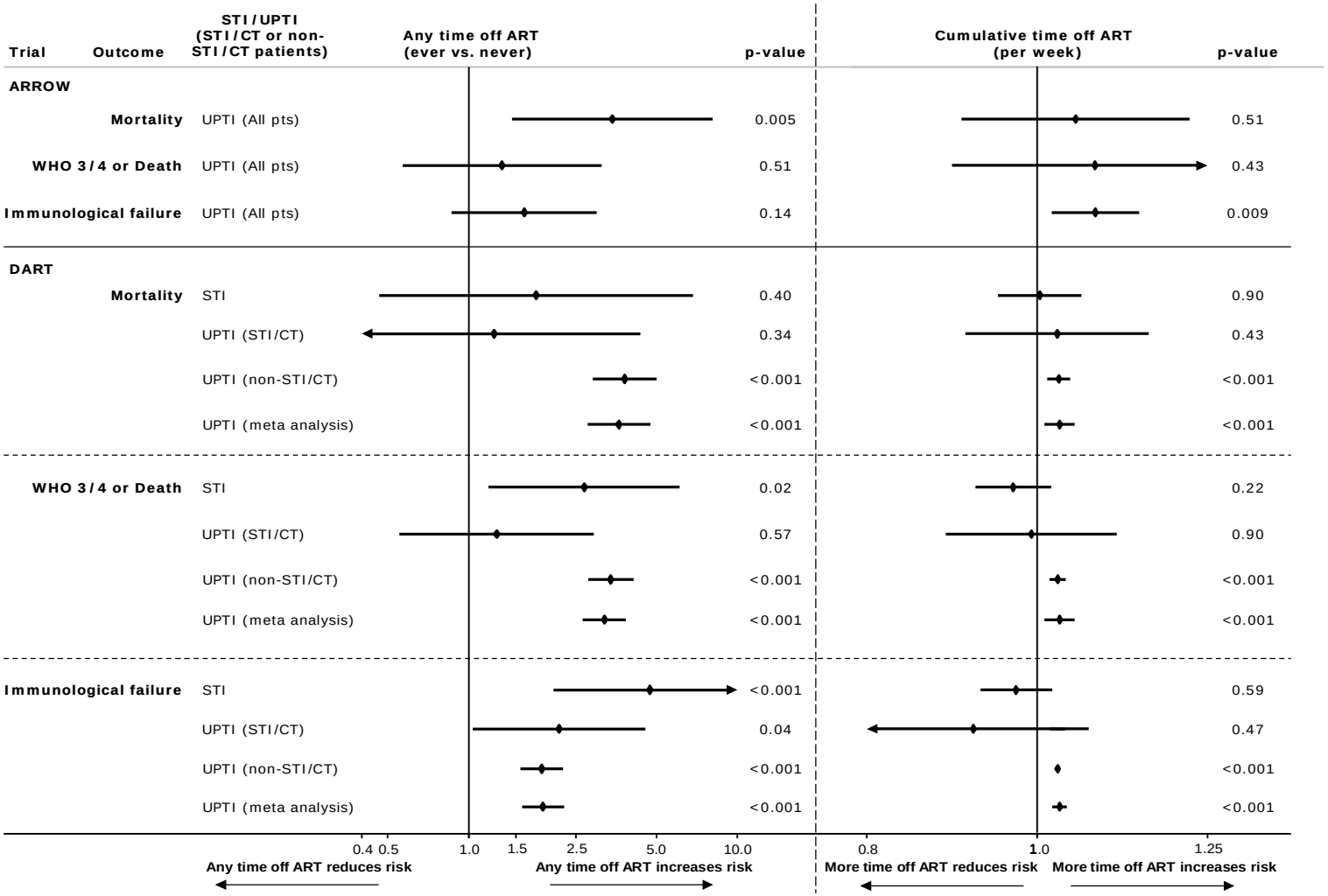
Variable*		HR (95%CI)	p-value
Any time off ART (ever vs. never)		1.61 (0.86, 3.00)	0.14
Cumulative time off treatment (per week)		1.06 (1.01, 1.10)	0.009
Sex:	Male	0	-
	Female	1.14 (0.70, 1.86)	0.60
Centre:			
	Entebbe, MRC/UVRI	0	-
	Kampala, JCRC	0.40 (0.18, 0.87)	0.02
	University of Zimbabwe, Harare	0.60 (0.29, 1.26)	0.18
	Paediatric Infectious Disease Clinic	0.58 (0.26, 1.26)	0.17
Monitoring strategy:			
	LCM (CD4 monitoring)	0	-
	CDM (no CD4 monitoring)	1.80 (1.09, 2.98)	0.02
First-line treatment strategy†:			
	Arm A	0	-
	Arm B	0.53 (0.29, 0.95)	0.03
	Arm C	0.60 (0.33, 1.10)	0.10
Pre ART CD4% (per 5% higher)‡		0.33 (0.25, 0.44)	<0.001
Age at ART initiation (per 5 years older)‡		1.49 (1.04, 2.14)	0.03

†Arm A: NNRTI+3TC+ABC -> NNRTI+3TC+ABC, Arm B: NNRTI+3TC+ABC+ZDV -> NNRTI+3TC+ABC, Arm C: NNRTI+3TC+ABC+ZDV -> 3TC+ABC+ZDV
‡Reference category: pre ART CD4%=10, Age=5 years
§Defined as in section 3.2.6 of the methods: aged 3-<5 years confirmed CD4%<10% and aged 1-<3 years confirmed CD4%<15%; children aged ≥5 years: confirmed CD4 50-99 cells/μL or single CD4 <50 cells/μL
*After model selection using BE(0.1) the following variables remain independent predictors of mortality: pre ART CD4% (p<0.001), cumulative time off ART, monitoring strategy and age (all p=0.02) and first-line treatment strategy vs Arm A (Arm B p=0.03, Arm C p=0.08))

In children, there was a significant evidence to suggest an increased risk of immunological failure after 48 weeks for increasing cumulative time off ART (HR=1.06 per week [1.01-1.10], p=0.009); however, there was only a modest non-significant trend towards an increased risk for having ever interrupted ART (HR=1.61 [0.86-3.00], p=0.14) (Table 3.3.24). There was a significant effect of monitoring strategy (increased risk of immunological failure after 48 weeks with no CD4 monitoring (HR=1.80 [1.09, 2.98], p=0.02) and first-line treatment strategy (increased risk of immunological failure after 48 weeks in Arm A compared to both Arm B and Arm C (although marginal/non-significant) (p=0.03 and p=0.10 respectively; global (2df) p=0.07). In addition there was a highly significant effect of pre-ART CD4% with a substantially decreased risk of CD4<100 cells/μL after 48 weeks with a higher pre-ART CD4% (0.33 per 5% higher [0.25, 0.44], p<0.001). There was evidence of an age effect with an increased risk of immunological failure after 48 weeks with older age at ART initiation (1.49 per 5 years [1.04, 2.14], p=0.03) but no effect of gender (p=0.60).

Figure 3.3.10 shows all estimates for effect of cumulative time off ART and any time off ART for STIs and UPTIs in STI/CT patients and non-STI/CT patients in DART and all patients in ARROW.

Figure 3.3.10 Effect of cumulative time off treatment on mortality, WHO 3/4 or death or immunological failure in DART and ARROW



3.4. Discussion

In this analysis I have assessed the effects of structured treatment interruptions (STIs) on CD4 counts and clinical endpoints after returning to continuous therapy (CT); extending results from the DART STI sub-study which was terminated early due a detrimental effect of STIs on clinical disease progression within the trial [128]. Interestingly, when assessing the number of cycles of STIs patients could have had and the number of cycles they did have, I found that, even if the trial had not been terminated, patients were stopping undergoing STIs. The main reasons for this were low CD4 counts and WHO 3/4 events which is in keeping with the detrimental effects of STIs shown in other studies [119-121, 123-127, 130, 131], and demonstrates that this was unlikely to be a feasible long-term strategy. In addition to STIs I have assessed the effects of unplanned treatment interruptions (UPTIs) on CD4 counts and clinical outcomes and have explored the reasons for these unplanned treatment interruptions. I have also compared the long term effects of STIs and UPTIs on clinical outcomes.

I started by investigating what happened to CD4 counts when patients had returned to CT. I found that after STIs, returning to CT increased a patient's CD4 count, consistent with other studies in both high- and low/middle-income settings as shown by Kranzer et al. in their systematic review [150]. The SMART study [126] found that CD4 guided ART interruption significantly increased the risk of opportunistic disease or death from any cause compared with CT and the trial was terminated early. As in DART those patients in the STI arm were advised to restart therapy and follow-up continued [129]. Similar to DART, CD4 cell counts increased with re-initiation of ART but unlike DART they found that CD4 cell counts recovered to that at randomisation in just 6 months. In DART I have estimated that this would take around 16 months. This difference between the trials could be due to the fact that the SMART data are from high-income settings where patients generally have better prognosis; however, it is most likely that this is related to the higher pre-interruption CD4 cell counts in SMART than in DART (597 cells/μL in SMART vs. 358 cells/μL in DART). Those DART patients who were off treatment at the time of sub-study closure had a steeper increase in CD4 count on returning to ART, consistent with that shown in naïve patients [151]. After 12 weeks on CT their CD4 had increased to the same levels as STI patients on ART when the sub-study was terminated, but both remained well below CD4 counts in CT. The first increase in CD4 after therapy is started is due to the redistribution of CD4 cells into the peripheral blood circulation. Several studies [150, 152, 153] have shown that the effects of ART on CD4 after restarting treatment follow a biphasic pattern and that the effects of ART are indeed greater in the first months of restarting ART. Mussini et al. [153] also found that subjects who resumed ART with a higher viral load experienced a more rapid rate of CD4 increase during the first 3 months after returning to treatment. I was not able to assess the change in viral load as those assays were not routinely done in DART; I am therefore unable to say whether CD4 increases were greater in those with higher VL in DART.

I found that the CD4 deficit associated with having undertaken fixed-length STIs persisted for at least 4-5 years when back on CT, even though patients did reconstitute immunologically. Interestingly, I did not find that undertaking progressively more STIs led to progressively greater CD4 deficits back on treatment – rather, having undertaken one or multiple STIs led to similar deficits. I also found that the effect of treatment interruptions on long term CD4 counts showed no increasing detriment for more time off treatment but having ever interrupted ART had a detrimental effect on CD4 cell counts. This emphasises the importance of maintaining ART supplies and accessibility. The fixed duration strategy of 2 months off, 4 months on used in the TRIVICAN trial (the only other trial in low/middle-income settings of planned treatment interruptions) was able to continue until the end of the trial but the 12 weeks on 12 weeks off strategy in DART stopped early. This raises the question as to whether CD4 would have taken less time to return to that of the CT arm in TRIVICAN, with shorter time off relative to on ART. Unfortunately there are no data to assess this. In DART, however, I showed that *any* time off treatment had a detrimental effect on CD4 and clinical outcomes and that there was little effect of duration of time off treatment, therefore the shorter relative time may have been unlikely to affect reconstitution. Patients in the TRIVICAN trial also had higher pre-ART CD4 cell counts (median 460 cells/μL) vs. pre-CT/STI randomisation CD4 of 358 cells/μL in DART which may have helped faster reconstitution. There was also a higher threshold for stopping a treatment interruption in TRIVICAN (250 cells/μL vs. 50 cells/μL in DART). Thus a more selected patient group will have had repeated interruptions in TRIVICAN, and so the impact of repeated STIs will be less.

As expected, CD4 monitoring strategy had a significant effect on mortality, WHO3/4 events/death and switch to second-line; however, it did not have an effect on immunological failure. This differential effect of monitoring strategy on immunological failure and clinical outcomes could be due to the fact that in the CDM group, patients did not have their CD4 monitored routinely, therefore clinicians were unable to anticipate when a patient is going to have an event; however, in the LCM group they did know the CD4 count and were able to anticipate a clinical event, making the effect of monitoring strategy different for endpoints that potentially rely on a clinician knowing a patients CD4. Specifically, in the LCM group observing CD4<100 cells/μL would likely result in a switch to second-line treatment, or focussed interventions to improve adherence, and hence a reduction in the risk of clinical events, whereas CDM patients would continue on (failing) first-line treatment. There is a clear benefit of identifying patients with immunological failure in terms of subsequent mortality [109]. However, until the point at which a patient's CD4 drops to below 100 cells/μL, LCM and CDM are in fact managed the same (simply taking someone's blood is not going to make them do better or worse), which likely explains the lack of effect of monitoring strategy on immunological failure as defined here.

In contrast to adults, I found that in children total time off treatment resulted in progressively decreasing CD4% and increased risk of immunological failure for more time off treatment, but there was no additional effect of having had any time off treatment. This suggests that children

may tolerate at least short interruptions immunologically; however, this should not be for too long. However, as in adults, in children there was an increase in the risk for all clinical outcomes for ever interrupting ART with no evidence of increasing risk for increasing time off ART, in contrast to the effects on CD4%. In contrast, PENTA 11 showed that children who underwent CD4 guided STIs achieved similar clinical outcomes compared to those who were on CT [134] both during and after STIs finished. However at the end of PENTA 11, participants who had had STIs had lower CD4%, lower CD4 cell counts and were less likely to be virologically suppressed than those in the continuous therapy arm. Two years post trial (when children in the STI arm had returned to CT), immunological and virological outcomes were similar in the two arms. [154] The lack of difference in clinical outcomes seen in PENTA 11 may be explained by the difference in trial populations: in ARROW (low/middle-income) median pre-ART CD4 was around 250 cells/μL (CD4% around 12%) whereas in PENTA 11 (high-income) it was 627 cells/μL (IQR 320-1050) (CD4% 18% (IQR10-27)), with median CD4 before interruption 966 cells/μL (CD4% 37%). It is known that CD4 recovery on ART is directly correlated with pre-ART CD4 [155]; this was also found in PENTA 11, and I have shown that there is a significant effect of pre-ART CD4 on long term CD4 count and clinical outcomes. In PENTA 11 the treatment interruptions were also guided by CD4 and patients returned to treatment when their CD4 had dropped below 20% or they returned to treatment at 48 weeks. It is possible in ARROW that a child's CD4 could have dropped to a lower than safe level before they restarted treatment. However, I found that the median time off treatment for UPTIs in ARROW was 13 days per patient and the median time for each UPTI was just 7 days so this is probably unlikely.

Having any time off ART may have less of an effect on children than adults as, being younger, they have lower incidence of traditional risk factors for mortality such as hypertension, diabetes and smoking; they are generally healthier. However, they are growing and so may be more sensitive to the effects of replicating HIV, for example in the brain, effects that will not be identified through the clinical outcomes I considered here. The main difference between PENTA 11 and ARROW is that the interruptions assessed in ARROW were unplanned and in PENTA 11 they were planned. I showed in DART that the effects of UPTIs and STIs were different, plausibly due to unmeasured confounding, so this could also contribute here.

In adults, I found no evidence that the relative harm from STIs vs. CT in terms of clinical events decreased after return to CT, although overall clinical event rates continued to decline in both STI and CT with longer time on ART, reflecting the enormous benefits of antiretroviral therapy. Interestingly, I did not find that undertaking progressively more STIs led to progressively greater CD4 deficits back on treatment – rather, having undertaken one or multiple STIs led to similar deficits, also independently of whether STIs were halted for clinical/CD4 reasons or sub-study closure. My analysis of unplanned treatment interruptions found that any interruption was associated with an increased risk of poorer clinical outcomes, and that increasing time off treatment had an additional effect, increasing risk. These results are similar to the doubling of risk of interruption in care or death associated with unstructured ART interruptions found by Pasquet et al. [119]. The effect of treatment interruptions on mortality was greatest for UPTIs in non-STI/CT patients; however there was little evidence that the effects of UPTIs on clinical

outcomes differed in the STI/CT patients, nor that the effects of STIs and UPTIs differed in STI/CT patients, demonstrating challenges with power to detect heterogeneity in effects. The overall effect was a three-fold increase in the risk of death for any time off treatment of any kind. For more time off ART, there was no increase in the detrimental effect on mortality for patients having STIs and those having UPTIs in the STI/CT group but for UPTIs in the non-STI/CT patients, I found evidence of an increase in the risk of death for more time off treatment. However, again, heterogeneity tests demonstrated that it was difficult to be certain that similar effects did not exist in other groups, rather the study was underpowered to detect them. These results are consistent with the EuroSIDA study [123] which showed that the risk of death was increased more than two-fold for patients stopping ART for ≥3 months for unplanned treatment interruptions. Results from Pasquet and EuroSIDA also showed an increase in opportunistic infections, mortality, immunological and virological changes associated with unplanned treatment interruptions highlighting the importance of drug and patient management in low/middle-income settings. In some support of what I have found, a review of trials and studies that have investigated structured treatment interruptions, CD4 count guided or fixed period interruptions [132], concluded that interrupting treatment results in a variable degree of harm; the authors concluded that some strategies are less harmful than others and that interrupting and restarting treatment at higher thresholds for CD4 guided treatment, and reducing the time off treatment in each interval could reduce the risks. It also concluded that the benefits of a short period of time off treatment may potentially outweigh the risks. This is reflected somewhat in my results showing different point estimates of the effects of TIs depending on whether or not they are planned or unplanned and whether or not patients are included in a STI sub-study or not, although, as above, I cannot exclude lack of power also playing a role. Based on my results, any interruptions need to be carefully considered and should really be avoided where possible.

In addition, I found that there was more switching to second-line in those adults randomised to STI vs CT, including after returning to continuous ART. This is likely due to the detrimental effects of interruptions on CD4 and the increase seen in immunological failures in the STI group compared to the CT group. This could also be due to an association between treatment interruptions and drug resistance creating the need for patients to switch from first-line regimens. Associations between treatment interruptions and drug resistance have been demonstrated in 86 patients in Kampala, Uganda [156], as well as in high-income settings [157, 158], particularly for NNRTI resistance. However, in DART, STIs were undertaken using a two week NRTI tail for nevirapine, which should be sufficient to cover the slow decline in blood level [159]. No tail was used with tenofovir-containing regimens (3 NRTIs), but there was no resistance observed in 18 STIs assayed [160]. However, unplanned interruptions of NNRTI-containing ART done without using an NRTI tail do likely increase the risk of resistance.

As part of the main DART and ARROW trials I have been able to assess the effects of UPTIs as these data were requested as part of the data collection process together with the reasons for these interruptions, which are less commonly collected in observational studies. 32% of adults

and 24% of children had a UPTI lasting more than 4 days, although more UPTIs might have been expected in the children as both the child and the carer have reasons not to adhere to treatment. The most common reason for missing treatment in both adults and children/carers was being unable to attend clinic. My results are consistent with Roura et al. [161] who found that in discussion with patients, structural factors were consistently ranked as posing the biggest barrier to clinic attendance and hence adherence. They also highlighted the importance of addressing sociocultural and psychosocial factors, all reasons that I also found for people missing ART. Kranzer et al. [150] carried out a systematic review of the frequency, reasons, risk factors, and consequences of unstructured ART interruptions. The review included 16 studies from Africa, 14 from North America, 32 from Europe and 8 from the rest of the world. Twenty-two of these studies investigated reasons for treatment interruptions (18 from high income countries and 4 from Africa). In two studies from Nigeria they found that costs were the main reason for treatment interruptions followed by pharmacy stockouts which were reported in three of the four studies from low/middle-income settings. Challenges of multiple formulations to accommodate changes in dose as children grow make supply chain management even more challenging in children. However, these reasons were not relevant to DART or ARROW as drugs were free and stockouts did not occur due to the nature of a randomised trial. Perhaps for this reason results from DART and ARROW lay somewhere in the middle of these two settings, with the most common reasons for interruption being unable to attend clinic due to transport issues. Tweya et al. [120] found that the most common reason for interrupting treatment in Malawi was running out of drug whilst travelling (46% of patients), other reasons included forgetting to attend clinic or take treatment (17%), also highlighting the importance of reminders to attend clinic. A study carried out in Tanzania reported that during participatory group discussions, structural factors were consistently ranked as the most hard to control barriers to clinic attendance [162]. However, whilst numerically smaller in number, my results also demonstrate the role of mental health issues; domestic problems including discouragement by friends and relatives; and ongoing challenges of pill burden and fatigue with treatment to treatment interruptions. Faith and divine healing were also amongst the reasons given; highlighting that education is still needed in to how important these drugs are in reducing deaths from HIV and AIDS. Similar barriers were also highlighted by Roura et al. [161] who concluded that whilst removing structural obstacles is essential, addressing sociocultural and psychosocial factors is also required.

In 2013 Mann et. al [163] assessed the impact of ART interruptions in the Kenyan post-election crisis. Here political crises led to UPTIs caused by supply disruption, population displacement, unsafe travel and the inability to obtain medications. They defined an UPTI to be an interruption of ≥1 week (slightly longer than the 4 days I used in DART). They showed that the interruptions were associated with virological failure; two years after the UPTI patients were 5 times more likely to have failed virologically than those who took ART continuously. However, they found that any effect of interruption on CD4 percentage disappeared when allowing for pre-crisis CD4 count.

One advantage of my analysis is that much of the adult interruption data come from a randomised comparison and hence patients were similar in all ways except receipt of STIs vs. continuous therapy. The early termination of the STI sub-study, after which patients returned to CT, allowed me to assess the long-term impacts of prior STIs on CD4 and clinical outcomes. I supplemented this with a parallel analysis of unplanned treatment interruptions in adults and children, for which there were detailed reasons for interruption, unlike in many studies. I found some challenges when fitting some of my models involving STIs and UPTIs suggesting selection biases in who interrupts may be very strong making observational analyses challenging.

However, the fact that I used data from randomised trials is also a limitation, since patients received travel refunds, drugs were free and drug stockouts did not occur, thus substantially reducing the barriers to avoiding treatment interruptions compared to national programmes. Nevertheless unplanned interruptions still occurred in a substantial proportion of adults and children, suggesting results should be generalisable. It is unlikely that more or longer interruptions in ART programmes would be associated with lower or no risk compared to the increased risk of poorer outcomes identified in my study. There is also no clear mechanism by which stockouts could have a different effect on CD4/clinical outcomes compared to STIs, since both involve ART interruption. If anything, use of the NRTI tail with NVP in STIs undertaken in DART should lead to less harm being observed than if UPTIs occur in treatment programs. A further limitation is the likely inability to adjust for all confounders when considering the impact of unplanned treatment interruptions on outcome. In fact the substantial impact of ever vs. never previously interrupting ART, together with the lack of additional impact of increasing time off ART does suggest that interrupting ART may also be associated with poorer adherence or poorer health-seeking behaviour, that is that the association may not be wholly direct, but may also reflect other indirect pathways to poorer outcome.

3.5. Conclusions

In conclusion, interrupting ART is associated with lower CD4 counts and poorer clinical outcomes over the long-term even after returning to ART. These findings suggest that substantially greater efforts should be devoted to reducing the risk of any treatment interruption.

Chapter 4: Discussion

In this thesis I have investigated cause specific mortality (Chapter 2) and the effects of planned and unplanned treatment interruptions on long term outcomes (Chapter 3) for patients with HIV who are on antiretroviral therapy (ART) in the DART (adult) and ARROW (paediatric) trials [54, 58]. In this chapter I will discuss the results from my thesis, what they mean for people with HIV who are on ART and the key remaining challenges for delivering HIV treatment in low- and middle-income countries (LMIC).

Mortality in DART and ARROW

I have shown that for all-cause mortality and major individual causes of death, including those that are not traditionally thought of as immunodeficiency-related, the hazard of death declines similarly and significantly with increasing CD4 count (with the possible exception of deaths from cardiovascular disease). I have also shown that the associations between CD4 and mortality are similar whether patients are monitored with or without CD4 counts, apart from tuberculosis where in the CD4 monitoring group there was a stronger association between dying from tuberculosis and CD4 than in the no CD4 monitoring group. After assessing the change in risk of TB death as CD4 increases, there was a quicker decline in the risk of TB death in the CD4 monitoring group as CD4 increases above ~50 cells/μL. This could be due to one of two reasons – first clinicians may keep patients in the CD4 monitoring group with TB and with higher CD4s on failing first-line regimen while they treat TB, as this has fewer drug-drug interactions and may allow more successful TB treatment. Second, in the no CD4 monitoring group they do not know the CD4 so may assume it is low and switch the patient to second-line treatment which makes the treatment of TB more complicated.

In DART, 47% of deaths occurred in the first year after ART initiation. Similarly, Etard et al. [71] showed that 51% of deaths occurred in the first 12 months after ART initiation in a Senegalese cohort. These relatively large proportions of deaths in the first 12 months are likely attributed to the low CD4 counts at ART initiation (all patients in DART had CD4 <200 cells/μL and the median CD4 at baseline in Etard et al was 128 cells/μL). The majority of deaths in DART were judged primarily related to HIV. Similar results were seen in ARROW with 74% of deaths occurring in the first year after ART initiation. One strength of these data from DART and ARROW is that they have come from randomised controlled trials, with more consistent and complete data collection (7% and 3% lost to follow-up respectively), particularly regarding ascertainment of death and its causes. There was also a more consistent drug supply, including concomitant medications, giving good 'on ART' estimates of mortality. However, one limitation in estimating the causes of mortality and the change over time is the relatively low number of deaths despite the size of the trials.

A few studies from my literature search investigated the change in hazard over time in overall mortality using smoothing non-parametric hazard estimates from Cox regression [71, 72, 74, 93, 94] or Weibull piecewise models [79, 92, 108]. In contrast to the relatively strong assumptions of

e.g. parametric Weibull models, the method I have used here, FPMs, is a much more flexible approach which allows for the continuous changing hazard without over fitting change points to the specific data.

In addition to the DART results shown in Figure 2.4.1, using flexible parametric models (FPMs) to model the changing hazard of death over time, I showed that the hazard of death dropped very quickly after a few months on ART; there is a very rapid decline in hazard around 30-60 days. This was also the case for individual causes of death, with septicaemia and tuberculosis having the highest early hazard of death. The varying estimates found by Etard [71] and Hoffmann [93] show that any modelling strategy that fixes change points on the basis of observed data runs the risk of over fitting, as it does not seem plausible that genuine changes in risk occur at different times in different studies. However, even though the FPMs are able to model the hazard continuously and they do not abruptly cut the data they are not without their limitations. As I found when modelling the individual causes of death, the models appeared to be very sensitive to when the first event occurred and the timing of subsequent events (discussed further below). I tried to overcome this by modelling from 1 year after ART initiation; however I still found instability in the boundaries of the models even after 1 year of ART. Looking at the changing effect of baseline predictors over time using these models may have given me more insight into the changing hazard over time.

I chose FPMs instead of the standard Cox proportional hazards model or the simple Poisson or piecewise exponential model because I wanted to give a more precise estimation of the cause-specific hazards for each cause of death over time. FPMs allowed me to obtain an estimate of the hazard function and its uncertainty which varied smoothly over time, but was not unduly restricted by the strong assumptions underpinning many parametric survival models (e.g. monotonicity). Lack of monotonicity in the underlying hazard was particularly important in my application, since I observed hazards increasing shortly after ART initiation (randomisation) then decreasing (discussed further below). When the goal of a survival analysis is to simply estimate hazard ratios (e.g. between CD4 vs no CD4 monitoring) then the precise form of the baseline function is less important, and a Cox model can then be used appropriately as the baseline hazard function is not directly estimated but treated as a nuisance parameter in the estimation of the other coefficients from the partial log likelihood. One assumption of the Cox model is that any hazard ratio predicted by the model is proportional over time, for example that the hazard ratio is the same at 1 week, 1 month, 1 year etc. An advantage of FPMs is that they do allow smoothly varying non-proportional hazards as can be seen in Figure 2.3.8 where the difference between CD4 and no CD4 monitoring is not constant over time. CVD is a good example of non-proportional cause-specific hazards and the FPM has been able to model this appropriately (Figure 2.3.6).

A recent review [164] of the only 12 prognostic studies that have used FPMs since their introduction in 2002 stated that the most common benefit of FPMs was improved model accuracy and the key advantage of FPMs was their ability to model the baseline log cumulative hazard flexibly with restricted cubic splines. They found only two studies that reported limitations

of the models, which included overfitting and difficulties in model interpretation when there was more than one time dependent effect. No study reported sensitivity to when the first events occurred as I have found here.

Miladinovic et al. [165] made a comparison between the Cox proportional hazards model and FPMs using a dataset from a hospice on patient survival. They used internal validation to assess the validity of the models (i.e. with the same data as with they fit their original models) and found that FPMs were closer in fit to the Kaplan-Meier curve than estimates of survival from the Cox model. They concluded that both the robust modelling of the baseline survival and overall model fit gave better survival estimation using FPMs.

As with all model selection there is the danger of overfitting; with FPMs this could be due to several factors, the number of knots chosen, there not being enough data or the splines being sensitive to outliers. FPMs are reasonably robust to changes in the knot positions and so the default knot positions used (similar to those outlined in Table 2.2.2) should not have effected this [98]. I chose the number of knots using the AIC ($-2\log L + 2k$, where k is the number of parameters in the statistical model, and L is the maximised value of the likelihood function for the estimated model). An alternative would have been the BIC ($-2\log L + k\log n$, where k is the number of observations in the model) which penalises the number of parameters (here knots) more in model selection. I may then have chosen different models with a smaller number of knots which may then have meant I did not estimate an initial peak in hazard.

The models appeared to be very sensitive to when the first events occurred, e.g. in Figure 2.3.6 those causes of death with very early deaths around for example 9 days had a very high early peak but when the first event was later at ~50 days the peak in hazard was much less pronounced. When comparing fitted models it is difficult to be confident that the estimated differences are real; for example septicaemia/neutropenia, neurological and cryptococcal causes of death all have similar timings of first events and yet the estimated hazards are very different. This may well be an artefact of the rather arbitrary timing of the first event, given the fact that provision of written informed consent to join the trial meant that patients who were essentially close to death should not have been enrolled. To understand whether the peak in risk was a result of the statistical modelling rather than being clinically meaningful I could have carried out a sensitivity analysis where I moved the time of deaths that occurred in the first 2 weeks to, for example 14 days post randomisation and assessed the effect this had on the estimated hazards. However, one important limitation is lack of data, despite the size of the trial. When analysing these data I considered individual causes of death which had at least 10 deaths; however in the future if I were to use these models again I would probably suggest having at least 20 events (approximately 10 in each randomised group).

Analysis of mortality risk changes using FPMs following admission in the FEAST trial found a similar pattern to my analyses, in that the baseline hazard rose to a high peak around 1.6 hours and then declined sharply through to 5.2 hours and then more slowly beyond and out to 48 hours with a steady slow decline past 31 hours (personal communication with L George). In

FEAST, this early high peak in hazard was investigated using simulations. A piecewise hazard model was fitted to the FEAST data assuming a high constant hazard for the first 1 hour from randomisation which then dropped. This model was then used to simulate datasets to which FPMs were then fitted. A number of estimated hazard profiles were found with some having a flatter peak and some having no peak at all or some with a sharp peak. Moving the knot positions did not remove the peak unless the first interior knot was at >48 hours compared to a median uncensored survival time of 8 hours. In addition, fitting the FPMs to the log hazard on the observed time scale rather than the log time scale showed a monotonically decreasing hazard which better reflected the piecewise exponential hazard. Together these findings also suggest sensitivity analyses checking both time scales are important in future applications.

Identifying and treating HIV-positive individuals

A higher percentage of deaths, a higher hazard of death and an association between low CD4 and a higher rate of deaths in the first year after ART initiation are consequences of late presentation to care which highlights the importance of identifying HIV-positive individuals early and making sure all HIV-positive individuals receive treatment. These are all challenges that still stand in delivering HIV treatment in LMIC, as discussed below.

UNAIDS 90-90-90 targets

In 2014 UNAIDS launched their 90-90-90 treatment target to end AIDS globally, which aims at 90% of people living with HIV knowing their status, 90% of HIV diagnosed people receiving treatment and 90% of those on HIV treatment achieving viral suppression by 2020 [20]. This is equivalent to 81% of people living with HIV receiving ART and 73% of people living with HIV being virally suppressed on ART. Progress was assessed at the end of 2016 based on the latest epidemiological and programme data from 168 countries worldwide. Approximately 37 million people were living with HIV, including 64% in sub-Saharan Africa. An estimated 70% [plausibility bound; 51-84%] knew their HIV status; among those who knew their HIV status, 77% [57->89%] were accessing antiretroviral therapy, and 82% [60->89%] of people accessing treatment had suppressed viral loads [40].

Current coverage of ART

Making sure as many people as possible are receiving ART is key to reducing deaths from AIDS, other HIV-related illnesses and even non HIV-related illnesses, as shown by my analysis of causes of death. At the end of 2016, an estimated 19.5 million people were receiving ART globally. However, in the majority of sub-Saharan Africa this was still less than 60% of people with HIV compared to more than 80% in high-income countries such as Australia, Italy and Sweden [166]. The global scale up of ART has been the primary contributor to a 48% decline in deaths from AIDS-related causes from 2005 to 2016. The number of children dying of AIDS-related illnesses has been cut in half in just six years from 210,000 (160,000-260,000) in 2010 to 120,000 (79,000-160,000) in 2016 with increased access to paediatric ART playing a significant role alongside improved coverage of effective prevention of mother to child

transmission. In addition increasing ART coverage has been shown to substantially reduce the incidence of new HIV infections among heterosexual serodiscordant couples [167]. Modelling suggests further reductions in HIV transmission are achievable with high uptake of regular HIV testing and universal ART initiation at diagnosis [168], although the practical issues and implementation barriers remain huge and further work is needed to realise the benefits (see below for discussion of the ANRS 12249 trial).

There has been a very successful scale up of treatment across sub-Saharan Africa; for example in Uganda, just 12% of HIV-positive individuals were receiving ART in 2006 compared to 69% in 2016 [169]. In eastern and southern Africa, there was an almost three fold increase in the number of people on ART in 2016 compared to 2010. Because the number of people living with HIV in sub-Saharan has increased and eligibility for ART has widened, this scale-up is equivalent to an increase in coverage of treatment of 23% (19-27%) in 2010 to 60% (48-68%) in 2016 [40]. However, as outlined above, this is still low compared to other parts of the world.

There is a significant problem with HIV-positive adolescents, worldwide, not just in LMIC. An estimated 2.1 million (1.4 million-2.6 million) adolescents were living with HIV in 2016, with 150 dying from AIDS-related causes every day. Between 2000 and 2015, AIDS-related deaths declined in all age groups apart from adolescents where mortality more than doubled (8,000 to 41,000) [170]. Adolescents remain vulnerable to sexual acquisition of HIV and have low rates of testing and linkage to care [171]. Additional challenges in this group of patients are higher rates of loss to follow-up and poor adherence as well as an increased need for psychosocial support [172-176]. Adolescents continue to be underserved by current HIV services and have significantly worse access to and coverage of ART.

Test and treat

Despite progress in ART scale-up there are still a significant number of people in sub-Saharan Africa who have tested HIV-positive but are not on treatment and a further number who do not know that they have HIV and therefore do not seek the treatment they need. In October 2015, the World Health Organisation (WHO) released new treatment guidelines that encouraged starting ART immediately, removing all limitations on eligibility for starting treatment and aiming to avoid the high losses from pre-ART care [177]. With this, the number of patients referred for treatment at the time of diagnosis has increased, but helping individuals overcome the immediate barriers to ART initiation remain critical. Further, these referrals increase the burden on the limited resources that already exist in LMIC. Sub-Saharan African countries do not always have the resources or funding to provide a higher percentage of ART coverage with these extra barriers that need to be addressed.

A number of trials have evaluated strategies for universal test and treat [178]. The test and treat approach was evaluated in Swaziland through the MaxART implementation study which found that some patients delayed initiating ART and had often tested positive for HIV several times before initiating ART [179]. This delay was associated with a desire to come to terms with their

diagnosis and prepare for lifelong treatment. Some participants had heard that ART was non-essential and taking care of one's self through other means was sufficient. People also worried about ART-related adverse events and disclosure and the impact on social and economic relationships. Whilst logistical barriers can potentially be avoided, if someone needs to come to terms with their diagnosis before starting treatment this can be hard to accommodate [180]. Other barriers to test and treat are access to HIV testing, the costs of the drugs and the persistent stigma of HIV infection [181].

HIV incidence in South Africa remains high with around 380,000 adults and children newly infected in 2016 [182]. Mathematical modelling had suggested that expanded use of ART could be the key to reducing the rate of new HIV infections at the population level. The hypothesis that universal test and treat would reduce HIV incidence was tested in the ANRS 12249 treatment and prevention trial [183]. The trial aimed to investigate whether universal ART initiation offered to all HIV-positive individuals reduced HIV incidence in a rural hyper-endemic region of South Africa by comparing standard of care (national ART initiation guidelines were to initiate treatment for those with CD4 count <350 cells/μL or WHO stage 3/4 or pregnancy until December 2014 and then <500 cells/μL since January 2015) with test and treat. The trial found no significant population-level effect of universal ART on incidence despite HIV testing uptake being very high and repeat testing acceptable. The issue identified by the trial was linkage to HIV care, which was slow and poor (only 30% of individuals had registered at the trial clinic within 6 months of home HIV diagnosis), therefore leading to lower than anticipated ART coverage with no significant difference in ART coverage between the two randomised groups. These findings suggest that the conditions required for a policy of test and treat to translate into a reduction in HIV incidence were not met. The overall HIV cascade did not vary between groups and fell short of the 90-90-90 target. There was a lower mortality rate in the test and treat group, however the difference was not significant. Delayed linkage to care was related to a number of factors; being young, more educated, newly diagnosed, not knowing anyone else with HIV, and increased distance between their home and the clinic. Either mobile clinics closer to people's homes or home initiation of ART may have improved this, although studies have shown that these often have lower rates of linkage to care than facility based testing (see below) [184, 185].

Although all HIV-positive individuals are now eligible for ART [177], large challenges remain in linking individuals to HIV care and treatment services. Studies prior to new recommendations to treat regardless of CD4 showed that between 32% and 50% of HIV-positive individuals assessed at public sector hospitals and clinics as ART-eligible did not initiate treatment. A study carried out at a South African clinic assessed demographic and psychological factors associated with ART readiness in patients referred for ART by an online health clinic in Cape Town [186]. They considered three key components, (i) an awareness that treatment will be beneficial, (ii) motivation to initiate treatment and (iii) the intention to start treatment soon. The study showed that ART readiness was significantly lower among individuals who reported good health (asymptomatic) and those who did not think they would test HIV-positive when tested. A positive diagnosis being a surprise can be associated with denial and this finding may explain

why denial is often identified as a barrier to ART initiation. Readiness was also associated with knowledge of ART or knowing someone already on ART who had had positive effects of treatment. Although not statistically significant they found a small association between stigma and readiness to treat. Rates of linkage to care and treatment are particularly poor from community-based HIV-testing services, such as mobile clinics and home-based testing services [184, 185]. While community-based testing services are effective at reaching previously undiagnosed HIV-positive individuals [187-189], it may be more difficult to ensure that those individuals begin ART. Self-testing is now recommended by WHO as a way to achieve the first of the 90-90-90 targets, in particular to reach first-time testers [190, 191]; encouraging results have been demonstrated in particular in Malawi where early trials were carried out [192]. However, further research is needed in how to help individuals overcome their lack of readiness to start treatment once they have received a positive diagnosis. Effective education is important along with removing stigma and uncertainty regarding side effects of treatment. Some people may think starting treatment is a greater risk than any benefit to a person's immediate quality of life. The SEARCH study which is a research project working in 32 rural communities in Kenya and Uganda has shown exceptional results, demonstrating that the 90-90-90 target can be achieved within these kind of populations [40]. They have a mobile and home based testing system, they hold fairs near where people live, which are for different diseases not just HIV and educators are able to provide health-related counselling. The systems they have in place mean that providers can reach a large proportion of the community in a short space of time. For those who test HIV positive they prioritise service delivery, they give people a friendly environment, a telephone hotline and texting service, and they also counsel people on viral load suppression. Whether the costs of sustaining this kind of system long-term are viable is however an important question.

Many countries were able to start the test and treat policy straight away but for LMIC this was harder due to funding. In sub-Saharan Africa it was started first in Kenya, South Africa and Malawi. Greater stigma and lower health literacy add barriers to the uptake of testing. However, in 2016, 45 LMIC had adopted the test and treat all recommendations and 31 other LMIC had said that they would start the test and treat during 2017 [193].

Late presentation to care with HIV infection

Late presentation to care with HIV infection is still a problem worldwide, even where ART has been readily available for years. COHERE defined late presentation as CD4 count <350 cells/µL [194], demonstrating that there are differing views about what is considered "late": in LMIC <200 cells/µL or <100 cells/µL is considered late [114]. In 2014, IeDEA and ART-CC showed that 20-25% of people in LMIC were still starting ART with CD4 counts <100 cells/µL [114], even though the median CD4 count at initiation is rising [40] and more people are initiating ART. Lablite also showed that in Zimbabwe, despite increased ART initiations across all patient groups after raising the CD4 threshold for initiation to 500 cells/ µL and introducing treatment for all pregnant/breastfeeding women, 24% men and 13% non-pregnant women had pre-ART

CD4<100 cells/ µL [195]. It is known that those who present late have a higher risk of death and poorer ART response [196]. Late presentation is also associated with an increased risk of HIV transmission, ART drug resistance [197] and healthcare expenses [198]. The prevalence of late presentation to HIV care ranges from around 30% in high-income countries to >70% in some studies in African countries [198-200], depending on definitions. The issue with early deaths seen in my analysis highlights the need to reduce the impact of late presentation on mortality which is where the results of the REALITY trial are key [201].

The REALITY trial looked at ways to reduce deaths in the early stages of treatment for people starting ART with low CD4 counts (<100 cells/µL). It tested three 12 week strategies, in addition to standard HIV treatment with 2NRTI+NNRTI. The three interventions were enhanced prophylaxis to prevent infections, increasing the potency of ART by adding the anti-HIV drug raltegravir to reduce the amount of virus in the blood faster and ready to use supplementary food to improve nutritional status. The trial found that giving enhanced prophylaxis reduced the risk of death by 25%, and those in this group were also less likely to have new WHO stage 4 events, new tuberculosis, new cryptococcal disease, grade 4 adverse events or require admission to hospital. Therefore the researchers recommend that people starting HIV treatment with low CD4 counts (that is late presenters) should be given enhanced prophylaxis for the first few weeks of treatment. Using this approach will hopefully go some way to reducing the risk of these early deaths. However, the other two strategies, supplementary food and increasing the potency of ART, did not show any benefit [202, 203].

As discussed above there are still several ongoing issues with identifying HIV-positive individuals. Once identified, the need for treatment is not always seen by the patient and in some LMIC is not always possible straight away. With not everyone with HIV being treated, there is still the issue of transmission to other people and a significant number of people are still dying from HIV. Treatment is key, meaning that there is the need for more resources to test, treat and keep people on their drugs.

Delivering long-term treatment

In chapter 3 of my thesis, I assessed the long-term effects of structured treatment interruptions (STIs) on CD4 counts and clinical endpoints after returning to continuous therapy; extending results from the DART STI sub-study which was terminated early due a detrimental effect of STIs on clinical disease progression within the trial [56]. In addition, I assessed the effects of unplanned treatment interruptions on CD4 counts and clinical outcomes, and explored the reasons for these unplanned treatment interruptions.

Impact of time off ART

When ART is stopped there is a drop in CD4 count which increases again with the re-initiation of ART [56]. I found it would take around 16 months back on continuous therapy for the CD4 counts to return to those at randomisation following the detrimental effect of the STIs in DART. Rather than the fixed interruptions in DART, the SMART trial [204] investigated CD4-guided ART interruptions, and found that these significantly increased the risk of opportunistic disease or death from any cause compared with continuous therapy. Similarly to the DART STI sub-study, SMART was also terminated early. As in DART those patients in the STI arm were advised to restart therapy and follow-up continued [129]. Similar to DART, CD4 cell counts in SMART increased with re-initiation of ART but recovered to that at randomisation in just 6 months. This difference between the trials could be due to the fact that the SMART trial was conducted in high-income settings where patients generally have better prognosis; most importantly the pre-interruption CD4 cell counts were greater in SMART than in DART (median: ~600 cells/μL in SMART vs. ~400 cells/μL in DART); as were the nadir CD4 counts (median: 250 cells/μL and 86 cells/μL, respectively). Those patients who were off treatment at the time of sub-study closure in DART had a steeper increase in CD4 count on returning to ART. This finding is consistent with that in naïve patients starting ART [151], most likely due to the redistribution of CD4 cells into the peripheral drug circulation; after 12 weeks on continuous therapy the CD4 count for patients in DART had increased to the same levels as STI patients on ART when the sub-study was terminated, but both remained well below CD4 counts with continuous therapy. Using mixed models I was able to predict that the CD4 deficit associated with having undertaken fixed-length STIs persisted for at least 4-5 years when back on continuous therapy, even though patients did reconstitute immunologically. I found that after sub-study closure rates of switch to second-line were greater than during the randomised phase in both the STI group and the continuous therapy group with a trend towards greater rates in the STI group; there were no switches to second-line in the continuous therapy group during the study so I was unable to estimate this rate.

I found that there was no increasing detriment in terms of CD4 cell counts for more time off treatment in unplanned interruptions but having ever interrupted ART in an unplanned way had a detrimental effect on CD4 cell counts. In contrast, ever interrupting treatment and the longer the time off treatment were generally associated with poorer clinical outcomes in adults (Figure 3.3.10). This supports how important it is to maintain ART supplies and accessibility to avoid patients in treatment programmes having to stop ART. Interestingly, I showed in DART that the effects of unplanned treatment interruptions and STIs were different, plausibly due to unmeasured confounding.

In contrast I found that in children there was an increasing detriment in terms of CD4% for more time off treatment due to unplanned treatment interruptions but no additional effect of any time off treatment. This suggests that children may tolerate at least short interruptions immunologically; however this should not be for too long, as demonstrated by generally poorer

clinical outcomes associated with interruptions, although power was low in these analyses. The PENTA 11 trial [154] (providing the only randomised evidence of the effects of planned treatment interruptions in children) showed that although interruptions could not generally be recommended, due to children's' greater potential for immune recovery they may be an acceptable option for children, particularly when there is a high risk of unplanned treatment interruptions. Children are at a lower risk compared to adults for cardiovascular, renal and liver complications due to their younger age. Children also have a lower incidence of traditional risk factors such as hypertension, diabetes and smoking; these are all factors whose risks increase substantially in HIV-positive adults stopping ART.

One limitation of my analyses is that VL was not measured routinely in either the DART or ARROW trials, but was measured only in specific subsets of patients to answer specific scientific questions. The effect of treatment interruptions on CD4 counts is mediated through its effect on VL, since stopping treatment means VL becomes unsuppressed and rises, and CD4 cells start to die, as in untreated HIV. Tong et al. showed that detectable VL (>300 copies/mL) was significantly associated with treatment interruption for more than two consecutive days among patients who had been on ART for at least 6 months. A negative effect on virological suppression increases the risk of transmission and has a detrimental effect on the plan to eliminate HIV infection, highlighting again the importance of maintaining drug supplies and keeping people on life-long ART.

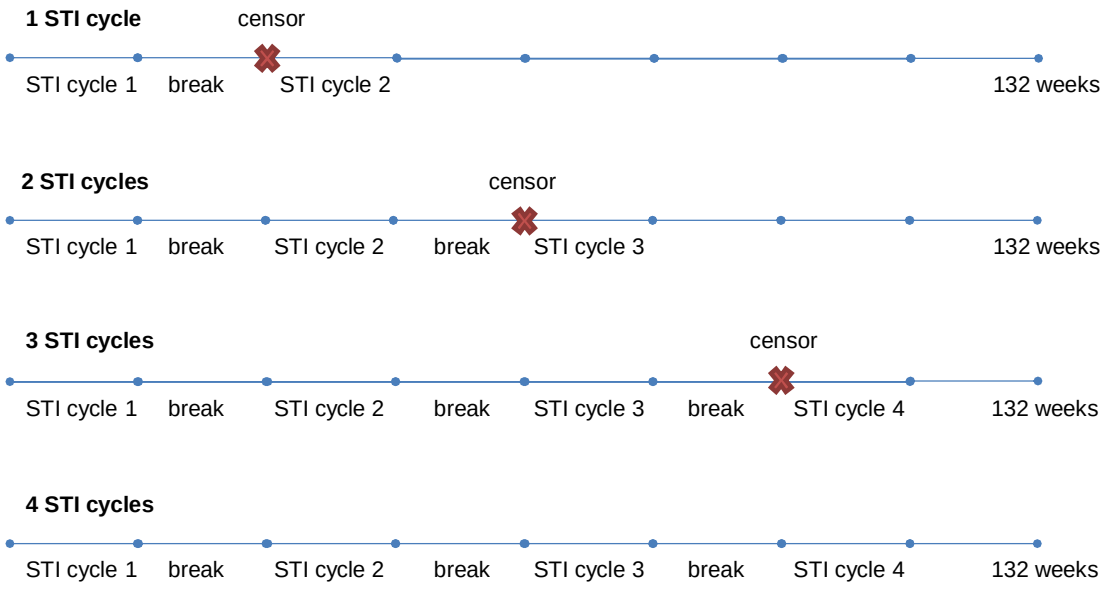
I assessed the effect of number of STIs on CD4 count at 132 weeks using standard regression models (e.g. Table 3.3.2), adjusting for factors at trial baseline and randomisation to CT/STI. These analyses assumed no unmeasured confounding; that is, that there are no common causes of receiving more or fewer STIs and having a higher or lower CD4 count at 132 weeks which are not included in my models. This assumption is valid for a randomised intervention, such as comparing CT vs. STI, but patients were randomised to a strategy of receiving repeated STIs, not to a specific number, so my exposure of interest is not randomised. I found a negative effect of any time off treatment on CD4 at 132 weeks but not an increasing effect of more time off treatment; that is, having 4 STIs was not any worse than having 1 STI. However, in practice patients had variable numbers of STIs (Figures 3.3.1 and 3.3.2), and this is therefore an average effect of STIs across the number of STIs that were actually undertaken. A key limitation of my analysis is that I did not account for the fact that some patients could have had more cycles but did not. In particular, I found that those who had 4 cycles were highly selected compared to those who had 1 or 2 cycles (Figure 3.3.2), and that those who received fewer cycles than they should have done generally did so due to low CD4 or WHO 3/4 events, suggesting that those who were more healthy received more STI cycles. This plausibly means that I may have missed a dose-response effect and that my estimate of the effect of STIs on CD4 at 132 weeks is an underestimate of the risk of repeated STIs due to unmeasured confounding from patients with low CD4/large CD4 decreases stopping STIs early. One possibility would have been to directly adjust for time-updated confounders between randomisation to STIs and sub-study closure such as CD4 as these would have affected whether the subsequent (2^{nd}, 3^{rd} or 4^{th} STI cycles) occurred. However, the problem with this

approach is that time-updated CD4 (and likely other confounders) are not only related to whether a patient has more cycles of STIs, but are also related to the outcome, that is CD4 at 132 weeks, and are affected by previous STI cycles.

To appropriately account for time dependent confounders affected by prior treatment (here STIs) I could have used a causal inference model. A number of causal inference methods have been developed over recent years including dynamic marginal structural models (MSMs) which aim to estimate the effect of a treatment regime by "artificially" censoring patients who become non-compliant with a given treatment regime and upweighting those who remain compliant using inverse-probability-of-censoring (or equivalently inverse-probability-of-treatment) weights and estimating risk in the weighted population; dynamic MSMs have most commonly been used to look at "when to start" treatment questions [205, 206].

I could in theory have used a similar approach to estimate the causal effect of regimes such as "one STI", "two STIs" on subsequent CD4 in the same way Crook et al. estimated the effect of contraceptive method on risk of HIV acquisition [207]. For example, in order to estimate the causal effect of having had four STIs I could have censored those in the STI arm who did not receive all four STIs at the first time at which they should have started the next STI cycle and did not as follows (Figure 4.1).

Figure 4.1 Estimating the causal effect of having had four STIs



Here patients are 'artificially censored' at the time at which they would have started their next cycle of STI (that they did not start), meaning that they do not contribute to the outcome model for CD4 at 132 weeks. Instead in the outcome model those who received 4 STIs are up-weighted to account for all patients in the STI arm using inverse-probability-of-censoring weights which reflect the probability that each person received 4 STIs given their baseline and time-updated covariates. Weights could potentially be estimated from a pooled model; alternatively separate models for each STI might be needed, essentially considering at each

start of an STI cycle what was the probability of each individual having the STI as planned vs. not having the STI as planned. The initial weights are 1 for everyone as everyone included had 1 cycle. But at the second cycle patients who had the second cycle will get upweighted to account for those that did not (with higher weights given to the less common patients (e.g. those who continued with STIs despite low CD4)) and similarly at the third and fourth cycles. The weighting models (but not the outcome model) would include time-updated covariates like most recent CD4 and disease stage, as well as baseline covariates. A similar approach could have been used to censor and upweight STI patients to look at 1, 2 or 3 STIs and I could have compared all 4 regimes to continuous ART in a single weighted outcome model.

This approach is problematic if there is a predictor or combination of predictors which is very strongly (or completely) associated with a regime (e.g. if no patients whose CD4 drops below x continue on STIs); this may have made the above theory difficult to apply in practice particularly within the DART trial where there was good adherence to a strict protocol [208]. Other causal methods are also difficult to use in this case although g-computation (where the relationships between all variables in the data are modelled) may be possible with extrapolation of models outside the data [206].

Another limitation of my work is that I only included a relatively small number of covariates up to STI randomisation in my standard regression models for the association between number of STI cycles and CD4 count at 132 weeks. Other factors that I could have included include socioeconomic status, missed trial visits prior to the STI randomisation, history of weight, BMI, haemoglobin, WHO 3/4 events and ART history since trial entry and prior to STI randomisation. In addition I could have included data available in DART from a symptoms checklist of 26 symptoms that were collected every 4 weeks. This could have reduced the impact of standard unmeasured confounding. I could also have included these factors in my weighting models.

In addition I assessed the effects of STIs and UPTIs (and total duration off treatment) on CD4/CD4% and clinical outcomes in both DART and ARROW patients. In DART I found the effects of STIs and UPTIs on CD4 at 4 years were different (Table 3.3.12) which is very likely due to unmeasured confounding. The results in Table 3.3.12 suggest that there is a decrease in CD4 at 4 years for more time off treatment due to UPTIs but I cannot distinguish whether this is a result of the time off treatment or a result of underlying reasons for the UPTIs; thus my effect estimate should not be interpreted as a causal effect but may still be useful for clinical management. While in theory, causal models could have been used to adjust for confounders associated with UPTIs and subsequent CD4 (or other outcomes), addressing the issues with UPTIs is more complicated than for STIs: firstly, the specific reasons UPTIs were undertaken were only recorded very broadly (and are unlikely to be captured as well as predictors of patient management); secondly, whereas STIs were a fixed length, UPTIs were variable in length thus the duration of the UPTI also needs to be considered. There are few examples in the causal literature which estimate the effect of an exposure which cannot be assigned [209, 210] as is the case with a UPTI or include intermittent exposure where (as here) the reasons for stopping exposure (here ending a UPTI) as well as starting exposure (starting a UPTI) are needed [211].

The most common reason for UPTIs was being unable to attend clinic, but this could be for several different reasons each with a different relationship with outcome, or no relationship at all. For example, there is no reason why you should be sicker or healthier just because you have no access to transport, but if the reason for not attending clinic is that you are too unwell to travel this would clearly have an effect on outcome. In contrast, if you are too busy to attend clinic this may be because you are well. Correctly constructing weighting models (or modelling relationships using a g-computation approach) to account for both starting and stopping UPTIs would require a larger number of more detailed covariates than were collected in the trial.

ART adherence in adults

As discussed above, the global targets for scaling up of ART therapy include ensuring that 90% of patients on ART achieve viral suppression. This new target emphasises the need to ensure optimal levels of adherence. Poor adherence includes both sporadic taking of ART (e.g. missed doses) and unplanned treatment interruptions. Structured treatment interruptions are no longer recommended in guidelines but unplanned treatment interruptions are common. My findings that the consequences of treatment interruptions include lower CD4 counts and poorer clinical outcomes even after returning to ART suggest that substantially greater efforts should be devoted to reducing the risk of any treatment interruption, including strengthening supply chain management to ensure continuous drug supplies, early contact of patients not attending drug refill visits (e.g. by mobile phone text, although the effects of this have been mixed with positive effects in some studies [212, 213] and negative effects in others [214, 215]) and making short supplies of ART available for patients who travel.

In both DART and ARROW, I found the most common reasons for unplanned treatment interruption were structural, such as being unable to attend clinic. The second most common reason for unplanned treatment interruptions was adverse events/toxicity; however, this accounted for only 22% of all unplanned interruptions in DART and in ARROW this was even lower with only 6 patients interrupting ART due to toxicity. In DART more than 50% of the adverse events were due to blood disorders and included neutropenia and anaemia, likely due to zidovudine and low CD4 counts at ART initiation. This relatively low rate of stopping treatment for adverse events is likely due to the nature of the trials with more regular assessment of patients and more patient support and might be higher in contemporary patients on the same/similar ART outside of trials. The drugs in DART are now old drugs which were given twice daily. The current WHO recommendation, as preferred first-line ART for adults, is a better once daily regimen containing efavirenz and tenofovir with either lamivudine or emtricitabine. Mouton et al. [216] carried out a review that focused on the key toxicity issues arising from the use of this WHO recommended first-line ART in adults. They assessed those toxicities that are seen both at the onset of ART, as well as the long-term toxicities seen after prolonged use in large cohorts. Where possible they focussed on sub-Saharan Africa. They found that it was uncommon for these drugs to result in toxicity and the benefits of ART far

outweighed the risk of toxicity. In addition, they found that recognising drug toxicity in HIV-infected persons is challenging as patients may have multiple comorbidities.

With even newer drugs, rates of adverse events/toxicity may be lower currently and in the future. The integrase inhibitor dolutegravir is a less toxic alternative to efavirenz. It was previously only recommended in high-income countries due to its high cost but with a new low cost deal [217] that has been put in place its use in LMIC is increasing with more than 20 LMIC having initiated dolutegravir procurement. It is expected that WHO will recommend it in the next treatment guidelines (revision planned during 2018) as a safe and well tolerated first-line agent that has a higher probability of virological suppression than other first-line agents [218, 219].

A recent study carried out in sub-Saharan Africa found that in adults in South Africa, poor ART adherence was related to patient and family factors, stigma and discrimination, substance abuse, socioeconomic challenges, health care and systems [220]. Another study carried out in 24 countries in sub-Saharan Africa including South Africa, Uganda, Ethiopia and Kenya found that the most frequently identified barrier to ART adherence was forgetting, followed by lack of access to adequate food, stigma and discrimination, side effects and being outside the house or travelling [221]. These are slightly different to the most important reasons I found for unplanned treatment interruptions in DART, perhaps because DART was a randomised controlled trial and patients came to clinic very frequently (4 weekly) for check-ups meaning they received continuous reminders. Further, as the trial was early in ART treatment (2003-2008), there was substantial reinforcement of the need for drugs. DART participants were therefore probably less like to simply forget; rather it was a case of running out of drugs when they were unable to attend clinic. Croome et al. [221] also reported on facilitators to adherence; these being, social support, reminders, feeling better after taking ART, disclosing their HIV status and having a good relationship with their healthcare provider. Many of these would have applied to DART trial participants who set up social clubs in clinic, which they attended frequently. In contrast, Merten et al. [117] highlighted that experiences of low quality services and access constraints can undermine trust in health care services, and that provider's attitudes, the time they spend with patients and low communication can also decrease adherence to ART. DART and ARROW participants were fortunate to receive ART within relatively well-funded trials.

Longer-term suboptimal adherence levels lead to negative outcomes including increased risk of disease progression [222], drug resistance [223], high viral load and consequent risk of transmission [224, 225] and death [121, 226]. At the end of 2016 only 50% of people living with HIV in southern and eastern Africa had achieved viral suppression [40]; this is equivalent to 83% of those who were receiving ART being virally supressed. Despite the high percentage of people receiving ART who are virally supressed, adherence to treatment is still a major challenge as the only way to achieve viral suppression is to take continuous ART. Patients on ART face multiple barriers to adherence and no single intervention is sufficient to ensure high levels of adherence. Following a systematic review carried out in 2016, Shubber et al. [227] suggested that healthcare providers should consider a triage approach that first identifies patients at risk of poor adherence and then seeks to establish the support that is needed for

them to overcome their most important barriers to adherence. The adherence literature often divides these barriers into structural barriers and social barriers. Tweya et al. [120] found that the most common reason for interrupting treatment in Malawi was running out of drug whilst travelling (46% of patients), other reasons included forgetting to attend clinic or take treatment (17%), also highlighting the importance of reminders to attend clinic. A study carried out in Tanzania reported that during participatory group discussions, structural factors were consistently ranked as the most hard to control, including barriers to clinic attendance [162]. In my analysis I also found that the most common reason for missing treatment in both adults and children/carers was being unable to attend clinic i.e. a structural reason. Similarly, Roura et al. [161] found that when discussing barriers to clinic attendance with patients, structural factors were consistently ranked as the main barrier, which ultimately leads to poor adherence. They also highlighted the importance of addressing sociocultural and psychosocial factors, all reasons that I also found for people missing ART. The DART data also demonstrate the role of mental health issues; domestic problems including discouragement by friends and relatives; and ongoing challenges of pill burden and fatigue with treatment to treatment interruptions. Faith and divine healing were also amongst the reasons given; highlighting that education is still needed into how important these drugs are in reducing deaths from HIV and AIDS.

Adolescents

Globally adolescents and young adults account for more than 40% of new HIV infections and HIV-related deaths amongst adolescents increased by 50% from 2005-2012. However, there is very little research on adherence amongst the growing population of adolescents in Southern Africa. Kim et al. [228] found that nearly half (45%) of all adolescents living with HIV in Malawi reported non-adherence to ART in the past month. Commonly reported barriers to adherence were in fact similar to adults and included forgetting (40%), travel from home (14%), busy with other things (11%), feeling depressed/overwhelmed (6%), feeling stigmatized by people outside (5%) and within the home (3%). Factors found to be independently associated with missing a dose in the past week were drinking alcohol in the past month, missed clinic appointment in the past 6 months, witnessed or experienced violence in the home and poor treatment self-efficacy. Other reasons included being away from home, too tired, didn't want others to know, couldn't deal with it that day or ran out of drugs. Unpublished data from the AALPHI cohort based in the UK found that 27% of adolescents reported missing any doses in the last 3 days and 31% reported missing more than 2 days in a row in the last month (40% reporting either outcome). Predictors of both outcomes were poorer quality of life (PedsQL), poorer self-perception of HIV, more years on ART and surprisingly an increase in the number of other young people they had told. They also found that adolescents with HIV found it harder to take doses of ART at the weekend, suggesting that perhaps less routine and being more social have an effect on adherence. Family background did not appear to predict adherence [229]. These results highlight that the issues around mental stability, stigma and the burden of long-term treatment in fact are very similar in adolescents, adults and children; rather the effects of these factors are

accentuated in adolescents. In ARROW I showed that the main reason for children interrupting ART was being unable to attend clinic, just as in adults, and the same reasons likely apply.

Issues with drug supply

Although having enough antiretroviral treatment was not highlighted as a reason for interruption in DART due to the nature of the trial, it was highlighted as a key issue by Kranzer et al. [150] in their systematic review and remains challenging in some LMIC. Tong et al. [230] investigated ART interruption for more than two consecutive days in 19 health service centres in Cameroon. They found that 52% of the HIV services that participated in their study recorded stock-outs of at least one of the three most prescribed ART regimens during the previous year. In another study in Cameroon, 85% of the health care facilities reported ART stock-outs [231]. There are also several studies that have reported ART stock-outs as the main reason for missing doses [119, 232-235]. Chan et al. showed that ART stock-outs were more common in paediatric care compared to adult care 23% vs. 7%, respectively [236]. Clinic staff in the study by Tong et al. reported several ways of dealing with stock-outs: switching regimens to use an available similar class drug, splitting a delivered dose or sending patients to another HIV service. These strategies were also reported by a study in Tanzania [237]. Worryingly, splitting delivered doses runs a serious risk of suboptimal drug levels which may lead to resistance.

Retention in care

In order for HIV-infected people to adhere to treatment, they have to be in care. However, recent estimates show that just over half of those diagnosed with HIV may be engaged in and retained in care at any one time. In fact, retention in care has been estimated to be as little as 67% in sub-Saharan Africa [238], highlighting that it is still a critical challenge for all HIV treatment programmes especially where resources are limited. Preparing patients for treatment and treating them for just a few months before losing them to follow-up is also an inefficient use of resources. Cost-effectiveness is important, as programmes seek to maximise the impact of limited resources; lack of attention to loss to follow-up is as costly as failures in ensuring good adherence. Patients who do not remain in care may need to move on to second-line treatment which is generally more expensive, or they may fall ill as a result of being off ART and therefore require hospitalisation. All of these things place further stress on an already overburdened health care system [239].

Reducing the burden on healthcare systems

When WHO broadened its guidelines to recommend that all identified people living with HIV should initiate ART irrespective of immunological or clinical status, this had a substantial impact on the already burdened health systems in sub-Saharan Africa. Current research is aiming to develop systems that should improve the efficiency of healthcare delivery by reducing unnecessary burdens on the health care systems, which could potentially increase retention in care. Several interventions such as tracing of patients lost to follow-up, task shifting and differentiated care all have implications on resources such as staffing and clinical capacities and

these may not easily be scaled up or contracted in response to changes in demand. Revill et al. [240] highlighted that in most cost-effectiveness studies, there is the assumption that unit costs of resources suitably reflect the value of those resources in the health care system and that it does not matter which particular resource is utilised or released in the delivery of alternative interventions. However, when the availability of those resources cannot easily be adjusted to the changes in demand because of local variability, these assumptions cannot be made. This has potentially important consequences for how economic evaluation is undertaken and the way in which results are used in formulating health policy and are implemented by programmes in health care delivery.

Frequency of clinic visits and differentiated care

Patients typically attend clinic every 1-3 months for clinic assessment and to collect drugs. This is largely historical but also exacerbated by the difficulties in keeping sufficient drug supplies to routinely issue drugs for longer time periods. Needing to frequently collect ART refills may lead to suboptimal adherence and disengagement from care due to the time spent waiting at clinics and cost to patients of frequent clinic visits, particularly for those who have to travel long distances [241]. Frequent clinic visits also place a high demand on the healthcare system due to the costs of providing personnel and infrastructure. Less frequent dispensing of ART and community-based ART-delivery models are potential strategies to reduce the load on overburdened healthcare facilities and reduce the barriers for patients to access treatment. Mathematical modelling has shown that reducing the frequency of clinic visits for stable patients is expected to be cost-effective in sub-Saharan Africa [242]. Observational research in Zambia has shown that 2- and 3-monthly visit intervals were associated with decreased loss to follow-up and decreased visit lateness compared to patients who attended monthly [243]. As a result, WHO has recommended that stable ART patients require less frequent medication pick-ups and clinic visits (3- to 6-monthly) [244, 245]. However, the available data supporting the effectiveness of this strategy is sparse and the quality of evidence is low due to potential for bias. A recent systematic review found that reduced frequency of clinic visits and medication pick-up for ART patients may lead to improvements in program retention and patient outcomes [246]. A single, pilot cluster-randomised trial that included a comparison of clinic visit frequencies (maximum 3-monthly) for ART patients has been conducted in low-income settings, but included only 96 participants in the intervention group [247]. No larger scale randomised trials have been conducted, and little cost data are available. Fatti et al. [248] plan to carry out a cluster randomised trial to investigate the effectiveness and cost-effectiveness of 3- vs. 6-monthly dispensing of ART for stable HIV patients in community ART-refill groups in Zimbabwe. They highlight that clinics that are relieved of stable ART patients may be able to increase the rate of new ART initiations and thus scale up ART coverage.

Differentiated care, whereby patients who are stable on ART attend clinic even less frequently (6-12 monthly and may collect their drugs at alternative pick-up points) has been recommended in the latest WHO treatment guidelines [171] to further reduce clinic burden; those who have

recently started ART or have any toxicity or indication of lack of virological suppression continue to attend more regularly [249]. CD4 count may be used to triage patients to different care pathways but there is an increasing emphasis on using viral load which provides a more direct measure of the current treatment effect on the virus. Over the last decade WHO recommendations in LMIC have moved towards using viral load for monitoring treatment, and since 2013 viral load monitoring has been recommended as the preferred monitoring approach to diagnose and confirm treatment failure [35]. In 2016 these guidelines were updated and WHO now recommends that routine viral load monitoring be carried out at 6 months, at 12 months and then every 12 months thereafter if the patient is stable on ART, to synchronise with routine monitoring and evaluation reporting. “Viral-load-informed differentiated care” tailors care so that those with suppressed viral load visit the clinic less frequently and attention is focussed on those with unsuppressed viral load to promote adherence and timely switching to a second-line regimen. Routine viral load testing has been available for some time in South Africa but is gradually being rolled out in a number of other African countries [250], mostly by collection of dried blood spot samples in clinic which are then tested in regional laboratories. Phillips et al. [242] used mathematical modelling to evaluate the cost-effectiveness of viral-load-informed differentiated care, accounting for the limitations of dried blood sample testing. They found that viral-load-informed differentiated care using dried blood samples was cost-effective (provided it led to reduced clinic visits) and recommended it as a strategy for patient management; however further real life evidence as the approach is rolled out is needed. The difference between models and real-world experience of “test and treat” discussed above emphasises the importance of this.

Community based ART delivery models

Community-based ART models are another approach to reduce burden on healthcare systems. Murray et al [251] carried out a systematic review on improving retention in HIV care among adolescents and adults in low- and middle-income countries. They found that interventions involving the delivery of community-based services had the strongest evidence based on the quality and the number of studies. Eight studies examined shifting the delivery of HIV care and treatment from health facilities to communities. These interventions relocated ART distribution to the community and engaged community health workers or peers to distribute ART and monitor symptoms, resulting in fewer patient visits to a clinic. Two community-based service interventions also incorporated directly observed therapy i.e. making sure someone takes their tablets by watching, or counselling. Overall, the results of community-based service interventions were positive: six found positive associations between the interventions and retention in care, and two found no association. Community ART-refill groups are a novel strategy that has been introduced with the intent to reduce barriers to patients accessing regular treatment and to limit health facility congestion [252]. Community ART-refill groups are groups consisting of 6-12 stable ART patients who meet at a community venue to receive ART refills, and who provide mutual support to each other. Retrospective observational studies of community groups receiving monthly ART refills have shown encouraging results [253, 254].

Follow-up between clinic visits

Another way to improve retention in care is to increase the amount of contact between patients and clinics between visits. Results from one of the first mobile health trials in Africa [212] (referred to in the section on importance of adherence), WelTel Kenya1, showed that patient–clinician text messaging significantly improved treatment adherence and viral suppression among individuals who had initiated ART [212]. The WelTel intervention involved sending weekly interactive text messages, which were followed up by telephone calls if a patient indicated that they had an issue with adherence. This was in addition to their usual care, which consisted of a clinic appointment two weeks after the positive HIV test followed by a once a month visit for the first six months, then if adherent a visit every two/three months, with those who were non-adherent continuing to be seen every month. Anyone who did not attend was called by clinic staff, twice if necessary. They found no effect of the intervention on retention, which may be due to the regularity of usual care. However, in addition to the benefits on adherence and viral suppression above, they did identify improvements in quality of life with text messaging.

Comorbidities

As treatment improves, HIV-positive people are living longer and are therefore experiencing other comorbidities found in the general population such as cardiovascular disease, malignancies, cognitive impairment and reduced bone mineral density, which impact disability and everyday functioning [255]. Improving the management of these comorbidities in LMIC is essential, including screening and diagnosis. Managing HIV as a long-term chronic condition alongside other comorbidities and providing integrated care is increasingly being recognised as key for the patient but also for healthcare providers [256].

4.1. Conclusions

In conclusion, delivering ART to people in low- and middle-income countries still has major challenges, including starting patients on treatment early in order to reduce the risk of early deaths, and retaining people on treatment to reduce the detrimental effects of treatment interruptions. The contribution of HIV-related-malignancies to deaths, likely triggered by pre-cancerous events occurring before ART initiation to longer-term ART mortality highlights the importance of earlier HIV diagnosis and access to care. Low but increasing risk of death from trauma/suicide in adults highlights the importance of long-term psychosocial support and empowering patients to manage their own treatment. Greater efforts should be devoted to reducing the risk of any treatment interruption, including strengthening supply chain management, early contact of patients not attending drug refill visits and making extra supplies of ART available for patients who travel. Access to care for people with HIV needs to be a priority; treatment needs to be started in a timely manner, patients need to be retained on treatment and sufficient monitoring is key if the 90-90-90 target is to be met by 2020.

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