The long term durability of combination antiretroviral therapy in HIV-positive patients across Europe

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Declaration

I, Joanne Reekie, 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

Abstract

Despite dramatic improvements in the quantity and quality of life for Human Immunodeficiency Virus (HIV) positive people with the introduction of combination antiretroviral therapy (cART), all-cause mortality rates remain higher than the general population. Furthermore, treatment is a lifelong commitment and a substantial burden on patient life.

The aim of this thesis was therefore to assess the long term durability of cART through assessing various clinical, virological and immunological outcomes including mortality in HIV-positive patients across Europe. The analyses were based on data from the EuroSIDA cohort, an observational cohort of more than 16000 HIV-positive patients from Europe, Israel and Argentina.

Results showed that HIV-positive patients on a well-tolerated and fully suppressive cART regimen have a small risk of treatment failure occurring over the next 6 months and could therefore be monitored less frequently. In contrast patients who have spent a low percentage of time with a suppressed viral load whilst on cART or who have recently rebounded may require more intensive monitoring after making a treatment switch. In patients who have achieved an initial response and tolerated the first three months of treatment, nevirapine efavirenz and lopinavir based cART regimens all have similar durability based on risk of all-cause discontinuation and development of serious clinical events. Starting cART earlier to reduce the proportion of patients with a low CD4 count may decrease the rate of developing many common non-AIDS related malignancies. Individuals in Eastern Europe had an increased risk of mortality from AIDS related causes in part due to differences in use of effective cART.

In conclusion results from this thesis provide evidence that could help improve the long term durability of cART for HIV-positive patients through different measures of healthcare capturing the wide aspects of treatment and outcomes.

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Chapter 1 Introduction

1.1 Introduction

Over the past 30 years the HIV epidemic has moved from a single report of a cluster of infections[1] to a worldwide pandemic[2]. HIV first appeared on the public health agenda in 1981, when young gay men started dying of obscure diseases[3]. At this time, very little was known about the disease, and infection with HIV was usually fatal within months or years of the appearance of AIDS defining symptoms[4]. Since then, our knowledge and understanding of the disease has increased dramatically, and huge advances in treatment in the mid-90's mean that HIV is now seen as more of a chronic and manageable infection in the developed world[5;6]. However, HIV remains among one of the leading causes of death worldwide, accounting for more lives than any other infectious disease[7], and the incidence of HIV is still increasing in the majority of regions in the world[8]. In the most heavily affected countries HIV has reduced life expectancy by 20 years[9], though treatment advances in the developed world have resulted in a dramatic decline in mortality rates from AIDS related causes[10]. However, in the majority of patients mortality rates remain higher than that of the general population[11-13]. Furthermore, with no significant development towards a cure or vaccine[14;15], treatment is a life-long commitment and a substantial burden on the patient's life, as well as being itself associated with a number of toxicities and adverse events[16-18]. Additionally, recent studies have also found that HIV-positive patients are at a greater risk of death from other illnesses not thought to be directly associated with HIV infection[19-21]. More research needs to be done to understand and help improve the long term treatment and outcome for patients infected with HIV.

1.2 The start of the epidemic

HIV first came to prominence in 1981 with a spate of deaths of young gay men in the United States attributable to obscure diseases[3;22] that had rarely been seen before, such as Pneumocystis carinii pneumonia (PCP)[23;24] and Kaposi's sarcoma, a rare cancer [1;6;22;25-29]. These diseases started to become very common in young, previously healthy homosexual men[23;25;27]. At first there was a lot of stigma surrounding the disease and names initially suggested for what is now called 'AIDS' included 'gay compromised syndrome', 'gay related immunodeficiency syndrome (GRID)' and 'gay cancer'[30;31].

However, similar symptoms were soon also reported in intravenous drug users[32-36], haemophiliacs and other patients who had received blood transfusions[36-38],as well as Haitians in the United States[36;39]. Similar symptoms were also seen in the same groups of patients in Europe[27;28;40-46]. Additionally, there were reports that children whose parents were in these risk groups were also experiencing these symptoms[47;48]. Reports in Europe of similar symptoms in previously healthy Africans began to emerge[49], and doctors in Africa also began to report an increase in cases of Kaposi's sarcoma[50-52], giving rise to the idea that there might be a second epidemic in Africa not confined to particular risk groups[52;53]. It quickly became apparent that the underlying cause of these diseases was severe immunodeficiency[3;23;24]. In 1982, Acquired Immunodeficiency Syndrome (AIDS) was named and defined by the CDC[36], in French it was referred to Syndrome d'immuno-depression acquise (SIDA). Although immunodeficiency diseases were not unheard of , the striking difference with AIDS was that it had a 100% mortality rate[3].

1.3 The origins of HIV

In 1983, France's Pasteur Institute discovered the virus believed to be responsible for AIDS, and named it Lymphadenpathy Associated Virus (LAV)[54;55]. Around the same time, Robert Gallo and his team discovered a virus which they named Human T-cell Lymphotropic Virus 3 (HTLV-III) and similarly provided evidence that it was this virus that was responsible for AIDS[56-58]. By 1985 it was recognised that these two viruses were the same. To avoid confusion, and because of its obvious detrimental effects on the human immune system, these viruses were renamed Human Immunodeficiency Virus (HIV)[3;59;60]. In the mid-80s it became apparent that there were two types of HIV, HIV-1 and HIV-2[3]. These two types, HIV-1 and HIV-2, are not closely related to each other and within these two types are a variety of subgroups, some of which have considerable genetic diversity [61].

Much speculation has existed over how humans became infected with the disease. One theory was that it came from a contaminated oral polio vaccine used in Africa in the late 1950's or that it was a biological weapon developed in America. These ideas have since been proven to be unfounded[62;63]. In 1999, a group of researchers announced that they had found a virus in chimpanzees that was almost identical to HIV[64], and it is now accepted the HIV-1 is closely related to a virus found in Chimpanzees [64-66] and HIV-2 to Simian Immunodeficiency Virus (SIV) found in sooty mangabey monkeys[61;67]. The wide range of subgroups in both of these types suggest that cross species transmission has occurred on several occasions[61;68;69]

HIV-1 is the predominant form of the virus worldwide[70;71] and the cause of the vast majority of infections. However, the spread of HIV-1 in the developed world is still mainly confined to those in high risk groups such as homosexuals, intravenous drug users, and sex workers[72]. It is thought that HIV-1 crossed into humans three times and this has resulted in three different subgroups M, N, and O[3;61;65]. M is the most common and widely spread across the world[61]. Within the M group so far 10 subtypes have been found, A-K, with B and D being the most similar to each other[61;72]. HIV-1 infections in North America and Europe are most likely due to group M subtype B, while subtype C is common in India and Southern Africa, and subtype E spread most rapidly through Southeast Asia[3;61]. Although HIV-1 group M subtype B accounts for fewer infections than subtype C, it is the more widespread[73], and no other subtype has been found in as many countries around the world[74]. No subtypes have been found thus far in groups O or N[3]. Group O is currently restricted to west central Africa, and N has only been identified in cases from Cameroon[3;61;75;76].

It is thought that HIV-1 was introduced to the human population 60-90 years ago[77] and there is now very little doubt that the disease originated in Africa, as many cases have now been found dating back to the 1960's[78]. A study analysing plasma samples from Africa dated from 1959 to 1982 found an HIV positive sample from as early as 1959[78]. Studies found that these samples had diversified substantially from the original strain that crossed over into humans, indicating that HIV had been circulating in the population for several years[79]. Further studies have since found that HIV-1 originated in central Africa, apparently around the 1930s[65;77], coinciding with when the first towns were emerging in the same region of Africa[71]. The earliest reported evidence of AIDS in Africa has been found to be in the late 1950s-early 1960's[78;80]. However, it is thought that a high number of tuberculosis-caused mortality could actually have been undiagnosed AIDS-related mortality [73].

Evidence has shown a high prevalence of HIV-1 among Haitian immigrants in the US[81]. There was debate over whether Haiti was the source from where the epidemic spread to the US[77;82;83] or whether the Haitian HIV-1 epidemic was caused by sex tourism from the US in the mid-1970s[84;85]. However, a recent study has found that Haiti has the oldest known HIV epidemic outside sub-Saharan Africa[73], where it then spread to the US.

We now know that HIV-1 was circulating in the USA for around 10 years before it was first recognised[73], with the date of the US epidemic estimated to have started around 1968-1969[73;86]. Studies describing the prevalence of AIDS found 4.5% of homosexual men in San Francisco and 6.6% in New York were estimated to have AIDS in 1978[87;88]. As there is a long interval between HIV seroconversion (initial infection with HIV) and symptomatic disease this evidence suggests that several thousand individuals in the US were already infected, therefore implying that the virus had already been circulating the US for several years[73].

Once established, HIV-1 was able to spread quickly through the pre-existing networks of gay men and injecting drug users it encountered in north America and Europe during the early 1980's[3]. One of the oldest recorded cases of AIDS in the developed world is thought to be that of a sailor in Manchester, UK, who was reported to have died from an AIDS like illness in 1959[89].

There are several theories as to why there was such a dramatic increase in the number of AIDS cases in the 1980s. One suggestion is that HIV had been spreading slowly in the heterosexual population before entering the homosexual male population, where it spread explosively[73]. There was a massive increase in the number of unsterile injections, mainly for antibiotics, in sub-Saharan Africa around in the mid twentieth century which likely aided the dispersion of HIV though the widespread re-using of needles and syringes[90]. Once established in the human population, overseas troops, overland trucks and increases in air travel enabled the rapid and widespread dispersion on the virus[91].

There is little debate over the origins of HIV-2. HIV-2 is less infectious than HIV-1 and spreads at a slower rate[3]. So far HIV-2 has been found to have 6 subtypes A-F. HIV-2 is much less prevalent and is predominately found in west Africa and India[3;72]. Wild sooty managabey monkeys, indigenous to the same region, are known to be infected with SIV. It is thought that humans first became infected with this type in rural west Africa where these monkeys are often eaten or kept as pets[61].

1.4 Biology of HIV: how it works in the body

1.4.1 The HIV virus

HIV and SIV are retroviruses, belonging to a subgroup called lentiviruses or 'slow' viruses[61;92]. These viruses are characterised by the long interval between initial infection and the onset of serious symptoms[7]. HIV within the living cells of the host[91]. The primary cellular targets of HIV are the lymphocyte cells that express the CD4 cell protein, these CD4 cells are a vital part of the human immune system[93]

Figure 1.1 The Life cycle of HIV[91]

Once the virus has been transmitted into the blood it hunts down the helper T-cells or CD4 cells, so that it can bind to the CD4 receptor present on the surface of these cells and usually either the CCR5 or the CXCR4 co-receptors[94]. Once the HIV virus has bound to these cells, the contents of the virus are released. Once inside the cell the HIV enzyme reverse transcriptase (RT) helps convert the HIV RNA into DNA so that it is compatible with normal human genetic material[95]. This is known as the provirus. The provirus is then able to integrate into the host's genomic DNA[3]. When the cell becomes active it treats the HIV DNA just like it would normal human genes. First it converts it to messenger RNA (using human enzymes) in a process called transcription. The messenger RNA is then transported outside the nucleus and is used as a blueprint for producing new HIV proteins, this process is called translation.

Among the strand of messenger RNA produced by the cell are complete copies of HIV genetic material. The genes produced are much larger than those in the final model, and the protease enzyme is vital at this point as it is responsible for cutting the long protein strands into smaller functional pieces. They gather together with newly made HIV proteins, enzymes, to form new viral particles just inside the cell membrane. The virus then 'buds' (peels off the cell) and these matured particles are ready to go on and infect other cells. A single cell can make thousands of infectious HIV particles[7]. The host cell dies soon after the release of the new virus particle as it is significantly weakened through this process, and this is the reason for the destruction of the immune system. If the cell is not active, then the provirus may lie dormant within the cell for several years[96]. This has complicated attempts to eradicate HIV and also why therapy is a lifelong commitment[97].

1.4.2 Immunology and Virology

The HIV-virus destroys mature CD4 T-cells and reduces the body's ability for them to be repalced[93]. The number of CD4 cell counts in a healthy person is between 500-1600 cells/mm^3 [98;99] and natural daily variability is large, regardless of HIV status[100;101]. A persons CD4 count can change due to a number of different factors such as fatigue, time of day, stress, exercise, acute infections, drug use and diurnal variation[101-103]. Woman tend to have higher CD4 counts than men[104].

It has been shown that HIV-positive individuals have much lower CD4 counts than that of HIV-negative individuals[99] and that the CD4 cell count gradually decreases during HIV infection, as shown in figure 1.2[105-107]. A lower CD4 count has been found to be associated with an increased risk of the development of AIDS and death[108-112]

A major hindrance into completely understanding and explaining of how the virus impacts the immune system, is the inherent difficulty of studying the immune system in living humans[93]. However, techniques for measuring CD4 count have improved and the cost is relatively cheap (around $5[113] in the developing world and $66 in the developed world) although there are still numerous sources of variability in the measured components[114].

CD4 counts were complimented by viral load measurements once this method became available for routine use in 1996. The method measures the amount of HIV genetic material (HIV-RNA) in the plasma (liquid part of the blood) and gives us the viral load[115-117]. During the initial stage of infection with HIV, the viral load can reach 10 million copies/ml

before it falls at the end of the acute phase following primary infection, (figure 1.2)[118]. Viral load has been shown to be a strong predictor of AIDS and death, independent of CD4 cell count[116;119-121] with higher viral loads associated with a faster rate of disease progression[115].

The HIV-RNA test in the developing world costs around $25[113] and in the developed world $119. Initially these tests were only able to detect quantitative levels of HIV-RNA in the plasma when the viral load was > 500 copies/ml. However, more recently assays with lower limits of detection below 50 copies/ml have been introduced[122;123].

Viral load and CD4 cell counts are both important predictors of HIV-related mortality[124]. HIV-RNA is considered to be a good long-term marker of risk of clinical progression, that is, a single value can determine the risk of AIDS or death 5-10 years thereafter[116;125-128]. However, CD4 count is considered to be a much better predictor of short-term risk of disease progression[127;129-133].

Figure 1.2 Progression of the HIV virus (HIV-RNA) and deterioration of immune system (CD4 count) over of the course of an untreated infection[118]

1.5 The stages of HIV disease progression

The World Health Organisation (WHO) and the Centres for Disease Control (CDC) have both developed classification systems for the stages of HIV disease and its progression to

AIDS. Table 1.1 details the two classification systems; the main difference is that the CDC assesses the severity of HIV disease progression by CD4 count and HIV-specific conditions. The WHO classification systems on the other hand, can be used without CD4 count measurements and allow for clinicians with varying levels of expertise and training to diagnose AIDS[134], this is especially helpful in resource constrained settings.

Table 1.1 Stages of HIV infection in Adults

WHO classification[135]	CDC stage classifications[136]	Clinical symptoms
1. Asymptomatic (HIV infection)	A. Asymptomatic (HIV infection)	No AIDS-defining condition and either CD4+ T-lymphocyte count of ≥500 cells/μL or CD4+ T-lymphocyte percentage of total lymphocytes of ≥29
2. Mild (HIV infection)	B. Symptomatic	No AIDS-defining condition and either CD4+ T-lymphocyte count of 200--499 cells/μL or CD4+ T-lymphocyte percentage of total lymphocytes of 14-28.
3. Advanced (advanced HIV disease)	B. Symptomatic	No AIDS-defining condition and either CD4+ T-lymphocyte count of 200-499 cells/μL or CD4+ T-lymphocyte percentage of total lymphocytes of 14-28.
4. Severe (AIDS)	C. AIDS	AIDS defining condition or CD4+ T-lymphocyte count < 200cells/μL

1.5.1 Primary HIV infection

The first stage of HIV infection is generally called seroconversion. In the first few weeks after infection quite high levels of the virus are found in the blood stream[137-139], and a patients viral load in this early stage can reach millions of virion per ml of blood[7]. After initial infection HIV replicates quickly, producing viral bursts that infect many CD4 cells. During this first stage of infection there is normally a slight dip in CD4 cell count and a substantial increase in viral load (figure 1.2). The majority of individuals experience a brief illness, in most cases mild flu-like symptoms[137;140]. About 6-8 weeks after primary infection the CD4 count increases, the viral load decreases and most individuals enter a relatively asymptomatic stage that can last for several years[139;140].

1.5.2 Chronic HIV infection

This initial phase is followed by a gradual deterioration of immune function (figure 1.2)[7]. The body normally manages to compensate for the depletion in the immune system for 10-12 years after infection, with some people infected for as long as 20 years before needing treatment[141]. In the absence of treatment, older patients have higher rates of progression than younger patients[7]. HIV-2 seems to be less pathogenic and progresses to AIDS more slowly than HIV-1[142].

The decrease in CD4 count has been estimated to be relatively slow at 50-90 cells/mm^3 per year, until 18 months prior to AIDS diagnosis where between a 3 and 5 fold increase in CD4 cell depletion has been observed[107;143]. Once the CD4 cell count drops below 500cells/mm^3 half the immune reserve has been destroyed[7]. Patients with a CD4 count above 200/mm^3 rarely have opportunistic infections, while those with a CD4 count of less than 50/mm^3 have a high risk of infections[100]. Plasma HIV-RNA levels are correlated with CD4 count depletion, with higher viral loads predictive of a quicker progression to AIDS and death[121;144].

1.5.3 Clinical AIDS

The final stage of HIV disease progression is the development of AIDS. Table 1.2 shows the list of AIDS-defining illnesses. In addition, in the CDC classification system a CD4 count < 200cells/mm^3 in the presence of HIV infection is also as an AIDS diagnosis. Survival time after an AIDS diagnosis varies according to the AIDS-defining events. In the absence of treatment, studies have reported median estimates for a single AIDS-defining condition of between 3-51 months and between a 1.5 and two-fold decrease in survival time after a second condition is diagnosed[145].

Table 1.2 CDC category C, list of AIDS defining illnesses, note diagnosis of one of these disease without the presence of HIV virus does not mean AIDS.[134;136]

AIDS defining illnesses

- Candidiasis bronchi, trachea, lungs or esophageal
- Cervical cancer (invasive)
- Coccidioidomycosis, disseminated or extrapulmonary
- Cryptococcosis, extrapulmonary
- Cryptosporidiosis, chronic intestinal (greater than 1 month's duration)
- Cytomegalovirus disease (other than liver, spleen, or nodes)
- Cytomegalovirus retinitis (with loss of vision)
- Encephalopathy (HIV-related)
- Herpes simplex (an infection lasting longer than 1 month or in an area other than the skin such as esophagus or lungs) or bronchitis, pneumonitis, or esophagitis
- Histoplasmosis, disseminated or extrapulmonary
- Isosporiasis chronic intestinal (greater than 1 month's duration)
- Kaposi's sarcoma (KS)
- Lymphoma Burkitt's (or equivalent term)
- Lymphoma, immunoblastic (or equivalent term)
- Lymphoma, primary, of brain
- Mycobacterium avium complex or M. kansasii, disseminated or extrapulmonary
- Mycobacterium tuberculosis, any site (pulmonary * or extrapulmonary)
- Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
- Pneumocystis carinii pneumonia
- Pneumonia, recurrent *
- Progressive multifocal leukoencephalopathy
- Salmonella septicemia, recurrent
- Toxoplasmosis of brain
- Tuberculosis
- Wasting syndrome due to HIV

1.6 Current Situation

1.6.1 Global HIV epidemic

The most recent UNAIDS epidemic update published in 2009[8] shows that the number of people living with HIV worldwide is continuing to grow. In 2008 this number was estimated to be around 33.4 million, a 20% increase on the estimate form 2000[8]. 2.0 million people were estimated to have died due to an AIDS-related illness worldwide in 2008[8]. Figure 1.3 below is from the WHO showing the prevalence of HIV infection across the world.

Figure 1.3: Global view of the HIV epidemic[146]

From Figure 1.3 it is apparent that sub-Saharan Africa is the most heavily infected region worldwide. In this region over two thirds of the population are infected with HIV, and AIDS is the leading cause of death[2]. In 2001 it was reported that AIDS was responsible for 1 in 5 deaths in sub-Saharan Africa, twice as many as the second leading cause of death[147]. In 2008 22.4 million adults and children were estimated to be living with HIV in sub-Saharan Africa, just under 70% of the entire HIV epidemic, and 1.4 million died from an AIDS-related death[8]. Prior to the HIV epidemic, life expectancy in this region had increased to 62 years and it was hoped it would soon approach that of the developed world. However, due to the high number of HIV related deaths, life expectancy has now fallen to 44 years[148].

Globally, the epidemic is mainly confined to those in particular risk groups: intravenous drug users (IDU), homosexual men, and sex workers. The exception is Sub-Saharan Africa, where the epidemic is so widespread that everyone is at risk[2;147]. Sub-Saharan Africa is the only region in the world where more women are infected with HIV than men (64%)[2], as transmission of HIV is dominated by heterosexual sex and also a high number of mother-to-child transmissions[147].

It is thought that the global incidence of HIV peaked in 1996, when 3.5 million new infections were estimated to have occurred[8]. In comparison, the estimated number of new infections in 2008 was thought to be 2.7 million, 30% lower than in 1996[8]. Although the epidemic appears to have stabilised in most regions, including Sub-Saharan Africa, the prevalence of HIV in Eastern Europe and Central Asia is still increasing due to a high rate of new infections [8].

1.6.2 European HIV Epidemic

In 2007, HIV/AIDS surveillance in Europe reported that 48,892 newly-diagnosed cases of HIV were reported across Europe, from 49 of the 53 countries in the WHO European region[149]. Figure 1.4 shows the number of new infections by region of Europe and year of diagnosis[149]. Within Europe, the HIV epidemic varies across different regions and also by country to country.

Figure 1.4 HIV/AIDS surveillance in Europe: Update 2007. HIV cases per million population in geographic areas of the WHO European region by year of diagnosis 2000-2007[149]

Figure not available due to copyright restrictions

1.6.2.1 Eastern Europe

In 2008, UNAIDS estimated that 1.5 million people were living with HIV in Eastern Europe and Central Asia, a 66% increase from 2001[150]. Eastern Europe and Central Asia are considered together because of both their physical proximity to each other and their common epidemiological characteristics. This is the only region worldwide where HIV incidence is clearly on the rise (figure 1.4), and 110 000 new infections were estimated to have occurred in this region in 2008[8].

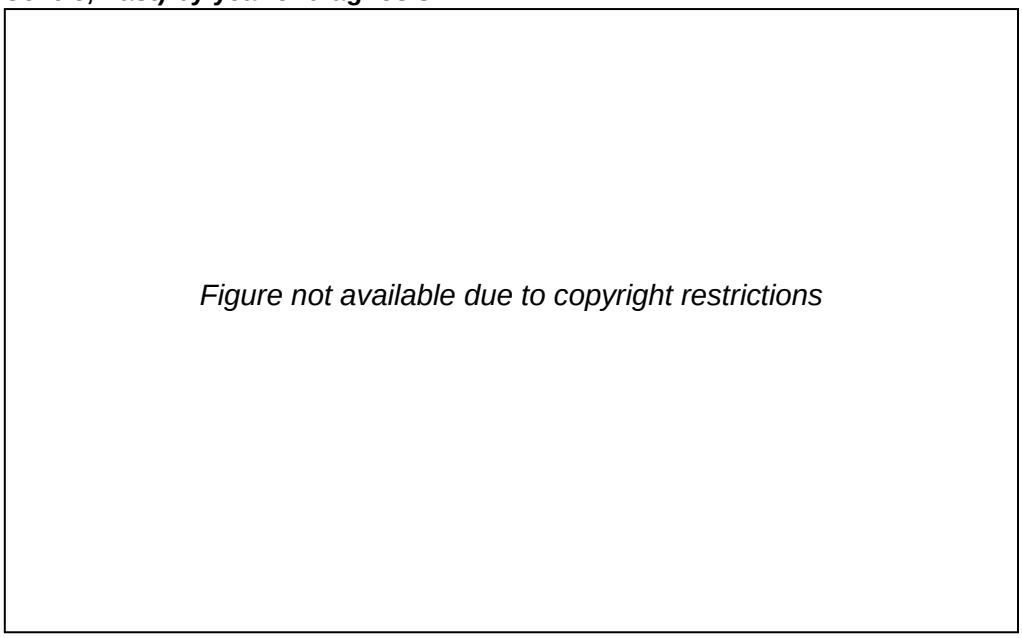
The prevalence of HIV among adults in this region was estimated to be 0.7% in 2008, an increase of 0.2% from 2001. Ukraine, Estonia and Russia are reported to have the highest infection levels in all of Europe[151]. In 2007, the prevalence of HIV infection in Ukraine among adults was estimated to be 1.6%[150], and for Estonia and Russia was estimated to be >1%[150].

The epidemic in these countries is mainly due to sex workers and intravenous drug users (IDUs)[147]. The overlap between these two groups has helped facilitate the spread of infection[150]. Nearly two-thirds (57%) of newly diagnosed infections in Eastern Europe were attributed to intravenous drug use and 42% from unprotected heterosexual sex[149]. An estimated 3.7 million people in Eastern Europe are current injection drug users, and one in four are thought to be HIV-positive[152]. The prevalence of HIV infection among IDUs in the Ukraine has been reported to be between 38.5% and 50.3%[151], and in Russia 38%[152]. In a number of countries in Eastern Europe, rates of AIDS have been reported to be increasing between 2000 and 2007 (figure 1.5), with the biggest increase in Belarus and the Republic of Moldova[149]. Treatment coverage is low in Eastern Europe, as the patients most at risk of HIV infection (IDUs and sex workers) are the ones least likely to receive antiretroviral therapy[153]. By December 2008, only 22% of adults in need of antiretroviral therapy were receiving it[8].

1.6.2.2 West and Central Europe

In West and Central Europe the prevalence of HIV infection was estimated to be 0.3% in 2008, with an estimated 850,000 people living with HIV alongside 30,000 new infections[8]. The total number living with HIV in this region is increasing. However, this is mainly due to antiretroviral therapy substantially increasing the life expectancy of patients as well as an increase in HIV testing in Western Europe leading to more diagnoses[2]. Within this region of Europe, rates of new HIV infection appear to be highest in Portugal[149]. In West Europe, 24,202 new HIV cases were diagnosed in 2007, with the majority of new infections diagnosed in homosexual men (40%)[149]. In contrast to Eastern Europe, only 8% of new infections were due to intravenous drug use[149]. In Central Europe, 1,897 new HIV cases were diagnosed in 2007, 53% of these were through heterosexual contact, 30% though homosexual male contact and 13% through injection drug use[149].

Figure 1.5 HIV/AIDS surveillance in Europe: Update 2007. Number of newly diagnosed AIDS cases per million population in the geographic regions of WHO European Regions (West, Centre, East) by year of diagnosis [149]



In West and Central Europe, although the number of new infections appears to be relatively stable (figure 1.5), the number of new infections among homosexual men has increased over the past decade, whilst there has been a decline in the number of new infections due to intravenous drug use[8]. In the UK, HIV diagnosis in homosexual men rose by 74% between 2000 and 2007[8], with the resurgence tied to an increase in sexual risk behaviour[8;154]. Trends in AIDS diagnoses (figure 1.5) in West and Central Europe continue to decline, mainly due to the effects of antiretroviral therapy[155].

There has been a significant decline in the number of AIDS-defining illnesses and deaths since the introduction of combination antiretroviral therapy (cART) in the mid 90s[10;156-159]. Figure 1.6 shows the declining incidence on AIDS in Europe from 1994-2001, based on data from the EuroSIDA study. Further progress in reducing AIDS events and HIV related mortality in this region will require an improved effort to diagnose HIV infection earlier. It is estimated that between 15% to 38% of patients in Europe are diagnosed after their disease has progressed to a level where they should have initiated treatment[160].

Figure 1.6 Decline in AIDS and death rates in the EuroSIDA study adjusted for, CD4 count, Data adjusted for CD4 count at recruitment, age, previous HAART treatment, AIDS status (yes or no)[10]

1.7 Transmission of HIV

As reported earlier, the continued rise in people living with HIV is due in part to the continued high rate of new infections[8]. HIV can be transmitted through unprotected sexual intercourse or oral sex with an infected person[161], via blood though the sharing of contaminated needles or syringes[162] or transfusions of contaminated blood[163]. It can also be transmitted from a mother to her baby during pregnancy, childbirth or through breastfeeding[164]. Risk of infection is largely dependent on the levels of the virus present[22;165;166]. For example, the virus can be found in a person's tear, but in such a small quantity that it is virtually impossible for a person to become infected in this manner[22]. Repeated exposure to the virus increases a person's risk of infection[167]. Additionally, the HIV virus dies quickly when outside the body so it cannot be transferred through shaking hands, or toilet seats for example[22].

1.7.1 Sexual transmission

The most common mode of HIV transmission worldwide is via sexual intercourse[22]. The risk of HIV infection by sexual transmission varies depending on sexual partner, sexual act and condom use[161]. It is greatly increased by infection of other sexually transmitted diseases, rough sex or a partner with a high viral load[7;168;169].

Woman are at a higher risk than men during heterosexual intercourse[7]. Worldwide, nearly half of all the individuals living with HIV are now women, who acquire the virus largely by heterosexual exposure[168]. Choosing a partner who has tested negative for HIV rather than a partner who has not been tested for HIV was found to be associated with a 47 fold reduced risk of transmission in heterosexual relationships[161] and the use of condoms a 20 fold reduced risk[161]. Regular condom use between heterosexual couples, where one individual is HIV-positive and the other is uninfected, leads to a very low risk of transmission[7]. However, because of limited economic options and gender inequality many woman cannot negotiate safe sexual encounters leaving them very vulnerable to sexually transmitted infections and unwanted pregnancy[168]. In addition a significant proportion of men do not consistently use condoms during anal sex with men or women[170]. This has led to topical microbicide formulations, applied vaginally or rectally being investigated as another strategy for the prevention of HIV transmission[7;168;171].

In July 2010 the Centre for AIDS Programme of Research in South Africa (CAPRISA) 004 study team reported the first evidence that a microbicide was associated with a significant reduction in HIV acquisition[172]. However, the effect size was relatively modest (woman using tenofovir gel were overall 39% [95% CI 60%-6%] less likely to become infected with HIV) and adherence was poor[173]. Male circumcision has been found to be an important intervention in reducing the risk of heterosexually-acquired HIV infection in men by around 60%[174-176], and is recommended by the WHO as an intervention to reduce HIV transmission[177]. Male circumcision does not however directly reduce male to female transmission and condom use is essential[178], but it can reduce the risk indirectly by reducing the risk of heterosexual men becoming infected[179]

In the developed world, male to male sexual contact is the highest reported risk factor for HIV infection[180]. The greatest risk of transmission in men who have sex with men (MSM) has been found to be receptive anal intercourse with an HIV-positive partner[161]. In the last few years there has been a resurgence in HIV infection among MSM in the developed world[8;181], as well as new epidemics in Asia, Africa and Latin America[182]. This increase, in some countries, is in part due to an increase in HIV testing[183]. However, there has been a reported increase in the number of MSM having unprotected anal intercourse[184], and recent outbreaks in other STIs support an increase in sexual risk taking[184-186]. Many MSM who know they are infected with HIV believe they have a responsibility to protect their partners from infection[187;188] and modify their behaviour accordingly[189]. However, some still engage in unprotected sexual behaviour[190] and there are many who remain unaware of their HIV status[191]. Some interventions within the MSM population including individual counselling, social and behavioural support discussing attitudes and beliefs, relationship support, and community interventions including workshops and empowerment activities have been found to lead to a significant reduction in the risk of transmission[192]. The potential role of circumcision among men who have sex with men has not been fully investigated[193]. However, in a recent study it was associated with a reduction in HIV incidence among participants who reported a preference for the insertive role in anal intercourse[194]. Despite these various interventions current efforts have been unable to contain or reduce the spread of infection among MSM and studies into the most effective intervention methods are rare[182;195].

1.7.2 Transmission via blood

HIV can also be spread through contact with infected blood[7]. The most common source of transmission via blood is through the reusing or sharing of syringes and needles in intravenous drug users (IDUs)[196]. Shared drug injection equipment can transmit HIV when residual infected blood remains on used syringes that are then reused by another person[162]. The number of HIV-positive IDUs and the number of IDUs at high risk continues to increase globally[197]. Incidence rates of transmission have been found to be between 10-50 per 100 person years at risk in many IDU populations around the world[197]. In countries where there are community outreach programmes and access to sterile injection equipment, such as the UK[198] and Australia[199], HIV prevalence has been kept low. Treating IDUs with respect and providing them with information on the modes of HIV transmission, sterile injection equipment through needle exchange programs, and condoms have proved successful intervention methods for reducing the risk of transmission[197].

Health care workers are also at risk through accidental needle stick injuries or mucosal splash with contaminated blood[7]. The average risk of transmission among healthcare workers is estimated to be 0.3% after a needle stick and 0.09% after mucous membrane exposure[200], although this varies according to the type of exposure i.e. depth of injury, viral load of person with HIV. Post exposure prophylaxis with antiretroviral therapy is now widely used after contact and thought to reduce the risk of HIV transmission by at least 80%[201] .

In the early stages of the epidemic no tests were available to screen for HIV, and therefore a number of people were infected through exposure to infected blood products, such as blood transfusions. Infection after a transfusion with HIV-1 infected blood, has been found to occur in 90%-100% of recipients[163;202]. Since 1985, blood in the USA, Canada and Europe has been routinely screened for HIV[7]. However, universal access to safe blood cannot be achieved without systems for ensuring quality and continuity of screening[203]. Poor access to safe blood has been associated with weak healthcare systems and rural settings[203]. Additionally, proper training of healthcare workers into the proper use of blood can lower the risk of HIV infection, as up to 50% of blood transfusions have been found to be unnecessary [204]. Ensuring the safety of blood transfusions has been found to be cost effective. For example the cost per HIV infection prevented through blood safety has been

found to be around $18[205], due to the high efficiency of transmission via infected blood. WHO recommends cost effective strategies for cost effective blood safety[206]. However, many countries still fail to screen all donated blood for HIV in accordance with minimum quality standards[203].

1.7.3 Mother to child transmission

HIV-positive pregnant woman have a significant risk of transmitting the virus to their child if effective interventions are not provided[164]. An estimated 2.1 million children under 15 were estimated to be living with HIV in 2008 and 430,000 new infections reported in 2008 were in children[8], with almost all of these new infections due to mother to child transmission[207]. Transmission can occur during pregnancy, labour, delivery or through breastfeeding after birth[164;208-211]. Without any intervention, the risk of transmission ranges from 15-40%[212]. Effective prevention requires access to testing early in pregnancy coupled with the ability to deliver antiretroviral therapy to mothers and infants[164].

Prevention of mother to child transmission is an area where there are huge differences in treatment and care across the world[164]. In developed countries, therapeutic and prophylactic antiretrovirals, and the avoidance of breastfeeding have reduced mother to child transmission from around 25% to between 1% and 5%[213]. In Europe, current treatment guidelines for pregnant woman are similar to non-pregnant patients, although the avoidance of some antiretroviral drugs is recommended[214]. Zidovudine is a preferred antiretroviral to be included in the regimen[214-216]. In many developed countries, such as the UK and USA, HIV testing in pregnant woman is part of their routine antenatal care[217;218].

In sub-Saharan Africa, where approximately two-thirds of the World's HIV-positive population live, woman make up approximately half of those infected and the majority are of child bearing age[164]. The rates of mother to child transmission remain high in this region, and breastfeeding is one of the key reasons for this[213]. About 40% of the total cases of mother to child transmission are due to breastfeeding[219]. Whilst significantly lower rates of transmission have been reported in non-breast fed compared to breast-fed populations, morbidity and mortality are often worse[164]. For the majority of women in resource poor countries, breast feeding is the only option[164]. Exclusive early breastfeeding has been associated with improved survival compared to other types of infant feeding[219;220]. Further work is needed to determine the optimal means to reduce transmission through breast milk.

Another key strategy is to increase testing, both early in pregnancy and at the time of delivery, as this is when mothers are likely to present for care[164]. Treatment and access to antiretrovirals is improving in the developing world. In 2008, 45% of pregnant women in low and middle-income countries received antiretrovirals to prevent transmission to their child. This has increased from 10% in 2004[221] and the number of new infections from mother to child transmission in 2008 was roughly 18% lower than in 2001[8]. In the past decade, the international community recognized the need for improved services for the prevention of mother to child transmission. The UN general assembly has set a target for 80% of pregnant woman and their children to have access to prevention, treatment and care by 2010 to reduce the proportion of infants with HIV by 50%[221].

1.8 Treating HIV

As well as the number of new infections, the other factor contributing to the rise in the number of people with HIV infection is the beneficial impact of antiretroviral therapy[8]. Although approximately one third of those in need of therapy don't have access to it, antiretroviral coverage in low and middle income countries rose from 7% in 2003 to 52% in 2010[222]. In 2008, approximately 4 million people in low and middle income countries were receiving antiretroviral therapy, a 10-fold increase in the last 5 years[8]. There are currently 25 antiretroviral drugs approved for the treatment of HIV, and current treatment guidelines recommend that patients are given three active drugs from at least two different classes[223]. There are 6 different classes of drugs available, each of which attack different stages of the HIV life cycle. The classes are nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI), Fusion inhibitors, CCR5 inhibitors, and integrase inhibitors although some of these drugs are only infrequently used because of their side effects, such as high pill burden, inconvenient administration schedules or drug interactions[224].

HIV treatment guidelines concerning what specific regimens to use are derived mainly from the results of short-term clinical trials with viral load outcomes[225]. Before the introduction of cART, the death rate of HIV-positive patients was much higher, and conclusions could be reached from trials using clinical outcomes within only a few years[226]. One of the first clinical trials by Fischl et al.[227] was terminated after only 7 months because of the obvious benefits of the drug, zidovudine. After the introduction of cART, to achieve the same power in clinical trials, the sample sizes and duration had to increase considerably, to provide statistically comparative power to evaluate clinical outcomes.

Although, a few early trials did use clinical outcomes[227;228] since 1996 clinical trials with surrogate outcomes, such as changes in CD4 count or viral load, have predominantly replaced clinical outcomes in trials of anti-HIV drugs[229]. Compared to clinical outcomes, using viral load or CD4 counts has many practical advantages, as the measurements are taken routinely in clinical practice and are fairly reliably quantified. However, the disadvantage of using short-term surrogate outcomes is that by definition they provide no direct information on the long-term implications of using ART[230]. Only a small percentage of clinical events occur in the first 24 or 48 weeks of a study, where there is a comparatively small risk of AIDS or death[229]. Observational research has played a key role in supplementing research from randomised controlled trials as patients are often followed over longer periods of time allowing for all stages of treatment to be observed, providing important information when randomised controlled trails are not feasible.

1.8.1 Antiretrovirals

1.8.1.1 Nucleoside reverse transcriptase inhibitors

Table 1.3: NRTI drugs

Generic name	Brand name	FDA approved[231]	Europe[232]
abacavir	Ziagen	17/12/98	**8/7/99**
didanosine	Videx	9/10/91	*
emtricitabine	Emtriva	2/7/03	**24/10/03**
lamivudine	Epivir	17/11/95	**8/8/96**
stavudine	Zerit	24/6/94	**8/5/96**
tenofovir	Viread	26/10/01	**5/2/02**
zalcitabine	Hivid	19/6/92	*
zidovudine	Retrovir	19/3/87	*

*not licensed by agency

NRTI are the earliest class of antiretroviral drugs approved for the treatment of HIV, with zidovudine being the first drug to be approved for the market, in 1987[231]. Nucleoside analogues are faulty versions of the building blocks necessary for HIV reproduction. When HIV's reverse transcriptase enzyme uses a nucleoside analogue instead of a normal nucleoside, reproduction of the virus's genetic material is halted. Nucleoside reverse transcript inhibitors attach themselves to the reverse transcriptase and prevent the RNA turning into DNA. Table 1.3 gives a summary of the NRTIs available for treatment of HIV, and when they were approved in the U.S. and Europe. Among NRTIs, the use of zalcitabine, stavudine and didanosine has dramatically declined or vanished, while zidovudine, lamivudine, abacavir and tenofovir have gained relevance[233].

To lighten patient's pill burdens, some drugs have been developed to contain combination of two or three NRTIs and these are listed in table 1.4.

Table 1.4: Combined NRTI drugs

Generic name	Brand name	FDA approved [231]	Europe [232]
abacavir and lamivudine	Epzicom/Kivexa	2/8/04	**17/12/04**
tenofovir and emtricitabine	Truvada	2/8/04	**21/2/05**
lamivudine and zidovudine	Combivir	27/9/97	**18/3/1998**
abacavir, zidovudine, and lamivudine	Trizivir	14/11/00	**28/12/00**

1.8.1.2 Protease Inhibitors (PI)

Table 1.5: Licensed PI drugs

Generic name	Brand name	FDA approved[231]	EMEA approved[232]
amprenavir	Agenerase	15/4/99	20/10/00
atazanavir	Reyataz	20/6/03	2/3/04
darunavir	Prezista	23/6/06	12/2/07
fosamprenavir	Lexiva	20/10/03	*
indinavir	Crixivan	13/3/96	14/10/96
lopinavir and ritonavir	Kaletra	15/9/00	20/3/01
nelfinavir	Viracept	14/3/97	22/1/98
ritonavir	Norvir	1/3/96	26/8/96
saqinavir (hard gel)	Fortovase	7/11/97	20/8/98
saquinavir (soft gel)	Invirase	6/12/95	4/10/96
tipranavir	Aptivus	22/6/05	25/10/05

*not licensed by agency

Currently there are 10 protease inhibitors approved for the market, and these are listed in table 1.5. The introduction of PIs into HIV therapy saw a dramatic decline in HIV-related morbidity and mortality[234]. At the end of the 90's ritonavir, saqinavir, indinavir and nelfinavir were the most widely used PIs[235]. Ritonavir had very potent activity against HIV [236], though, frequent adverse events were reported when used at the recommended dose [235], so ritonavir is currently used only at low doses as an enhancer to other PIs[235]. Many clinical trials have shown the effectiveness of PIs in HIV-positive patients[235]. Protease inhibitors restrict the function of the protease enzyme, so although the HIV virus is created it is unable to go on and infect other cells[237]. Indinavir and nelfinavir are now very rarely used because of their lower efficacy (nelfinavir) and greater toxicity (indinavir)[235]. Lopinavir is currently recommended as a first choice PI[214;223] and is the most widely tested PI in treatment naïve individuals[235]. The M97-720 study has demonstrated patients on lopinavir have sustained efficacy and no PI resistance after 7 years of follow-up[238]. In drug-naïve subjects, regimens based on atazanavir have shown non-inferiority compared to lopinavir or fosamprenavir, generally with improved tolerance[239]. In developed countries, atazanavir and lopinavir are the PIs most commonly prescribed[233], whilst tipranavir and darunavir are the two most recently approved PIs[231;232].

From current evidence, tipranavir is regarded as a very potent antiretroviral with a high genetic barrier to resistance and is recommended for highly treatment experienced patients[235]. However, it has been linked with a number of toxicities[240]. Darunavir also has high activity against other PI-resistant strains[241]. The recent ARTEMIS study found that darunavir showed non-inferiority compared to lopinavir and also had a more favourable safety profile[242]. As a result of this study, darunavir is currently approved in the US and Canada for both treatment experienced and treatment naïve patients[243].

1.8.1.3 Non-nucleosides reverse transcript inhibitor (NNRTI)

Table 1.6 Licensed NNRTI drugs.

Generic name	Brand name	FDA approved[231]	EMEA approved[232]
delavirdine	Rescriptor	4/4/1997	*
efavirenz	Sustiva	17/9/98	28/5/99
etravirine	Intelence	18/1/08	28/8/08
nevirapine	Viramune	21/6/96	5/2/98

*not licensed by agency

NNRTIs in combination with other antiretroviral drugs have been used for over a decade[224]. Table 1.6 shows the 4 drugs in this class that have been approved for treatment of HIV, two other NNRTIs loviride and caprivirine reached the Phase III and Phase II trials but development was discontinued due to disappointing results[244]. Nevirapine, efavirenz and delavirdine are all classed as first generation NNRTIs and were approved in the late 90s[231;232] . Efavirenz is often used in first line treatment regimens in the developed world[223;233], whereas nevirapine is recommended for first line therapy in resource-limited countries[224]. Studies have shown that resistance to NNRTIs will emerge quickly if viral load is not kept below 50 copies/ml. Side effects associated with both nevirapine and efavirenz include hepatotoxicity, and severe rash[224]. The most recent of this class to be granted drug approval is etravirine which was granted FDA approval in 2008[231;232]. It was developed as part of a new generation of NNRTIs with a better resistance profile[224]. The development of etravirine was conducted exclusively in treatment experienced patients[245;246], and recently published results found a superior response in the etravirine arm[247]. The approval of etravirine has meant that patients with NNRTI resistance now have another durable NNRTI treatment option[248;249]. Rilipivirine is another new NNRTI currently in phase III trials that has been shown to be active in NNRTI resistant patients, and may offer some advantages over etravirine. A once daily 25mg dose is being trialled, whereas etravirine is currently a 200mg dose twice daily[250].

1.8.1.4 Entry/fusion inhibitors

Table 1.7 shows the licensed entry and fusion inhibitors. Maraviroc is an entry inhibitor, it was approval from the FDA and European commission in 2007[231;232]. Specifically, maraviroc blocks the chemokine co-receptor 5 (CCR5) which HIV uses as a co-receptor to bind and enter a human helper T cell[251]. It is the first example of an anti-HIV drug that blocks the cellular rather than viral function[252]. However, its use is limited to viruses that use the R5 co-receptor. Vicriviroc was another new entry inhibitor that reached phase III trials, but in July 2010 development was discontinued after trials failed to show superiority over existing treatments[253]. Enfurvitide, on the other hand, is a fusion inhibitor. It is costly and has an inconvenient dosing regimen, thus is used predominantly in salvage therapy. Enfurvitide was granted FDA approval in 2005. Integrase in an enzyme that inhibits the strand transfer activity of HIV-1 and integration into the host DNA[254]. Raltegravir selectively inhibits the activity of HIV integrase while avoiding interference with the normal cellular function[254].

Table 1.7 Licensed entry/fusion inhibitors

Drug type	Brand name	Generic name	FDA approved[231]	EMEA approved[232]
Fusion inhibitor	Fuzeon	Enfuvirtide	13/3/03	27/5/03
Entry inhibitor	Selzentry/celsentri	Maraviroc	6/8/07	18/9/07
Integrase inhibitor	Isentress	Raltegravir	12/8/07	20/12/07

1.8.1.5 Immune based therapies

Most of HIV drug research has focused on the development of antiretrovirals to stop HIV replicating inside the body. However, there has also been some interest in another type of treatment called immune based therapy that boosts the immune system so it can fight HIV on its own. Interlukin-2 was one of the first immune-based therapies used to treat HIV. Early studies found that interlukin-2, given intravenously or subcutaneously, in combination with antiretroviral therapy increased the CD4+ cell count significantly compared with antiretroviral therapy alone. However, two large clinical trials, the Subcutaneous Recombinant, Human Interleukin-2 in HIV-Infected Patients with Low CD4+ Counts under Active Antiretroviral Therapy (SILCAAT) study and the Evaluation of Subcutaneous Proleukin in a Randomized International Trial (ESPRIT), found that despite substantial increases in CD4 count compared to antiviral therapy alone Interlukin-2 plus antiretroviral therapy yielded no clinical benefit in either study[255].

The exact reason why no difference was found in terms of deaths or AIDS diagnoses, despite producing CD4 cell increases, is unclear. While other immune based therapies are currently in the research phase, to date none have shown a clear clinical benefit.

1.8.2 Treatment history

1.8.2.1 Monotherapy

When the first cases of HIV were diagnosed there was no treatment available for patients. In the mid-80's the first antiretroviral was approved for treatment against HIV[231]. Zidovudine (AZT) was initially developed as an anti-cancer drug in the 1960's[256]. The first large multicentre clinical trial was initiated in February 1986, and included 282 adult patients with AIDS. By September 1986, major differences in survival were apparent, and an independent data safety and monitoring board concluded that the study should be terminated and the placebo patients offered AZT[256]. The trial found that administering AZT could decrease mortality and the frequency of opportunistic infections[227] over the 24 week study period, although serious adverse reactions were found in a higher frequency in patients receiving AZT rather than the placebo[257]. AZT was the first antiretroviral shown to lower the rates of AIDS death significantly in a placebo controlled trials [228;258;259] and was licensed in the United States in March 1987[231]. Until 1991, AZT was the only approved antiretroviral drug available and AZT mono therapy became the standard of care for all patients infected with HIV[231]. However, benefits were found to be transient [260;261]. In 1989 the first studies began reporting resistance mutations in patients taking AZT for more than 6 months[262-264]. AZT was found to only prolong the life of patients by 6 to 18 months[224], and other alternatives that were less toxic with greater or equal efficacy were sought[265].

Phase I trials of didanosine (ddI) were reported in 1990 and found that there was activity against HIV, and that the toxicities associated with ddI differed from those associated with AZT[266-268]. ddI was licensed in the US in 1991[231] closely followed by zalcitabine (ddC) in 1992[231]. Studies found that in patients showing clinical progression on AZT switching to ddI showed significant benefit [269].

1.8.2.2 Dual therapy

To try to overcome the development of resistance, studies into combinations of drugs were instigated. Using ddI or ddC in combination with AZT was found to delay death, and the development of new AIDS defining illnesses compared to AZT monotherapy [270].

ddI used in combination with AZT was found to be more effective than ddC and AZT [270]. Studies using ddI and ddC in combination found that they had similar toxicities and cross resistance[271] and therefore did not do as well in combination therapy. The introduction of a second class of drugs, Protease Inhibitors, in 1995 changed HIV treatment dramatically.

1.8.2.3 The cART era

Combination antiretroviral therapy (cART) was introduced in 1995 and remains the current standard of care for HIV-positive patients. cART initially involved a combination of three drugs; two NRTI's and one PI. Shortly after the first protease inhibitors were approved, a third class of drugs (non-nucleoside reverse transcript inhibitors) was introduced[224], leading to a choice of cART options. Current cART involves a combination of three or more antiretriovirals from at least two classes[214]. With this new treatment there was a dramatic decline in the development of AIDS-defining illnesses and death[159;234;272]. The risk of death for an HIV-positive patient in the cART era has been estimated to be >85% lower than in the pre-cART era[272]. A recent study in the developed world found that life expectancy in HIV-positive patients treated with combination antiretroviral therapy increased between 1996 and 2005, and the average number of years remaining to be lived at age 20 years was about two-thirds of that in the general population in these countries[273].

One of the first trials comparing dual therapy with cART was the ACTG 229 trial[129]. The authors compared saquinavir taken in combination with zidovudine and zalcitabine, to the dual therapy of zidovudine and zalcitabine alone. The results found the treatment with the three drug combination was well tolerated, reduced viral load and increased CD4 count to a greater extent than those on dual therapy[129]. The PISCES study found similar findings[274]. Indinavir in combination with 2 NRTIs was also shown to be superior to dual therapy of 2 NRTIs [275;276]. Indinavir quickly became the most frequently prescribed PI, but long term success was limited due to strict intake regulation and renal toxicity[277]. Although early studies found that ritonavir was potent against HIV[278-280] it was found to have a high toxic profile at the standard dose [258;281;282]. Clinical trials comparing ritonavir boosted PI based cART regimens found that the boosted PI regimen was superior to a non-boosted PI regimen and generally safe [283;284].The introduction of lopinavir boosted with ritonavir was demonstrated to be superior to nelfinavir [285] and saquinavir [286].

As with PIs in combination antiretroviral therapy, several clinical trials demonstrated the superiority of NNRTIs in combination with 2NRTIs over dual therapy. The first NNRTI licensed was nevirapine[231;232]. Trials comparing dual therapy with triple-drug therapy including nevirapine and 2 NRTIs found that the addition of nevirapine was more effective at suppressing viral load[287-290]. The combined study compared NNRTI-based cART (nevirapine) with PI-based cART (nelfinavir) and found that nevirapine-based cART was at least as effective as nelfinavir-based cART[291]. Several studies evaluated the effect of switching from a PI-based cART regimen to nevirapine-based cART[292-294], and found that patients could maintain a suppressed viral load, with some benefitting from improved quality of life and reduced toxicity.

Efavirenz was licensed a couple of years later[231;232] and was also found to be a viable treatment option[295-297]. Several studies have compared the efficacy of efavirenz and nevirapine[298-300]. The results from the 2NN clinical trial found that nevirapine and efavirenz showed similar efficacy, but did report differences in the safety profile of the two drugs[300]. Efavirenz is used in first line treatment regimens in the developed world[223;233], although it is not recommended in pregnant woman[214], whereas nevirapine is recommended for first line therapy in resource limited countries[224], although caution is recommended in patients with higher CD4 counts[214].

1.8.2.4 Current treatment

With so many different treatment options available, the optimal sequencing of cART regimens is important to ensure long term benefit. In the early cART era, a significant proportion of patients did not fully benefit from cART. Those who had been exposed to sub-optimal mono or dual therapy had accumulated resistance[224]. Moreover, new drugs were often added sequentially to their regimen, thus leading to substantially lower responses, compared to simultaneous administration of the same drug[258]. In the beginning of the cART era, treatments for HIV involved taking 10 tablets every 8 hours, thus daily, patients were taking up to 30 drugs. High pill burden, strict dietary restrictions, and serious toxicities associated with these early treatments led to poor tolerability[6]. In 1998, with the introduction of lamivudine /zidovudine combined and efavirenz, the standard was 5 tablets taken twice daily[6]. In 2002, this changed again to 2-3 tablets twice daily, and then again in 2004, once daily treatment options were introduced. These drug combinations help improve adherence and tolerability. However, some problems with toxicities still remain[6].

Atripla (table 1.8) is the first co-formulated drug approved for the market that provides the treatment option of one pill once a day[301]. It contains efavirenz co-formulated with tenofovir and emitricitabine.

Table 1.8: Licensed multi-class drugs

Generic name	Brand name	FDA approved[231]	EMEA approved[232]
efavirenz, emtricitabine, and tenofivir	Atripla	12/6/06	13/12/07

Three long term studies ACTG 320, INITIO and Community Programs for Clinical Research on AIDS (CPCRA) Flexible Initial Retrovirus Suppressive Therapies (FIRST)[86;302-306], have all tried to address the question of which combination of antiretrovirals is the optimal choice when initiating cART. All three studies detected a difference in virological response, preferring to initiate cART with a NNRTI rather than a protease inhibitor based regimen. However, none of the studies found a difference in CD4 count between treatment groups. The CPCRA FIRST study[306], the longest study at 5 years, showed that even a consistent difference in viral suppression rates over a long period did not translate into a difference in AIDS-defining events.

Current treatment guidelines recommend that either efavirenz or lopinavir, boosted with ritonavir, should be taken with two NRTIs as a first line regimen[214;223]. A recent clinical trial found that efavirenz plus two NRTIs was more effective than lopinavir plus 2 NRTIs for initial therapy, but that the margin of superiority was marginal, and that patients failing on efavirenz had a higher rate of NNRTI resistance than lopinavir resistance in patients falling lopinavir[307]. Advantages of NNRTI over a PI based regimen include better gastrointestinal tolerability, fewer metabolic side effects, lower pill burden, and avoidance of potentially dangerous inhibitory drug interactions[250]. However, studies have shown that resistance to NNRTIs will emerge quickly if viral load is not kept below 50 copies/ml. Due to many considerations, there is still a lot of debate over choice of first line regimen, though one of the most important factors of a first line cART regimen is its long-term durability.

Recommendations for the initiation of treatment are based on CD4 T lymphocyte counts and plasma HIV-RNA levels[124]. Current guidelines recommend treatment should be started at a CD4 count of around 350cells/mm^3 [223]. However, there is currently some debate over the optimum time to initiate cART. Viral load levels tend to change only moderately over time in untreated individuals, with most at approximately 0.1 log copies/ml increase per year[308] .

Early therapy brings immediate treatment to the virus, decreasing the risk of early CD4 cell depletion and lowering the viral load, which in turn, decreases the risk of infection to others. Delayed therapy on the other hand, means avoiding the risk of toxic drug effects and relying on more potent and tolerable drugs becoming available by the time treatment begins. Phillips et al [309] found no strong evidence to suggest that starting therapy at a lower CD4 cell count or higher viral load was associated with a poorer outcome. However, they did find a slower rate in time to initial suppression in those with higher viral loads. The START (Strategic Timing of AntiRetroviral Therapy) study [310] is a randomised control trial of ART-naïve patients with a CD4 count greater than 500/mm^3, that aims to compare patients randomised to start cART immediately with those who defer treatment until CD4 declines to 350/mm^3 . The objective of this study is to hopefully answer the question of when is the best time to start treatment. The entire study is expected to take about 6 years, with follow-up ending December 2015.

The main aim of cART is to prevent clinical disease progression. Treatment guidelines suggest that suppression of viral load below a level of quantification for as long as possible is one of the key goals of cART and one of the deciding factors when planning a patient's treatment strategy[243;311;312]. Patients experience different immunological and virological responses after initiating cART[313-315]. After a patient has initiated cART, it is more clinically relevant to use the most recent laboratory markers and information on a patient to determine prognosis, as cART can reverse many of the pathological processes induced by HIV infection[316-318]. In clinical practice, 70-80% of patients starting cART achieve undetectable viral load[319] . This number has increased in recent years[320;321], although viral replication is still not fully controlled in large number of patients rendering the virus capable of developing resistance[322].

1.8.3 Limitations of cART

Although the impact of treatment has transformed HIV from a terminal disease to a chronic illness[6], cART remains a life-long commitment. Treatment failure is normally due to drug toxicity, poor adherence or drug resistance, and the three are very closely interlinked[323;324].

1.8.3.1 Toxicities

Differentiating between complications of HIV disease and ART toxicity is often very difficult. EuroSIDA reported that the most frequent reason for discontinuation of a first line regimen was ARV toxicities[19].

Toxicities may either be specific to a particular drug or to the class of drugs, and the adverse event caused by the toxicity can vary in severity from mild to fatal (table 1.9). Regardless of the severity of the adverse event, it may have an impact on adherence. Therefore, discussing potential side effects before patient and clinician decide on the treatment is important. Patients also need to learn how to recognise the symptoms and signs of severe toxicities.

Table 1.9: Severity of toxicity[325]

Grade 1: Mild	Transient or mild discomfort; no limitation in activity; no medical intervention/therapy required
Grade 2: Moderate	Limitation in activity- some assistance may be needed; no or minimal medical intervention/therapy required
Grade 3: Severe	Marked limitation in activity, some assistance usually required; medical intervention/therapy required hospitalization possible.
Grade 4: Severe life threatening	Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required; hospitalization or hospice care

Due to the urgent need for treatment at the beginning of the epidemic, drugs were developed and licensed quickly, often with little known about their long term toxicity. Subsequently, the sustained benefits of cART have led to more patients being on cART for longer periods of time. Drug-related toxicity is being increasingly recognised because of the declining incidence of opportunistic infections[17], and the type and timing of antiretroviral therapy is often influenced by potential toxicities[17]. In the current HIV-era, most ARV toxicities have been found to be moderate and can be well managed in out-patient clinics[326]. Table 1.10 lists some of the toxicities commonly associated with ARVs.

Table 1.10: Common ARV toxicities[325]

Haematological toxicity	Drug-induced bone marrow suppression most commonly seen with AZT (anaemia, neutropenia)
Mitochondrial dysfunction	Primarily seen with the NRTI drugs, including lactic acidosis, hepatic toxicity, pancreatitis, peripheral neuropathy, lipotrophy, myopathy
Renal toxicity	Nephrolithiasis, commonly seen with indinavir. Renal tubular dysfunction is associated with tenofovir
Other metabolic abnormalities	More common with PIs. Include hyperlipidaemia, fat accumulation, insulin resistance, diabetes and osteopenia
Allergic reactions	Skin rashes and hypersensitivity reactions more common with the NNRTI drugs but also seen with certain NRTI drugs, such as abacavir and some PI's

Clinical research has shown that different combinations of drugs have substantially different short and long term toxicities[327]. NRTI toxicities over medium and long term are mitochondrial, including myopathy (zidovudine), neuropathy (stavudine, didanosine, zalcitabine), hepatic steatosis and lactic academia (didanosine, stavudine, zidovudine), and lipoatrophy (possibly all predominately stavudine) and pancreatitis (didanosine)[17]. Drug hypersensitivity in HIV-positive patients is about 100 times more common than in the general population[328]. All NNRTIs (nevirapine, delavirdine, and efavirenz), abacavir and amprenavir are common antiretroviral drugs that cause hypersensitivity[17]. The risk factors for lipodystrophy syndrome, peripheral fat loss and central fat accumulation, include low body weight before therapy, total duration of cART, use of the dual PI combination ritonavir and saquinavir and use of stavudine[16;17]. PI use can result in increased rates of bleeding in haemophiliacs[329] and most antiretrovirals have been associated with hepatic toxicity[17].

With the dangers of toxicities, the Strategies for the Management of Antiretroviral Therapy (SMART) study[317] was set up to compare in a randomized fashion the use of episodic cART according to CD4 count with the recommended practice of continuous therapy on the risk of new or recurrent opportunistic disease or death. Originally, the trial was planned to include 6000 patients to be followed for at least 6 years. The study was terminated early after only an average of 16 months of follow-up due to an increased risk of disease progression when using ART episodically. The rates of non-opportunistic disease and death were also higher in the group using ART episodically, contrary to what was expected.

1.8.3.2 Adherence to treatment

Without adequate adherence, antiretroviral agents are not maintained at a sufficient concentration to suppress HIV replication in infected cells and to lower plasma viral load[330]. Patients who are more adherent to treatment are more likely to achieve sustained viral suppression[331;332] and are less likely to show signs of disease progression[333]. Hogg et al. found that after adjusting for other prognostic factors, patients who used antiretroviral therapy <75% of the time in the first year were threefold more likely to die [334]. The impact of adherence on change in CD4 count tends to be delayed and less apparent than the impact on viral load[335]. Patients have been found to take on average 70-75% of their prescribed medication [336;337].Paterson et al.[331] found that adherence of 95% or more was necessary to achieve optimal viral suppression. However, other studies looking into disease progression have found that even adherence of 50% significantly decreases a

patients risk of progression to AIDS[333;337]. Reported differences in the degree of adherence required to achieve and maintain a successful virological response is probably due to differences in study design, the population and antiretroviral regimen studied and the difficulty in reliably measuring patient adherence[335]. All studies are in agreement though, that emphasis should be placed on the importance of attaining 100% adherence, as relatively small declines in patient adherence can lead to an increase in treatment failure[327].

Adherence is also important to help prevent the development of resistance to certain regimens, as poor adherence has been linked to an increased risk of the development of drug resistance[330]. Different treatment regimens are affected in different ways[336]. For example, NNRTIs regimen are much less forgiving of missed doses than PI-based regimens because NNRTIs have a much lower genetic barrier to resistance and have a much longer half-life than the NRTI backbone. Patients on NNRTI regimens have been found to show greater signs of adherence to treatment as their regimens are generally simpler (decreased pill burden) and have less severe toxicities associated with them.

Older patients have been found to adhere better to treatment [331]. Whereas patients with a high pill burden[331] and patients infected via IDU are less likely to be adherent to treatment[330]. The number of appointments missed by a patient has also been linked to poor adherence[338].

Different methods have been used to assess adherence to therapy[330], including more straight forward measures such as patient or clinician reports[339], to more advanced systems such as MEMS caps (Medication Events Monitoring System) which incorporate a counter on the pill bottle to track how often the bottle is opened, as well as the time and date, DOT (directly observed therapy) where patients take their medication whilst being observed by a clinician or nurse, and tests to measure the drug levels in the blood. All these methods have limits in the accuracy of the measurements[331;339]. Counselling of individual patients on the need for adherence has also been shown to be beneficial and affordable to individual patients[340] Clinicians are recommended to emphasize the importance of adherence at scheduled clinic visits. Consideration needs to be made to assess the adherence abilities of different patient groups as they may have different reasons and concerns about adherence to treatment[330;341].

1.8.3.3 HIV Drug Resistance

Effective sustained cART is complicated by the prevalence or emergence of drug resistance[342]. In the context of HIV, drug resistance is the ability of the virus to adapt so that it can multiply in the presence of a drug that would normally suppress it. Any antiretroviral drug can select for resistance if given alone. The advantage of cART is that with multiple drugs from different classes, the virus is much less likely to develop resistance since the other drugs reduce the ability of the mutant virus to replicate[343]. However, a significant minority of patients are likely to develop resistance to at least one class of drug[252]. The primary cause of drug resistance is poor adherence to a regimen [7]. Much of the drug development in recent years has been focussed on the discovery of drugs within new classes, or drugs within old classes that are active against resistant viruses[252].

Technological advances have made it possible to detect resistance, even when they are present as low-level minority species[252]. There are two different type of resistance test, the genotype and phenotype. Phenotypic assays measure the ability of a virus to grow in different concentrations of antiretroviral drugs, whereas genotypic assays detect drug resistance mutations when present in HIV genes. From a blood sample, a genotypic test pinpoints exactly where on the HIV gene a mutation (or mutations) has occurred, and what the new mutation is. However, interpretation of genotypic drug resistant tests is not always straightforward. The Rega algorithm is one of many algorithms that are used to interpret the genotypic drug resistance profile for a patient[344]. This method looks at the site where drug resistant mutations have been found, and codes each drug as either 1, 0.5, and 0 for sensitive, intermediate resistance, and resistant to that particular drug[344]. This can help clinicians decide which treatment a patient should be on. Drug resistance testing is not recommended in patients with a viral load <1000copies/l[345]

Recently, several new antiretroviral drugs (such as etravirine and tipranavir), have been licensed in old classes of drugs that show a better resistance profile and do not appear to have cross resistance with first generation ARVs[231;232]. The first generation PIs had low bioavailability resulting in high pill burden and short half-life often necessitating in multiple daily dosing[277]. Due to these factors poor adherence was often observed and viral replication was not durably suppressed in a number of patients[346]. When failure occurred, multiple protease resistant mutations were detected[277].

To combat this, PIs were combined with a low dose of ritonavir to boost the level of PI in the patient's plasma, so the virus has to acquire more mutations to achieve resistance[277]. This led to improved bioavailability and half-life of the PIs[347].

It is rare for patients failing on lopinavir to show development of resistance mutation in the protease gene[252]. Most recently, darunavir and tipranavir have been licensed and are active against viruses with acquired resistance to earlier PIs and also have a high genetic barrier to the development of resistance[252]. Similarly, although nevirapine or efavirenz have a long half-life, they have a low genetic barrier to resistance with only one mutation required to confer high level resistance[250;252]. Etravirine, a second generation NNRTI, has been found to show activity against viruses resistant to the first generation NNRTIs[252] and has a relatively high genetic barrier. Rilpivirine, another new NNRTI currently in phase III clinical trials, also retains activity against HIV viral strains resistant to other NNRTIs[250].

In addition, several new classes of drugs have been developed. Maraviroc, a CCR5 inhibitor, has been found to be active against both B and non-B subtypes[348]. Resistance to maraviroc occurs either through the development of mutations that allow HIV-1 to use CXCR4 co-receptors, or mutations that continue to allow HIV to continue using the CCR5 co-receptors. Currently the frequency of co-receptor switching is unknown, but it is of concern as the use of CXCR4 is associated with more rapid disease progression[250]. Raltegravir has a relatively low genetic barrier[250], resistance to raltegravir has been shown to emerge rapidly if other components of the patients regimen are suboptimal[252], and it is therefore important to combine it with two fully active NRTIs[349].

It is thought that certain HIV-1 subtypes may be more susceptible to developing antiretroviral resistance than others[70]. Most drugs are developed in North America and Western Europe, where subtype B is the most prevalent strain, thus the development of drugs is based on this strain and it may be that other strains have different responses. When individuals develop resistance to one antiretroviral agent, they often develop resistance to others with in the same class[323]. This is known as cross-resistance and further complicates the selection of appropriate drugs for treatment[7], a big problem with NNRTI treatment[252]. Another complication is the transmission of drug resistant strains to treatment naïve individuals[7].

Triple class resistance means a patient is resistant to the three original classes of antiretrovirals, NRTIs, NNRTIs and PIs. It is most commonly found in heavily treatment experienced patients. However, it has also been found in treatment naïve individuals[350], although the incidence of triple class resistance has been reported to be low and fairly consistent over time[351]. Recent progress in drug development has improved patient treatment options considerably, with novel compounds being developed with reduced toxicity and enhanced susceptibility to resistant HIV[277]. Heavily treatment experienced patients with resistance to several classes of drug now have several different options available for salvage therapy. An optimal treatment regimen must be tailored to the patient based on antiretroviral history, genotype resistance, side-effects profile, drug interactions, pharmokinectics, schedule preferences and perceived adherence[352].

1.9 Non-AIDS Events

Mortality rates remain high relative to the HIV-negative population[155]. However, with fewer AIDS-related deaths there has been an increase in the proportion of deaths in HIV-positive patients from non-AIDS related illnesses. This does not mean however, that there has been an increase in the risk of patients dying from non-AIDS related causes[353]. In fact, the introduction of cART has seen a decrease in the number of non-AIDS illnesses, as well as AIDS[10;354], suggesting that perhaps HIV may play a role in other diseases that has not been fully investigated or understood[353]. As the HIV population ages, non-AIDS related illnesses have become an increasingly important factor in the long term treatment and care of HIV-positive patients, as age is a significant risk factor for illnesses such as cardiovascular disease, liver disease and many cancers, as in persons without HIV[355]. There is a strong relationship between decreasing CD4 count and increasing risk of non-AIDS defining illnesses[356;357], leading to the hypothesis that starting treatment earlier could possibly reduce the risk of many non-AIDS illnesses as well as AIDS events[353]. In patients on cART, more than 50% of deaths are from causes other than AIDS[354;358-360]. The three main causes of death in HIV-positive patients, after AIDS, have been reported to be cardiovascular disease, non-AIDS related malignancies and liver related disease[359;361-363]

1.9.1 Cardiovascular disease

Cardiovascular disease contributes approximately 10% of the deaths among HIV-positive individuals[361;364]. The main risk factors for cardiovascular disease in the general population also appear to be associated with an increased risk in the HIV-positive population[18;365]. These risk factors include older age, male, smoking, family history, high cholesterol, hypertension (high blood pressure) and diabetes mellitus[365-371], and studies have shown that there is a higher incidence of these risk factors in the HIV-positive population than in the general population[372;373].

Additionally, it has been reported that the use of cART is associated with an increased risk of cardiovascular disease[18]. In particular, the use of PIs have been found to be associated with an increased risk of heart disease in HIV-positive patients[374;375]. A study comparing an HIV-negative cohort of patients with HIV-positive patients who initiated a PI containing regimen found that there was higher risk of cardiovascular disease in the HIV-positive patients[372]. It is thought that risk factors for cardiovascular disease can be increased by the use of PI containing cART regimens[376;377], as some of the side effects of PIs include hyperglycemia (high blood sugar), hyperlipidemia (raised lipids) and lypodystrophy (abnormal fat redistribution)[373;378]. Furthermore, two NRTI's, didanosine and abacavir, have been found to increase the risk of cardiovascular disease in patients receiving cART, although the biological reasoning behind this increased risk is not fully understood[379]. Although an increased risk of cardiovascular disease has been associated with some antiretroviral drugs, the absolute risk has been found to be relatively low and must be balanced against the clear benefits of cART[18].

1.9.2 Liver related disease

Liver disease is now a leading cause of death among HIV-positive patients[361]. A study of 13 cohorts found that 7% of all deaths were due to liver related disease[362]. A high proportion of liver related death is attributable to Hepatocelluar Carcinoma (HCC)[380], a non-AIDS defining malignancy (NADM).Similarly to risk factors for cardiovascular disease patients, HIV-positive patients have a higher incidence of liver related risk factors than the general population. High alcohol consumption[360;381;382], being female[381;383], older age[382], intravenous drug use, and hepatitis B and C co-infection have all been associated with an increased risk of liver disease[361;362;384].

Liver related deaths in HIV-positive patients have been found to be largely a consequence of Hepatitis B and C co-infection[360;364;385-388]. In the DAD study, more than 70% of liver related deaths were associated with Hepatitis B or C infection[361]. Progression to liver cirrhosis in patients infected with hepatitis C has been estimated to occur over 20 years[389;390]. However, co-infection with HIV has been found to accelerate the progression of HCV disease progression[391;392], probably due to a decrease in immune function.

Immunosuppression is associated with more rapid progression to liver fibrosis and cirrhosis[19;361] and the risk of liver related death has been found to decrease with increasing CD4 count. One study reported that per 100 cell increase in CD4 count, there was a 33% decreased risk of death from end stage liver disease[363]

The use of cART has been found to improve immunodeficiency by suppressing the rate of viral replication. However, increased exposure to cART has been found to be associated with an increased risk of liver related deaths in patients with similar CD4 cell counts[19]. One reason for this is that some cART regimens have been found to lead to hepatotoxicity[393-395] which has become more prevalent in recent years[396]. In a study looking at mortality due to liver related disease, discontinuation due to hepatoxicity increased from 6% in 1996 to 32% in 1999[397]. The reported incidence of severe liver related toxicity after cART initiation varies from 2-18%[396]. In particular hypersensitivity reactions have been reported in patients on nevirapine and abacavir[398-401]. The risk of these liver related toxicities in patients receiving cART has been found to be higher in those co-infected with Hepatitis B or C[394;402;403]. It is recommended that if any patient is experiencing severe liver toxicity, antiretroviral therapy should be discontinued[396].

1.9.3 Non-AIDS malignancies (NADM)

Three different types of cancer have been classified as AIDS defining malignancies (ADM); Kaposi's sarcoma (KS), Non-Hodgkin's lymphoma (NHL), and more recently, cervical carcinoma[136]. These three cancers all occur at significantly higher rates in HIV-positive patients than in the general population[404]. In the context of HIV-positive patients, all other cancers are classed as non-AIDS defining malignancies (NADM). In developed countries, where the use of cART has decreased the incidence of many ADM[405], NADM are now more common than ADM[406-408] and NADM now account for more morbidity and mortality in HIV-positive patients than ADM[409]. Many of these NADM have been found at higher rates in the HIV-positive population than in HIV-negative patients[410].

Again, like cardiovascular and liver related disease, at least part of the increased risk of NADM in HIV-positive patients is explained by the higher prevalence of associated risk factors. Smoking, high alcohol consumption and co-infection with hepatitis B and C have been found at an increased level in HIV-positive patients[18;372;411-413], and some studies have attributed the increased incidence to some NADM to these factors[414-419].

Some studies have shown immunodeficient patients have a higher risk of developing some NADM, regardless of HIV-infection status[420-422]. There is growing interest in the effects of immunodeficiency on the risk of NADM, and whether the use of cART, though improving immune function, may reduce the risk of HIV-positive patients developing particular NADM.

1.10 Aims and objectives of this thesis

In summary, since the introduction of cART around 15 years ago there have been dramatic improvements in the quantity and quality of life for HIV positive people. A consequence of this is that the focus of HIV research is changing to address different, but equally important, questions. Once initiated, treatment for HIV is a lifelong commitment, and in order for patients to maintain a high quality of life it is important that patients are initiating cART on the most effective, and safest, treatment regimen available to them. Equally they should be monitored by clinicians closely enough to receive effective treatment, but not too closely that it impacts unnecessarily on the patient's life. Additionally, as a result of durable effective treatment, we now have an ageing HIV-positive population who appear to be at an increased risk of developing a variety of different clinical events not traditionally seen as AIDS related, but that seem to occur at a higher rate than in the HIV-negative population. It is important to understand what factors are related to the development of these events and what impact these new clinical events are having on mortality rates across the HIV positive population in Europe. The results of this thesis attempt to address a number of different issues within one study, the EuroSIDA study, with an emphasis on the long term treatment and care of HIV positive people across Europe. The details of this study, the patients included and data collected are described in detail in chapter 2. This chapter also gives details on the statistical methods used to analyse the data.

Chapter 3 investigates whether current treatment guidelines recommending that patients on antiretroviral therapy are seen at clinics every three months are necessary for all patients. In particular, the analysis focuses on patients that have been able to maintain a well-tolerated and fully suppressed cART regimen, and whether it might be safe to increase the time between clinic intervals in these patients. This would make the long term commitment of treatment less intrusive on patients' lives, and also free up valuable resources for the increasing HIV population and those who do require closer monitoring, such as patients presenting for care late. The idea for this initially came from Professor Brian Gazzard who was interested in looking at reducing the frequency of monitoring ART-naïve patients who had started cART and were doing well on therapy. After I had performed an initial feasibility analysis and found there was insufficient data to focus the analysis on solely on ART-naïve patients. Therefore I developed the analysis plan for the analysis presented in this chapter including both ARV-naïve and experienced patients who were responding well to treatment.

Chapter 4 focuses on patients patterns of virological suppression after initiating cART. As discussed earlier, viral load is associated with disease progression and the main aim of cART is to maintain a suppressed viral load for as long as possible. There are many studies that have looked at virological suppression. This chapter investigates whether a patient's history of virological suppression affects the durability of future cART regimens, and whether patients are able to re- suppress and maintain the suppression on a new treatment regimen. The idea for chapter 4 was mine and followed on from the work and supplementary analyses I had performed as part of the exploratory analyses in chapter 3.

Chapter 5 compares the long term durability of nevirapine, efavirenz and lopinavir in clinical practice across Europe focussing on the development or progression to serious non-AIDS clinical events. Treatment guidelines in Europe recommend that treatment naïve patients initiating cART start either a nevirapine, efavirenz or lopinavir based cART. Each of these regimens have been shown to be effective in clinical trials, but these are over a relatively short period of time and the focus has been on virological suppression and AIDS related events. For treatment to be effective in the long term, it is also important to monitor the risk of developing serious events not thought to be AIDS related that may impact on whether it is safe for patients to maintain the regimen. The original idea for chapter 5 was proposed by Boehringer Ingelheim who were interested in comparing the long term

durability of patients on nevirapine to those on efavirenz and lopinavir. I began by drafting the analysis plan based on this proposal, but took the initiative to expand it to not just look at rates of discontinuation but also long term serious non-AIDS related events and mortality, as this is a particular interest of mine.

Chapter 6 investigates the role of immunodeficiency caused by long term HIV infection on the development of non-AIDS defining malignancies. In addition to the risk of non-AIDS events in patients on specific cART regimens, it has been speculated that HIV infection may itself play a role in development of certain clinical events not classed as AIDS events. In particular, several malignancies not classed as AIDS related have been found at a higher rate in HIV-positive people than in age matched HIV-negative people.
It is important to understand what factors may affect the development of these malignancies, and where some are more likely to occur than others, so that patients at a higher risk can be effectively treated to prevent, or screened to detect the development of any of these malignancies. Before analysing this data, Dr Csaba Kosa, based at the Copenhagen HIV Program (CHIP), helped by cleaning the data on non-AIDS defining malignancies. I wrote the proposal to investigate the role of immunodeficiency caused by long term HIV infection on the development of non-AIDS defining malignancies after discussion at meetings with CHIP. After I presented a poster of this work at 16th Conference on Retroviruses and Opportunistic Infections, I decided to group the malignancies by whether or not they were virus related after Professor Andrew Grulich suggested at the conference that this may make the data easier to interpret.

Chapter 7 looks at the mortality rate of HIV positive people under routine follow-up in Europe in the current treatment era. In particular, the chapter investigates the proportion of deaths related to both AIDS and non-AIDS related causes and whether there are differences across regions of Europe. The focus of this analysis is whether differences in treatment and patient monitoring are having an impact on mortality rates and whether these have changed over time. EuroSIDA has previously looked at the all-cause mortality rates of the HIV positive individuals enrolled in the study however the last report was in 2002. Through discussion with CHIP I decided it would be interesting to compare AIDS and non-AIDS related mortality rates across the different regions of Europe, and developed the proposal and analysis plan. Crucially, as the study now has collected a lot more data from East Europe, and EuroSIDA has developed an algorithm to enable all

causes of death could be coded as either AIDS or non-AIDS related, I thought the work could significantly add to pervious publications and volunteered to lead the project.

For each chapter I presented an analysis plan, which I drafted with some guidance from my supervisor, Amanda Mocroft, for approval to the EuroSIDA steering committee. After approval of each of the analysis plans I was then responsible for analysing the data, interpreting the data, both with some supervision, and was solely responsible for drafting the first version of the manuscript upon which each chapter is based. The first draft was then discussed among a core group of 4 or 5 people who were working on each project, including Amanda Mocroft, Jens Lundgren, Ole Kirk, Justyna Kowalska, and Daria Podlekereva. The clinicians involved provided help with clinical interpretation of the data, and also provided relevant background information. After the initial discussions I performed any additional analysis or sensitivity analyses that may have been suggested. After agreement within the core group, I the results were then circulated for round the co-authors and steering committee members for further comments and suggests.

Chapter 8 summarises the findings and conclusions from each of the chapters in this thesis with a discussion of the overall limitations of these analyses, and the implications and applications of these results.

Chapter 2 Data and Methodology

2.1 Data

2.1.1 Overview

EuroSIDA is one of the largest European multicentre prospective observational cohort studies, and includes Argentina and Israel. The primary objective of EuroSIDA is to follow the long term clinical prognosis for the general population of HIV-positive people living in Europe, and to assess the impact of antiretroviral drugs on the outcome of HIV infection. It was initiated in May 1994 as the successor of a study called AIDS in Europe. AIDS in Europe was a retrospective study of 6,572 HIV-positive patients in Europe from 1979 until the 31st December 1989, and was one of the first studies to look at the spread of AIDS across Europe. There are now more than 16,000 patients enrolled in EuroSIDA, with patients from 103 centres across Europe (including Israel and Argentina), in 34 different countries as shown in figure 2.1.

Figure 2.1 The European countries involved in EuroSIDA (Israel and Argentina are non-European participants)

EuroSIDA was set up primarily to look at the long term effects of antiretrovirals in HIV-patients across Europe. However, like other observational studies, EuroSIDA is useful for assessing the natural history of disease, effects of different treatment strategies and long term side effects. One of the unique features of EuroSIDA, is that it has now been running for more than 15 years and has follow up data for more than 16000 patients. Therefore, EuroSIDA has been able to help track the course of the HIV epidemic across Europe, the effects of the introduction of cART, the use of cART in different areas of Europe and changes in the epidemic over time. Due to its size, relatively rare events, such as non-AIDS malignancies, can be studied. Further, EuroSIDA has relatively low rates of loss to follow-up, <5 per 100 person years of follow-up (see section 2.1.5) and high data quality (see section 2.1.6). In addition, its collaboration with other studies has led to other research questions being addressed that EuroSIDA could not answer alone.

EuroSIDA on its own, and in collaboration with cohort studies, has played an important role in helping increase our understanding HIV, the impact of cART, and influencing treatment guidelines across Europe. The study has published extensively, celebrating its 100th publication in the autumn of 2008. EuroSIDA has also presented abstracts at most major HIV related conferences since 1997. Full details of all publications and presentations can be found at www.cphiv.dk.

2.1.2 Enrolment of Patients

Patients aged 16 years and over are enrolled into EuroSIDA. Patient inclusion was performed at predetermined time periods at outpatient clinics, and patients were required to have a routine booked outpatient appointment to be recruited, thus patients recruited are already under routine follow-up and care at the individual centres, ensuring as much as possible that the selection of patients is unbiased to gain a representative sample from each clinic. The EuroSIDA follow-up form is given in Appendix I.

There have now been 8 periods of enrolment into EuroSIDA, and a total of 16,599 patients are currently enrolled in EuroSIDA (table 2.1). In each cohort, patients were enrolled until a predefined number was attained. The number of patients recruited from each centre is determined from the total number of patients under follow-up at the centre, the ability of the centre to provide follow-up data, and to ensure regional representation. The first enrolment of patients began on the 2nd May 1994 and continued until a predefined number of patients had been enrolled.

There were 3,115 patients enrolled in this cohort (cohort I), and it was originally defined as EuroSIDA I. In November 1995, enrolment began on the second cohort. 1,363 patients had been enrolled when it stopped in April 1996. 2837 patients were recruited in the third cohort between February 1997 and September 1997. Since then, a further 5 cohort enrolments have been complete (table 1). For cohort I-III, patients were only eligible if they had a CD4 count below 500 cells/mm^3 measured in the 4 months prior to enrolment. From cohort IV, onwards this restriction was removed as cART was widely introduced across Europe and patients had higher CD4 counts[423]. In more recent years (cohort V onwards), there has been an effort to recruit more patients from Eastern Europe as these patients were less well represented, and it became clear the extent of the HIV epidemic in this region[424]. The most recent cohort, cohort VIII, began enrolment in November 2007 and enrolled 2,259 new patients. More than half of the new patients enrolled were from Eastern Europe.

New cohorts are added to ensure an update of the patient population and to replace those who have died or been lost to follow-up.

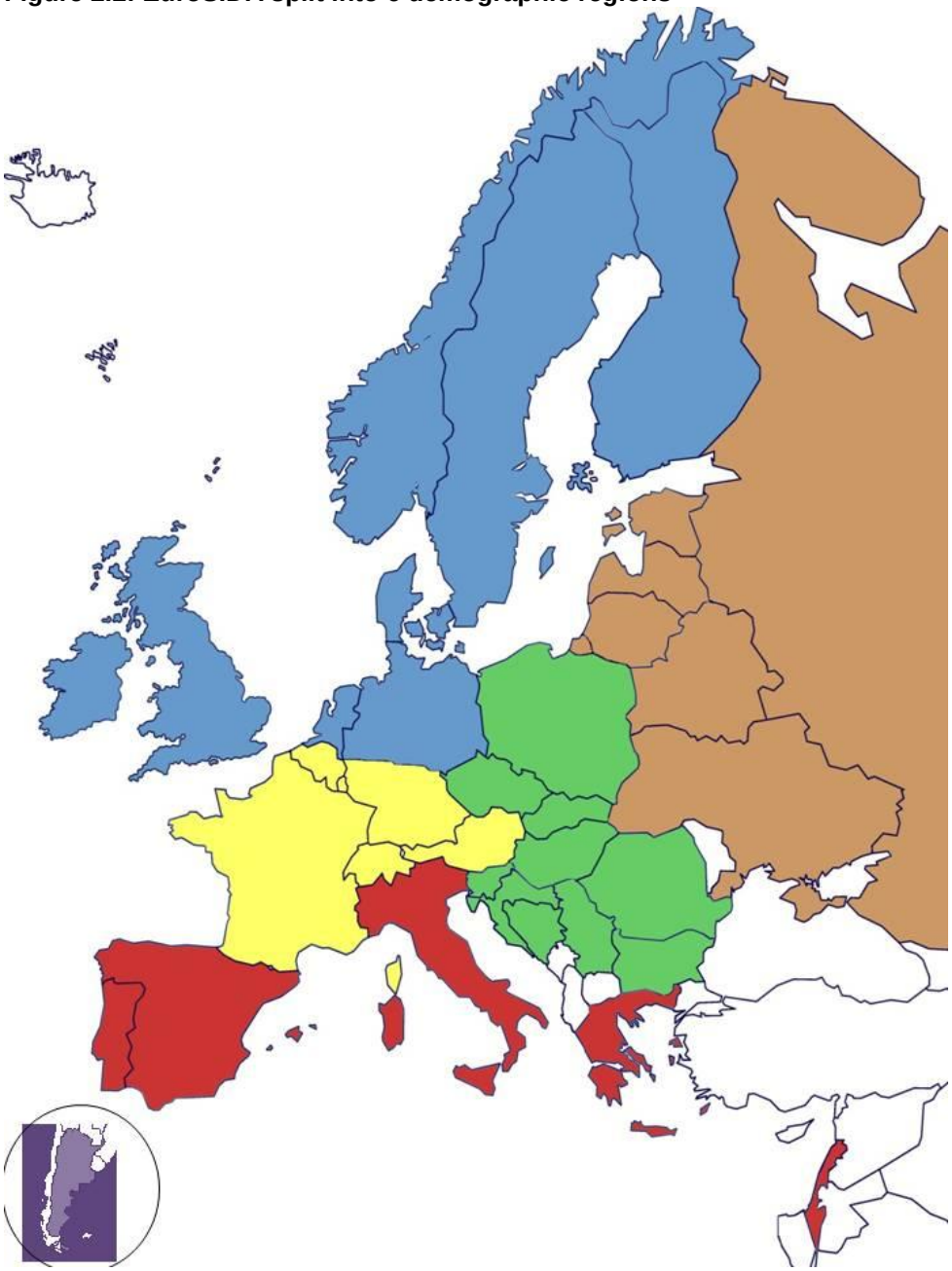
Table 2.1 Number of patients enrolled in EuroSIDA

Cohort	N of patients	Enrolment date
I	3115	Spring 1994
II	1363	Winter 1995
III	2837	Spring 1997
IV	1225	Spring 1999
V	1223	Autumn 2001
VI	2119	Spring 2004
VII	2458	Winter 2005
VIII	2259	Winter 2007

As shown in figure 2.2, for the purpose of analysis EuroSIDA is often split into 4, 5 or 6 demographic regions, depending on the research question of interest. There are 30 centres included in Southern Europe from Spain, Portugal, Italy, Greece, Serbia, Montenegro, Israel, and Argentina. Although where sufficient numbers allow, Argentina is classed as its own region. There are 20 centres included in West Central Europe from France, Belgium, south Germany, Luxemburg, Switzerland, and Austria. There are 23 centres included in North Europe from, United Kingdom, Ireland, Netherlands, north Germany, Denmark, Sweden, and Norway.

There are 31 centres included from East Europe from, Belarus, Bulgaria, Croatia, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Romania, Russia, Serbia, Slovakia, and Ukraine. From Cohort VII onwards there has been sufficient data available to split East Europe into two separate geographical regions East and East central Europe. In this case 17 centres from East Europe, Estonia, Latvia, Lithuania, Belarus, Ukraine and Russia and there are 14 centres included from East Central Europe Poland, Czech Republic, Slovakia, Hungary, Romania, Bulgaria, Croatia, and Serbia.

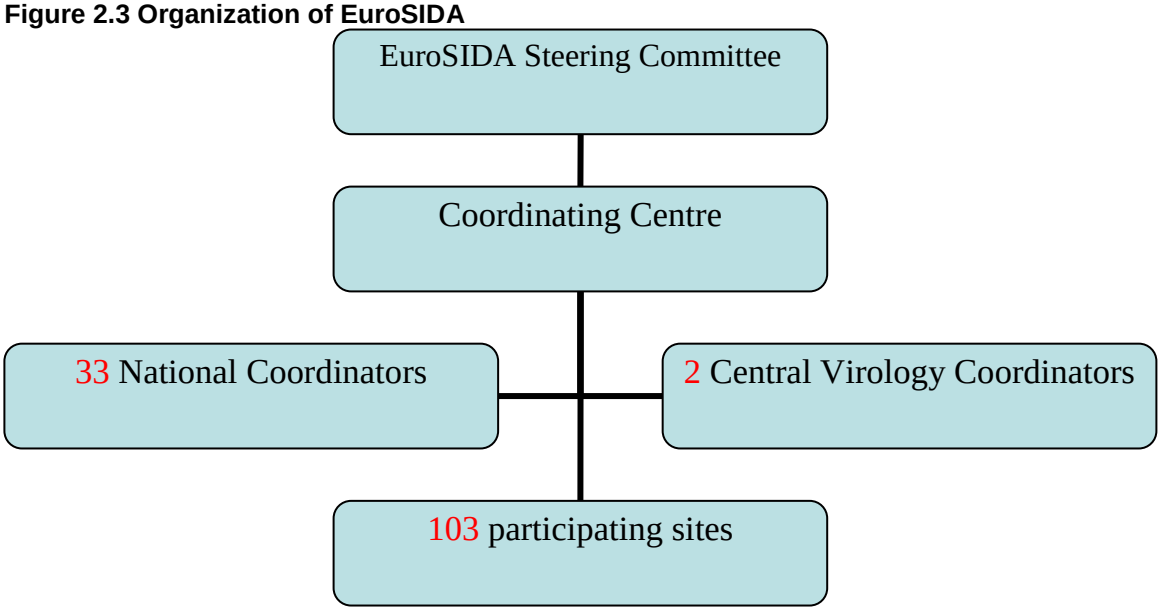
Figure 2.2: EuroSIDA split into 6 demographic regions



2.1.3 The EuroSIDA organization

Figure 2.3 gives an overview of the organization of the EuroSIDA study. The study is headed by a steering committee which consists of 14 members. The steering committee meets, on average, by telephone conference every 2 months to discuss the progress of current projects, the approval of new project proposals, and the overall running of EuroSIDA, funding and sponsorship. The steering committee consists of regional representatives from across Europe. A full list of the members of the EuroSIDA study group is given in Appendix II.

Figure 2.3 Organization of EuroSIDA



The overall co-ordination of EuroSIDA is provided by the Copenhagen HIV program (www.cphiv.dk). The coordinating centre is responsible for the management of the EuroSIDA database. The statistical centre for EuroSIDA is based at the Royal Free Hospital, University College London Medical School, Royal Free Campus, London and works very closely with the coordinating centre. Additionally, there are three working groups, Virology, Hepatitis and Pharmacokinetics. A core group of clinicians and statisticians meet 3-4 times a year to discuss future projects and work priorities. This active collaboration between clinicians, statisticians, epidemiologists, HIV virologists, hepatologists, pharmacologists is crucial for the running and the development of the study but is not necessarily reflected by the members on the steering committee.

The Virology group is focused on research into all aspects of HIV drug resistance. In addition to the main EuroSIDA clinical database there is an extensive database on any HIV resistance tests that have been performed locally and reported to the coordination centres. A plasma sample is requested on all patients every 6 months. This plasma sample is stored locally (in general) at minus 80 degrees Celsius, and intermittently shipped to the central repository at the coordinating centre. The central repository contains more than 36,000 samples. There are also additional stored samples available for some patients which can be utilized for specific HIV projects as required. The database can be checked to see whether there is a sample available for each patient that would be eligible for inclusion into a specific analysis, extracted from freezers and analyzed. The EuroSIDA Virology Laboratory Group is constituted by two interactive centres one in the UK: ICVC International Clinical Virology Centre, London (up to 2004) and another one in Spain: IrsiCaixa Foundation, Badalona. The main objective is to evaluate the genotypic resistance as a factor associated to the virological, immunological and/or clinical outcome. Other objectives included supporting the development of the plasma bank, defining the prevalence of resistance in untreated patients across Europe, and understanding the impact of chronic viral co-infections on virological and clinical terms. Additionally, EuroSIDA has set up a computerised system to make the results of any additional samples tested available to the clinicians.

As mentioned in the introduction, hepatitis co-infection is common within the HIV-positive population[384;388]. The EuroSIDA hepatitis group is built around the two central laboratories in Bonn and Madrid, and the group is headed by Jürgen Rockstroh, Vincent Soriano and Lars Peters. EuroSIDA includes the largest cohort of chronic viral hepatitis/HIV coinfected patients worldwide. Data on hepatitis B and C serology, viral load and genotype are reported on all patients once annually. Furthermore, in 2006, all patients with available plasma samples have been investigated at a central reference laboratory for relevant hepatitis B and C serology, viral load and genotype. Of all patients enrolled in EuroSIDA, around 25% were anti-HCV IgG positive. Of 1,940 seropositive patients with available plasma samples, 1,496 (77%) had detectable serum HCV-RNA. Around 7% of all patients in EuroSIDA were HBsAg positive. Of 474 HBsAg positive patients with stored plasma samples available 315 (66.5%) were HBV-DNA positive. The main objectives are to study the natural history of chronic hepatitis B and C in HIV patients, how these co-infections influence the outcome from and tolerability to ART, the roll-out and outcomes from hepatitis treatment and regional differences in these parameters.

Until now the EuroSIDA study has published eight papers in peer-reviewed journals on the epidemiology and clinical outcomes of HIV/hepatitis co-infection.

The pharmokinetics group is run by David Burger at the Department of Clinical Pharmacy, Radboud University Medical Centre, Nijmegen in the Netherlands. In their lab, they analyse the pharmacokinetics of plasma samples, which measures the amount of drugs in the blood using a validated reversed-phase HPLC method[425]. So far this group has published one paper in EuroSIDA, looking at whether patients with high efavirenz plasma concentrations have an increased likelihood of toxicity-driven efavirenz discontinuations, no association was found[426]. They are currently working on a new project 'Relating PI therapy failure with drug exposure' to determine the role of non-adherence in patients (measured by the level of PI in the blood) presenting with virological failure.

Primary support for EuroSIDA is provided by the European Commission BIOMED 1 (CT94-1637), BIOMED 2 (CT97-2713), the 5th Framework (QLK2-2000-00773) and the 6th Framework (LSHP-CT-2006-018632), and the 7th Framework (FP7/2007-2013, EuroCoord n° 260694) programmes. Current support also includes unrestricted grants by Gilead, Pfizer, BMS, Merck and Co. The participation of centres from Switzerland was supported by The Swiss National Science Foundation (Grant 108787).

2.1.4 Data collection

At recruitment, in addition to demographic and clinical information, a complete antiretroviral history is collected, together with the 8 most recent CD4 counts and viral load measurements. This is done through a EuroSIDA enrolment form.

EuroSIDA is an observational study and the data collected by EuroSIDA is that which is already available. The study does not have any influence on the treatment or monitoring of the patients being followed. The patients are therefore followed and treated in agreement with requirements in the individual clinics. At 6 monthly intervals, relevant data are extracted from patient clinical charts onto follow-up forms (see Appendix I). This data is then used to create 6-monthly updates of the EuroSIDA data set so that the most recent and up to date information can be used for all analysis. Table 2.2 gives a summary of the data collected at enrolment, and at subsequent follow-up visits.

EuroSIDA patients also participate in the Data collection on Adverse Events of anti-HIV Drugs study (D:A:D study). EuroSIDA is one of the main contributors of data to the D:A:D, study whose main aim is to assess the incidence of myocardial infarction among HIV/AIDS patients who are receiving anti-retroviral therapy. Over time, the data collection form has been modified to capture additional information. Variables collected include the date of diagnosis, date of any serious illness of interest, such as MI, non-AIDS defining cancer, liver disease or end stage renal disease, any cholesterol measurements or blood pressure measurements, as well as information on the use of any drugs to treat any of these illnesses, such a lipid lowering drugs or diabetic medication. Information on smoking status is also collected.

Table 2.2 Summary of data collected in EuroSIDA

Demographics	**Laboratory values**
Gender	Serum total cholesterol
Date of Birth	Serum HDL cholesterol
Mode of infection	Serum triglycerides
Country of origin	S-creatinine
Race	Heamoglobin
Basic clinical information	Platelet count
Height	ALT
Weight	AST
Blood pressure	INR
Smoking	Bilirubin
Family history of MI	S-lactate (not LDH)
Clinical events	S-amylase
Cardiovascular events	CD4 counts
Metabolic events	HIV RNA
Other organ events	**Hepatitis virology/serology**
Pregnancy in woman	Hepatitis B antigen
Outcome of pregnancy	Hepatitis B antibody
Antiretroviral treatment	HBV-DNA
Dates of starting or stopping	Hepatitis C antibody
Reason for discontinuation	HCV-RNA
Adherence rating	HCV-genotype
Treatment against infection	Toxoplasma antibody
Dates of starting and stopping drugs	CMV antibody
Treatment related to cardiovascular risk	**Severe opportunistic infections**
Dates of starting and stopping drugs	Dates and diagnosis (definitive presumptive, autopsy)
Patients who have died	**Other severe infections**
Date of death	Dates and diagnosis (definitive presumptive, autopsy)
Autopsy performed	**AIDS defining malignancies**
Presumed cause	Dates and diagnosis (definitive presumptive, autopsy)
CoDe case report	**Non-AIDS defining malignancies**
	Dates and diagnosis (definitive presumptive, autopsy)

EuroSIDA has recently begun collecting adherence data, although this has proved to be problematic. As the data is taken from patient notes and transferred onto the follow-up form, information on adherence is only collected if a comment has been made in the notes.

Additionally, there are only 3 categories of adherence, poor (<70%), perfect (>95%) and anything in between, and no information is collected on how adherence has been measured. The EuroSIDA follow up form is reviewed every 6 months by the coordinating centre and the core group to see if there are any extra variables that need to be collected. The steering committee also review the data collected every time they reapply for funding.

2.1.5 Loss to follow-up

An essential component of any observational cohort is complete follow-up of as many patients as possible[427]. It is important to consider loss to follow-up (LTFU) when analyzing data as it is a potential source for selection bias[428]. LTFU can be defined in a variety of different ways. Within EuroSIDA, LTFU occurs when no follow-up form is returned to EuroSIDA and no CD4 cell count or viral load measured at the clinic for over 1 year. LTFU within EuroSIDA is less than 5 per 100 PFYU and does not appear to have changed over time[427]. There are a number of initiatives within EuroSIDA to minimize LTFU, including educating sites on the importance of continued follow-up and reporting, annual monitoring at selected sites to check accurate data collection, and seeking further information from the clinic if the patient is thought to be lost to follow-up. If a patient has moved care, follow-up can sometimes be continued at another EuroSIDA site. In addition, sites with high LTFU are approached to help investigate what the underlying reasons are and to provide extra support and strategies that may help reduce the level of LTFU.

2.1.6 Data quality

An extensive quality assurance programme has been established to secure correct patient selection and to verify that accurate data are supplied. This includes monitoring visits and data quality control at the coordinating centre. Members of the coordinating office visit all centres to ensure correct patient selection and that accurate data are provided, by checking the information provided against case notes for all reported clinical events and a random sample of 10% of all other patients. Centres have ethical approval according to their own local and national requirements, and the coordinating centre is required to have copies on file of the approvals.

2.1.7 Data summary

As the data is updated every 6 months, several versions of the data set are used in this thesis (see table 2.3). In general, each chapter is based on the most recent version of the database available at the time the manuscript was published or submitted for publication. Table 2.3 gives an overview of the number of patients included in each chapter and the inclusion criteria.

Table 2.3: Characteristics of the updated data sets

Chapter	EuroSIDA dataset	N in dataset	Median follow-up date (IQR)	Brief summary of inclusion criteria	N included in analysis	Primary end points
3	D27*	14241	5/07 (9/02-11/07)	All patients who have maintained a stable and fully suppressed cART regimen for one year, with CD4 count and viral load measurements available prior to cART initiation and during the year of stable therapy.	2237	All cause failure defined as either: CD4 count <200 cells./mm3 CD4 count below CD4 count at cART Two viral load > 500 copies/ml AIDS defining event Non-AIDS defining event Death
4	D29	16599	6/08 (3/04-12/08)	All who were on cART for a least 6 months prior to initiating a new cART regimen after 1/1/2000, with at least 6 months follow-up available after the treatment change	2721	Virological failure defined as viral load >500 copies/ml at 4 months after treatment switch
5	D30	16599	1/09 (3/04-6/09)	All patients who initiated either efavirenz, nevirapine or lopinavir based cART regimens, who had not been exposed to any of the three drugs previously , who achieved suppression and remained on the regimen for more than 3 months	2906	Discontinuation of treatment regimen, development of serious non-AIDS clinical event last visit or recorded measurement for laboratory events
6	D29	16599	6/08 (3/04-12/08)	All patients enrolled in EuroSIDA with some prospective follow-up and a CD4 count available prior to recruitment into EuroSIDA	14453	Development of a non-AIDS defining malignancy
7	D32	16599	11/09 (4/04-6/10)	All patients enrolled in EuroSIDA with some prospective follow-up after 1/1/ 2002	13280	Death

*** Cohort VIII was added in D28**

2.1.8 Ethical approval

EuroSIDA is a non-intervention data collection study. The data obtained are collected from clinical sites participating in the EU Commission funded project and governed by the EU Grant Agreement, which includes ethical requirements. All sites have a contractual obligation to ensure that data collection and sharing is done in accordance with national legislation. In the case that EuroSIDA sites have no requirement for ethical approval of observational research studies, the principal investigator of the site has to document this in a written statement. The Co-ordinating Centre ensures that the holding of data complies with the local and national protections acts. All participating patients have to read a Patient Information Letter describing the study set-up and study details, including the fact that anonymised samples and data will be collected centrally and used in accordance with the Scientific Programme of EuroSIDA. All participants sign an Informed Consent Form confirming participation before enrolment into the study.

2.2 Methodology

Throughout this thesis a variety of different statistical methods have been used. A methods section at the start of each analysis chapter will describe the methods that were used to perform the analysis in that chapter. The inclusion criteria will also be described in detail in each of the methods sections, as well as summarized in table 2.3. The rest of this chapter will give an overview of the main statistical methods used throughout this thesis. All statistical analysis in the thesis has been performed using SAS version 9.1 (SAS institute, Cary, North Carolina, USA).

2.2.1 Initial analysis

When analysing data, it is important to provide summary statistics. Summary statistics give an overview of the population being analysed. They are also useful to check for any obvious data errors, and can be used to compare different groups of patients before any formal analysis is performed.

For categorical variables, the total number of patients (N) and the percentage (%) in each category is reported and for continuous variables the mean and standard deviation (SD) are reported if the data are normally distributed and the median and interquartile range (IQR) if the data are skewed. If the data are approximately normally distributed then the mean and median will be similar. If the data are highly skewed then the mean and median

can be quite different. In the EuroSIDA study the majority of the continuous variables that describe patient characteristics are highly skewed therefore in most cases the median and the inter quartile range are given.

To compare the characteristics of different groups of patients, the Pearson's chi-square test is used when the variable of interest is categorical. However, if there are less than 5 patients in any category then Fishers exact test is used. For normally distributed continuous data, un-paired t-tests are used to compare different groups. To compare two sets of values between the same patients, such as viral load at starting cART and at 6 months, then a paired t-test could be used. When comparing continuous variables that are skewed the Wilcoxon rank sum test is used if two groups are being compared. If three or more groups are compared then the Kruskall-Wallis test is used, which is an extension of the Wilcoxon rank sum test. These tests are non-parametric and do not make any assumptions about the underlying distribution of the data

All tests performed were two sided, and a p-value of less than 0.05 was taken to be statistically significant i.e. there was a significant difference between the groups and the null hypothesis of there being no difference could be rejected. Additionally, the clinical significance of results was also considered by looking at the size of the effect and the width of the confidence intervals.

2.2.2 Statistical modelling

Statistical models are developed to help quantify the relationship between the outcome of interest and various explanatory variables. They provide a mathematical representation of how the variability of a response can be explained in terms of explanatory variables. They also incorporate a random component to account for the deviation of the observed response values from the predicted values.

In addition, statistical modelling can allow us to adjust for confounding from other variables. Before attributing any difference in outcome between the exposure groups to the exposure itself, it is important to examine whether the exposure-outcome association has been affected by other factors that differ between the exposure groups and which also affect the outcome. Such factors as said to confound the association of interest[429]. When looking at the effect of a variable on an outcome, another variable is a confounder if it is correlated with the variable of interest and it is also associated with the outcome. For example, in HIV epidemiology research we may be interested in assessing whether

patients on a PI based regimen have a lower risk of death than those on an NNRTI based regimen. The CD4 count at starting treatment is likely to be a confounder as it may be related to what treatment a patient is started on, and CD4 count is also known to be associated with risk of death. In randomized clinical trials the randomization should ensure that any factors that affect the outcome are equally distributed between the groups and it therefore controls for both known and unknown confounders. In cohort studies, such as EuroSIDA, this is not the case. Therefore, statistical methods have been developed to allow us to account for at least some of the confounding in the analysis by using multivariable statistical models. By including other variables in a model and 'adjusting' for the effect they have, we can remove most of the bias, so that we can see the effects of a particular treatment or intervention in a similar way to if we were comparing similar patients whose only difference was exposure to the intervention. The main limitations to analysing cohort studies are discussed in detail in the final conclusion (section 8.2.)

Univariable models contain only one explanatory variable, and are used to investigate what effect this variable alone had on the response. They do not account for any confounding, showing the unadjusted relationship between one variable and the outcome of interest.

In multivariable models, the impact of each explanatory variable is adjusted for all other variables in the model, e.g. in the above example the adjusted comparison is the effect of PI based regimen versus NNRTI among people with similar CD4 counts.

Unfortunately, there are some variables that are not routinely collected in certain cohorts, and other variables that simply cannot be measured. This can, in some cases, lead to biases or confounding by indication. This is a particular limitation when analysing data from cohort studies, and will be discussed in detail in chapter 8.

Three main methods of statistical modelling were used in this thesis: logistic regression, Poisson regression and survival analysis. Each of these methods are described in detail below

2.2.2.1 Logistic regression

Often in HIV analysis, we wish to investigate whether or not the person experiences an event of interest, for example, virological suppression, AIDS or death.

The risk on an event occurring by a certain time point (t) can be calculated by

$$\text{Risk of event by time t} = \frac{\text{Number of people with an event by time t}}{\text{Total number of people followed}}$$

In a similar way the odds of an event can be calculated by

$$\text{Odds of event} = \frac{\text{Number of people with an event}}{\text{Number of people without an event}}$$

Logistic regression is most commonly used for data where the outcome is binary, i.e. there are two possible outcomes, such as success/failure, alive/dead. In general, the outcome is referred to as an event, and we are interested in the probability of an event occurring. When looking at probability, (p) is a number between 0 and 1, but in order for us to perform regression analysis it has to be transformed so it takes the value between minus infinity (-∞) and infinity (+∞). Thus, p is used to calculate the odds of the event occurring, and logistic regression is used to model the log of the odds. The name logistic comes from the fact that we use the log transformation to do the analysis. When the outcome is rare, the odds of an event is similar to the probability of an event.

Equation 2.1 Logistic regression model

$$\text{Log-odds (outcome)} = \beta_0+\beta_1x_1+\beta_2x_2+\ldots\ldots+\beta_nx_n$$

The $\beta_0\ldots\ \beta_n$ are regression coefficients, $x_1 \ldots x_n$ are explanatory variables.

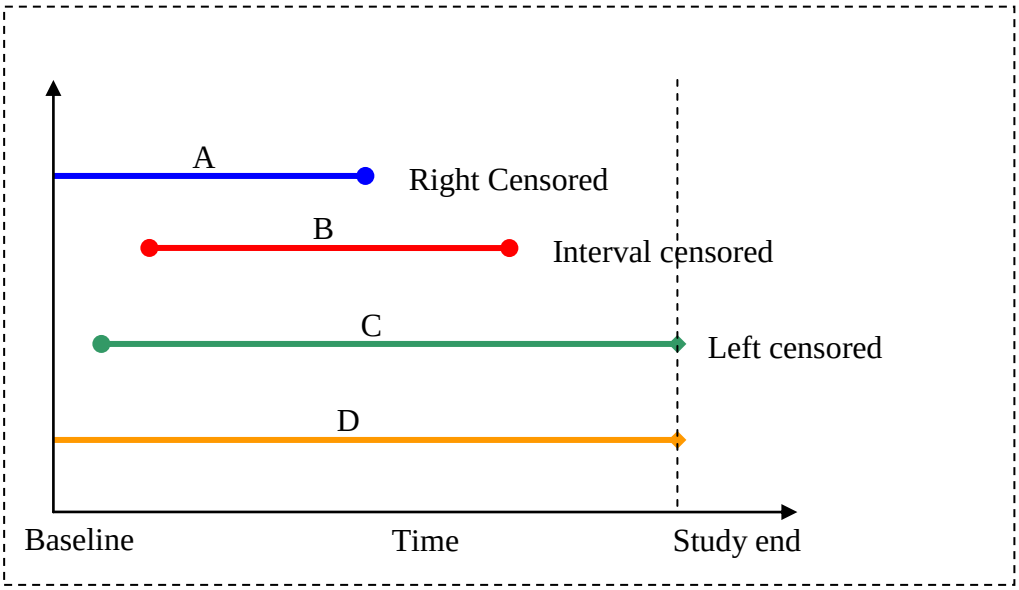
In equation 2.1, the coefficient (β_i) from the model reflects the independent effect of the variable (x_i) that is not explained by the other factors. Anti–logging β_i gives an estimate of the odds ratio association between x_i and the event. The equation can be used to predict the probability that an event occurs for each individual.

As you can see from equation 2.1, this type of model assumes a linear relationship between the explanatory variables and the log odds of an event. It can be used to estimate the odds ratio that compares the outcomes from two groups of patients. An odds ratio is defined as the ratio of the odds in one group compared to the odds in a second group. A value greater than one indicates the first group had greater odds of an event than the second, the opposite is true if the value is less than one. One limitation to investigating the risk of an event, or the odds, is that we cannot account for the fact that not all people are followed for the same amount of time.

2.2.2.2 Time to event data

Poisson regression analysis and survival analysis are commonly used when dealing with time to an event data. They allows us to deal with data that has been censored. An observation is censored if the period of observation has stopped before the event of interest has occurred, so the time to an event is unknown. In the context of HIV, and in particular in cohort studies, many events of interest involve looking at censored data. Data can be right censored, left censored or interval censored. Figure 2.4 shows the three different kinds of censoring.

Figure 2.4: Examples of different types of censoring



The most common type of censoring, and the type found in HIV research, is right censoring, which is where some patients have not experienced the event of interest by the end of the period of follow-up. In EuroSIDA we also have left censoring. There is substantial data available about the patients history prior to recruitment, but all analyses are left censored at recruitment (or some other relevant time-point depending on the analysis in question). If censoring was ignored and only complete data considered, a lot of important information would be disregarded and could bias the conclusion on the distribution of event times. For example, if the analysis was survival following an AIDS event, and persons with AIDS events prior to recruitment were included, we would likely over estimate survival time because patients diagnosed with AIDS prior to recruitment are likely to have different survival times to those diagnosed with AIDS during prospective follow-up, as, by definition, they have survived long enough to be recruited to the study.

2.2.2.3 Poisson regression analysis

Poisson regression analysis is similar to logistic regression, but the outcome variable is a rate. The incidence or event rate per year of follow up (PYFU) is shown in equation 2.2.

Equation 2.2

$$IR = \frac{\text{number of events}}{\text{PYFU}}$$

To get the incidence rate per 100 PYFU the incidence rate is multiplied by 100. For rarer events, the IR per 1,000 PYFU is sometimes used. For example, if the incidence rate was X per 100 PYFU, this means that if we followed 100 people for 1 year we would expect X number of events to occur. An incidence rate is a number between 0 and infinity. If more than 20 events are observed then 95% confidence intervals are calculated using the normal approximation to the Poisson distribution. Where the number of events is ≤ 20 then the exact Poisson distribution is used to calculate the 95% confidence interval.

Poisson regression analysis is used to model the rate ratios and again allows for adjustment of numerous different variables. As the assumptions of linear regression again are not met, the log of the rate, rather than the rate itself, is modelled and then it is transformed. The model is interpreted in a similar way to logistic regression, but instead of odds ratios the estimates are relative rates or rates ratios.

Incidence rate ratios (IRR) are used to compare different groups of patients; one group is taken as the reference group and then the IR in each of the other groups is divided by the reference group. The value of IRR can take any value greater than 0. An IRR > 1 implies that there is an increased rate in the comparison group whereas if 0< IRR < 1 then there is a decreased rate compared to the reference group. This type of analysis is called time to event ,as it can account for the fact that some data may be censored.

2.2.2.4 Kaplan Meier survival analysis

Kaplan-Meier curves can be used to compare two groups but this is without adjustment for any other variables. This can be useful as it allows you to see the risk to each group at different time points, and thus to see whether there is a difference between the two groups and if the relationship changes over time or remains constant.

The survivor function S(t) is used to find the probability that an individual survives beyond a particular time. The survivor function takes the value 1 at t=0 and decreases to 0 at t=infinity. The survival function is normally highly skewed, and the most appropriate central summary of the distribution is by the median survival time, which is the value of t when S(t)=0.5.

The most commonly used non-parametric estimator of the survivor function is in Kaplan Meier survival analysis. It is commonly used in time to event data, when the data to be analysed is censored. For example, the time to stopping a particular drug, time till viral load reaches a certain point, or time till death.

Equation 2.3

$$S_{KM}(t) = \prod_{J=1}^{k} (s_j / r_j) \qquad \text{for } t_{(k)} \leq t < t_{(k+1)},\ k=1,2,\ldots,m$$

Where rj is the number of individuals alive before time t(j) for j=1,2,..m and dj the number of individuals who die therefore s(j) = 1- dj/rj

$$\frac{\text{Number surviving beyond t}}{\text{Number in the sample}}$$

2.2.2.5 (Cox) Proportional Hazards Model

The hazard function or hazard rate is defined as the rate at which an individual is likely to experience an event, in the next small interval of time, given that they have already survived up to that point.

The proportional hazards model is defined in equation 2.4 for a baseline (i.e. at z = 0) hazard function $h_0(t)$ and where β is a set of unknown parameters.

Equation 2.4

$$h(t,z) = h_0(t) * \exp(\beta^T z)$$

It does not assume that the data fit any defined distribution or shape, it only assumes that the hazards are proportional i.e. they remain the same over time. To check the assumption of proportional hazards is true we test for an interaction, or graphically the Kaplan Meier plot can be used.

This method is similar to logistic and Poisson regression, but the outcome variable is the hazard of an event occurring. Again it is the log of the hazard, and the anti-logged

parameter estimates are now relative hazards. The main difference between Cox regression and Poisson is that in Cox regression we need to have a time scale of interest, e.g. time from first viral suppression. However, this means that we cannot easily consider whether this rate increases or decreases over time. Further, with Cox proportional hazards models you assume the hazards are proportional over time.

2.2.2.6 Competing risks

In time to event analysis, the occurrence of an event of interest is often precluded by another event that prevents the event of interest from being observed. For example, looking at the time to discontinuation of an antiretroviral due to toxicities, an individual may discontinue the drug due to another reason, such as virological failure, before discontinuation due to toxicity. Competing risks are events that prevent an event of interest from occurring, rather than just preventing you from seeing it happen (censoring). Therefore, when looking at time to discontinuation of an antiretroviral due to toxicity, discontinuation of the drug due to virological failure is a competing risk.

Any analysis where “censored” does not just mean end of follow-up or lost to follow-up is actually a competing risks analysis. In this example, patients are observed at study entry and followed until either the event of interest (discontinuation due to toxicities), a competing event (i.e. discontinuation for another reason), or censoring[430]. When competing events are censored, the complement of the Kaplan Maier survival curve may not appropriately estimate the cumulative incidence[430]

A number of nonparametric and regression methods exist for analysing data with competing risks[430]. In this thesis, an approximation of the sub distribution hazard has been used for performing competing risks analysis in Kaplan Maier survival and Cox Proportional Hazards models. In this method, all patients who experience a competing event remain in the risk set and the individual is then censored administratively at the last study visit. For the competing risks analysis in this thesis, patients with a competing event remained in the data set and were censored at the median date of last follow-up visit. In order to do this, where information was missing for the patient with the competing event, the last observation was carried forward.

2.2.3 Performing analysis

2.2.3.1 Model selection

In this thesis, all the explanatory variables are defined prior to starting the analysis. The majority of the variables are already well established in HIV research. The explanatory variables of interest are first entered into univariable models and any that are significant at the 10% level i.e. p value < 0.1 (to be conservative) are entered into the multivariable model. A stepwise selection method was then used to confirm whether or not, after adjustment, other variables might be confounders, and all additional potential confounders were added. Details of the model building approaches used in each chapter are described in further detail in the methods section of each chapter.

2.2.3.2 Fixed or time updated variables

Most analysis in this thesis uses fixed variables, i.e., variables measured at baseline that remain unchanged over time. These fixed variables consider long-term association between the variable at the outcome of interest. In time updated analysis, the short-term outcome between two variables is considered. For example, CD4 count can be included in a model in a variety of different way. There is the CD4 count at baseline, or the nadir CD4, which are both fixed variables, but it can also be included as an updated variable that changes depending on the most recent CD4 count measurement.

In order to include time-updated covariates in the models developed in this thesis, the follow-up time was split into monthly intervals. At each new month, the value of each variable was assessed and updated accordingly. Monthly intervals were chosen as a number of the dates recorded in EuroSIDA in the early period of data collection were only recorded to the nearest month. Generalised estimating equations were used to account for the fact that repeated measures of the same subject were then included in the models and measurements from the same individual over time will be more correlated than those between different individuals.

2.2.3.3 Manipulating data

For certain variables that are highly skewed and do not follow a normal distribution for statistical analysis, it is often sensible to transform variables. In HIV the viral load distribution is highly skewed, therefore it is often transformed using the logarithmic transformation to the base 10, if $x = 10^u$ then by definition u is the logarithm (base10) of x, i.e. $100 = 10^2$, $2 = \log_{10}(100)$[431]. So a viral load of 50 copies/ml = 1.69 $\log_{10}$ copies/ml and a viral load of 5000 copies/ml = 3.69 $\log_{10}$ copies/ml.

Another common transformation in HIV analysis is to use $\log_2$ (base2) when looking at CD4 count as predictor of an event. For example, if risk of death is estimated to be 20% lower per $\log_2$ increase in CD4 count, this means that patients with a CD4 count of 100/mm^3 have a 20% lower risk of death than patients with a CD4 count 50/mm^3 and similarly patients with a CD4 count of 200/mm^3 have a 20% lower risk of death than those with a CD4 count of 100/mm^3. When the $\log_2$ transformation is used it is often described as per doubling or 2 fold increase.

Another option for continuous variables is to redefine them as a categorical variables, based on commonly used cut-offs. For example, CD4 count could be split into 5 categories i.e. ≤50, 51-200, 201-350, 351-500 and >500. The advantage of this is that it is easily understood and interpreted by clinicians, the disadvantage is that the actual risk of the event for someone with a CD4 count of, for example, 51/mm^3 will not be greatly different from the risk of someone with a CD4 count of 49/mm^3, yet the risks produced may be significantly different for different categories.

2.2.3.4 Statistical interactions

The statistical modelling methods described above assume that the effect of each variable in the model does not depend on the value of other variables. If this is not true, for example if the effect of CD4 count on risk of discontinuation of treatment is bigger in males than in females, then there is a statistical interaction. The first step in dealing with statistical interactions, is to test for interaction by incorporating an interaction term into the model. If the interaction term is significant then the model should be fitted separately for the different levels of the factor. However, test for interaction are typically underpowered.

2.2.3.5 Endpoints

Clinical endpoints in HIV are normally defined as either disease progression or death. Many clinical trials, because of time constraints, use surrogate end points. In HIV, these are often CD4 counts or viral load. A summary of the endpoints used in each of the chapters is given in table 2.3 and also in the methods section of each of the analysis chapters. A variety of sensitivity analyses using slightly different versions of endpoints are also used to check the robustness of the results. For example there are a variety of different endpoints that can be used to define virological failure, such as one viral load measured above 500copies/ml, or 2 consecutive viral loads measured above 500copies/ml, or a viral load above 50copies/ml in patients with a lower limit of detection ≤50copies/ml.

2.2.4 Summary

A number of different methods commonly used for analysing observational data have been described in this chapter. These methods are not without disadvantages and the biases in cohort studies can be great, as discussed in more detail in chapter 8. There is a detailed methods section at the start of each chapter that provides further details on the patients selected, the specific statistical methods used, and the endpoints analysed in that chapter.

Chapter 3 Investigation into whether frequency of monitoring can be reduced in a subgroup of HIV-positive people?

3.1 Introduction

As discussed in chapter 1, the introduction of cART in the early 90's had a dramatic effect on the treatment of HIV-positive patients and their survival rates[6;10;156;157;159;272]. Since then, huge developments have been made in the treatment and care of patients. Drug regimens are now less toxic, once daily regimens have been developed[432-434], drugs have been made in combination to decrease pill burden[435;436], and more sensitive monitoring tools have been developed[437;438].

The improvements made in technology have meant that viral load and CD4 testing are common practice for all HIV-positive patients in the developed world although they are still relatively expensive. Routine diagnostic tests in the developed world such as viral load and CD4 counts cost around $119 and $66 respectively per test[439-441]. Treatment guidelines such as the WHO, BHIVA and EACS, are constantly updated and modified in order to keep as up to date as much as possible with the newest drugs and treatment strategies so that patients get the best possible treatment available to them based on current evidence. For example, looking at when to start treatment, guidelines stated in 1997 that patients should start with a CD4 count <200/mm^3 and that treatment should be considered in those with a CD4 count 200-350/mm^3 depending on their viral load[442]. However, as studies found that patients starting treatment earlier had a better prognosis[443], treatment guidelines have subsequently been changed to recommend treatment is started in all patients with a CD4 count <350/mm^3 [214;223]. A new randomized control trial, START, has been designed to investigate whether there are benefits in starting cART at even higher CD4 cell counts (>500/mm^3) compared to delaying the start of cART until the CD4 count falls to below 350/mm^3 as current treatment guidelines recommend[444].

Populations of patients with HIV under routine care have increased dramatically over the past decade. For example in 1997, 16,645 patients were treated in the UK, and in 2007 this number had more than tripled to 55947[445]. Similarly, in a Southern Alberta cohort study which includes all HIV-positive patients living in the area, the number of patients increased by 74% from 526 to 920 from 1997 to 2006[446].

There are several reasons for this increase. There has been an increase in number of patients diagnosed with HIV; for example in the UK just under 3,000 patients were newly diagnosed in 1997 compared to over 7,000 in 2005[183]. This is, in part, due to an increase in the number of HIV tests being performed[183]. Further, the introduction of cART has meant that patients infected with HIV are living longer, with lower rates of AIDS defining illnesses. EuroSIDA found that there was an 8% decrease in the incidence of AIDS or death per 6 month period after 1998[10]. Patients are living longer so are remaining on treatment longer; this means the number of patients in care and on treatment is larger[183]. Also, an increase in immigrants infected with HIV has added to the UK HIV-positive population. In the UK in 1998, the number of black Africans infected heterosexually was 2,557. This has increased 16-fold to 19,013 in 2007[445]. A similar pattern has been reported in other countries[446].

However, a large number of infected patients in both the developed and the developing world remain unaware of their HIV status, with estimates suggesting that approximately one quarter of patients in the developed world infected with HIV are unaware of their HIV status[447]. These patients are at a higher risk of transmitting the virus to uninfected individuals than those aware of their HIV status[448], and patients presenting for care late in their infection tend to have poorer outcomes and make larger demands on clinical resources[449] than those who start treatment according to guidelines. Attempts are being made to increase the number of patients infected with HIV who know they are infected, and if these are successful then patient populations will increase further.

The increase in patient populations has in turn meant that there has been an increase in the number of patients who need to be seen routinely for management and care of HIV. With patients living longer, and the number of patients infected with HIV rising, there is a growing burden being placed on clinical services, health care providers and financial resources.

HIV-positive patients are seen at clinics for a whole variety of reasons, such as testing of viral load and CD4 cell count, complete blood count liver and renal function, lipid profile and glucose levels[311;312;450;451]. Some treatment regimens can be quite complex, with different toxicities and dietary requirements depending on the regimen. Patients on salvage therapy require particularly close monitoring[452].

In addition to emphasizing the importance of adherence and discussing any side effects of treatment, clinicians may also discuss other issues and risk factors associated with cART, including advice on smoking cessation and the benefits of and having a healthy lifestyle, which may particularly benefit patients at high risk of other diseases such as cardiovascular disease[452]. Patients may also be counselled on practicing safer sex and the risk of transmission[452].

Currently, treatment guidelines in developed countries such as Britain and across most of Europe and the US recommend patients on antiretroviral therapy should be seen every 3-4 months for CD4 count and viral load monitoring[311;453-455]; this has been the case for several years now. These guidelines are not based on results of clinical trials but have been based on the expert clinical opinion[456]. CD4 count was first recognized as a predictor of short term risk of opportunistic infections and death[129;457], but some studies reported that it had limited ability to predict disease progression over prolonged period of time[100;458]. Then, after improvements in HIV-RNA testing[459-461], both baseline viral load and CD4 count were found to independently predict clinical events[462;463] and viral load monitoring was added to the treatment guidelines[456;464]. The frequency of viral load monitoring, recommended at 3-4 monthly intervals, was chosen as that was the frequency already recommended by expert clinical opinion for CD4 count monitoring [465].

Although there has been an increase in patient populations, fewer patients are being admitted to hospital, developing AIDS defining illnesses or serious opportunistic infections[10;234;297;466], which means the costs associated with in-patient care have decreased. In the cART era, the total cost per patient has not increased substantially over time[446;467]. The average monthly cost of patients in care in developed countries has been estimated to be between $1,000 -$2,000[439;468], and the total life time cost of care over $380 000[439]. The annual cost of outpatient visits for patients on cART in Western and Central Europe is around $240 per patient per visit[469]. A Canadian study reported that between 1997 and 2006, the cost of care for all patients within the region increased by 69%[446]. They also reported that the cost of outpatient costs had increased slightly from $169 to $186 with patients in poorer health requiring more disease management and more clinic visits[446].

Despite the improvements in antiretroviral regimens and constant updating of treatment guidelines, recommendations for monitoring a patient after starting cART are the same regardless of how they respond to treatment or how advanced the disease is[312;450;451;470].

Patients who respond poorly to treatment or have newly initiated cART may require particularly close monitoring, to ensure good adherence and check for toxicities[452]. This may not be true for patients who have remained on a stable regimen for several years. Previous modelling work, using a simulated cohort based on initial clinical presentation of HIV-positive adults in the US, has shown that a strategy of monitoring patients less frequently (e.g. every 12 months) prior to a pre specified warning point where monitoring is then increased (e.g. every 3 months) confers the same clinical benefit to constant 3 monthly monitoring, but at a much lower cost[440].

Previous studies have shown that the greatest risk of treatment failure is in the first few months after starting treatment[471;472]. The risk of clinical disease progression (new AIDS/death) was also found to be significantly higher in patients starting cART with low CD4 counts[473] and those who could not maintain an undetectable viral load[466]. Thus we hypothesized that there may be a group of patients, who have responded well to treatment and are on a well-tolerated and fully suppressed cART regimen, where it may be safe to reduce the frequency of clinic visits to every 6, 9 or 12 months. This would potentially save time for both patients and health care providers. It could also help reduce the impact of infection with HIV and intrusion into people's lives, as the current 3-monthly monitoring frequency may act as a regular reminder of their illness, and an inconvenience to daily living, including having to take time off work to attend clinic appointments.

3.2 Aim

The aim of this chapter was to investigate whether the current recommendation of monitoring patients at 3-monthly intervals is necessary for all patients, or whether there is a sub group of otherwise healthy patients, who have responded well to therapy and are on a well-tolerated and fully suppressive cART regimen, who could safely have their visit interval extended to 6-monthly.

3.3 Methods

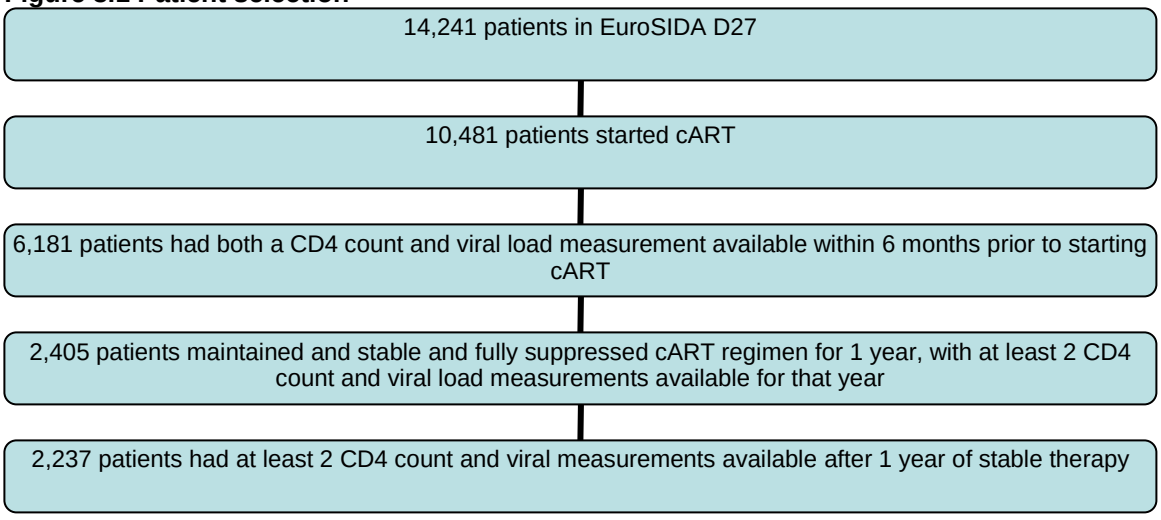
3.3.1 Patient selection

There were 14,241 patients in the D27 EuroSIDA update. Figure 3.1 shows how patients were selected for inclusion. All patients must have maintained a stable fully suppressive cART regimen for a period of at least 1 year. A stable and fully suppressed cART regimen was defined as a period of at least one year during which all CD4 counts were above 200/mm^3 and at least 100/mm^3 above the CD4 count at starting cART, and all viral loads were <500 copies/ml.

cART was defined as a regimen containing at least 3 antiretrovirals of which at least 2 must be nucleosides or nucleotides and 1 must be either a protease inhibitor or a non-nucleoside reverse transcript inhibitor. 3,760 patients were excluded who had not started cART. Patients without a CD4 count measured in the 6 months prior to starting cART were excluded (4,300 patients). Patients were required to have at least 2 CD4 counts and 2 viral loads measured during the 1 year period over which the regimen was considered to be stable and fully suppressed. Patients were additionally required to have made no changes to the cART regimen (stopping or starting any drugs) during the 1 year period, and for no serious illness to have occurred during the 1 year period. A serious illness was defined as an AIDS defining illnesses, a non-AIDS defining malignancy, other serious opportunistic infection (OI), cardiovascular disease (CVD), diabetes, hypertension or grade III/IV liver failure. However, non-AIDS events were only systematically collected in EuroSIDA after 1st January 2000.

Each patient was included in the analysis only once, after the first year they maintained a stable and fully suppressive cART regimen. 3776 patients were excluded who did not meet the requirements of 1 year on a stable and fully suppressed cART regimen. A further 168 patients were excluded as they had insufficient follow-up after the year of stable therapy (at least 2 CD4 count and viral load measurements recorded). 2,237 patients maintained a stable and fully suppressive cART regimen for at least a year with sufficient follow-up data and thus were included in the study.

Figure 3.1 Patient selection



3.3.2 Statistical analysis:

For each patient, baseline was defined as the end of the first year the patient maintained a stable a fully suppressed regimen, as defined above.

Kaplan Meier survival analysis was used to find the probability (risk) of disease progression within the next 3, 6, and 12 months.

Disease progression was defined as

- CD4 count below 200/mm^3
- CD4 count decrease to lower than the CD4 count at starting cART
- Two consecutive viral load measurements above 500 copies/ml
- Development of any AIDS defining illnesses
- Development of any non-AIDS defining illnesses or serious opportunistic infections
- Death

Non-AIDS defining illnesses included any non-AIDS related malignancies, pancreatitis, end stage renal disease, other liver related disease, myocardial infraction, stroke or other cardiovascular disease.

The risk of failing using each of the 6 categories above was investigated separately, and as a composite end point. For immunological events, patients were censored at the date of their last recorded CD4 count if they did not experience an event. Similarly, for virological failure events, patients were censored at their last recorded viral load measurement if they had not experienced an event. For each other event (AIDS event, non-AIDS event and death) patients were censored at their last recorded visit. For the composite end point, failure was the time of the first one of any of the events above occurring, and follow-up was until the date of last recorded visit.

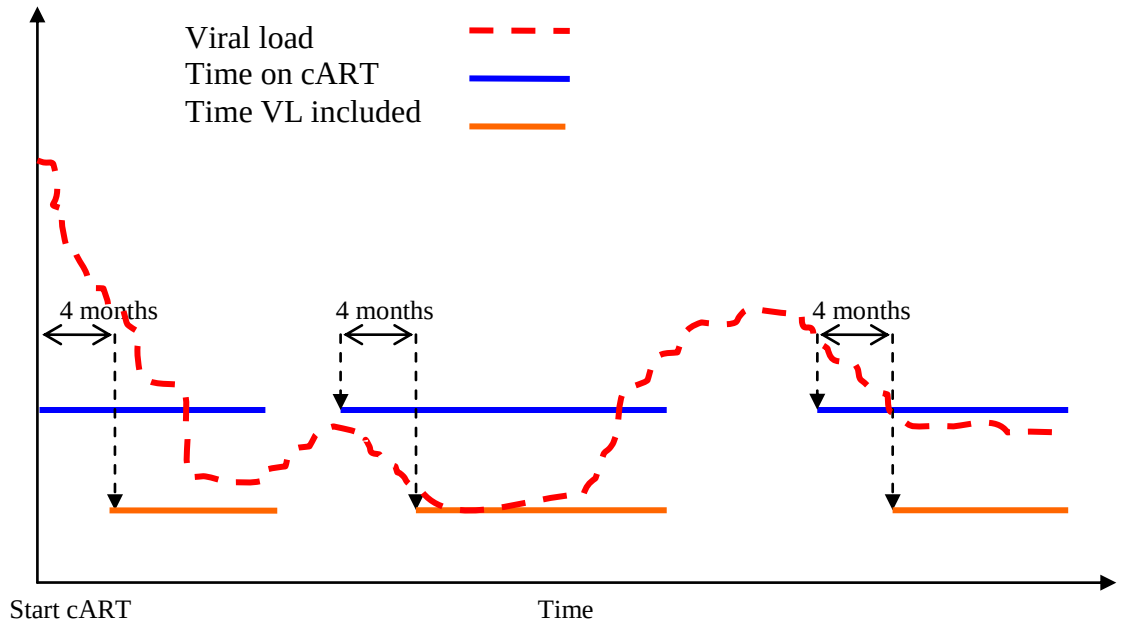
Univariable Cox Proportional Hazards models were used to identify factors associated with disease progression in the 12 months after baseline. These included CD4 and viral load at starting cART and at baseline, in addition to the diagnosis of any previous AIDS defining illnesses. Also tested were age, gender, race, HIV exposure group, hepatitis B and C status, the length of time the patient was on cART before a stable and fully suppressed regimen was found, which cART regimen (NNRTI, single PI or ritonavir-boosted PI) the patient was on, the number of treatment changes prior to baseline, and time a patient

spent on cART with viral load <500 copies/ml. Individuals were classed as Hepatitis B positive if they had a positive HBV surface antigen test recorded and Hepatitis C positive if they had a positive HCV antibody test. A treatment change was defined as stopping or starting at least one antiretroviral, apart from dose modification.

When looking at the time a patient spent on cART with suppressed viral load, the first 4 months after starting a new cART regimen were excluded to allow time for the patient to respond to treatment, as were any periods when the patient was off treatment (see Figure 3.2).

Changes to the cART regimen after baseline, specifically decreasing the number of antiretrovirals included in the regimen, or stopping the regimen, was included as a time-updated binary variables. Any factors with a p-value <0.1 were included in the multivariable Cox Proportional Hazard models. All Cox proportional hazards models were stratified by centre.

Figure 3.2 How time on cART with a suppressed viral load was calculated



3.4 Results

3.4.1 Patient characteristics

10,481 patients started cART but only 2,237 patients who maintained a stable and fully suppressed regimen for one year were included in the analysis (figure 3.1). In general, demographic characteristics were similar between patients who were excluded and those included. However, a higher proportion of patients whose mode of transmission was IDU were excluded (24% vs. 17%, p<.0001) and a higher proportion of patients from East Europe were excluded (20% vs. 14% p<.0001). Table 3.1 describes the baseline characteristics of the patients included in this study. Patients were mainly male 76% (1,703), white 86% (1,933), and were thought to have been infected with HIV via homosexual sex, 49% (1,091). The median age was 40 (interquartile range [IQR] 35-47). 52% of patients were antiretroviral-naïve at starting cART and 25% had a previous AIDS diagnosis. Median CD4 count at starting cART was 218/mm^3 (IQR 108-327), and viral load 4.64 log_{10}copies/ml (IQR 3.79-5.24). At baseline, the median CD4 count was 520/mm^3 (IQR 396-693) and median increase in CD4 count from starting cART to baseline was 290/mm^3 (IQR 412-208). 4% of patients had had a positive test for Hepatitis B and 14% for Hepatitis C. The median time between start of cART and baseline was 2.8 years (IQR 1.9-4.3). Median time between successive CD4 and viral load measurements was 3 months (IQR 3–4 months) in addition the median time between baseline and the next CD4 or viral load measurement was 2 months (IQR 1-3 months).

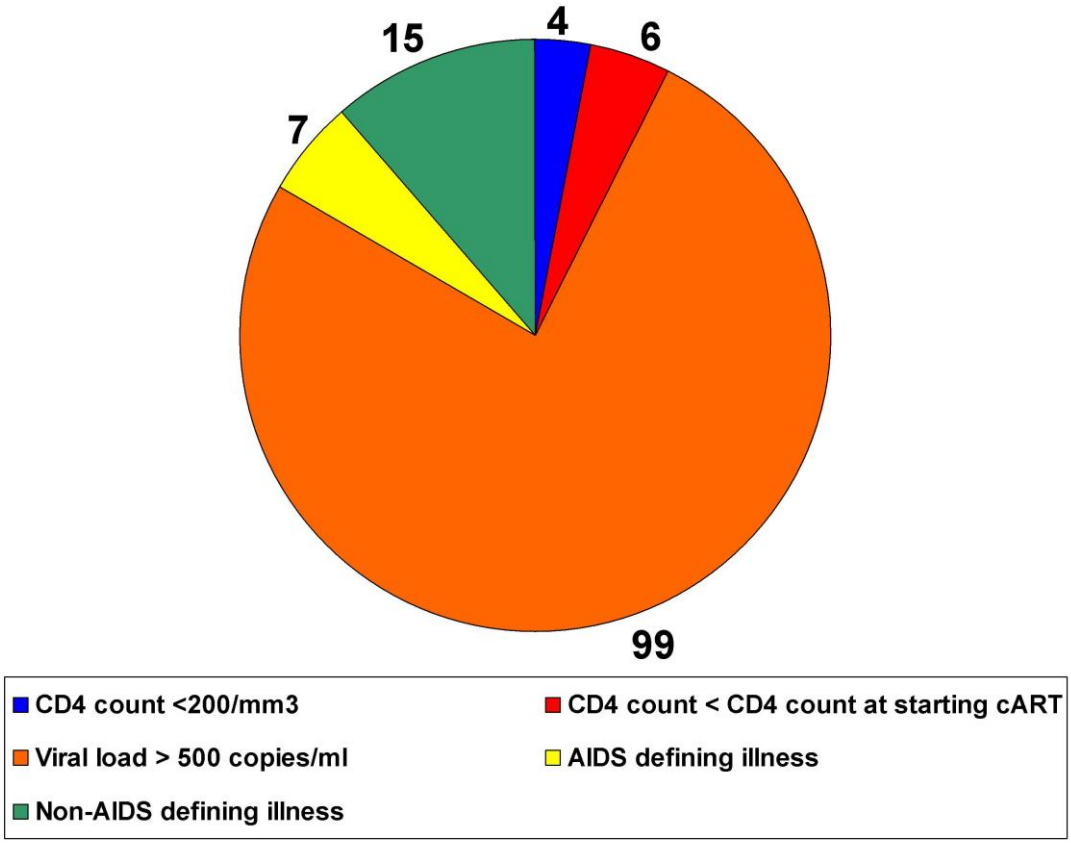
Table 3.1: Baseline characteristics of 2237 included patients

		All Patients		Event within 1 year		No event after 1 year		Chi-squared
		N	**%**	**N**	**%**	**N**	**%**	**p-value**
Total		2237	100.0	131	5.9	2106	94.1	
Gender	Male	1703	76.1	95	72.5	1608	76.4	0.01
Race	White	1933	86.4	111	84.7	1822	86.5	0.86
Exposure group	Homosexual	1091	48.8	56	42.8	1035	49.2	0.06
	IDU	372	16.6	30	22.9	342	16.2	
	Heterosexual	624	27.9	36	27.5	588	27.9	
	Other	150	6.7	9	6.9	141	6.7	
Hepatitis B Status	HBV negative	1558	69.7	80	61.1	1478	70.2	0.07
	HBV positive	90	4.0	5	3.8	85	4.6	
Hepatitis C Status	HCV negative	1140	51.0	55	42.0	1085	51.5	0.10
	HCV positive	325	14.5	23	17.6	302	14.3	
Region of Europe	South/Argentina	646	28.9	58	44.3	588	27.9	0.0003
	Central	507	22.7	29	22.1	478	22.7	
	North	801	35.8	38	29.0	763	36.2	
	East	283	12.7	6	4.6	277	13.2	
Treatment at baseline	PI	777	34.7	55	42.0	722	34.3	0.13
	PI boosted	633	28.3	37	38.2	596	28.3	
	NNRTI	827	37.0	39	39.0	788	37.4	
Naïve	Yes	1161	51.9	55	42.0	1106	52.5	0.01
Prior AIDS	Yes	562	25.1	35	26.7	527	25.0	0.66
		Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Kruskal-Wallis**
Age	Years	40.3	35.2-47.3	38.8	34.2-45.1	40.4	35.3-47.8	0.07
CD4	/mm^3	520	396-693	546	376-691	520	397-693	0.91
Time since started cART	Years	2.8	1.9-4.3	2.8	2.0-4.3	2.8	1.9-4.3	0.51
CD4 at starting cART	/mm^3	218	108-327	232	106-376	218	108-324	0.32
Viral load at starting cART	$\log_{10}$copies/ml	4.64	3.79-5.24	4.65	3.78-5.24	4.04	3.80-5.24	0.57
% time on cART with RNA<500 copies/ml		95.0	83.3-100.0	91.7	66.0-100.0	95.2	84.2-100	0.62
nadir CD4	/mm^3	157	66-252	167	69-273	156	66-252	0.27
Peak viral load ($\log_{10}$copies/ml)		4.96	4.39-5.44	5.00	4.37-5.47	4.96	4.39-5.44	0.63
Baseline date (month/year)		05/02	04/00-10/04	03/02	05/00-03/04	05/02	04/00-10/04	0.27
Time between CD4 measurements (Days)		94	91-121	92	78-115	95	91-121	0.001
Time between viral load measurements (Days)		98	91-122	92	89-115	98	91-122	<.0001

3.4.2 Disease Progression

131 patients (6%) experienced disease progression in the first year after baseline. Figure 3.3 shows the first disease progression event experienced by each patient i.e. those included in the composite end point. 99 patients (4.6%) experienced virological failure, 4 patients (0.2%) experienced a CD4 count less that 200/mm^3, 6 patients (0.3%) experienced a CD4 count >100/mm^3 below pre-cART levels, 7 patients (0.3%) developed a new AIDS defining illness (2 developed tuberculosis, 1 cryptosporidiosis, 1 toxoplasmosis, 2 non-Hodgkin's Lymphoma, and 1 oesophageal candidiasis). 15 patients (0.7%) experienced a non-AIDS defining malignancy or serious OI, of which 10 were non-AIDS defining cancers and the remainder were serious OIs and there was one death.

Figure 3.3: Number of disease progression events observed (cumulative failure end point)



Of these 131 patients, 121 patients experienced only 1 event in the year after stable therapy and 10 of these patients experienced 2 events in the year after stable therapy. In addition to failing virologically, 3 patients had CD4 count < 200/mm^3, 4 had a CD4 count <100/mm^3 above their CD4 count at starting cART, and 1 died. In addition to a non-AIDS event, 1 patient also had a CD4 count <200/mm^3 and 1 had an AIDS event. Therefore the total number of events observed was 141.

From the baseline characteristics in table 3.1 there is very little difference between those patients that experienced disease progression within 1 year and those that did not. Median baseline CD4 count was 520 (IQR 376-691) in patients who did not experience an event and 546 (IQR 376-691) in those who did (p=0.91). Patients had been on cART for a median of 2.8 years in both groups (p=0.51), although a higher proportion of treatment naïve patients did not experience disease progression, 53% vs. 42% (p=0.01). Patients who had an event had spent a lower percentage of time on cART with a suppressed viral load, median 92% (IQR 66%-100%) of the time compared to median 95% (IQR 84%-100%) of the time in patients who did not experience disease progression, though these differences were not significant (p=0.62)

Table 3.2 describes the characteristics of the patients who experienced disease progression at the time of their first event. Median CD4 count at the time of first event was higher than the median CD4 count at starting cART: median increase 224cells/mm^3 (IQR 101-328). There was a median decrease of 78cells/mm^3 (IQR -217 – 58) in CD4 count from baseline to disease progression. Patients had been on cART for a median of 3.5 years prior to disease progression. Additionally, the majority (64.2%) of patients experiencing disease progression had a viral load > 1000 copies/ml at the time of first event.

Table 3.2: Characteristics at time of disease progression (cumulative failure end point)

		N	Percentage
Patients experienced an event		131	100
Viral load at first event	<500copies/ml	32	24.4
	500-999copies/ml	15	11.5
	1000-9999 copies/ml	45	34.4
	≥10000copies/ml	39	29.8
		Median	**IQR**
Age at first event		39	35 – 45
CD4 count at first event (/mm^3)		436	336 – 597
CD4 count change from starting cART		224	101 – 328
CD4 count change from baseline		-78	-217 – 58
Time on cART at first event		3.50	2.7– 4.9
Year of failure		2002	2000 – 2004

Figure 3.4 shows the Kaplan Meier figure of the probability of disease progression in the year after baseline. At 3 months after baseline, there was an estimated 0.3% risk of **any** disease progression (95% CI 0.1, 0.5), which increased to 2.2% at 6 months (95% CI 1.6, 2.8) and 6.0% (95% CI 5.0, 7.0) after 12 months. As mentioned previously, virological failure was the main reason for disease progression. There was an estimated 1.5% (95% CI 1.0, 2.0) risk of virologic failure after 6 months, which increased to 4.6% (95% CI 3.7, 5.5) after 12 months. In contrast, there was less than a 1% probability of any of the other disease progression events occurring in the 12 months after baseline. The risk of non-AIDS failure is shown in figure 3.4 in addition to the risk of overall disease progression and virological failure. The 4 other disease progression events in Kaplan Meier analysis had a lower risk than developing a non-AIDS defining illnesses and are not shown.

Figure 3.4: Kaplan Meier estimates of disease progression after a year of stable cART: the probability of having a CD4 count < 200/mm^3 or < CD4 at initiation of cART, developing new AIDS, or dying, one year after baseline, were all less than the probability of developing a non-AIDS defining illness and are not shown.

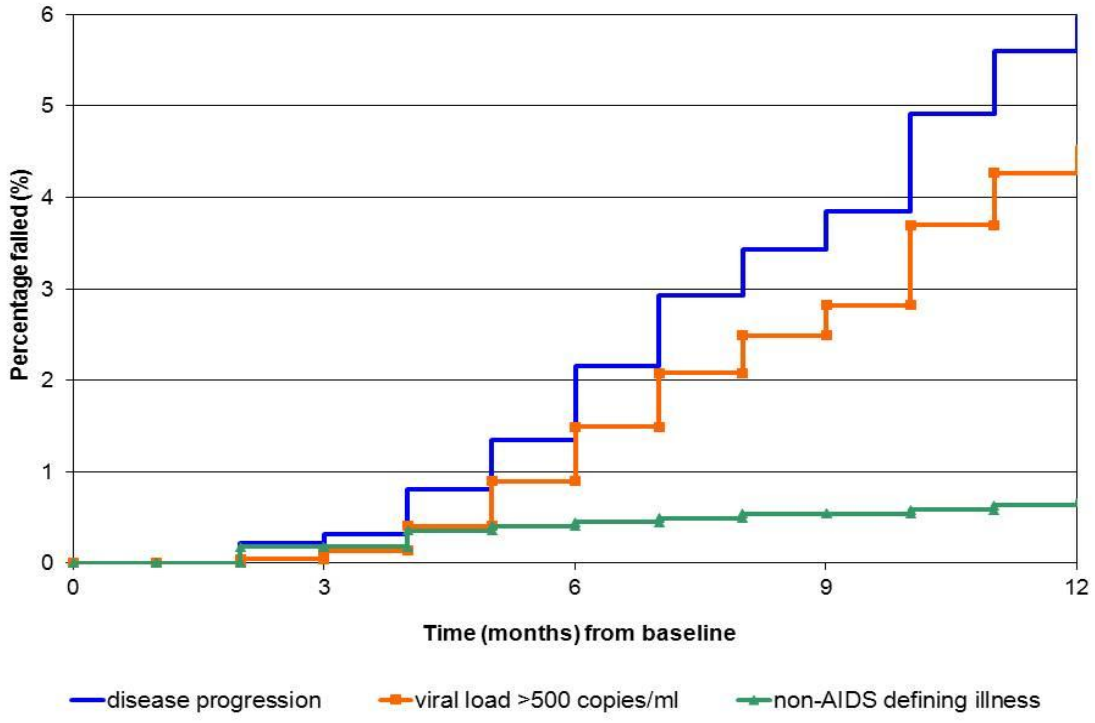


Table 3.3 gives the number of patients who failed, and the number of patients remaining under follow-up at 3, 6 and 12 months, for each disease progression event. After 3 months no patient had failed immunologically (all CD4 counts above 200/mm^3 and above pre-cART levels), only 1 patient had developed an AIDS defining illness (TB), 4 patients developed non-AIDS defining illness, (3 cancers and 1 bacteremia) and 3 patients failed virologically (viral load > 500 copies/ml). After 6 months, although still quite low, the number of patients that had failed virologically had increased to 33. At 6 months, patients had a 0.04% risk of having a CD4 count < 200 mm^3 and 0.1% risk of a CD4 count 100/mm^3 below the CD4 count at starting cART. In addition, patients had a 0.1% and 0.4% risk of developing AIDS or a non-AIDS defining illness after 6 months. The risk of immunological failure, developing AIDS or non-AIDS defining illness or death in the 12 months after a year of stable therapy were less than 1%.

Table 3.3: Percentage of patients experiencing disease progression at 3, 6, and 12 months after baseline

	% with an event	95% CI	N events	N remaining under follow-up
Disease progression: CD4<200				
3 months	0	-	0	2238
6 months	0.04	0-0.1	1	2199
12 months	0.4	0.1-0.7	8	2088
Disease progression: CD4 < CD4 at initiation of cART				
3 months	0	-	0	2238
6 months	0.1	0-0.2	3	2197
12 months	0.5	0.2-0.8	10	2086
Disease progression: Confirmed HIV-RNA>500				
3 months	0.1	0-0.3	3	2234
6 months	1.5	1.0-2.0	33	2166
12 months	4.6	3.7-5.5	99	1986
Disease progression: *AIDS Defining illness*				
3 months	0.04	0-0.1	1	2237
6 months	0.1	0-0.3	3	2208
12 months	0.4	0.1-0.7	8	2113
Diseases progression: *Non AIDS Defining illness*				
3 months	0.2	0-0.4	4	2234
6 months	0.4	0.1-0.7	10	2201
12 months	0.7	0.4-1.0	15	2106
Disease progression: *Death*				
3 months	0	-	0	2235
6 months	0.05	0-0.1	1	2208
12 months	0.05	0-0.1	1	2116
Disease progression: (any of the above)				
3 months	0.3	0.1-0.5	7	2228
6 months	2.2	1.6-2.8	48	2161
12 months	6.0	5.0-7.0	131	1992

3.4.3 What baseline variables were predictive of disease progression?

Cox proportional hazards models were used to investigate the factors associated with a lower risk of 'disease progression events' in patients who have maintained a stable and fully suppressed cART regimen for at least 1 year. Table 3.4 show the results from the univariable Cox proportional hazards models.

Table 3.4: Cox proportional hazards model: Any disease progression

		Univariable		
		Hazard Ratio	**95% CI**	**p-value**
Gender	Male	1.00	-	-
	Female	1.27	0.85-1.90	0.25
Race	White	1.00	-	-
	Other	1.13	0.65-1.98	0.67
Age	Per 10 yrs older	0.83	0.68-1.03	0.08
Exposure Group	IDU	1.00	-	-
	Homosexual	0.64	0.39-1.05	0.08
	Heterosexual	0.83	0.50-1.39	0.47
	Other	0.78	0.35-1.71	0.53
Hepatitis B status	Negative	1.00	-	-
	Positive	0.99	0.40-2.49	0.99
	Unknown	1.54	1.01-2.35	0.04
Hepatitis C status	Negative	1.00	-	-
	Positive	1.21	0.71-2.04	0.49
	Unknown	1.09	0.63-1.88	0.75
Treatment group	PI	1.00	-	-
	PI boosted	1.09	0.70-1.69	0.70
	NNRTI	0.72	0.47-1.11	0.13
Naïve at starting cART		0.87	0.59-1.29	0.49
Previous AIDS		1.06	0.72-1.58	0.76
Year started cART	Per year	1.02	0.93-1.11	0.76
Time since started cART	Per year	1.01	0.92-1.01	0.89
Treatment at starting cART	PI	1.00	-	-
	PI boosted	0.89	0.54-1.48	0.65
	NNRTI	1.05	0.66-1.68	0.82
Baseline CD4	per 2 log higher	1.00	0.73-1.37	0.99
CD4 at starting cART	per 2 log higher	1.04	0.92-1.17	0.52
CD4 nadir	per 2 log higher	1.06	0.96-1.17	0.27
Viral load at starting cART	Per 1 log higher	1.13	0.96-1.35	0.14
Peak viral load	Per 1 log higher	1.19	0.96-1.47	0.11
Time to first suppression after initiation of cART	Per 3 months	1.00	0.96-1.05	0.96
% time with RNA <500 whilst on cART prior to baseline	Per 10%	0.85	0.78-0.93	0.0002
Number of treatment changes due to toxicities (from starting cART to baseline)	Per treatment change	0.91	0.78-1.07	0.26
Reducing number of ARV's in regimen after baseline	Time updated from baseline	1.87	0.25-14.09	0.54
Stopping all ARV's after baseline	Time update from baseline	21.44	8.22-55.99	<.0001

From table 3.4, patients are at an increased risk of a 'disease progression event' if they stop all antiretrovirals (IR 21.44, 95% CI 8.22-55.99, p<0.0001). The percentage of time a patient has spent on cART prior to baseline with a viral load < 500 copies/ml was also highly significant. The more time spent with a suppressed viral load the less likely the patients were to experience a 'disease progression event'. Age may be a significant predictor of future disease progression with older patients being at a higher risk of disease progression. However, this difference was marginally significant p=0.08. No other factors were significant at the10% level, and thus only age, time with suppressed viral load whilst on cART, and stopping all ARV 's were included in the multivariable model.

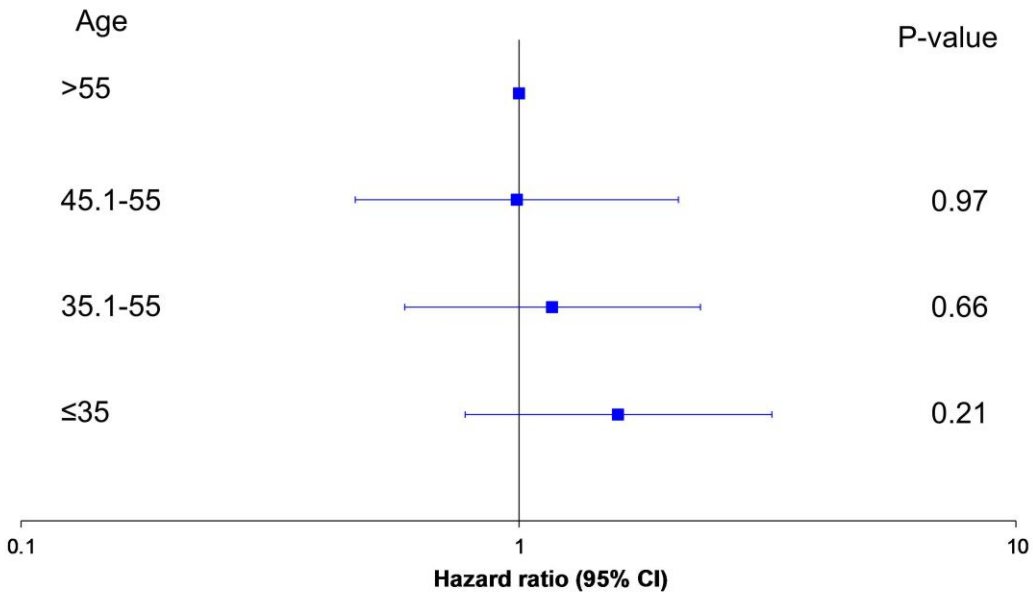
Table 3.5 Cox proportional hazards model: Any disease progression (% time on cART included as a continuous variables).

		Multivariable		
		Hazard Ratio	**95% CI**	**p-value**
Age	**Per 10 yrs older**	0.83	0.67-1.03	0.08
% time with RNA <500 whilst on cART prior to baseline	**Per 10%**	0.84	0.77-0.92	0.0002
Stopping all ARV's after baseline	**No** **Yes**	1.00 20.94	- 7.93-55.29	<.0001

Table 3.5 shows the results of the multivariable Cox proportional hazards model. After adjustment, stopping all antiretrovirals in the regimen after baseline increased the risk of disease progression (hazard ratio [HR] 20.94, 95% CI 7.93, 55.29, p<0.0001). In addition, there was a 16% reduction in the risk of disease progression per 10% increase in the proportion of time spent on cART with viral suppression < 500 copies/ml prior to baseline (HR 0.84; 95% CI 0.77, 0.92, p=0.0002). Age was also associated with a marginally significant 17% lower risk of disease progression per 10 years older (HR 0.83, 0.67-1.03, p=0.08).

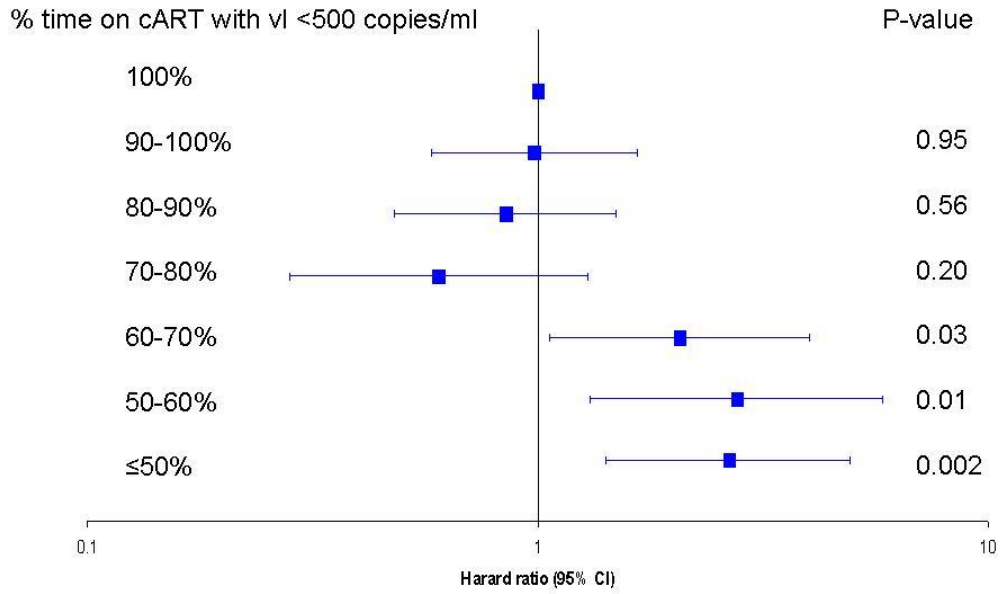
Age was also investigated as a categorical variable. The results of the univariable cox-proportional hazards model are shown in figure 3.5. The marginal trend of an increased rate of disease progression with decreasing age appears to be roughly linear; therefore the continuous variable was used for the remainder of this analysis.

Figure 3.5 Univariable cox proportional hazards model age and rate of disease progression



Similarly, results might be easier to interpret and apply to individual patients if the proportion of time with HIV-RNA < 500 copies/ml prior to baseline is categorized. This also helps to suggest whether there may be a useful cut-off value for the proportion of time suppressed, to which the risk of a disease progression event is significantly different from the next category of proportion of time suppressed.

Figure 3.6: Univariable analysis percentage of time with viral load <500 copies/ml whilst on cART prior to baseline (excluding the first four months after starting cART or making a treatment switch to allow for patients to re-suppress)



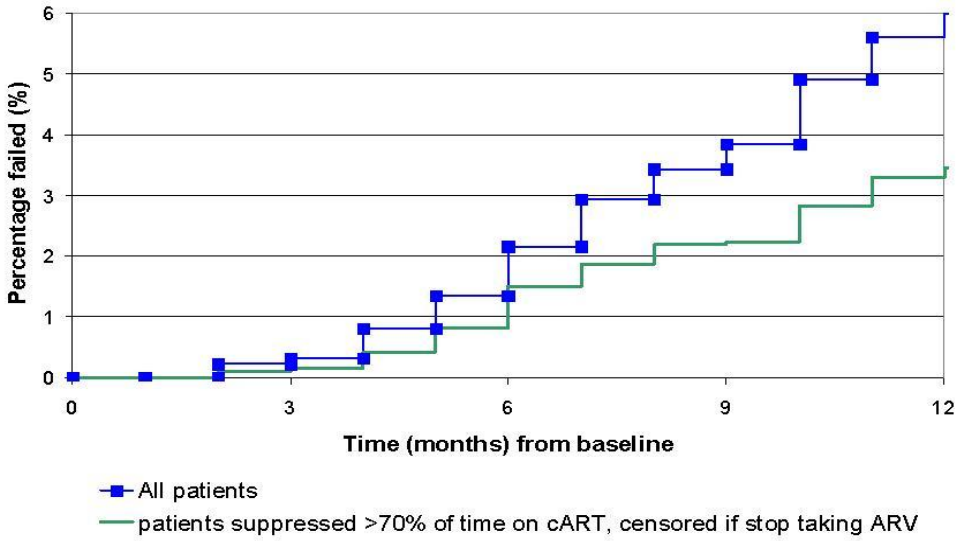
From figure 3.6 it appears that patients who are suppressed more than 70% of the time they are on cART prior to baseline have a lower risk of disease progression in the 12 months after maintaining a year of stable therapy. Table 3.6 gives the results of the multivariable analysis when % time suppressed is included in the model as a binary variable (suppressed more than 70% vs suppressed less than 70%). Patients were at almost 3 times the risk of disease progression within the next 12 months if they have spent less than 70% of their time on cART prior to baseline with a viral load <500copies/ml, (HR 2.82, 95% CI 1.87-4.26, p<0.001).

Table 3.6 Cox proportional hazard model: Any disease progression (% time on cart included as a categorical variable).

		Multivariable		
		Hazard Ratio	95% CI	p-value
Age	Per 10 yrs older	0.82	0.66-1.01	0.06
% time with RNA <500 whilst on cART prior to baseline	≥70%	1.00	-	-
	<70%	2.82	1.87-4.26	<.0001
Stopping all ARV's after baseline	Time update from baseline	22.03	8.33-58.27	<.0001

Figure 3.7 shows the probability of disease progression in all patients compared to the subset of 1,972 patients who spent more than 70% of the follow-up on cART with suppressed viral load prior to baseline, of whom 57 (2.9%) patients experienced disease progression in the 12 months after baseline; 40 of the disease progression events were due to virologic rebound. The proportion of patients estimated to have experienced disease progression for any reason at 6 months was 1.5% (95%CI 0.9, 2.1) and 3.3% (95%CI 2.6, 4.4) after 12 months.

Figure 3.7: Kaplan Meier risk of disease progression in 12 months after baseline



N remaining under follow-up

2237	2228	2161	2093	1992
1972	1865	1679	1520	1339

3.4.4 Sensitivity analysis

Treatment switches were not included as a 'disease progression event' in the main analysis. This decision was made after discussion with clinicians; patients in the study population had been on their current regimen for at least 1 year, had responded well and had been able to tolerate it well. Most drug switches would presumably be due to convenience rather than toxicity or treatment failure, which would have been captured by viral rebound. However, identifying treatment switches due to treatment failure are important to detect viral failure as early as possible in patients who are seen regularly. It is possible that treatment switches may have been made before the confirmatory viral load measurement above 500copies/ml, and this information would not be captured as quickly if patients were seen less often. Thus the analysis was repeated with treatment switches due to insufficient virologic response (i.e. switched treatment with a single viral load > 500copies/ml) included as a 'disease progression event'.

A total of 22 patients made a treatment switch in the 12 months following a year of stable therapy. However, this resulted on only 11 additional patients being classed as having a 'disease progression event' as the other 11 had already experienced a different 'disease progression event'. Thus, 142 patients were classed as failing due to any 'disease progression event' when treatment switches were included. The estimated probability of a treatment switch following viral rebound at 3, 6 and 12 months was 0%, 0.3% (95% CI 0.0, 0.4) and 1.0% (95% CI 0.6, 1.4) respectively. The cumulative estimated probability of disease progression increased to 0.3% (95% CI 0.1, 0.5), 2.3% (95% CI 1.7, 2.9) and 6.4% (95% CI 5.4, 7.4) at 3, 6 and 12 months respectively when this definition of treatment failure was added to the cumulative definition of disease progression.

Analyses were also repeated with a stable cART regimen defined on the basis of all viral loads < 50 copies/ml. As not all patients have a viral load measured with a lower limit of detection (LOD) of 50 copies/ml or less, the sample size was substantially reduced in this analysis. 1053 patients had viral load measurements that were consistently measured at a LOD ≤50 copies/ml and thus were included in the analysis.

A total of 76 patients experienced at least one ‘disease progression event’ in the 12 months following a year of stable therapy, of whom 64 patients failed due to 2 consecutive viral loads above 50 copies/ml. The cumulative estimated probability of disease progression was 0.5% (95% CI 0.1, 0.9), 2.5% (95% CI 1.5, 3.5) and 7.5% (95% CI 7.9, 9.1) at 3, 6 and 12 months respectively.

As there has been debate about when to start therapy, and many treatment guidelines now state that treatment should be initiated at CD4 counts around 350/mm^3, the inclusion criteria were redefined to include only a subset of 1871 patients on a stable regimen with CD4 counts consistently 350/mm^3 or above. Disease progression events based on CD4 counts were defined as a CD4 count < 350/mm^3; all other definitions of failure remained the same. The results were similar to the main analysis. In the 12 months following baseline 3 patients failed due to CD4 count falling below 350/mm^3 and 102 patients experienced any ‘disease progression event’. The risk of the CD4 count falling below 350/mm^3 was 0, 0.1(95% CI 0, 0.2), and 0.2 (95% CI 0, 0.4) and of any ‘disease progression event’ was 0.3 (95% CI 0.1, 0.5), 1.9 (95% CI 1.3, 2.5) and 5.6 (95% CI 4.6, 6.6) at 3, 6, and 12 months respectively.

The results from the sensitivity analysis are summarised in table 3.7. For each of the three sensitivity analysis effect of the predictors on rate disease progression were similar. Although, the proportion of time on cART with a suppressed viral load was no longer significant when stable cART was defined as a maintaining a viral load <50 copies/ml.

Table 3.7 Summary of sensitivity analysis results

Sensitivity analysis	N included	N events	Multivariable cox proportional hazard model		Hazard Ratio	95% CI	p-value
Including treatment changes as disease progression	2237	142	Age	Per 10 yrs older	0.83	0.68-1.03	0.08
			% time with RNA <500 whilst on cART prior to baseline	≥70%	1.00	-	-
				<70%	2.85	1.92-4.24	<.0001
			Stopping all ARV's after baseline		22.17	8.94-54.99	<.0001
Stable cART defined as a viral load <50 copies/ml	1053	76	Age	Per 10 yrs older	1.09	0.97-1.23	0.16
			% time with RNA <500 whilst on cART prior to baseline	≥70%	1.00		
				<70%	1.35	0.90-2.01	0.14
			Stopping all ARV's after baseline		9.36	5.78-15.15	<.0001
Stable regimen defined as CD4 count >350 cells/mm^3	1871	102	Age	Per 10 yrs older	0.83	0.65-1.06	0.14
			% time with RNA <500 whilst on cART prior to baseline	≥70%	1.00	-	-
				<70%	2.90	1.80-4.69	<.0001
			Stopping all ARV's after baseline		12.85	4.61-35.78	<.0001

3.5 Discussion

Patients in this study were found to have a low chance of disease progression, measured in a variety of different ways, occurring in the next 3-6 months. The most common reason for disease progression was viral rebound. Very few of the disease progression events observed were 'serious'. The probability of developing an AIDS defining illness, non-AIDS defining illness, serious opportunistic infection or dying in the next 12 months was less than 1% among patients on a stable and fully suppressed cART regimen. The probability of the CD4 count dropping substantially to either below 200/mm^3 or below the CD4 at starting cART was also less than 1%. Our findings suggest that the interval between clinic visits in HIV-1 positive patients that have tolerated a fully effective cART regimen for extensive periods of time may be extended to 6 months.

It was surprising that more variables were not significant in the univariable analysis. However, as all patients in the study population were required to be on a stable treatment regimen for at least a year, with a suppressed viral load (<500 copies/ml) and a CD4 count above 200/mm^3, the reason for the lack of significant factors may be due to the initial selection criteria. A recent study analysed gender, age, level of education, marital status, mode of HIV acquisition, viral load, and CD4 cell count and found only gender and marital status to be significant predictors of treatment failure in patients who had already achieved suppression[474]. Another study looking at the risk of treatment failure after viral suppression for a year found that both gender and choice of regimen (PI vs NNRTI) were independent predictors of virological failure[475]. From the baseline characteristics (table 3.1) there did appear a lower proportion of males experiencing a disease progression event. Overall very few events were observed; repeating the analysis in a larger study population that is not predominately male, would increase the power and our ability detect if gender and other variables were significance predictors of a disease progression event. No treatment difference was found in our analysis, this again maybe due to our choice of patients.

Older age was found to lower the risk of experiencing a 'disease progression event', this is consistent with other studies that have reported that older patients are less likely to modify or discontinue cART[476;477], more likely to be more adherent to treatment[331] and have virologic success[478].

Patients who have spent less time with uncontrolled viremia whilst on cART were less likely to experience disease progression, and we identified that less than 70% of follow-up time with virological suppression was associated with an increased risk of disease progression. A previous study, by Lapadula et al.[479], considered disease progression in HIV positive patients with CD4 counts above 200 cells/mm^3 and found that time with undetectable viremia was a significant predictor in clinical progression. The authors speculated that the length and extent of viral replication may induce immune dysfunction which is not completely captured by CD4 cell count or completely reversed by cART.

The time a patient spends on cART with incomplete viral suppression could also be an indicator of a patient's adherence to treatment[480;481]. A strong relationship has been found between virological suppression and different measures of adherence[480;482;483]. Several studies have been published reporting the optimal level of adherence necessary to maintain virologic suppression, with varying results, from greater than 95% to 53%[331;337;482;484;485]. EuroSIDA has only recently begun collecting data on adherence and so this could not be studied directly in these analyses. However, all the patients had controlled viremia for over 1 year, and so must have been adherent to their regimen for some time. Previous studies have found a variety of reasons why patients may not be adherent to therapy. Common reasons are side effects, depression, relationship with physician or simply forgetting[330]. Different patient groups have also been shown to have different concerns about treatment and non-adherence[341]. Younger patients[331], regimens with a high pill burden[331] and patients with a history of intravenous drug use[330] have been found in some studies to be less adherent. Studies have found that including the patient in treatment decisions and trying to tailor their care to suit the individual patient are beneficial in improving patient adherence[330;341].

Treatment changes were not included as disease progression events in the main analysis. One of the inclusion criteria was to have been on a stable regimen for at least one year with no treatment changes.

Most short-term treatment related toxicities emerge within 6 months of starting treatment such as hypersensitivity, CNS, dyslipideamia, and mitochondrial toxicity[486;487]. Therefore the included patients were those who were not only on stable therapy, but also tolerating the chosen therapy well. Thus, treatment discontinuations not captured by virological failure were most likely to be a change due to convenience or concern for long term toxicity, and can be made at the next clinic visit in 3 or 6 months time. There may always be a risk of unexpected toxicities arising between clinic visits and patients with concerns should of course continue to present themselves immediately for assessment at the clinic responsible for their care.

Previous studies have investigated the required frequency of visits. A clinical trial investigated the risk of increasing the visit times in clinical trials associated with missing important laboratory related toxicities from every 8 weeks to every 16 or 24 weeks[488]. They concluded that study visits could not be safely increased without potentially harming the subject's health. However, the study population was predominately patients with a low CD4 count (median CD4 count at entry was 41cells/mm^3), who were not necessarily virologically suppressed and were on investigational drug regimens that require close monitoring. The authors speculated from their findings that it may be possible to safely increase the time between clinic visits in patients with a higher CD4 count. They found that a higher CD4 count at entry was associated with fewer drug toxicities being missed as those subjects were healthier in general with fewer opportunistic infections and better able to tolerate the medications. Additionally, the effect of an increase in missed drug toxicities was found to be more dramatic in the case of protease inhibitors. Another study investigated the interval between viral load monitoring and found that frequent HIV RNA monitoring (every 2 months) resulted in better treatment management, measured by improvement in HIV viral load suppression, compared with infrequent monitoring (twice yearly)[489]. However, differences in CD4 counts and overall survival were not statistically significant. This study included patients with low CD4 counts, high viral loads >5,000copies/ml and patients who had recently started treatment.

In contrast, our study focused on a very select patient population who would be expected, a priori, to have a low risk of disease progression and where the risks associated with less frequent clinical monitoring were much lower than those in previous studies.

Extending the time between clinic visits for example to 6 months would benefit the patients in several ways. Patients would save time and, if working, would take less time off for appointments. There would be fewer reminders to patients of their illness; reinforcing the view that HIV-1 can be thought of as a chronic long term illness in some patients. Considerable resources in the out-patient clinic setting could be saved by a less frequent monitoring of the stable patients, which is warranted due to increasing numbers of HIV-positive patients in most settings, be it in the developed or the developing world[2]. This would also allow clinicians to allocate more resources to those patients at greatest risk of treatment failure and clinical disease progression.

Finally, halving the number of tests performed each year for selected patients could substantially reduce costs for the monitoring of patients who are on stable therapy. For example, in EuroSIDA approximately 15% of the patients were maintaining a stable and fully suppressed cART regimen. Potentially, the cost of monitoring these patients would be cut by 50% each year, resulting in a substantial decrease in cost and resources that could be redistributed to patients in need of closer monitoring.

The current recommendation of monitoring every 3 months[311;450;451;453] is not necessarily to prevent clinical disease. Clinical diseases in this group of patients are now comparatively rare[490]. Rather, it is used to help identify virologic failure as early as possible after it first occurs to prevent the accumulation of drug resistance which limits future treatment options[491]. However, a recent study looking into cost effective monitoring strategies in developing countries found that the use of antiretroviral therapy without viral monitoring did not have marked detrimental effect on a patients development of resistance compared to CD4 count or clinical monitoring[492]. A consequence of increasing the time between clinic visits, is patients may spend longer with detectable viremia before it is identified, thus increasing the risk of developing resistance[322].

The importance of patients presenting for care as soon as they develop any new symptoms or have worries about any aspect of treatment should continue to be emphasized to patients. Clinicians may also choose to see patients on a more regular basis to consider comorbidities, detect/monitor long term adverse events, discuss risk reduction behaviours to reduce onward transmission or to emphasize adherence[452]. These results highlight the need for the individualization of the clinical management of patients.

There are a few limitations to this study which should be noted. We have no information on what is discussed between patient and clinician during each clinic visit, or the extent to which this is standardized across Europe. Part of the consultation might include counselling about adherence and minimizing the risk of transmission to others. The impact on adherence or risk of HIV transmission if patients were counselled at 6 monthly or 12 monthly intervals cannot be estimated from this study. It is possible that less frequent monitoring will result in poorer adherence, in turn leading to a higher risk of treatment failure[335;493]. In addition, there may be other factors which benefit the patient from more regular clinic visits that are unknown and therefore cannot be accounted for. Additionally, as mentioned earlier there were very few events observed, repeating the analysis in a study with a bigger sample size would increase the power. Also, the EuroSIDA cohort predominately consists of white men, looking at the frequency of monitoring required in other patients groups would also be of benefit.

To conclude, we have shown HIV-positive patients who are on a well-tolerated and fully suppressive cART regimen who have at least 12 months of complete viral suppression have a small risk of disease progression occurring over the next 6 months. Therefore in this subgroup of otherwise healthy patients it may be reasonable to consider increasing visit intervals from 3 months to 6 months.

3.6 After completion of analysis and publication results

Current treatment guidelines published in January 2011[494] recommend that, in patients with consistently suppressed viral loads whose CD4 cell count has increased well above the threshold for opportunistic infection risk, the CD4 count may be monitored every 6 to 12 months, unless there are changes in the patient's clinical status. The recommendations also state that adherent patients with suppressed viral load and stable clinical and immunologic status for >2–3 years, the interval between HIV RNA monitoring may be extended to every 6 months. However, at this time the recommendation was only based on expert opinion. This analysis provides some direct evidence to support these recommendations.

A manuscript of this analysis was published in AIDS in November 2008 and can be found in Appendix IV.

Chapter 4 History of viral suppression on cART as a predictor of virological failure after starting at least one new antiretroviral

4.1 Introduction

In the previous chapter considering the frequency of monitoring required for healthcare in an HIV-positive patient, the proportion of time a patient had spent with a suppressed viral load on cART prior to maintaining a stable and fully suppressed cART regimen was found to a strong predictor of the risk of future treatment failure. In addition, studies have found that increasing time with viral suppression decreases the risk of viral rebound[495;496], and that with increasing numbers of episodes of viral failures, the goal of viral suppression becomes harder to achieve[497]. Figure 4.1 is from Benzie et. al and shows the relative rate of viral rebound per additional regimen failed, stratified by duration of viral load suppression. An increased rate of viral rebound was found per additional regimen failed, but this association was reduced the longer patients had maintained suppression. Patients who had remained virologically suppressed for less than 1 year had a 43% increased chance of viral rebound per extra regimen failed. This decreased to 23% in those who remain suppressed for 1–2 years.

Figure 4.1 Relative rate of viral rebound (95% confidence interval), per additional antiretroviral regimen failed. Source Benzie et al.[496]

The aim of cART is the suppression of viral load for as long as possible[223;498]. Results from observational studies show that over 70% of those initiating cART from antiretroviral naïve achieve complete virological suppression within 6 months[499]. However, a significant proportion of patients fail to achieve viral suppression in the first 6 months of starting cART and many others go on to experience viral rebound sometime thereafter[500]. Many studies have looked into the risk factors and reasons for virological failure, either due to poor initial virological response to treatment or virological rebound. Table 4.1 summarises some of the factors these studies have identified.

Table 4.1 Various factors that have been found to be associated with virological failure

Factors investigated	Studies that found an increased risk of poor virological response	Studies that found an increased risk virological rebound
Female		Geretti et al.[475]
Higher Baseline HIV-RNA	Paredes et al.[478] Wood et al.[501] Phillips et al.[309] Moore et al.[502] Ledgergerber[466]	Le Moing et al.[503] Mocroft et al.[472]
Lower baseline CD4 count	Miller et al.[504] Moore et al.[502] Ledergerber et al. [466]	Benzie et al.[496] Miller et al.[504] Abgrall et al.[490] Le Moing et al.[503] Miller et al.[505]
Younger age	Paredes et al.[478] Weintrob et al.[506] Silverberg et al.[507]	Benzie et al.[496] Le Moing et al.[503] Mocroft et al.[472] Smith et al.[508]
Black ethnicity		Benzie et al.[496] Smith et al.[508]
Antiretroviral experienced at starting cART	Ledergerber et al[466]	Sungkanuparph et al.[509] Phillips et al.[495] Abgrall et al. [490] Collaboration of HIV Cohorts[510]
Less time with viral suppression		Phillips et al.[495] Benzie et al.[496]
Increasing number of previous treatment failures		Benzie et al.[496]
Starting cART earlier (calendar year)	Moore et al.[502] Ledergerber et al.[466]	Benzie et al.[496] Mocroft et al.[472] Smith et al.[508]
Previous treatment interruptions		Bansi et al.[511]
Poor adherence	Pharm[352] Paterson et al.[331] Liu[512] Mannheimer[513] Oette[332]	Walsh et al.[491] Bangsberg[514] Glass et al. [515] Le Moing[503]
Persistent low level viremia		Sungkanuparph et al.[509] Easterbrook et al.[516]

In clinical practice, around 70-95% of patients starting cART achieve an undetectable viral load[319;517;518], and this proportion has increased in recent years[320;321;502;518]. This increase is partly due to a high proportion of treatment failure in the early cART era being observed in patients who had previously been exposed to mono or dual therapy[131;495], who had already developed resistance to nucleoside drugs prior to initiating cART[519]. Additionally, newer antiretrovirals that are less toxic, easier to tolerate, have less stringent dietary requirements and have less complex dosing regimen have resulted in better adherence from the patients[520-523]. Furthermore, some new antiretrovirals are available in the same class without cross resistance to other antiretrovirals in the class[524;525]. In a retrospective study of 542 antiretroviral naïve patients initiating therapy at the University of Alabama, two periods of antiretroviral initiation were identified, prior and after August 2004[526]. The median durability, defined as duration of initial regimen, of cART regimens introduced after August 2004 was found to be 263 days longer than those who started prior to 2004. Once daily regimens had the longest durability; 543 days longer than twice daily regimens. Regimens were primarily discontinued due to toxicity rather than virologic failure[526]. Another study by the Swiss HIV cohort found that the durability of initial regimens has not significantly improved over time[518]. They reported that 48.8% of 625 patients during 2000–2001, 43.8% of 607 during 2002–2003, and 44.3% of 634 during 2004-2005 changed cART within 1 year (p=0.15). However, they did observe improvements in virological (viral load <50copies/ml) and immunological (increases in CD4 counts) outcomes[518].

Despite these improvements viral replication is still not fully controlled in all patients at all times. The three main reasons for this are interlinked, including treatment limiting toxicities, poor adherence, and the development of resistance, which are all discussed in detail in Chapter 1 section 1.8.3 ‘Limitations of cART’. Different combinations of drugs have substantially different short and long term toxicities[327], and in the current HIV-era most have been found to be moderate and can be well managed in outpatient clinics[326]. However, regardless of the severity of the adverse event experienced due to toxicities it may have an impact on adherence[330]. Patients who are more adherent to treatment are more likely to achieve sustained viral suppression[331;332;352;512;513] and are less likely to show signs of disease progression[331;333;336;337;527;528].

Poor adherence has been linked to an increased risk in the development of resistance[330]. However, certain regimens maybe more susceptible to development of resistance than others at differing levels of adherence[481].

Previous analyses have found that a patient is more likely to fail in the first few months after initial viral suppression[472]. Smith et al.[529] found that the risk of viral rebound 1-2 years, 2-3 years and >3 years after viral suppression was reduced by 70%, 79% and 86% respectively compared to the first year of viral suppression. One possible explanation for this finding is a selection effect, in which patients who were more likely to experience virological rebound have been selected out as the time from initial response has increased[472]. Those that experience more or potentially serious toxicities, are less adherent, or have developed a greater degree of resistance may thus have a rebound in viral load more quickly. Additionally, treatment interruptions with detectable viral load increase the risk of rebound [511], as does pre-cART exposure to nucleoside reverse transcript inhibitor (NRTI) regimens[495;530]. These factors all affect a patient's risk of treatment failure on a particular treatment regimen. With increasing numbers of episodes of viral failures the goal of viral suppression becomes harder to achieve[497] as on-going viral replication while receiving cART generally promotes the emergence of drug resistance which in turn compromises treatment options[531]. Further, having failed multiple lines of therapy may be an indicator that a person is more likely to be chronically poorly adherent[532].

In the previous chapter, treatment failure was defined as either, virological (viral load >500 copies/ml), immunological (CD4 count <200 cells/mm^3 or CD4 count < 100 cell/mm^3 CD4 count at starting cART), or clinical (the development of any AIDS defining illness, non-AIDS defining illness or death). However, the main reason for treatment failure was virological, with an estimated 4.6% (95% CI 3.7, 5.5) risk of virological failure after 12 months and less than a 1% probability of any of the other treatment failure defining events occurring in the 12 months after baseline.

It is important to understand what role prior virological suppression has on a patients' risk of future virological failure after a treatment switch. As mentioned earlier, the main aim of cART is the suppression of viral load for as long as possible, but this is just a means of achieving the real goal, which is ultimately to reduce the risk of patients developing AIDS or non-AIDS events and dying prematurely. Numerous studies have demonstrated the link between uncontrolled viral replication and the development of AIDS, non-AIDS defining illnesses and death[533-537]. Additionally, patients successfully treated with cART, achieving and maintaining a suppressed viral load, have been found to have the lowest mortality rates, which although higher than the general population are comparable to patients with other chronic conditions[13;538-542]. Therefore, if we can identify markers that may be used to help reduce the risk of future virological failure we can hopefully indirectly reduce the risk of mortality in HIV-positive patients on cART.

4.2 Aim

The aim of this chapter was therefore to investigate whether a patient's prior history of viral suppression on cART was predictive of future virological failure. In particular the aim was to investigate, in patients already on cART, whether after starting at least one new antiretroviral (ARV), for any reason, a patient's risk of future virological failure was associated with their previous history of viral suppression and whether different patterns of virological suppression and rebound after cART initiation were associated with differing risks of future virological failure. This may help guide clinicians as to whether certain patients are at an increased risk of virological failure after a treatment change, and may require closer monitoring and adherence counselling.

4.3 Methods

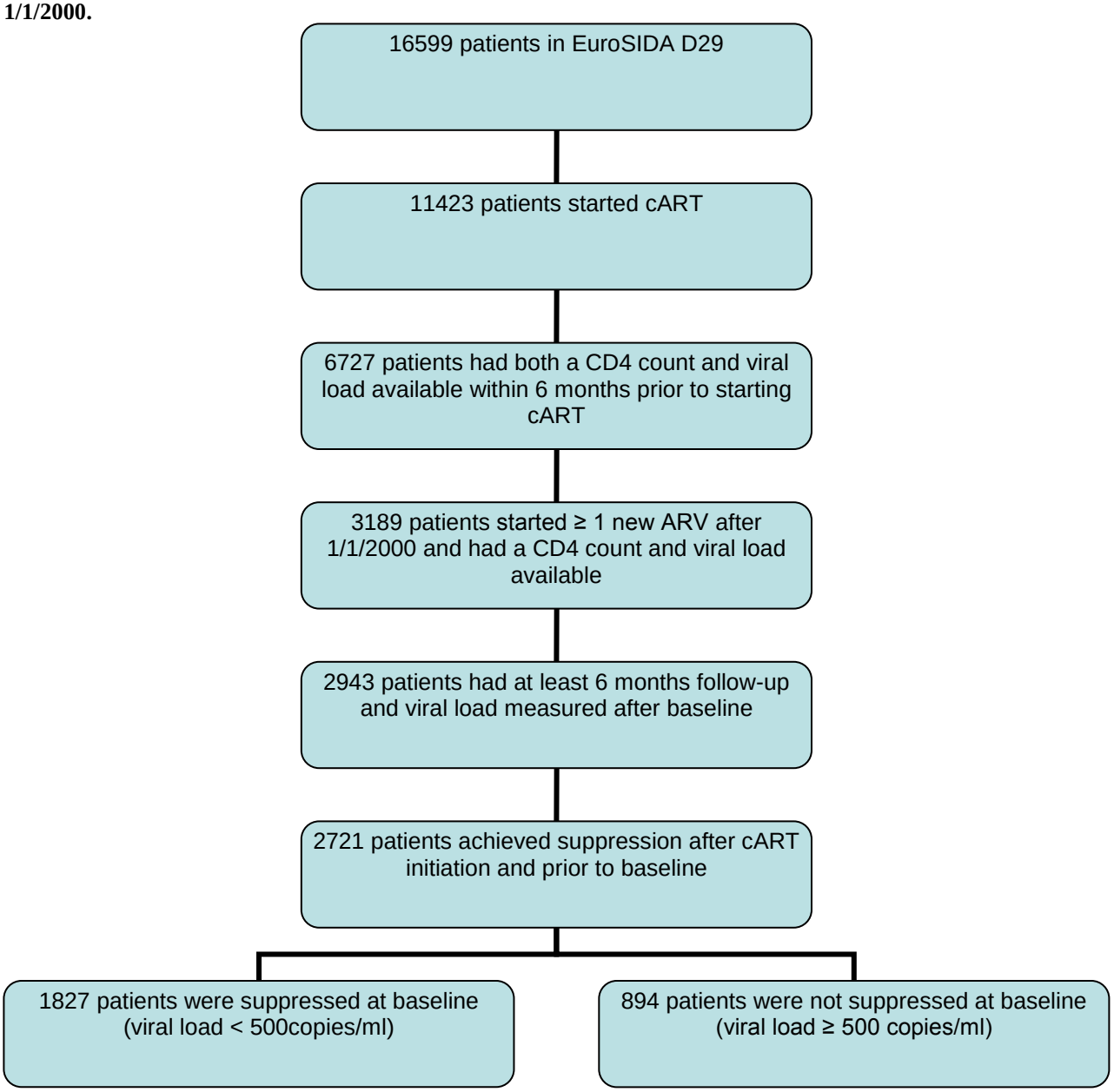
4.3.1 Patient selection

There were 16599 patients included in the D29 EuroSIDA update. Figure 4.2 shows how patients were selected for inclusion. All patients who were on cART and started any new ARV(s) $\geq$ 1st January 2000, during prospective follow-up, were included in the analysis. As this analysis is looking at prior patterns of virological suppression on cART, baseline was defined as the date of starting new ARV(s). cART was defined in the same way as the analysis in chapter 3, a regimen containing at least 3 antiretrovirals of which at least 2 must be nucleosides or nucleotides and 1 must be either a protease inhibitor or a non-nucleoside reverse transcript inhibitor. The 1st January 2000 was

chosen to ensure that all patients were on a contemporary cART regimen. Starting new ARV(s) could be for any reason apart from dose modification or formulation change i.e. switching from taking 2 lopinavir/ritonavir tablets (400mg/100mg) twice daily to 4 tablets (800mg/200mg) once daily or switching from taking lamivudine and zidovudine as individual drugs to taking combivir would not count as starting new ARV(s) changes. Patients could start as little as one new antiretroviral e.g. from one NNRTI to another because of toxicity, up to changing their whole regimen, e.g. the NRTI backbone and an NNRTI could be replace with a new NRTI backbone and a ritonavir boosted PI.

To ensure there was sufficient data to look backwards from baseline (time of starting new ARV(s)) at the patients history of viral suppression on cART up to the time starting new ARV(s), patients had to have been on cART for > 6 months prior to starting the new ARVs. Additionally, to allow for patients to have experienced virological failure after starting the new ARVs patients had to have at least 6 months follow-up and at least one viral load measurement after starting the new ARV(s). Furthermore, as we were interested in prior patterns of virological suppression, patients were required to have achieved viral suppression (viral load< 500 copies/ml) at least once after cART initiation and prior to starting new ARV(s). 2,721 patients started at least 1 new antiretroviral after 1/1/2000 and were included in the analysis. Patients starting new ARV(s) whilst virologically suppressed and those starting new ARV(s) whilst virologically failing were analysed separately. 1,827 patients were virally suppressed at the time of starting a new antiretroviral and 894 were virologically failing.

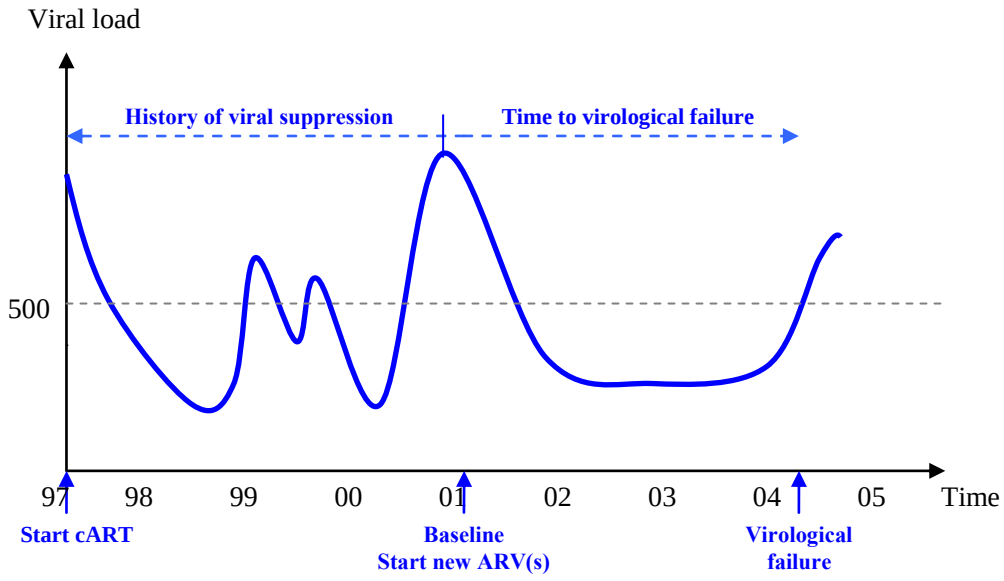
Figure 4.2: Inclusion criteria; baseline is defined as the date of first starting new ARV(s) ≥ after 1/1/2000.



To help explain the inclusion criteria, figure 4.3 gives an example of a patient (Mr Blue) included in the analysis. Mr Blue started cART in 1997 with an unsuppressed viral load. Mr Blue achieved a suppressed viral load around 8 months after starting cART. In 1999 he experienced two small viral rebounds before re-suppressing his viral load. He then experienced a third viral rebound in late 2000 and in early 2001 he started a completely new cART regimen. On this new cART regimen, Mr Blue quickly suppressed his viral load and it remained suppressed until spring 2004 when he experienced a viral rebound.

Baseline for Mr Blue is January 2001 and he would be in the unsuppressed group. His history of viral suppression is the period from starting cART prior to baseline. The time to virological failure after baseline would be 3 years and 2 months.

Figure 4.3: Mr Blue's pattern of viral suppression (an example of a patient included in the study)



4.3.2 Statistical analysis

Virological failure was defined as a viral load measured >500 copies/ml at least 4 months after baseline. Patient follow-up was measured from baseline to date of virological failure or date of last viral load measurement, whichever occurred first. Poisson regression analysis was used to investigate factors associated with virological failure after starting new ARVs. Potential explanatory variables included age, gender, year of starting cART, antiretroviral-naïve at starting cART, risk group, ethnicity, region of Europe, baseline CD4 count, CD4 nadir, peak viral load, previous AIDS diagnosis, time on cART, current treatment regimen, number of previous treatment regimens, time spent on cART prior to baseline, the number of antiretrovirals previously exposed to and the reason reported for stopping the previous antiretroviral in the regimen.

In addition to the traditional explanatory variables investigated above, variables that summarised the history of viral suppression after cART initiation prior to baseline were investigated. The variables used to summarise the history of viral suppression after cART initiation were:

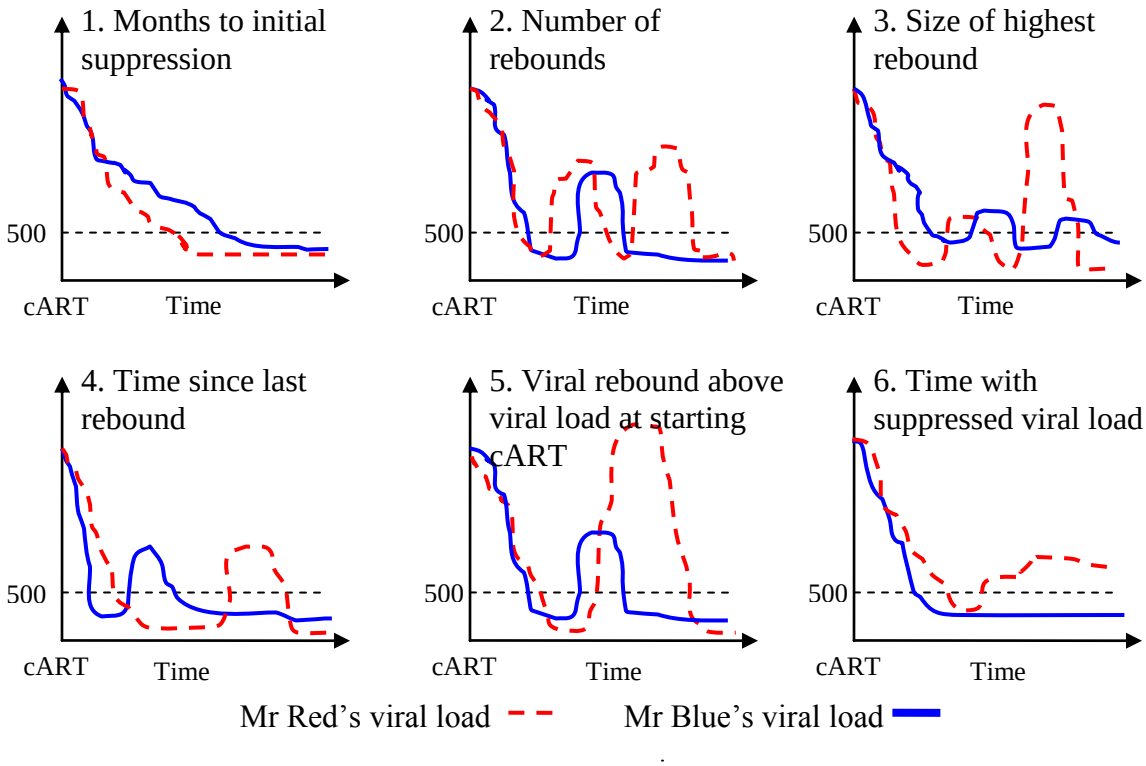
1. Months to initial suppression (HIV-RNA ≤ 500 copies/ml) after starting cART
2. Number of viral rebounds after initial suppression
3. Size of the highest viral rebound
4. Time since most recent viral rebound (for patients virologically suppressed at baseline)
5. Viral rebound above viral load at first starting cART (yes versus no)
6. Proportion of time spent with a viral load ≤ 500 copies/ml while receiving cART

Viral suppression was defined as a single measurement of HIV-RNA ≤ 500 copies/ml. Viral rebound was defined as a single viral load > 500copies/ml measured after a period of suppression prior to the regimen change. Variable 3 was investigated as a numerical variable and categorically as either no-rebound, low (500-1,000 copies/ml), medium (1,000-1,0000copies/ml) and high (>10,000). Variable 6, the proportion of time on cART with a suppressed viral load, was defined in the same way as in the previous chapter, any period of time where the patient was off cART or the first four months after starting/restarting a new cART regimen were excluded (see figure 3.2). Thus only time when the patient was on cART and should have been suppressed were included.

Any variable that was significant at the 10% level in the univariable model was then included in a multivariable model.

To explain why these variables were chosen figure 4.4 shows some of the different patterns of viral suppression that could be observed after cART initiation. Graph 1, for example, shows that after starting cART Mr Blue takes longer to suppress his viral load than Mr Red (variable 1). Graph 2 shows that Mr Blue experienced one viral rebound prior after starting cART, Mr Red experienced two viral rebounds (variable 2) and graph 6 shows that Mr Blue spent a longer time after starting cART with a suppressed viral load than Mr Red (variable 6). Variables 1-6 were thought to best describe the most commonly occuring different patterns that could be observed.

Figure 4.4: Summary of possible different patterns of viral suppression that could be observed in two fictional patients Mr Red and Mr Blue.



Sensitivity analysis considered confirmed virological failure after baseline (i.e. two consecutive viral load measurements above 500 copies/ml) and virological failure after baseline defined as a viral load above 50 copies/ml in the subgroup of patients who have viral load measurements, using an assay with a lower limit of detection of 50 copies/ml. Additionally, the analyses were also repeated in a sub-group of patients who had resistance data available at baseline. In this sensitivity analysis, the multivariable Poisson regression models were additionally adjusted for the patients genotypic sensitivity score (GSS) at baseline. Data from the most recent resistance test available prior baseline was used and the GSS was calculated using the REGA algorithm, version 7.1[344].

4.4 Results

4.4.1 Patient characteristics

2,721 patients were included in the analysis, 1827 (67%) patients were virologically suppressed and 894 (33%) were virologically failing at the time of starting new ARV(s). Table 4.2 describes the characteristics of these patients at baseline. There were significant differences between these two groups of patients. A higher percentage of patients virologically suppressed at baseline were ARV naïve at starting cART, 878 patients (48%) compared to 321 patients (36%) virologically failing, p<.0001. For those virologically suppressed at baseline, median CD4 count at baseline was 500cells/mm^3 (Inter-quartile range [IQR] 350-690) whereas those virologically failing had a lower median CD4 count 301cells/mm^3 at baseline (IQR 201-444), p<.0001.

Table 4.2: Baseline characteristics, baseline was defined as the date of starting new ARV(s) after 1/1/2000

		Virologically failing		Virologically suppressed		p-value
		N	%	N	%	Chi squared
Total		894	32.9	1827	67.1	
Gender	Male	639	71.5	1394	68.6	0.007
Race	White	132	14.8	246	13.5	0.36
Exposure group	Homosexual	317	41.5	841	46.0	0.006
	IDU	208	23.3	315	17.2	
	Heterosexual	261	29.2	550	30.1	
	Other	54	6.0	121	6.6	
Region of Europe	South/Argentina	309	34.6	433	23.7	<.0001
	Central	252	28.2	438	24.0	
	North	243	27.2	739	40.5	
	East	90	10.1	217	11.9	
Hepatitis B Status	HBV negative	758	84.8	1505	82.4	0.28
	HBV positive	51	5.7	123	6.7	
	Unknown	85	9.5	199	10.9	
Hepatitis C Status	HCV negative	556	62.2	1197	65.5	0.0008
	HCV positive	237	26.5	373	20.4	
	Unknown	101	11.3	257	14.1	
Naïve	Yes	321	35.9	878	48.1	<.0001
Previous AIDS	Yes	233	26.1	517	28.3	0.22
		Median	IQR	Median	IQR	Kruskal wallis
Age	years	40	36-47	42	37-50	<.0001
CD4	per mm^3	301	201-444	500	350-690	<.0001
base VL	log_{10}copies/ml	4.22	3.48-4.92	1.69	1.60-1.69	<.0001
cART CD4	per mm^3	245	130-375	220	102-335	<.0001
cART viral load	log_{10}copies/ml	4.43	3.56-5.06	4.54	3.60-5.20	0.07
CD4 nadir	per mm^3	150	60-240	142	54-238	0.17
Peak viral load	log_{10}copies/ml	5.13	4.67-5.60	4.94	4.34-5.42	<.0001
ARVs taken previously	Number	6	4-8	5	4-7	<.0001
Time since started cART	years	4.5	3.2-5.9	4.4	2.5-6.2	0.22

Table 4.3 shows the reasons for starting new ARV(s) and the regimens patients were on after starting new ARV(s). The majority (56%) of patients virologically suppressed at baseline were on a NNRTI after starting new ARV(s), whereas the majority (51%) of patients who were virologically failing at baseline were on a boosted PI regimen after starting new ARV(s). The main reason reported for starting new ARV(s) was toxicity or patient/physician choice in those who were virologically suppressed at baseline. In those virologically failing at baseline, 1/3 reported the reason for starting new ARV(s) as treatment failure and 1/3 toxicity or patient/physician choice. Of those virologically suppressed at baseline, 932 (51%) started only 1 new ARV at baseline and 349(19%) started a completely new cART regimen (≥3 ARVs). Of those virologically failing at baseline the majority of patients started a completely new cART regimen (67%), 167 patients (17%) virologically failing started only one new antiretroviral. The majority of additions without stopping any ARV(s) were due to patients adding ritonavir to a PI regimen.

Table 4.3: Regimen change characteristics

		Virologically failing		Virologically Suppressed		p-value
Total (N,%)		894	32.9	1827	67.1	
Baseline date (Median, IQR)	Month/Year	02/05	01/04-12/05	12/03	04/02-10/05	<.0001
Treatment started (N, %)	NNRTI	311	34.8	1029	56.3	<.0001
	PI	130	14.5	214	11.7	
	PI+ri	453	50.7	584	32.0	
Number of new antiretrovirals started (N, %)	1	167	16.7	932	51.0	<.0001
	2	124	13.9	546	29.9	
	3	453	50.7	295	16.1	
	4	150	16.7	54	3.0	
Reason for starting new ARV(s) (N, %)	TF	287	32.1	108	5.9	<.0001
	TOXPC	303	33.9	1089	59.6	
	Other/unknown	304	34.0	630	34.5	

* TF- treatment failure, TOXPC-toxicity or patient/physician choice,

4.4.2 Incidence of virological failure after starting new ARV(s) according to viral suppression history after cART initiation prior to baseline

After starting new ARV(s), 451(24.7%) patients virologically suppressed at baseline experienced virological failure, over median 3.0 years follow up, with an incidence rate [IR] of 7.3 per 100 person year of follow-up [PYFU] (95% confidence interval [CI] 6.7-8.0). 543 (60.7%) patients virologically failing at baseline experienced virological failure, over a median 1.28 years follow-up, with an IR of 28.3 per 100 PYFU (95% CI 25.9-30.7). Patients virologically suppressed at baseline had a 74% lower risk of virological failure after baseline compared to those virologically failing at baseline (unadjusted IRR 0.26, 95% CI 0.23-0.29, p<.0001).

Figure 4.5 Kaplan Meier risk of virological failure after baseline

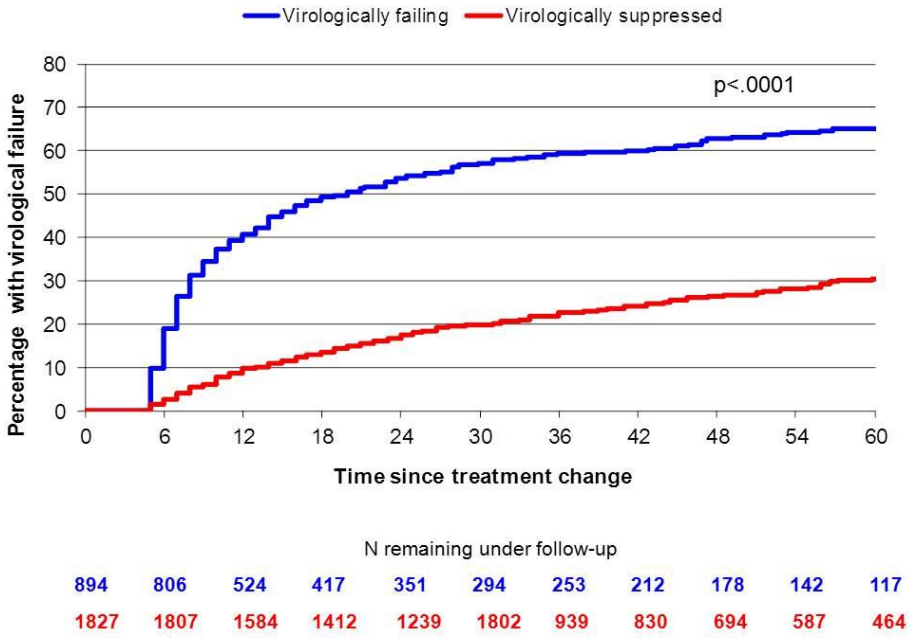


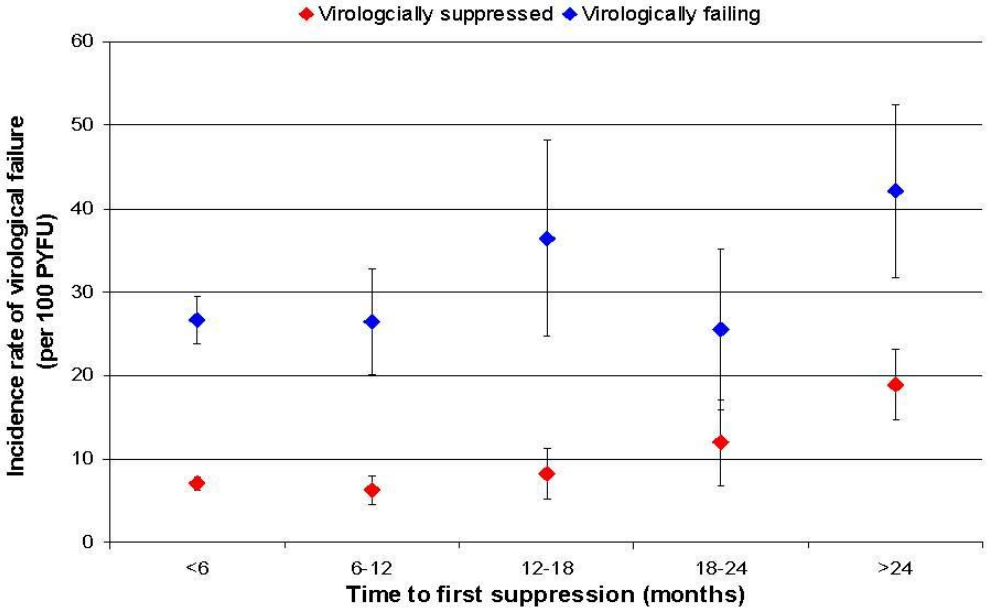
Figure 4.5 shows the Kaplan Meier risk of virological failure after baseline, patients virologically failing at baseline had a higher risk of virological failure after starting new ARV(s) (p<.0001). At 12 and 48 months respectively, those virologically failing had a 48.4% (95%CI 45.1-51.7) and a 54.6% (95% CI 51.2-58.0) risk of virological failure after starting new ARV(s). Patients virologically suppressed at baseline had a 9.8% (95% CI 8.74-11.2) and an 18.0% (95%CI 16.2-19.8) risk of virological failure, 12 and 48 months after starting new ARV(s).

4.4.3 Variables describing the prior history of virological suppression

4.4.3.1 Months to initial suppression (HIV-RNA ≤ 500 copies/ml) after starting cART

Those who were virologically suppressed at baseline had a median time to first suppression after cART initiation of 3.0 months (IQR 1.3-7.4). Patients who were virologically failing at baseline took slightly longer time to achieve suppression after first initiating cART, median 3.6 months (IQR 2.0 -10.1), p=0.001. Figure 4.6 shows the incidence rate of virological failure by time to first suppression. Patients who took longer to achieve initial suppression after cART initiation had an increased rate of virological failure after baseline, IRR 1.04 per 6 months longer to achieve suppression (95% CI 0.99-1.09, p=0.14) in those suppressed at baseline and IRR 1.06 per 6 months longer to achieve suppression (95% CI 1.02-1.10, p=0.003) for those virologically failing at baseline. This increased rate was only significant in patients who were virologically failing at baseline. However the incidence rate ratios were similar for both groups.

Figure 4.6: Incidence rate of virological failure and 95% confidence intervals for time to first suppression after cART initiation

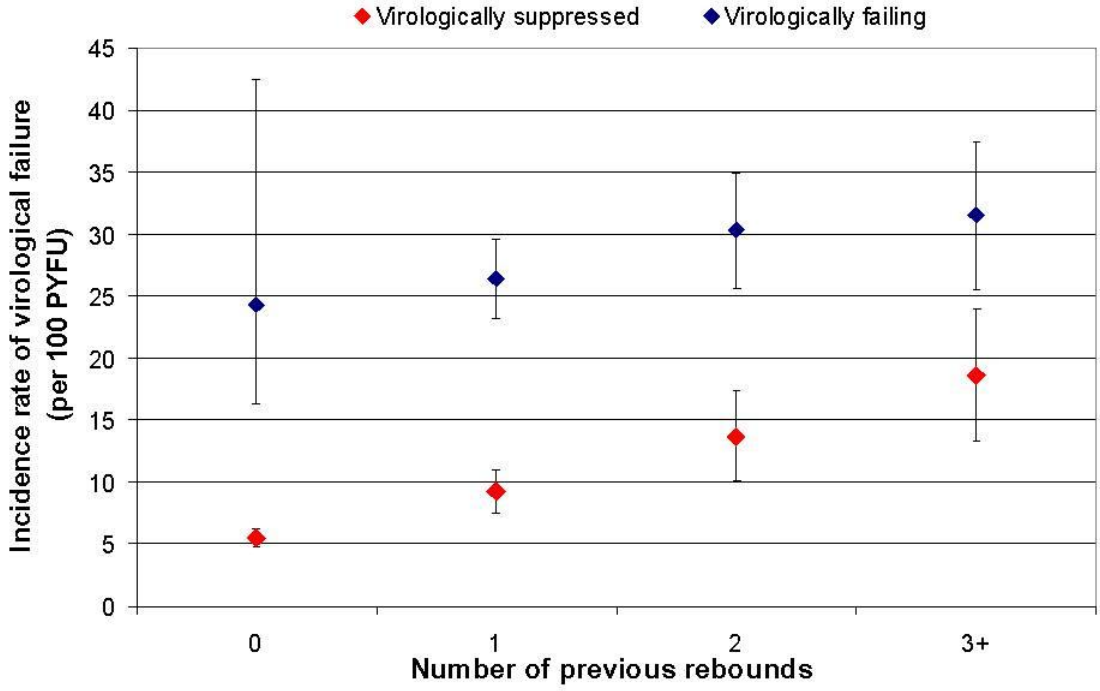


Virologically suppressed (N,%)	1288 (70.5%)	237 (13.0%)	111 (6.1%)	55 (3.0%)	136 (7.4%)
Virologically failing (N,%)	582 (65.1%)	122 (13.6%)	55 (6.2%)	47 (5.3%)	88 (9.8%)

4.4.3.2 Number of viral rebounds after initial suppression

1,238 (67.8%) patients virologically suppressed at baseline had not experienced a viral rebound prior to baseline after initial suppression from cART initiation. 30 (3.4%) patients virologically failing at baseline experienced their first rebound at the time of starting new ARV(s). Figure 4.7 shows the rate of virological failure after baseline by the number of viral rebounds the patient had experienced prior to baseline. There was a 41% increased rate of virological failure after baseline for each viral rebound experienced prior to baseline (IRR 1.41, 95%CI 1.31-1.51) in those virologically suppressed at baseline. In those virologically failing at baseline there was a 6% increased rate of virological failure after baseline for each viral rebound experienced prior to baseline (IRR 1.06, 95%CI 0.99-1.14, p=0.11).

Figure 4.7 Incidence rate of virological failure and 95% confidence intervals by number of viral rebounds experienced prior to baseline

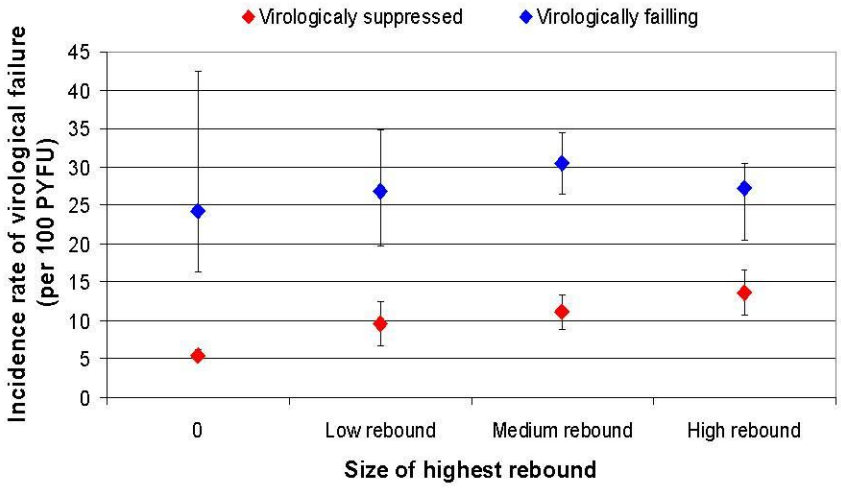


Virologically suppressed (N,%)	1238 (67.8%)	358 (19.6%)	130 (7.1%)	101 (5.5%)
Virologically failing (N,%)	30 (3.4%)	455 (50.9%)	248 (27.7%)	101 (5.5%)

4.4.3.3 Size of the highest viral rebound

Of those patients virologically suppressed at baseline who experienced a rebound, 206 patients (35%) had experienced a high viral rebound (>10,000 copies/ml) a higher proportion, 434 (49%) of patients virologically failing at baseline had experienced a high viral rebound, p<.0001. In patients virologically suppressed at baseline those who had a low viral rebound (501-1,000 copies/ml) prior to baseline had a 30% lower rate of virological failure after baseline (IRR 0.70, 95% CI 0.49-1.01, p=0.06) and those who had a medium viral rebound (1,001-10,000 copies/ml) had a 18% lower rate (IRR 0.82, 95%CI 0.60-1.10, p=0.19) compared to patients who had experienced a high viral rebound (>10,000 copies/ml) prior to baseline (Figure 4.8). The median maximum rebound in those virologically supressed at baseline who had experienced a prior rebound was 3,400 copies/ml (IQR 1,100-25,600), compared to 10,250 copies/ml (IQR 2,301-51,000) in those virologically failing at baseline. Fitted continuously rather than categorically, patients suppressed at baseline had 21% higher rate of virological failure per log10 increase in size of highest rebound (95% CI 1.15-1.26, p<.0001). There was no significant difference in the size of the highest rebound patients virologically failing at baseline had experienced and the rate of future rebounds either fitted categorically (p=0.53) or continuously (p=0.70).

Figure 4.8 Incidence rate of virological failure and 95% confidence intervals by size of highest rebound

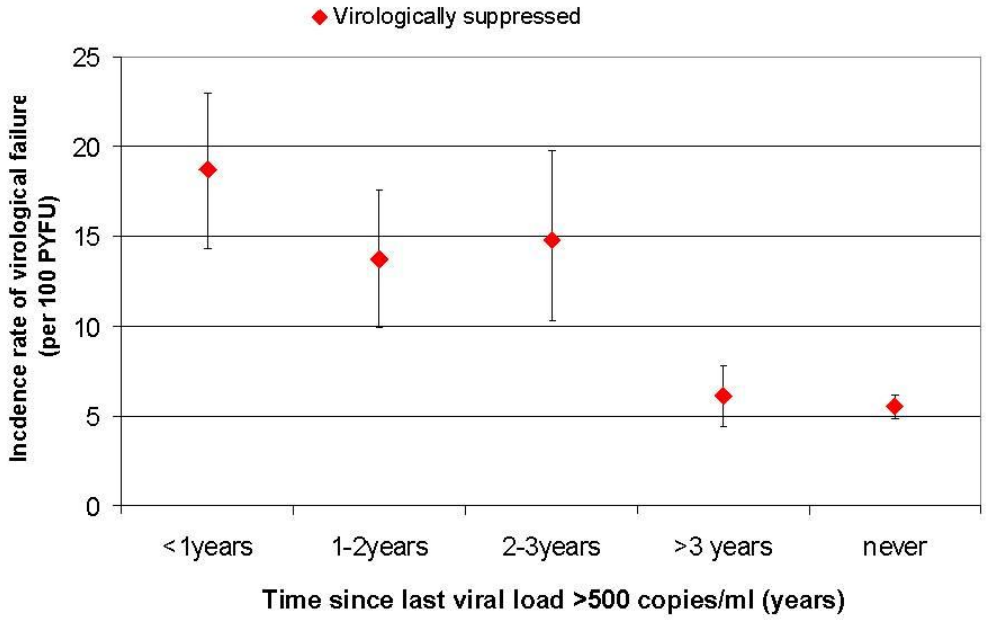


Virologically suppressed (N,%)	1238 (67.8%)	136 (7.4%)	247 (13.5%)	206 (11.3%)
Virologically failing (N,%)	30 (3.4%)	87 (9.7%)	343 (38.4%)	434 (48.6%)

4.4.3.4 Time since most recent viral rebound (for patients suppressed at baseline)

Time since last rebound was not investigated as a predictor of virological failure after baseline in patients with virological failure at baseline, as by definition they were all failing at baseline. In those who were virologically suppressed at baseline, 137 (23.2%) patients had experienced a viral rebound in the year prior to baseline. In those who had experienced a rebound after cART initiation, the median time since last viral rebound was 2.3 years (IQR 1.1-4.0). There was a higher rate of virological failure in patients who had virally rebounded more recently before baseline (figure 4.9). For example, patients who had virally rebounded in the year prior to baseline had a 3.4 times higher rate of virological failure compared to patients who had never virally rebounded (IRR 3.37, 95% CI 2.59-4.39, p<.0001), whereas there was no significant difference in the rate of virological failure in patients whose last viral rebound was more than 3 years prior to baseline and those who had never rebounded (IRR 1.10, 95%CI 0.81-1.49, p=0.54).

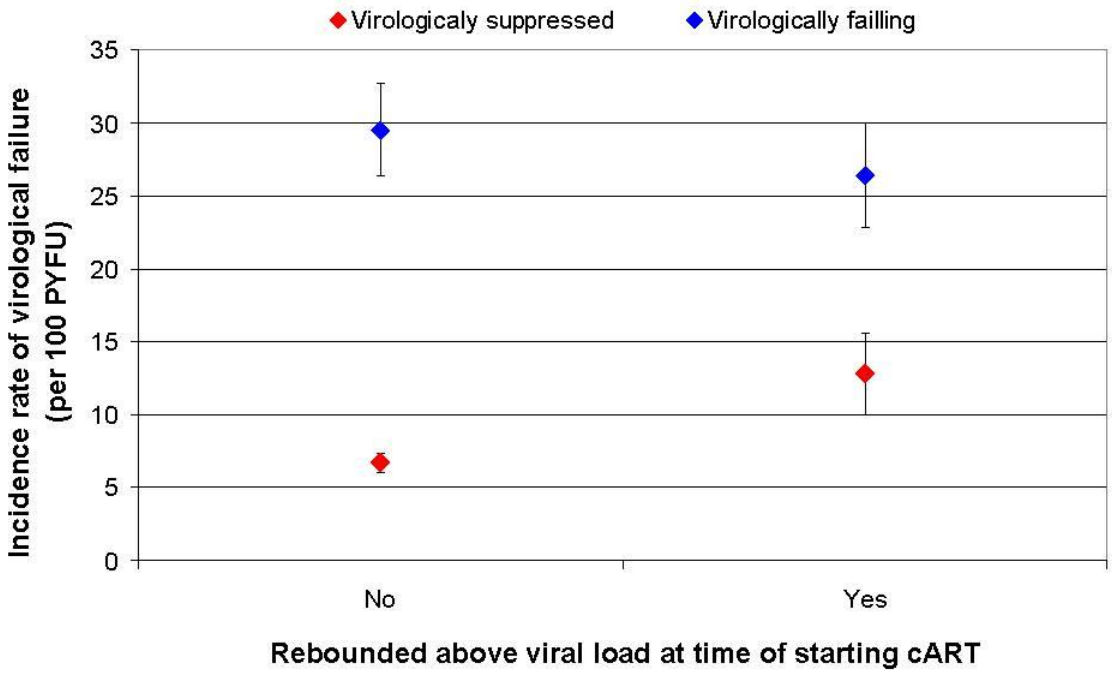
Figure 4.9 Incidence rate of virological failure and 95% confidence intervals by time prior to baseline since last viral rebound



4.4.3.5 Viral rebound above viral load at first starting cART (yes versus no)

212 patients (11.6%) virologically suppressed and 353 patients (39.5%) virologically failing at baseline had experienced a viral rebound above their viral load at the time of starting cART. Figure 4.10 shows that patients virologically suppressed at baseline had almost double the rate of virological failure after baseline if they had experienced a rebound above their viral load at starting cART (IRR 1.91, 95% CI 1.51-2.42, p<.0001). However, restricting this analysis to only those who had rebounded prior to baseline this effect was no longer significant (IRR 1.18, 95%CI 0.90-1.56, p=0.23). There was no significant difference between those who had experienced a viral rebound above their viral load at starting cART and those who had not in patients who were virologically failing at baseline (IRR 0.89, 95%CI 0.75-1.06,p=0.21).

Figure 4.10 Incidence rate of virological failure and 95% confidence intervals by whether or not the patients had experienced a rebound above their viral load at starting cART

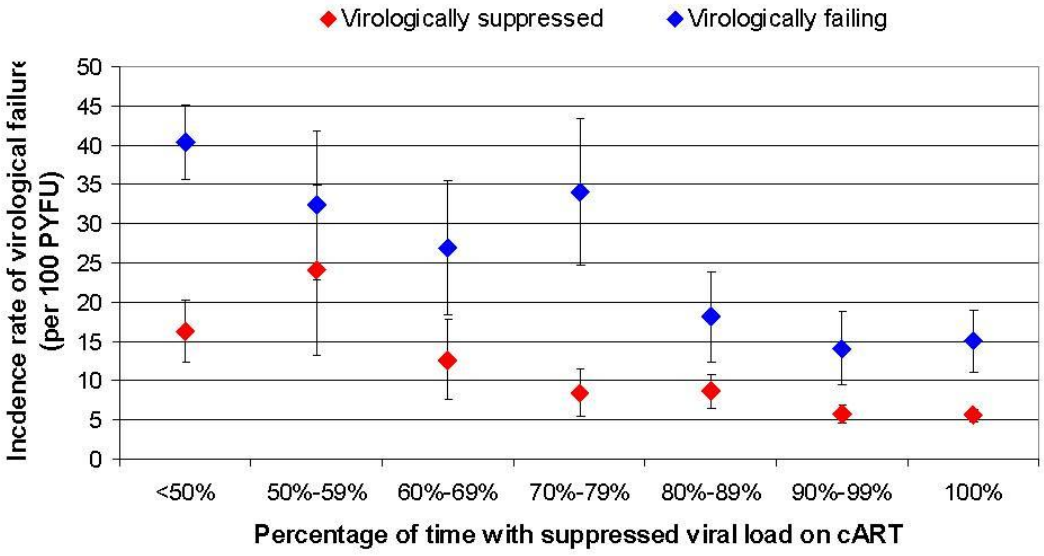


Virologically suppressed (N,%)	1615 (88.4%)	212 (11.6%)
Virologically failing (N,%)	541 (60.5)	353 (39.5%)

4.4.3.6 Proportion of time spent with a viral load ≤ 500copies/ml while receiving cART

Overall, virologically suppressed patients had spent a median of 98% (IQR 86%-100%) of time on cART suppressed (viral load <500 copies/ml) after cART initiation. Patients virologically failing at baseline had spent a lower percentage of time on cART suppressed (viral load <500 copies/ml) after cART initiation, median 58% (IQR 22%-90%), p<.0001. There was a decreased risk of virological failure with increasing percentage of time a patient had spent with a suppressed viral load whilst on cART prior to baseline (figure 4.11). Of those suppressed at baseline, patients who were suppressed for <50% of the time they were on cART had almost a 3 times higher rate of virological failure compared to patients suppressed more than 90% of the time they were on cART (IRR 2.91, 95% CI 2.23-3.81, p<.0001). Similarly, in those who were virologically failing at baseline patients who were suppressed for <50% of the time they were on cART had almost a 2.5 times higher rate of virological failure compared to patients suppressed more than 90% of the time they were on cART (IRR 2.57, 95% CI 2.07-3.25, p<.0001).

Figure 4.11 Incidence rate of virological failure and 95% confidence interval by the percentage of time with a supressed viral load



Virologically suppressed (N,%)	146 (8.0%)	31 (1.7%)	63 (3.5%)	96 (5.3%)	209 (11.4%)	445 (25.4 %)	837 (45.8%)
Virologically failing (N,%)	386 (43.2%)	68 (7.6%)	61 (6.8%)	73 (8.2%)	77 (8.6%)	90 (10.1%)	139 (15.6%)

4.4.4 Demographic predictors of virological failure after starting new ARV(s)

In addition to the variables describing the patients' history of viral suppression prior to baseline, demographic variables found in analysis to be associated with rate of virological failure were also included in the analysis. Table 4.4 shows the results of the univariable analysis and multivariable Poisson regression analysis for patients virologically suppressed at baseline. Demographic variables associated with rate of virological failure after baseline in patients virologically suppressed were, gender, age, HIV exposure group, region of Europe, Hepatitis C status, being naïve at starting cART, having been diagnosed with AIDS previously, CD4 nadir, CD4 count at starting cART, time on cART prior to baseline, number of ARVS exposed to prior to baseline, date of baseline, treatment regimen on at baseline, the reason for starting new ARV(s) at baseline and the number of new drugs started.

Similarly, table 4.5 shows the results of the univariable and multivariable Poisson regression analysis for patients who were virologically failing at baseline. Demographic variables associated with rate of virological failure after baseline in patients virologically failing were, region of Europe, Hepatitis C status, being naïve at starting cART, age, baseline CD4 count, viral load at starting cART, date of baseline, date of starting cART, number of ARVS exposed to prior to baseline, the reason for starting new ARV(s) at baseline and the number of new drugs started.

These two multivariable models were used to find the best fitting model including the variables describing the patients history for virological suppression.

Table 4.4 Poisson regression analysis investigating demographic predictors of virological failure in patients virologically suppressed at baseline

		Virologically suppressed					
		Univariable			Multivariable		
		IRR	95% CI	Global p-value	IRR	95% CI	p-value
Gender	Male	1.00		<.0001	1.00		
	Female	1.54	1.26-1.88		0.99	0.77-1.27	0.93
Race	White	1.00		0.36			
	Other	1.13	0.87-1.47				
Exposure group	Homosexual	1.00		<.0001	1.00		
	IDU	2.34	1.84-2.97		1.26	0.90-1.76	0.18
	Heterosexual	1.74	1.40-2.17		1.51	1.15-1.97	0.002
	Other	0.99	0.64-1.53		0.90	0.57-1.42	0.65
Region	South	1.00		<.0001	1.00		
	West	0.55	0.43-0.71		0.60	0.46-0.78	0.0001
	North	0.40	0.32-0.51		0.52	0.41-0.66	<.0001
	East	0.46	0.32-0.67		0.54	0.37-0.80	0.001
Hepatitis B status	Negative	1.00		0.85			
	Positive	1.06	0.75-1.51				
	unknown	0.94	0.70-1.26				
Hepatitis C status	Negative	1.00		<.0001	1.00		
	Positive	2.05	1.66-2.52		1.55	1.15-2.08	0.004
	Unknown	0.57	0.43-0.76		0.66	0.47-0.92	0.01
Naïve at start cART	No	1.00		0.0003	1.00		
	Yes	0.71	0.58-0.85		1.02	0.81-1.29	0.84
Prior AIDS diagnosis	No	1.00		0.04	1.00		
	Yes	0.80	0.64-0.98		0.90	0.72-1.14	0.38
Regimen	Single PI	1.00		0.002	1.00		
	Boosted PI	0.74	0.56-0.98		0.87	0.65-1.16	0.33
	NNRTI	0.62	0.48-0.81		0.70	0.54-0.92	0.009
Reason for stopping	TF	1.00		0.002	1.00		
	TOXPC	0.58	0.41-0.81		1.11	0.66-1.87	0.68
	Other/unknow	0.73	0.52-1.04		0.87	0.62-1.23	0.43
Age	per 10 years	0.76	0.69-0.84	<.0001	0.82	0.73-0.92	0.0005
Base CD4	per 2 fold increase	1.02	0.91-1.14	0.75			
cART CD4	per 2 fold increase	1.09	1.04-1.15	0.0008			
CD4 nadir	per 2 fold increase	1.15	1.08-1.23	<.0001	1.13	1.05-1.21	0.001
cART viral load	per $\log_{10}$ increase	0.96	0.89-1.04	0.31			
Peak viral load	per $\log_{10}$ increase	0.94	0.85-1.04	0.25			
Baseline date	per year	0.90	0.85-0.95	0.0002	0.92	0.86-0.97	0.004
Date started cART	per year	0.98	0.94-1.02	0.29			
Time on cART	per year	0.96	0.92-1.00	0.07	1.00	0.95-1.05	0.90
Number new drugs started	per drug	1.10	0.99-1.22	0.07	1.03	0.92-1.14	0.65
Number of drug exposed to	per drug	1.10	1.06-1.14	<.0001	1.11	1.06-1.17	<.0001

* IDU – intravenous drug user, TF- Treatment failure, TOXPC – toxicity or patient/physician choice

Table 4.5 Poisson regression analysis investigating demographic predictors of virological failure in patients virologically failing at baseline

		Virologically failing					
		Univariable			Multivariable		
		IRR	95% CI	Global p-value	IRR	95% CI	p-value
Gender	Male	1.00		0.40			
	Female	1.08	0.90-1.30				
Race	White	1.00		0.66			
	Other	1.05	0.84-1.32				
Exposure group	Homosexual	1.00		0.15			
	IDU	1.28	1.04-1.58				
	Heterosexual	0.98	0.80-1.21				
	Other	1.15	0.79-1.69				
Region	South	1.00		0.002	1.00		
	West	0.84	0.68-1.03		0.78	0.63-0.97	0.02
	North	0.66	0.53-0.81		0.61	0.49-0.76	<.0001
	East	0.88	0.64-1.20		1.07	0.76-1.51	0.69
Hepatitis B status	Negative	1.00		0.20			
	Positive	1.01	0.70-1.46				
	unknown	1.30	0.99-1.72				
Hepatitis C status	Negative	1.00		0.01	1.00		
	Positive	1.31	1.08-1.58		1.16	0.95-1.42	0.15
	Unknown	0.96	0.73-1.27		1.23	0.92-1.64	0.16
Naïve at start cART	No	1.00			1.00		
	Yes	0.69	0.57-0.83	<.0001	0.81	0.64-1.02	0.06
Prior AIDS diagnosis	No	1.00		0.25			
	Yes	1.12	0.93-1.35				
Regimen	Single PI	1.00		0.38			
	Boosted PI	1.01	0.79-1.29				
	NNRTI	0.89	0.68-1.15				
Reason for stopping	TF	1.00		0.24			
	TOXPC	1.10	0.89-1.35				
	Other/unknow	0.92	0.75-1.13				
Age	per 10 years	0.87	0.79-0.96	0.004	0.86	0.78-0.95	0.003
Base CD4	per 2 fold increase	0.91	0.85-0.97	0.003	0.94	0.89-1.00	0.06
Base viral load	per $\log_{10}$ increase	1.02	0.93-1.12	0.63			
cART CD4	per 2 fold increase	0.98	0.93-1.02	0.28			
CD4 nadir	per 2 fold increase	0.96	0.91-1.01	0.14			
cART viral load	per $\log_{10}$ increase	1.09	1.01-1.18	0.02	1.18	1.08-1.28	0.0001
Peak viral load	per $\log_{10}$ increase	1.08	0.96-1.23	0.22			
Baseline date	per year	0.89	0.84-0.94	<.0001	0.91	0.85-0.96	0.001
Date started cART	per year	0.95	0.91-0.99	0.03	1.03	0.98-1.08	0.27
Time on cART	per year	0.97	0.93-1.01	0.11			
Number new drugs started	per drug	0.81	0.74-0.88	<.0001	0.86	0.79-0.94	0.0008
Number of drug exposed to	per drug	1.11	1.08-1.15	<.0001	1.12	1.08-1.16	<.0001

* IDU – intravenous drug user, TF- Treatment failure, TOXPC – toxicity or patient/physician choice

4.4.5 Risk of virological failure after starting new ARV(s) in patients virologically suppressed when starting new ARV(s)

The results of the univariable Poisson regression analysis investigating each variable describing the history of virological suppression as a predictor of future virological failure are shown in table 4.6. Each variable describing the history of virological suppression was then entered separately into the multivariable model, developed in section 4.4.4, that included all the demographic variables. The results of these models are shown in table 4.6.

In each of the 6 models, after adjustment for demographic variables: gender, HIV exposure group, region of Europe, hepatitis C status, naïve, prior AIDS diagnosis, baseline treatment regimen, reason for starting new ARV(s), age, CD4 nadir, baseline date, time on cART, number of new antiretrovirals started, and number of antiretrovirals previously exposed to the variable describing history of virological suppression was significantly associated with the rate of future virological failure (table 4.6).

These 6 variables describing the history of virological suppression were highly correlated with each other. Therefore, the best fitting model was selected using stepwise selection. This model contained variable 4: the time since last rebound, and variable 6: the percentage of time on cART with a suppressed viral load, in addition to the demographic variables already selected. The results of this model are shown in figure 4.12.

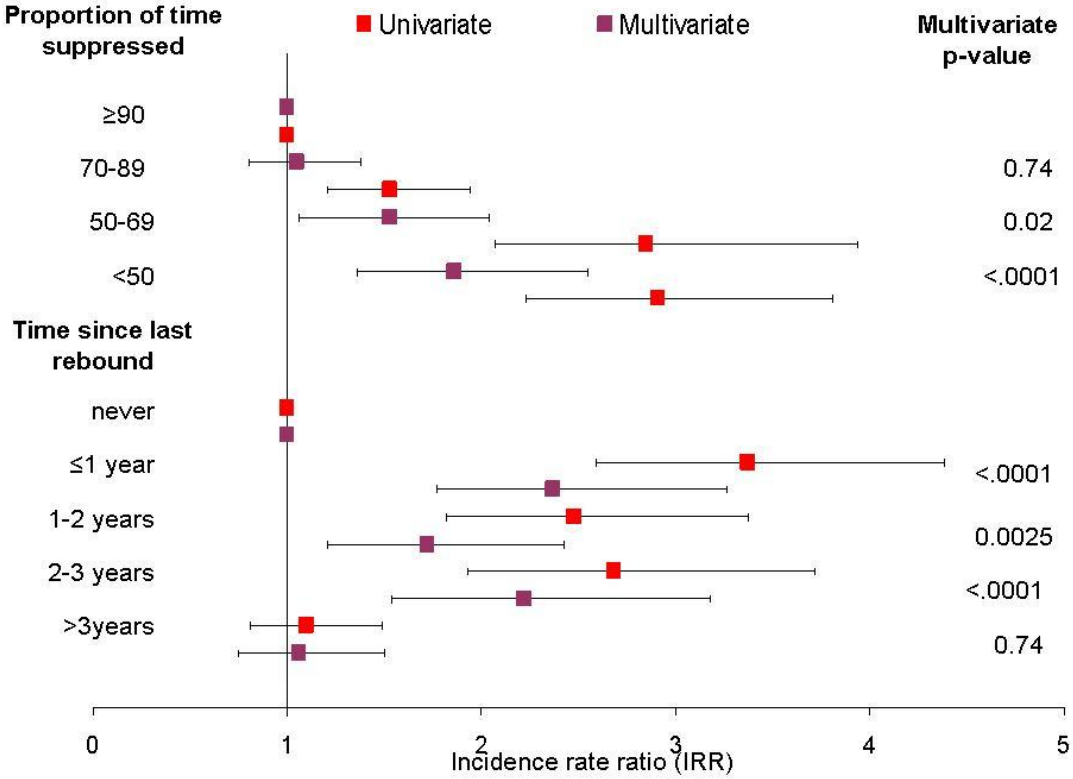
Table 4.6 Poisson regression analysis investigating history of virological suppression as a predictor of virological failure in patients virologically suppressed at baseline

		Virologically suppressed					
		Univariable			Multivariable*		
		IRR	95% CI	Global p-value	IRR	95% CI	p-value
1. Months to initial suppression	Per 6 months	1.04	0.99-1.09	0.13	1.05	1.00-1.11	0.05
2. Number of rebounds after initial suppression	No rebound	1.00		<.0001	1.00		
	1	1.67	1.34-2.10		1.62	1.27-2.06	<.0001
	2	2.48	1.85-3.34		2.52	1.82-3.49	<.0001
	≥3	3.37	2.47-4.59		3.16	2.21-4.53	<.0001
3. Size of highest rebound	>10000	1.00		<.0001	1.00		
	1001-10000	0.82	0.60-1.10		0.70	0.52-0.96	0.02
	500-1000	0.70	0.49-1.01		0.65	0.44-0.94	0.02
	No rebound	0.41	0.32-0.52		0.39	0.30-0.53	<.0001
4. Time since last rebound	Never	1.00		<.0001	1.00		
	≤1year	3.37	2.59-4.39		2.69	2.02-3.56	<.0001
	1-2 years	2.48	1.82-3.37		1.98	1.43-2.74	<.0001
	2-3 years	2.68	1.93-3.72		2.31	1.63-3.29	<.0001
	>3years	1.10	0.81-1.49		1.10	0.78-1.54	0.57
5. Viral rebound above viral load at starting cART	No	1.00		<.0001	1.00		
	Yes	1.91	1.51-2.43		1.46	1.12-1.89	0.004
6. Percentage of time with suppressed viral load	≥90	1.00		<.0001	1.00		
	70-89	1.53	1.21-1.94		1.35	1.05-1.73	0.02
	50-69	2.85	2.06-3.94		2.35	1.66-3.28	<.0001
	<50	2.91	2.23-3.81		2.25	1.66-3.04	<.0001

***Multivariable model also adjusted for gender, HIV exposure group, region of Europe, hepatitis C status, naïve, prior AIDS diagnosis, baseline treatment regimen, reason for starting new ARV(s), age, CD4 nadir, baseline date, time on cART, number of new antiretrovirals started, and number of antiretrovirals previously exposed to.**

Figure 4.12 shows the adjusted models describing risk of virological failure after starting new ARV(s) in patients who were virologically suppressed at baseline. There was no significant difference in the rate of virological failure in patients whose last viral rebound was more than 3 years prior to baseline (IRR 1.06, 0.75-1.50, p=0.74), whereas patients who had virally rebounded in the year prior to baseline had a 2.4 times higher rate of virological failure after baseline compared to patients who had never rebounded (IRR 2.37, 95%CI 1.74-3.21, p<.0001). The lower the percentage of time a patient had spent suppressed prior to baseline, the higher the rate of virological failure. Patients who had spent less than 50% of the time they were on cART prior to baseline with a suppressed viral load had a 86% (95% CI 1.36-2.53, p<.0001) higher rate of virological failure after baseline compared to patients who were suppressed more than 90% of the time they were on cART. Additionally, older patients had a lower rate of virological failure (IRR 0.84 per 10 years older, 95% CI 0.75-0.94, p=0.002). Patients with a higher CD4 nadir had an increased rate of virological failure (IRR 1.13 per 2 fold increase, 95%CI 1.05-1.22, p=0.0008). Furthermore, the more antiretrovirals a patient had been exposed to prior to baseline the higher the rate of virological failure (IRR 1.06 per drug, 95%CI 1.01-1.12, p=0.03). Patients on a boosted PI containing cART regimen had a 24% (IRR 0.76, 95% CI 0.57-1.01, p=0.05) and patients on a NNRTI regimen had a 30% (IRR 0.70, 95% CI 0.54-0.91, p=0.008) lower rate of virological failure compared to patient on a non-boosted PI regimen. Patients in north Europe had a 39% lower rate of virological failure compared to patients in the south Europe (IRR 0.61, 95%CI 0.417-0.78, p<.0001). After adjustment for these variables, none of the other variables describing the patients history of viral suppression and rebound prior to baseline were independently associated with the risk for future virologically failure after starting new ARV(s).

Figure 4.12 Univariable and multivariable Poisson regression models for patients virologically suppressed at baseline



Multivariable model also adjusted for gender, HIV exposure group, region of Europe, hepatitis C status, naïve, prior AIDS diagnosis, baseline treatment regimen, reason for starting new ARV(s), age, CD4 nadir, baseline date, time on cART, number of new antiretrovirals started, and number of antiretrovirals previously exposed to.

4.4.6 Risk of virological failure after starting new ARV(s) in patients virologically failing when starting new ARV(s)

Table 4.7 shows the results of the univariable and multivariable Poisson regression analysis for each variable describing the history of virological suppression, in patient who were virologically failing at baseline. Each variable describing the history of virological suppression was entered separately into the multivariable model, developed in section 4.4.4, that included all the demographic variables.

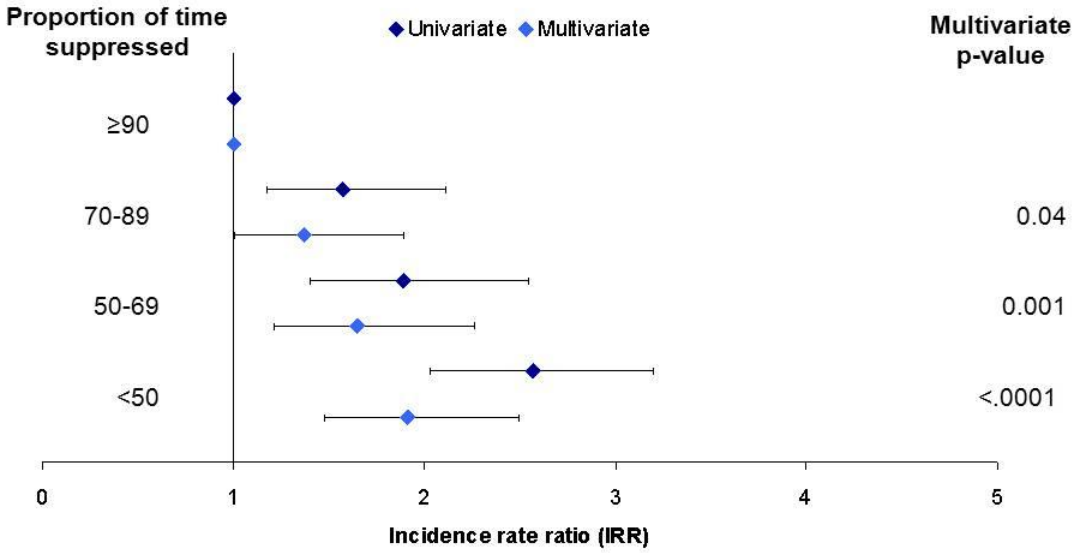
In patients virologically failing at baseline, similarly to those virologically suppressed at baseline, the lower the percentage of time spent with a suppressed viral load whilst on cART prior to baseline the higher the rate of future virological failure (figure 4.13). Compared to patients suppressed more than 90% of the time they are on cART, patients suppressed between 70-90% had a 1.37 times higher rate of virological failure (95%CI 1.01-1.86, p=0.04). Those suppressed between 50-70% of the time had a 1.65 times higher rate of virological failure (95% CI 1.21-2.26, p=0.001) and those suppressed less than 50% of time they were on cART had almost double the rate of virological failure compared to those suppressed more than 90% of the time (IRR 1.91, 95%CI 1.46-2.49, p<0.0001). In addition, older patients had a lower risk of virological failure (IRR 0.87 per 10 years older, 95%CI 0.79-0.96, p=0.007) as did patients with a higher viral load at starting cART (IRR 1.13 per log10 increase, 95%CI 1.04-1.23, p=0.003). Patients who had been exposed to more ARVs prior to baseline had a 9% increased rate of virological failure per additional ARV (95%CI 1.05-1.14, p<.0001). The more new drugs a patient stated at baseline the lower the rate of virological failure (IRR 0.88 per additional drug started, 95%CI 0.81-0.96, p=0.004). Patients from north Europe had a 33% lower rate of virological failure compared to patients in south Europe (IRR 0.67, 95%CI 0.54-0.84, p=0.0005). No other variables describing patterns of virological suppression after baseline were independently associated with risk of virological failure after adjustment (table 4.7).

Table 4.7 Poisson regression analysis investigating history of virological suppression as a predictor of virological failure in patients virologically failing at baseline

		Univariable			Multivariable*		
		IRR	95% CI	Global p-value	IRR	95% CI	p-value
Months to initial suppression	Per 6 months	1.06	1.02-1.10	0.002	1.02	0.97-1.07	0.37
Number of rebounds after initial suppression	No rebound	1.00		0.13	1.00		
	1	1.09	0.67-1.78		1.08	0.65-1.79	0.76
	2	1.25	0.76-2.06		1.11	0.65-1.87	0.70
	≥3	1.30	0.78-2.17		1.07	0.62-1.85	0.80
Size of highest rebound	>10000	1.00		0.53	1.00		
	1001-10000	1.12	0.94-1.34		1.16	0.96-1.39	0.12
	500-1000	0.99	0.74-1.33		1.08	0.80-1.46	0.69
	No rebound	0.89	0.55-1.46		0.98	0.59-1.64	0.95
Viral rebound above viral load at starting cART	No	1.00		0.20	1.00		
	Yes	0.89	0.75-1.06		1.02	0.82-1.28	0.86
Percentage of time with suppressed viral load	≥90	1.00		<.0001	1.00		
	70-89	1.58	1.18-2.11		1.33	1.00-1.78	0.04
	50-69	1.89	1.40-2.54		1.65	1.22-2.25	0.001
	<50	2.57	2.03-3.26		1.90	1.47-2.44	<.0001

***Multivarible analysis also adjusted for region of Europe, hepatitis C status, naïve, age, baseline CD4 count, viral load at starting cART, baseline date, date of starting cART, number of new antiretrovirals started at baseline, number of antiretrovirals previously exposed to.**

Figure 4.13: Univariable and multivariable Poisson regression models for patients virologically failing at baseline



Multivarible analysis also adjusted for region of Europe, hepatitis C status, naïve, age, baseline CD4 count, viral load at starting cART, baseline date, date of starting cART, number of new antiretrovirals started at baseline, number of antiretrovirals previously exposed to.

4.4.7 Sensitivity analysis

A number of different sensitivity analyses were performed from varying the definition of virological failure to only including patients who were naïve at starting cART. Table 4.8 summarises the number of patients included in each of the sensitivity analyses.

Table 4.8 Summary of patients included in sensitivity analysis

Sensitivity analysis	Virologically suppressed at baseline	N Included (% included from main analysis)	N experienced virological failure
Virological failure defined as 2 consecutive viral loads >500 copies/ml	Yes	1827 (100%)	278 (15%)
	No	894 (100%)	456 (51%)
Lower limit of detection <50 copies/ml	Yes	901 (49%)	369 (41%)
	No	389 (43%)	273 (70%)
Restistance data available at baseline	Yes	544 (29%)	135 (25%)
	No	479 (53%)	291 (61%)
Naïve at cART initiation	Yes	878 (48%)	168 (19%)
	No	321 (36%)	156 (49%)

4.4.7.1 Virological failure defined as two consecutive viral loads >500 copies/ml

The analyses were repeated with virological failure defined as 2 consecutive viral loads measured >500 copies/ml. In patients suppressed at baseline, 278 (15%) experienced confirmed virological failure after baseline with an incidence rate of 4.2 per 100 PYFU (95% CI 3.7-4.7). 51% (456) of patients virologically failing at baseline experienced confirmed virological failure after baseline with an incidence rate of 20.5 per 100 PYFU (95% CI 18.6-22.4). The results of the multivariable model are shown in table 4.9 and were consistent with the main analysis.

Table 4.9 Multivariable Poisson regression analysis where virological failure was defined as 2 viral loads >500 copies/ml

		Virologically suppressed*			Virologically failing+		
		IRR	95%CI	P-value	IRR	95%CI	P-value
Time since last rebound	Never	1.00					
	≤1year	3.11	1.84-5.25	<.0001			
	1-2 years	1.61	0.98-2.65	0.05			
	2-3 years	1.77	1.04-3.02	0.03			
	>3years	1.08	0.65-1.79	0.77			
Percentage of time with suppressed viral load	≥90	1.00			1.00	-	-
	70-89	1.17	0.83-1.65	0.38	1.33	0.94-1.88	0.11
	50-69	2.12	1.34-3.34	0.001	1.82	1.28-2.60	0.0009
	<50	2.36	1.58-3.53	<.0001	2.47	1.84-3.32	<.0001

* also adjusted for gender, HIV exposure group, region of Europe, hepatitis C status, naïve, prior AIDS diagnosis, baseline treatment regimen, reason for starting new ARV(s), age, CD4 nadir, baseline date, time on cART, number of new antiretrovirals started, and number of antiretrovirals previously exposed to and genotypic sensitivity score

+also adjusted for region of Europe, hepatitis C status, naïve, age, baseline CD4 count, viral load at starting cART, baseline date, date of starting cART, number of new antiretrovirals started at baseline, number of antiretrovirals previously exposed to, and genotypic sensitivity score.

4.4.7.2 Sub-group analysis in patients with viral load measured at a lower limit of detection of 50 copies/ml

The analyses were also repeated using a lower limit of detection for viral load of 50copies/ml; 901 patients virally suppressed at baseline were included in this analysis and 41% of those experienced virological failure (defined as a viral load >50 copies/ml) with an incidence rate of 14.3 per 100 PYFU (95% CI 12.8-15.8). The results of the multivariable Poisson regression analysis are shown in table 4.10. Those who had virally rebounded in the year prior to baseline had 85% higher rate of virological failure compared to patients who had never virally rebounded, and patients suppressed <50% of the time they were on cART had a 13% higher rate of virological failure (95% CI 0.79-1.64, p=0.50) compared to those suppressed more than 90% of the time, although this

was not statistically significant after adjustment. 389 patients virologically failing at baseline had viral load measurements available using a lower limit of detection of 50 copies/ml. Of these virologically failing patients 273 (70%) experienced virological failure after baseline with an incidence rate of 37.5 per 100 PYFU (95%CI 33.1-42.0). After adjustment, similarly to the main analysis, the lower the proportion of time a patient had spent virologically suppressed whilst on cART the higher the rate of future virological failure (table 4.10).

Table 4.10 Multivariable Poisson regression analysis in patients with viral load measured at a lower limit of detection of 50 copies/ml

		Virologically suppressed*			Virologically failing+		
		IRR	95%CI	P-value	IRR	95%CI	P-value
Time since last rebound	Never	1.00					
	≤1year	1.85	1.33-2.57	0.0003			
	1-2 years	1.37	1.00-1.88	0.04			
	2-3 years	1.01	0.67-1.53	0.96			
	>3years	0.60	0.40-0.91	0.01			
Percentage of time with suppressed viral load	≥90	1.00			1.00	-	-
	70-89	0.98	0.75-1.29	0.89	1.06	0.63-1.78	0.83
	50-69	1.11	0.78-1.57	0.56	1.59	0.97-2.59	0.06
	<50	1.13	0.79-1.64	0.50	2.15	1.38-3.32	0.0006

* also adjusted for gender, HIV exposure group, region of Europe, hepatitis C status, naïve, prior AIDS diagnosis, baseline treatment regimen, reason for starting new ARV(s), age, CD4 nadir, baseline date, time on cART, number of new antiretrovirals started, and number of antiretrovirals previously exposed to and genotypic sensitivity score

+also adjusted for region of Europe, hepatitis C status, naïve, age, baseline CD4 count, viral load at starting cART, baseline date, date of starting cART, number of new antiretrovirals started at baseline, number of antiretrovirals previously exposed to, and genotypic sensitivity score.

4.4.7.3 Sub-group analysis in patients with resistance data

1023 (37.6%) patients had some resistance data available at baseline (544 patients (29%) virologically suppressed at baseline and 479 (53%) virologically failing at baseline). In those virologically suppressed at baseline the median time since the most recent resistance test was 4.00 years (IQR 2.23-5.76) and in those virologically failing at baseline the median time since last resistance test was more recent, only 0.75 years (IQR 0.12-2.98) prior to baseline.

Of those virologically suppressed with data available, 405 (75%) had a GSS score ≥ 3 for their baseline cART regimen. 135 (25%) patients virologically suppressed at baseline

experience virological failure. The results of the multivariable Poisson regression analysis are shown in table 4.8. Consistent with the main analysis, a higher rate of virological failure was associated with a lower percentage of time spent with a suppressed viral load. The trend for time since last rebound is less clear, probably due to lack of power. Further, there was no significant difference in rate of virological failure in patients with a GSS <3 compared to those with a GSS ≥ 3 (IRR 1.41, 95%CI 0.89-2.23, p=0.14) after adjustment for all demographic variables, percentage of time suppressed and time since last rebound.

Similarly, in those virologically failing with some resistance data available, 356 (74.3) had a GSS score ≥ 3 for their baseline cART regimen. 291 (61%) patients experienced virological failure. The results of the multivariable Poisson regression analysis are shown in table 4.8 and are consistent with the main analysis. Additionally, there was no significant difference in the rate of virological failure in patients with a GSS <3 compared to those with a GSS ≥ 3 (IRR 0.96, 95%CI 0.72-1.27, p=0.75).

Table 4.11 Multivariable Poisson regression analysis in patients with resistance data available

		Virologically suppressed*			Virologically failing[+]		
		IRR	95%CI	P-value	IRR	95%CI	P-value
Time since last rebound	Never	1.00					
	≤1year	2.73	1.46-5.09	0.0016			
	1-2 years	0.95	0.50-1.82	0.87			
	2-3 years	3.60	1.99-6.51	<.0001			
	>3years	0.89	0.48-1.62	0.69			
Percentage of time with suppressed viral load	≥90	1.00			1.00		
	70-89	1.39	0.80-2.41	0.24	1.44	0.91-2.27	0.11
	50-69	1.39	0.69-2.83	0.35	1.88	1.18-3.00	0.008
	<50	2.17	1.23-3.83	0.007	1.84	1.21-2.80	0.004

* also adjusted for gender, HIV exposure group, region of Europe, hepatitis C status, naïve, prior AIDS diagnosis, baseline treatment regimen, reason for starting new ARV(s), age, CD4 nadir, baseline date, time on cART, number of new antiretrovirals started, and number of antiretrovirals previously exposed to and genotypic sensitivity score

[+]also adjusted for region of Europe, hepatitis C status, naïve, age, baseline CD4 count, viral load at starting cART, baseline date, date of starting cART, number of new antiretrovirals started at baseline, number of antiretrovirals previously exposed to, and genotypic sensitivity score.

4.4.7.4 Sub-group analysis in patients antiretroviral naïve at starting cART

1,199 patients were treatment naïve at starting cART. Of these patients 878 (73.3%) were virologically suppressed at the time of starting a new ARV(s) and 168 (19%) experienced virological failure after baseline. In adjusted analysis the time since last virological rebound remained a significant predictor of virological failure and an increased rate of virological failure was also observed with less time spent suppressed however the trend was not as clear in this group of patients. 321 (26.7%) patients naïve at starting cART were virologically failing at baseline of those 158 (49%) experienced virological failure. These results were consistent with the main analysis with patients suppressed less than 50% of the time having double the rate of virological failure compared to those suppressed > 90% of the time.

Table 4.12 Multivariable Poisson regression analysis in patients with resistance data available

		Virologically suppressed*			Virologically failing+		
		IRR	95%CI	P-value	IRR	95%CI	P-value
Time since last rebound	Never	1.00					
	≤1year	2.84	1.63-4.97	0.0002			
	1-2 years	3.13	1.72-5.72	0.0002			
	2-3 years	1.92	0.92-4.02	0.08			
	>3years	0.99	0.52-1.90	0.98			
Percentage of time with suppressed viral load	≥90	1.00			1.00		
	70-89	1.03	0.67-1.60	0.88	1.30	0.79-2.16	0.30
	50-69	2.46	1.30-4.64	0.0005	0.98	0.25-1.12	0.95
	<50	1.48	0.82-2.67	0.19	1.98	1.28-3.05	0.002

* also adjusted for gender, HIV exposure group, region of Europe, hepatitis C status, naïve, prior AIDS diagnosis, baseline treatment regimen, reason for starting new ARV(s), age, CD4 nadir, baseline date, time on cART, number of new antiretrovirals started, and number of antiretrovirals previously exposed to and genotypic sensitivity score

+also adjusted for region of Europe, hepatitis C status, naïve, age, baseline CD4 count, viral load at starting cART, baseline date, date of starting cART, number of new antiretrovirals started at baseline, number of antiretrovirals previously exposed to, and genotypic sensitivity score.

4.5 Discussion

A patient's history of viral suppression can provide important information about the risk of viral failure after a change in antiretrovirals. The variables describing the history of suppression after cART initiation, but before a change in regimen, were highly predictive in addition to the traditional baseline predictors in predicting future virological failure. The most important factor was the percentage of time spent with a suppressed viral load whilst on cART prior to starting a new ARV(s), both in patients virologically suppressed and those virologically failing at the time of starting new ARV(s). In addition, in patients who were virologically suppressed at baseline, time since last viral rebound prior to starting

new ARV(s) was also an important factor in predicting the risk for future virologically failure. After adjustment for these factors none of the other markers of previous patterns of suppression were significant predictors of virological failure after baseline.

Table 4.13 Comparison of rates of virological rebound observed in different studies with varying inclusion criteria

Study	Patient inclusion	Definition of viral rebound	Viral rebound rate per 100 PYFU (95% CI)
This study	All patients on cART who had achieved a viral load <500 copies/ml and starting new ARV(s)	One viral load >500 copies/ml	12.3 (11.5-13.1)
Benzie et al.[496]	All patients on cART who achieved a viral load <50copies/ml	two consecutive viral load >400 copies/ml	9.3 (8.9-9.7)
Bansi et al.[511]	All patients on cART who achieved a viral load <50copies/ml	Two consecutive viral load >400 copies/ml	8.1 (7.8-8.4)
Phillips et al.[495]	All patients who started cART and achieved viral load < 400 copies/ml by week 24	Two consecutive viral load >400 copies/ml	11.1 (9.1-12.3)
Smith et al.[508]	Patients achieving a viral load <50 copies/mL for the first time	Two consecutive viral loads >500 copies/mL	6.3 (5.6-6.9)
Smith et al.[529]	Patients who had failed ≥1 antiretroviral regimen in all three main drug classes and ≥3 previous ARV regimens and subsequently achieved viral load < 50 copies/mL	Two consecutive viral load >400 copies/ml	15.8 (12.9-18.7)

Table 4.13 summarises the rate of virological rebound observed in different studies with varying inclusion criteria. In the majority of the studies follow-up was from first suppressed viral load after initiating cART and therefore the rate of virological failure in these studies is lower than in ours[495;496;508;511]. Smith et. al[529] reported the rate in highly treatment experienced patients and this was higher than the rate observed in our study. However, patients virologically suppressed at the time of starting new ARV(s) were analysed separately to those virologically failing as the risk of future virological failure in these two groups was quite different. Patients virologically suppressed at the time of the starting new ARV(s) had an incidence rate of virological failure of 7.3 per 100 PYFU, which is very close to the rate observed in those studies following patients from first viral suppression. Unsurprisingly patients virologically failing at baseline had a higher rate of virological failure of 28.3 per 100 PYFU.

There was a clear relationship with increasing time suppressed prior to baseline, and decreasing risk of future virological failure. This variable only includes the time patients are recorded as receiving cART, therefore time spent with uncontrolled viraemia could be an indicator of poor adherence. In the previous chapter, patients who were on a stable and

fully suppressed cART regimen had an increased risk of a treatment failure event if they were suppressed less than 70% of the time they were on cART compared to those suppressed more that 70% of the time. The analysis in patients suppressed at the time of starting new ARV(s) is consistent with this. A study in patients with CD4 counts above 200 cells/mm^3 found that time with undetectable viraemia was a significant predictor in clinical progression[479]. In addition, previous studies have found that patients with a history of persistent low level viraemia (51-1000 copies/ml) were more likely to experience virological failure[509], as were those with intermittent viraemia above 400copies/ml compared to those who sustained an undetectable viral load[516]. However, some studies have shown that although moderate viraemia and viral rebounds may increase your risk of future rebounds, this may not translate into an increased risk of clinical disease progression[466;543].

In those who were virologically suppressed at baseline, after adjustment, time since last viral rebound was highly predictive of virological failure after starting new ARVs, consistent with findings from other studies. For example, Benzie et al.[496] reported that up to four years of sustained viral suppression was necessary in patients with previous treatment failures for them to achieve rebound rates similar to those with no prior treatment failures.

As mentioned in the introduction to this chapter, the greatest risk of viral rebound has been shown to be in the first few months after initial suppression[472], therefore it follows that increasing time since last virological rebound decreased the risk of virological failure after baseline. One potential explanation is that patients who were more likely to experience virological rebound have been selected out as the time from initial response has increased.

In contrast to previous findings[490], the association with virologic failure for size of each viral rebound prior to baseline was not significant after adjustment. The number of previous viral rebounds before baseline was important, and consistent with other studies[544]. However, after adjustment for the percentage of time a patient had spent suppressed and the time since last rebound, this variable added very little additional information. These analyses would suggest that a patient with 3 or more viral rebounds prior to baseline would have a very high rate of viral rebound. Palella et al. found that successive cART regimens were progressively less effective in suppressing viral load, and were generally shorter in duration[545]. In addition, treatment interruption strategies that have been used to combat the risk of long term drug toxicities and the cost of therapies, have been found to result in rapid viral rebound[546]. In the UK CHIC study, patients with undetectable viral load who had

previously interrupted ART had a raised risk of future viral rebound[511;534] compared with those who had not interrupted. Our results highlight the need for patients to be placed on a suitable regimen when initiating cART, emphasising the importance of adherence, and that consideration should also be given to future treatment strategies in order to decrease the risk of future viral rebounds.

In contrast to the previous chapter, where a subgroup of patients who had a low risk of disease progression in the next 12 months was identified, who could be monitored less frequently, these results may help identify patients who are at a higher risk of viral failure and may need to be monitored closely.

The reason for starting new ARV(s) was defined using information recorded on EuroSIDA follow-up forms (see chapter 2 section 2.1.4 'Data Collection' and Appendix I). If a patient has stopped an antiretroviral since their last follow-up visit, the date of stopping the antiretroviral is recorded. There is also a box for recording the reason for stopping. Only one reason is given per drug, and no information is collected on the reasons for starting new ARV(s). For this analysis, the reason for starting new ARV(s) was therefore defined as the reason recorded for stopping their most recent ARV either at, or prior to baseline.

The reason for starting new ARV(s) was not a significant predictor of future virological failure in either multivariable models. The most likely reason is that starting ARV(s) due to virological failure of the previous regimen was the most important factor, and the analyses were already stratified by this.

Earlier EuroSIDA studies have found differences in virological response to cART across Europe[320;424;547]. However, improvements in response have been observed over time most notably in East Europe[320;424]. In this study, in both patients who were suppressed and those who were virologically failing at a baseline a significantly lower rate of virological failure was observed in North compared to South Europe. There was no significant difference between South Europe and the other regions.

One limitation to this analysis, was the definition of virological suppression and failure. Defining virological failure is complicated. Viral load levels generally need to be between 250-500copies/ml to detect the presence of antiretroviral drug resistantance mutations[522]. Additionally, there is random variation in the results of viral load assays[548]. Low level viraemia or blips often defined as a single viral load measured above 50copies/ml but

below 1000copies/ml is often an isolated event and has not been found to be associated with treatment failure or accumulation of drug resistance[549]. In all the main analyses in this thesis, viral load suppression is defined using a viral load cut-off of 500copies/ml and virological failure a viral load >500 copies/ml as this was the lower limit of detection for many of the assays used. Table 4.13 shows that across different studies several different definitions are used, making comparisons between studies harder to interpret. In current clinical practice a viral load less than 50 copies/ml is the aim of cART[312;550]. The sensitivity analysis performed on the subgroup of patients with assays consistently measured with a lower limit of detection ≤ 50 copies/ml and viral suppression defined as a viral load <50 copies/ml showed consistent results with the main analysis.

Another limitation is that in the main analysis the patients' resistance profile was not accounted for. As discussed in the chapter 1 (section 1.8.3.3) effective sustained cART is complicated by the prevalence or emergence of drug resistance. Patients may be infected with a drug-resistant virus or drug resistant mutation which may lead to lower sensitivity to antiretroviral agents and subsequent rebound in viral load[342]. On average, resistance mutations are seen in 50-60% of individuals with detectable viral load on antiretroviral therapy[551]. Resistance data were available in a minority of patients; however the majority of those with data available were predicted to be on a fully active regimen.

The patient's resistance profile at baseline was not independently associated with the risk of future virological failure, regardless of whether the patient was virologically suppressed at baseline or not. However, as some resistance tests were performed several years prior to baseline these patients could have acquired new mutations. This analysis may also be limited by power, or the variables that were significant in our main analysis captured information that was also measured by the availability of resistance data. As the patients resistance profile was not found to be associated with the risk of virological failure, with most patients on a fully active regimen it is reasonable to speculate that in the majority of cases poor adherence may be the reason for the rebound.

The importance of good adherence is also discussed in more detail in chapter 1 (section 1.8.3.2). In brief, without adequate adherence, antiretrovirals are not maintained at a sufficient concentration to suppress HIV replication in infected cells and to lower plasma viral load[330]. Patients have been found to take on average 70-75% of their prescribed medication[336;337], and those who are more adherent to treatment are more likely to achieve sustained viral suppression[331;332] and are less likely to show signs of disease

progression[333]. Studies looking into disease progression have found that even adherence of 50% significantly decreases a patients risk of progression to AIDS[333;337]. As mentioned in chapter 2 (section 2.1.4) EuroSIDA has only recently begun collecting data on adherence and the data are very limited. The data currently collected consist of two questions. The first is whether any comment has been made on adherence in the patient notes. If the answer to this is yes the second section records the date of comment and also the opportunity to tick whether adherence as poor/inadequate (defined as <70%), excellent/full (defined as >95%) or anything in between. The main limitation to this data is that we do not collect any information on how adherence was measured, and thus it is hard to know how accurate or reliable this data is. However, the portion of time a patient has spent with an undetectable viral load since starting cART could help serve as an indicator to a patient's adherence, as the initial 4 months after starting or changing a cART regimen, when the viral load would not be expected to be undetectable, was excluded from analyses. Thus, patients who are suppressed for longer must be adherent to their therapy, and those with a poor history of viral suppression are those with poor adherence.

To summarise, when considering future treatment and monitoring strategies after a patient has started a new ARV(s), the previous response to cART regimens may provide an indication of the risk of future virological failure. Patients making a change to their cART regimen while maintaining a suppressed viral load have an increased risk of virological failure if they have spent a low percentage of time on cART with suppressed viral load, or experienced a viral rebound close to the time of starting new ARV(s). Patients with a low percentage of time suppressed whilst on cART, and those who have recently rebounded may require more intensive monitoring after starting new ARV(s), and consideration should also be made to increasing adherence counselling. The history of patterns of viral response to cART regimens should be an integrated component in deciding monitoring strategies and adherence counseling for patients whenever a change in cART is made.

A manuscript of this analysis was published in HIV Medicine in August 2010 and can be found in Appendix V.

Chapter 5 A comparison of the long term durability of nevirapine, efavirenz and lopinavir in routine clinical practice across Europe

5.1 Introduction

One of the findings of the analysis described in the previous chapter was that, in individuals on cART who made a treatment switch, those who had spent a low percentage of time on cART with a suppressed viral load and those who had experienced a viral rebound close to the time of the switch were at an increased risk of virological rebound after the treatment switch, compared to those whose viral load was previously mostly supressed. Virological and immunological outcomes to cART have improved in recent years[321;518]. In a recent Swiss study including patients enrolled between 2004-2005, virological failure was a reason for regimen change in only 7% of individuals compared to 51% changing due to tolerability issues[518]. Additionally, an Italian cohort reported that virological failure rates, defined as a viral load > 400 copies/ml, had declined from 42% in 1997 to 11% in 2004 after 12 months of therapy[552]. In order to significantly reduce the risk of morbidity and mortality, cART regimens should show short-term virological potency as well as durability.

5.1.1 Current treatment guidelines and common side effects of third drugs used in initial regimens

In Europe, nevirapine, efavirenz and lopinavir are currently recommended for initial therapy of HIV-positive naïve patients in combination with two nucleosides[214;223]. BHIVA guidelines state that efavirenz should be considered as the first choice for all patients[223]. The most commonly observed adverse events associated with efavirenz are rash and central nervous system (CNS) symptoms[553]. CNS symptoms have been reported in 25%-70% of patients receiving efavirenz[295;554-556]. Symptoms include dizziness, abnormal dreaming, headache, difficulty sleeping, anxiety and confusion. These symptoms normally occur within the first few days of treatment and can lead to early discontinuation of efavirenz in a small proportion of patients[556;557].

However, if therapy is continued the prevalence of these symptoms declines within a few weeks[554;555;557]. Efavirenz has a low genetic barrier to resistance, it has been reported that 6-8% of patients on efavirenz plus 2 NRTIs for 2-3 years develop resistance[558].

Nevirapine instead is mainly recommended for women trying to become pregnant and patients with mental health problems[223]. However, serious drug related toxicity has been associated with nevirapine[300] especially at high CD4 counts in female patients[559]. The most common side effects of nevirapine are rash, hypersensitivity reactions, headache, nausea, Stevens-Johnson syndrome, hepatitis and liver problems and these are experienced in between 1 and 10 patients in 100[560]. Analyses of the EuroSIDA database have previously shown that treatment experienced patients starting nevirapine at high CD4 counts had a significantly lower risk of discontinuation due to toxicity or patient/physician choice than those who were ARV-naïve and had high CD4 counts[400], and other studies have supported the finding that in treatment experienced patients there is no increased risk of discontinuation due to hypersensitivity reaction in patients with high CD4 counts and undetectable viral loads[401;561;562].

Due to problems with accumulation of drug resistance in people not fully adherent to NNRTI-based therapies, a PI based regimen may be recommended as in initial choice of regimen in patients where adherence is thought to be a problem[223]. Lopinavir boosted with ritonavir is one of the most widely used PI combinations. The most common side effects with lopinavir/ritonavir are diarrhoea, and an increase in cholesterol and triglycerides. These are normally seen in 1 out of 10 patients[563].

5.1.2 Comparison of virological outcomes between nevirapine, efavirenz and lopinavir

Evidence from clinical trials suggests that efavirenz provides similar virological response to nevirapine in treatment naïve patients. For example the 2NN study found that, based on the proportion of patients with treatment failure (defined as less than a 1 log_{10} decline in plasma HIV-1 RNA in the first 12 weeks or two consecutive measurements of more than 50 copies per mL from week 24 onwards), disease progression (defined as a new Centers for Disease Control and Prevention grade C event or death), or change of allocated treatment, nevirapine was comparable to efavirenz[300]. Additionally, the FIRST study[564] found that in treatment naïve patients, starting either efavirenz or nevirapine based cART, there was no significant difference in the primary outcome, defined as a viral load ≤50 copies per ml after 8 months or death. Rates of CD4 cell count recovery, or clinical outcomes were also similar, although the risk of virological failure, and virological failure with resistance, to any class of antiretroviral was lower in those on efavirenz[564].

However, data from observational studies have observed some difference in virological outcome. EuroSIDA has previously investigated differences in viral load outcome between patients on efavirenz and those on nevirapine[298]. This analysis included 2,203 patients enrolled up to cohort 4 who had started nevirapine or efavirenz based cART regimen, had not been exposed to any NNRTI previously and had CD4 count and viral load measurement available within the 6 months prior to starting cART. Virological failure was defined as the first of 2 consecutive viral loads >500 copies/ml 6 months after starting the regimen, and clinical outcome was measured in terms of new-AIDS or death. Only 6% of those starting nevirapine and 4% of those starting efavirenz were treatment naïve. Patients on efavirenz were found to have a 43% lower risk of virological failure compared to nevirapine (HR 0.57, 95%CI 0.47-0.69, p<.0001), and 51% lower risk of clinical disease progression (HR 0.49, 95%CI 0.46-0.96, p=0.03) after adjustment for previous antiretroviral use, previous AIDS, year started NNRTI, CD4 cell count (baseline [treatment initiation], nadir), and viral load (baseline, maximum). A follow up to this analysis investigated genotypic resistance profiles and virological response of patients on nevirapine or efavirenz[565].

After adjustment for the same factors listed previously and baseline drug resistance patients on efavirenz had a 50% lower hazard of virological failure compared to efavirenz (HR 0.50, 95% CI (0.39-0.65, P < 0.001). Results from other cohort studies have also reported superior virological response associated with efavirenz compared to nevirapine[299;566-570].

Another study, looking at pre-treated patients with a suppressed viral load (<200 copies/ml), switching from a PI based regimen to efavirenz, nevirapine or abacavir found no difference in outcome, defined as death, development of AIDS or virological failure (viral load ≥200 copies/ml) between efavirenz and nevirapine after 12 months of follow-up[571]. After three years of follow-up, there was still no significant difference in the probability of virological failure between patients on nevirapine and efavirenz[572].

Efavirenz has been found to have a favourable outcome compared to some boosted and unboosted PI regimens or triple NRTI regimens in clinical trials[295;573]. The AIDS clinical trial study group (ACTGS) recently compared the virological efficacy of efavirenz to boosted lopinavir in ART naïve patients and found that virological failure was less likely to occur in the efavirenz group compared to the lopinavir group[307]. Additionally, several observational studies have reported a superior virological response in patients receiving efavirenz compared to those starting PI based regimens[296;574]. In particular, compared to a boosted lopinavir regimen, analyses of the data of cohort studies have shown that efavirenz has virological efficacy at least as high[575] or superior to that of lopinavir[576]. Immunological outcome and clinical outcome has been reported to be similar[296;574;575] between efavirenz and lopinavir, with one study favouring lopinavir when immunological outcome was compared over 48 weeks[576].

As mentioned in the previous chapter, one of the main goals of antiviral therapy is the maximal suppression of viral load for as long as possible[223;498], and the virological potency of nevirapine, efavirenz and lopinavir is well documented. However, the ultimate goal of any HIV treatment is to prevent clinical progression and death.

5.1.3 Viral load as a Surrogate endpoint vs. Clinical endpoints

In the beginning of the cART era, the advances in therapy were progressing faster than new results could be published[577], and therefore since 1996 clinical trials with surrogate outcomes have predominately replaced clinical outcomes in trials of anti-HIV drugs. Only a small percentage of clinical events occur in the first 24-48 weeks of a study, particularly in antiretroviral naïve patients[229], and therefore the use of a surrogate outcome was advocated.

A surrogate outcome is an outcome that reliably predicts the effects of a drug on the clinical outcome[578]. Surrogacy requires that a valid test of the difference between the treatment groups based on the marker is also a valid test based on clinical endpoints[579]. A perfect surrogate should not only be a good predictor of clinical outcome but should also completely explain the treatment effect on clinical outcome[580]. They can allow for trials to run for a much shorter time and therefore reduce the cost and the time from initial development of a drug to government approval[230].

Both CD4 count and viral load have been shown to have independent prognostic values for the development of new AIDS or death in HIV-positive persons, in the natural history of HIV infection, and have been applied as surrogate markers in treatment trials[226]. Currently, clinical trials are typically powered to detect between regimen differences in short-term suppression of HIV-RNA rather than clinical outcomes[225].

However, there are problems with using surrogate outcomes as, by definition, they provide no direct information on the long term efficacy of cART[230]. For example, one problem with using HIV-RNA as a surrogate marker is that viral rebound can occur sometime after suppression, so with relatively short trial periods the benefits of the drug may be overestimated[581].

The SMART study provided good evidence of the need for long term studies with clinical endpoints[317]. Several small studies had indicated it might be possible to safely interrupt treatment for varying periods of time[582;583].

The SMART study was set up to compare the use of episodic cART according to CD4 count with the recommended practice of continuous therapy with respect of the risk of new disease (AIDS and non-AIDS related) or death[317]. Contrary to what was expected, rates of non-opportunistic disease and death mainly occurred when people were off ART and therefore were higher in the group using cART episodically. This study helped to illustrate that much remains to be understood about the detrimental effects of uncontrolled HIV replication and the benefits of ART[584]. The contrasting results from small short studies and the long term SMART trial highlight that there remains a need for adequately powered trials to look at the long term effects of cART based on clinical outcomes in addition to virological outcomes.

Other studies have found that virological differences do not necessarily translate into clinical differences, thus questioning the role of surrogate markers. The Antiretroviral Cohort collaboration (ART-CC) found that in ART-naïve patients, differences found in short term virological failure between regimens did not necessarily translate into differences in clinical outcome[570]. The Community Programs for Clinical Research on AIDS (CPCRA) investigated the optimum sequencing of cART regimens to ensure long-term benefit, and found that although there were differences in virological suppression rates these differences did not translate into a difference in AIDS defining events[306]. Similarly, a meta-analysis including 178 randomised clinical trials found that differences observed in CD4 count and HIV RNA between treatments did not result in meaningful differences in AIDS/death during relatively short time periods[585]. These studies show that there is a difference between looking at viral response of the first regimen initiated, and the success of a long term treatment strategy that includes the first treatment regimen.

Long term trials provide vital information not only on the risk of clinical disease progression, but also to our fundamental understanding of the disease and how the drug works. The development of clinical adverse events other than AIDS defining illnesses, such as long-term toxicities, liver failure and heart disease cannot be captured in 24-48 week trials. Additionally, viral load has not been shown to be related to many non-AIDS events experienced by patients in the modern cART era[18;586]. These factors can only be investigated over long-term studies that monitor clinical outcomes.

They require the collaboration of a large number of investigators and are very time consuming. Further, many drugs are now available that differ in toxicity, adverse events, their ability to suppress viral replication, development of resistance and patient adherence[587]. These newer drugs will not have been included in the early studies looking at using CD4 count and viral load as surrogate markers.

5.1.4 Assessing the long term durability

Choosing an antiretroviral treatment regimen for patients therefore requires consideration of a number of factors in addition to virologic and immunologic potency, including comorbidities, likely adherence, convenience, adverse events and the potential for drug interactions with other treatment[345].

Adverse effects have been reported with all antiretrovirals and are one of the most common reasons for discontinuation of treatment[16;588;589], even after the initial months of therapy [590]. Mocroft et al. reported a high rate of discontinuation of cART in the first 12 months due to toxicities, patient choice or poor compliance[476]. Some adverse events, such as gastrointestinal problems and hypersensitivity occur rapidly, within the first few months of starting treatment, while other adverse events, such as cardiovascular disease and pancreatitis, can take much longer to develop[18;399;591;592]. Such long-term adverse events can influence the durability of a regimen.

Many cohort studies have compared the short-term and long-term efficacy of different cART regimens[566;593-596], but less is known about the durability of different regimens, particularly in patients who have started a cART regimen more recently. If a regimen is virologically effective, durability can then be assessed as the time to discontinuation of the regimen, discontinuations due to toxicity or the rate at which potential markers of toxicity, such as increasing liver transaminases and cholesterol changes occur to assess the risk of future toxicities.

As mentioned above, nevirapine, efavirenz, or lopinavir boosted with ritonavir together with two NRTIs are the recommended choices for first line cART regimens[214;223].

Studies have previously investigated the virological efficacy and incidence of AIDS related events in patients on these three regimens, but less is known about the long term durability of each regimen, in particular the differences in the rate and reasons for treatment discontinuation and the risk of developing long term adverse event or non-AIDS related diseases.

5.2 Aim

The aim of this chapter was to compare the long term durability of nevirapine based cART regimens with that of efavirenz and lopinavir-based cART regimens to provide additional information to help aid the choice of initial cART regimen. Long term durability was measured in terms of:

1. Rate and time to discontinuation of either nevirapine, efavirenz or lopinavir and the reasons for discontinuation
2. Incidence of severe liver-related events, pancreatitis, non-AIDS defining malignancies, end stage renal disease and MI/stroke
3. Risk of deterioration in surrogate markers for clinical disease, such as risk of developing (or worsening of) anaemia, losing >10% of body weight, increase in total/HDL cholesterol ratio (>6.5), change in ALT or AST >2 times the upper limit of normal
4. Incidence of all-cause mortality

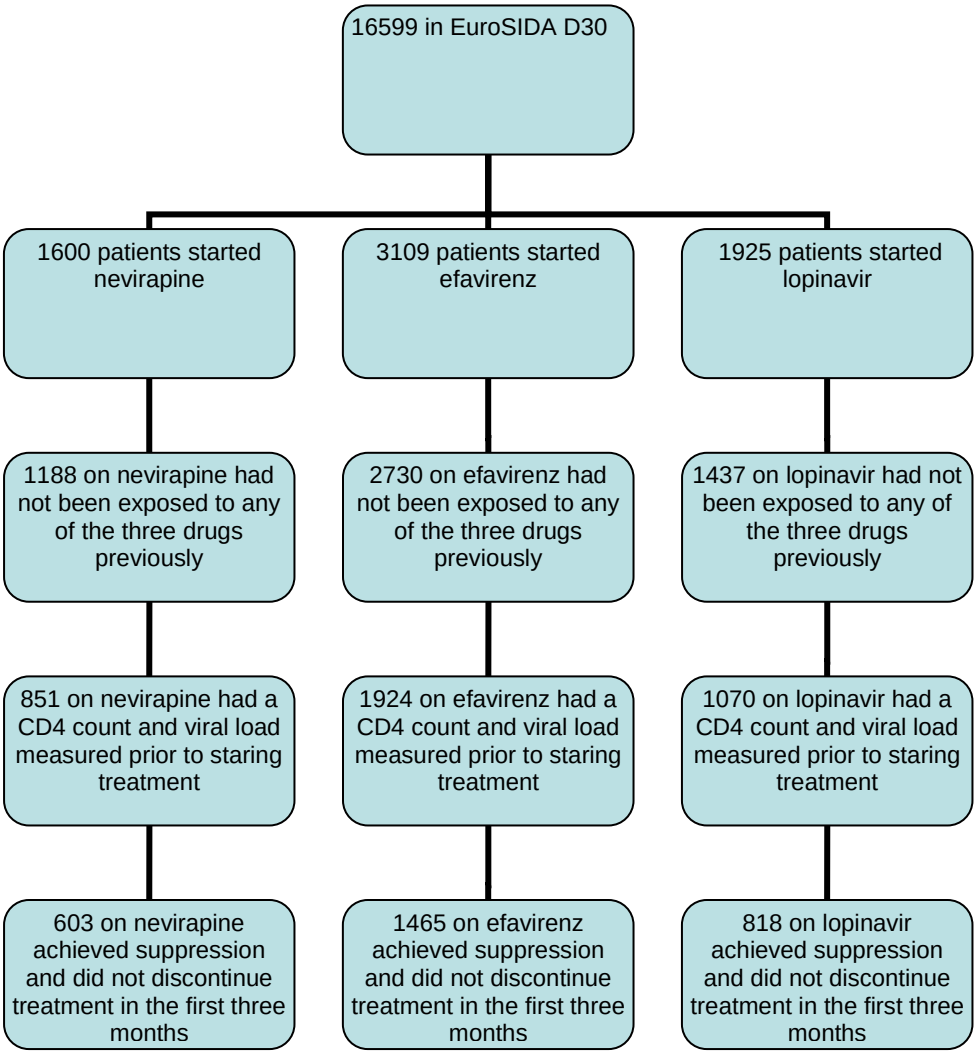
5.3 Methods

5.3.1 Patient selection

There were 16,599 patients in the D30 update. Figure 5.1 shows how patients were selected for inclusion into this analysis. Patients starting nevirapine, efavirenz or lopinavir together with exactly 2 nucleosides or nucleotides after 1 January 2000, during prospective follow-up, were included in the analysis, as by this time, all three drugs were routinely available in Europe. Baseline was defined as either the date of first virological suppression (defined as a single viral load < 500 copies/ml), or 3 months after the date of starting treatment, whichever occurred later. As the focus was on long term durability, patients were only included once virologic suppression had been achieved, and after at least 3 months exposure to the drug to exclude discontinuations due to early-onset, potentially treatment limiting toxicities. Patients were excluded if they did not have a CD4 count or viral load measured in the 6 months prior to starting the new regimen or if they did not have any prospective follow-up. Treatment experienced patients were included provided they had not previously been exposed to any of the drugs of interest.

Of the 6,634 potentially eligible patients, 1,600 started nevirapine (24%), 3,109 efavirenz (47%) and 1,925 lopinavir (29%), based cART regimen after 1/1/2000. 1,279 (19%) patients were excluded because of previous exposure to any of the three drugs, 412 on nevirapine (26%), 379 on efavirenz (12%) and 488 on lopinavir (25%). 1,510 (28%) patients were excluded because they had no CD4 count or viral load measurement prior to starting treatment, 337 (28%) on nevirapine, 806 (30%) on efavirenz, and 367 (26%) on lopinavir. 959 (25%) patients did not achieve suppression, had stopped treatment within the first three months or did not have sufficient follow-up and therefore were excluded, 248 (29%) on nevirapine, 459 (24%) on efavirenz, and 252 (24%) on lopinavir. Therefore a total of 2886 patients were left to be included in the analysis, 603 (21%) patients were on a nevirapine based cART regimen, 1,465 (51%) on an efavirenz based regimen, and 818 (28%) on a lopinavir based cART regimen.

Figure 5.1 Patient selection



5.3.2 Statistical analysis

Logistic regression was used to identify factors associated with starting nevirapine compared to efavirenz; patients starting lopinavir were excluded from this analysis. A similar analysis was used to identify factors associated with starting nevirapine compared to lopinavir, after excluding those taking efavirenz.

Nevirapine was chosen as the comparator in this analysis as the original idea for this work came from a request for a safety report by Boehringer Ingelheim who were interested in comparing the long term durability of patients on nevirapine to those on efavirenz and lopinavir.

The rate and time to discontinuation was investigated in a number of different ways:

1. Time to discontinuation of the *third* drug (nevirapine, efavirenz, or lopinavir) in the regimen
2. Time to discontinuation of *any* drug in the regimen

The incidence of discontinuation of the third drug was calculated and the reasons for discontinuation were compared in those who stopped between the 3 regimens. Time to discontinuation was determined using Kaplan-Meier methodology. Patients were followed until discontinuation or their last recorded clinical visit in EuroSIDA.

Consistent with previous EuroSIDA work[400;589], in addition to discontinuation for any reason, analyses considered separately discontinuation due to toxicities and patient/physician choice, or because of treatment failure. Reasons given for discontinuation were taken from patients notes and reported on standardised EuroSIDA follow-up forms (see Appendix I). One reason for discontinuation per antiretroviral is collected (the main reason according to the treating physician). Discontinuation due to reported treatment failure included virological, immunological or clinical failure and it was not based on a predefined definition but at the clinician's discretion as to whether they felt this was the reason for stopping treatment.

Again two different methods were adopted when investigating discontinuations due to specific reasons

1. Follow-up for patients discontinuing for a reasons different to the one of interest was censored at the time of discontinuation
2. Follow-up for patients discontinuing for reasons different to the one of interest was censored administratively at the time of last follow-up visits (competing risks analysis).

Cox proportional hazards models, stratified by centre to minimise biases due the different clinical experience in different centres, were used to compare the risk of discontinuation between the 3 regimens. Variables significant in univariable analysis ($p< 0.1$) were included in the multivariable model. Additionally a sub-group analysis investigated rates of discontinuations in treatment naïve patients separately.

The rate of development of any serious non-AIDS clinical events, changes in clinical markers, or death was compared between the 3 treatment groups using Poisson regression. For each event variables were selected for inclusion into the multivariable anslysis if they were significant ($p<0.1$) in the univariable model. A non-AIDS clinical event was defined at the date of the development of a non-AIDS defining malignancy, pancreatitis, end stage renal disease, grade III or IV hepatic encephalopathy, myocardial infarction, stroke or other cardiovascular disease[356;597]. Changes in major clinical or laboratory markers were defined as; developing or worsening anaemia, losing >10% of body weight at baseline, increasing total cholesterol to >6.2mmol/l or decreasing HDL cholesterol to <0.9mmol/l, AST or ALT levels increasing to > 2 times the upper limit of normal (ULN). Anaemia was defined as a haemoglobin level ≤ 12 or ≤14 (mg/dl) for females and males respectively[598]. Patients could either develop anaemia, or for those with anaemia at baseline, worsening anaemia was defined as a haemoglobin level ≤ 8 mg/dl[598]. For the liver function tests, 40IU/L was taken as ULN[599].

This analysis was an intent to treat analysis in that patients were followed from baseline until they experienced an event or the date of their last measurement for each clinical or laboratory marker in EuroSIDA, regardless of whether they were still receiving the initial third drug. It should be noted that not all patients in all groups had information on these markers available for all analyses; therefore the number of patients included in each analysis differed according to the availability of data. Patients with the event at baseline were excluded from analyses. For example individuals whose total cholesterol was >6.2mmol/l at baseline were excluded from that analysis.

5.4 Results

5.4.1 Comparison of included versus excluded patients

2886 (44%) patients who had started on nevirapine, efavirenz or lopinavir based cART were included in the analysis and 3748 (56%) were excluded. Table 5.1 compares the characteristics of the patients who were excluded to those who were included. Patients excluded had similar characteristics to those included, but were more likely to have previous cART exposure (64% versus 57%, p<.0001) and to have a prior AIDS diagnosis (32% versus 26%, p<.0001). A very low proportion of patients from East Europe were included; 214 patients, compared to 638 patients excluded (p<0.0001). The main reason for patients from East Europe being excluded was due to missing CD4 counts and viral loads at starting treatment, 477 patients in East Europe had a missing CD4 count or viral load. These patients could have been included by modelling CD4 count and viral load as categorical variables each with an unknown category. However, as the focus of this analysis was on long term durability, it was therefore important to have a viral load measurement available to know that the individual had initially responded to their regimen and was not discontinuing due to lack of initial virological response.

Table 5.1 Characteristics of the patients included compared to those excluded from the analysis

		Included in analysis		Excluded from analysis		P-value
N (% of total)		2886	43.5	3748	56.6	
Gender (N,%)	Male	2122	73.5	2590	69.1	<.0001
Risk (N,%)	Homosexual	1168	40.5	1236	33.0	<.0001
	IDU	593	20.6	935	24.9	
	Heterosexual	931	22.3	1227	34.0	
	Other	194	6.7	300	8.0	
Region of Europe (N,%)	South	934	32.4	1183	31.5	
	West Central	483	16.7	815	31.6	<.0001
	North	712	24.7	680	18.1	
	East Central	543	18.8	432	11.5	
	East	214	7.4	638	17.0	
Race (N,%)	White	2586	89.6	3250	86.7	0.0003
Prior AIDS (N,%)	Yes	754	26.1	1198	32.0	<.0001
Hepatitis B status (N,%)	Negative	2061	71.4	2354	62.8	<.0001
	Positive	139	4.8	193	5.2	
Hepatitis C status (N,%)	Negative	1555	53.9	1641	43.8	<.0001
	Positive	588	20.4	854	22.8	
Nave (N,%)	Yes	1036	35.9	1129	30.1	<.0001
Age (median, IQR)		40	34-46	39	33-45	0.0004

5.4.2 Patient characteristics

Table 5.2 compares the characteristics of the patients in each group at the time of starting their new regimen. A lower proportion of patients starting nevirapine were treatment naïve, 28% compared to 38% of patients starting efavirenz and 38% of patients starting lopinavir (p=<.0001). Patients on nevirapine had a higher median CD4 count 359 cells/mm^3 (IQR 230-583) and a lower viral load 2.70log_{10} copies/ml (IQR 1.70-4.56) compared to those on efavirenz, median CD4 count 323cells/mm^3 (IQR 190-535) and viral load 3.59log_{10} copies/ml (IQR 1.70-4.95) and lopinavir, median CD4 count 252cells/mm^3 (IQR 131-439) and viral load 4.05log_{10} copies/ml (IQR 2.07-5.14) (p= <0001 for both). A high proportion of patients infected via IDU were on lopinavir, 25% compared to 19% on efavirenz and nevirapine (p=0.004). Lopinavir was started, on average, more recently than the other two drugs: median date March 2004 compared to October 2001 for nevirapine and October 2002 for efavirenz. The majority of patients in the three treatment groups were on a NRTI backbone of zidovudine (AZT) and lamivudine (3TC), 46%, 46% and 48% on nevirapine, efavirenz and lopinavir respectively. 24%, 18% and 14% respectively were on stavudine (D4T) and lamivudine, this was the second most common NRTI for those on nevirapine and efavirenz. For patients on lopinavir the second most common NRTI backbone was tenofovir with 1 other NRTI. The median follow-up time was 2.6 years (IQR 1.1-4.8) when the endpoint of interest was discontinuation of the third drug for any reason.

In multivariable logistic regression analysis comparing the odds of starting efavirenz to nevirapine, after adjustment for gender, region of Europe, CD4 count and viral load at stating regiment and date of starting regimen patients starting nevirapine were 33% more likely to be female and have a higher CD4 count at starting their regimen (table 5.3). Further, patients from North Europe had lower odds of starting nevirapine compared to those from South Europe (OR 0.55, 95% CI 0.42-0.71, p<.0001). Similarly, after adjustment for HIV exposure group, race, region of Europe, hepatitis B and C status, CD4 count and viral load at stating regiment and date of starting regimen, compared to patients starting lopinavir, patients starting nevirapine had higher CD4 counts (OR 1.44 per doubling, 95% CI 1.30-1.58, p<.0001), and compared to patients from South Europe patients had lower odds of starting nevirapine in West Central (OR 0.66, 95% CI 0.46-0.94, p=0.02), North (OR 0.28, 95%CI 0.20-0.40, p<.0001), and East (OR 0.55, 95%CI 0.39-0.76, p=0.0004) Europe (table 5.3).

Table 5.2 Patient characteristics at time of starting regimen

		Nevirapine		Efavirenz		Lopinavir		P-value
N (% of total)		603	20.8	1465	50.7	818	28.3%	
Gender (N,%)	Male	423	70.2	1102	75.2	597	73.0	0.05
HIV exposure group (N,%)	Homosexual	241	40.0	596	40.7	331	40.5	0.004
	IDU	113	18.7	280	19.1	200	24.5	
	Heterosexual	213	35.3	494	33.7	224	27.4	
	Other	36	6.0	95	6.5	63	7.7	
Ethnic origin (N,%)	White	550	91.2	1309	89.4	727	88.9	0.31
Region of Europe (N,%)	South/Argentina	255	42.3	501	34.2	178	21.8	<.0001
	West Central	128	21.2	228	15.6	127	15.5	
	North	99	16.4	392	26.8	221	27.0	
	East central	96	15.9	229	15.6	218	26.6	
	East	25	4.1	115	7.8	74	9.0	
Prior AIDS (N,%)	Yes	146	24.2	369	25.2	239	29.2	0.05
Hepatitis B status (N,%)	Negative	417	69.2	1047	71.5	597	73.0	0.01
	Positive	28	4.6	58	4.0	53	6.5	
Hepatitis C status (N,%)	Negative	320	53.1	804	54.9	431	52.7	<.0001
	Positive	106	17.6	272	18.6	210	25.7	
Prior ARV treatment (N,%)	Naive	166	27.5	561	38.3	309	37.8	<.0001
	ART	57	9.5	93	6.3	38	4.6	
	cART	380	63.0	811	55.4	471	57.6	
NRTI backbone (N,%)	AZT & 3TC	275	45.6	677	46.2	396	48.4	<.0001
	DDI & D4T	47	7.8	117	8.0	55	6.7	
	D4T & 3TC	142	23.6	259	17.7	110	13.5	
	tenofovir + 1	46	7.6	144	9.8	127	15.5	
	abacavir + 1	47	7.8	147	10.0	59	7.2	
	Other	46	7.6	121	8.3	71	8.7	
Age (median, IQR)		40	34-47	40	34-46	40	33-46	0.74
CD4 count (median, IQR) cell/mm^3		359	230-583	323	190-535	252	131-439	<.0001
Nadir CD4 (median, IQR) cells/mm^3		190	98-287	170	70-258	114	48-210	<.0001
Viral load (median, IQR) log_{10} copies/ml)		2.70	1.70-4.56	3.59	1.70-4.95	4.05	2.07-5.14	<.0001
Regimen start date (month/year)		10/01	9/06-10/03	10/02	4/01-10/04	03/04	7/02-3/06	<.0001
Time to baseline* (days)		91	91-131	91	91-144	91	91-153	0.26

*baseline was the date of first virological suppression or three months after starting treatment, whichever occurred later

Table 5.3 Multivariable logistic regression investigating the odds of starting nevirapine compared to either efavirenz or lopinavir

		Odds of starting nevirapine compared to efavirenz			Odds of starting nevirapine compared to lopinavir		
		Odds Ratio	95% CI	P-value	Odds Ratio	95% CI	P-value
Gender	Male	1.00					
	Female	1.33	1.07-1.65	0.01			
HIV exposure group (N,%)	Homosexual				1.00		
	IDU				0.73	0.46-1.15	0.17
	Heterosexual				1.52	1.12-2.05	0.006
	Other				0.76	0.46-1.25	0.27
Region	South	1.00			1.00		
	West Central	1.11	0.85-1.45	0.42	0.66	0.46-0.94	0.02
	North	0.55	0.42-0.71	<.0001	0.28	0.20-0.40	<.0001
	East	1.11	0.83-1.48	0.48	0.55	0.39-0.76	0.0004
Hepatitis B status	Negative				1.00		
	Positive				0.67	0.39-1.14	0.13
	Unknown				0.98	0.65-1.49	0.93
Hepatitis B status	Negative				1.00		
	Positive				0.77	0.49-1.19	0.23
	Unknown				1.44	0.86-2.42	0.17
CD4 count at starting regimen	Per doubling	1.14	1.04-1.25	0.006	1.44	1.30-1.58	<.0001
Viral load at starting regimen	Per log10 increase	0.96	0.89-1.03	0.23			
Calendar year of starting regimen	Per year later	0.87	0.83-0.91	<.0001	0.67	0.63-0.71	<.0001

Variables included in the mulitvariable models were significant (p<0.1) in univariable analysis

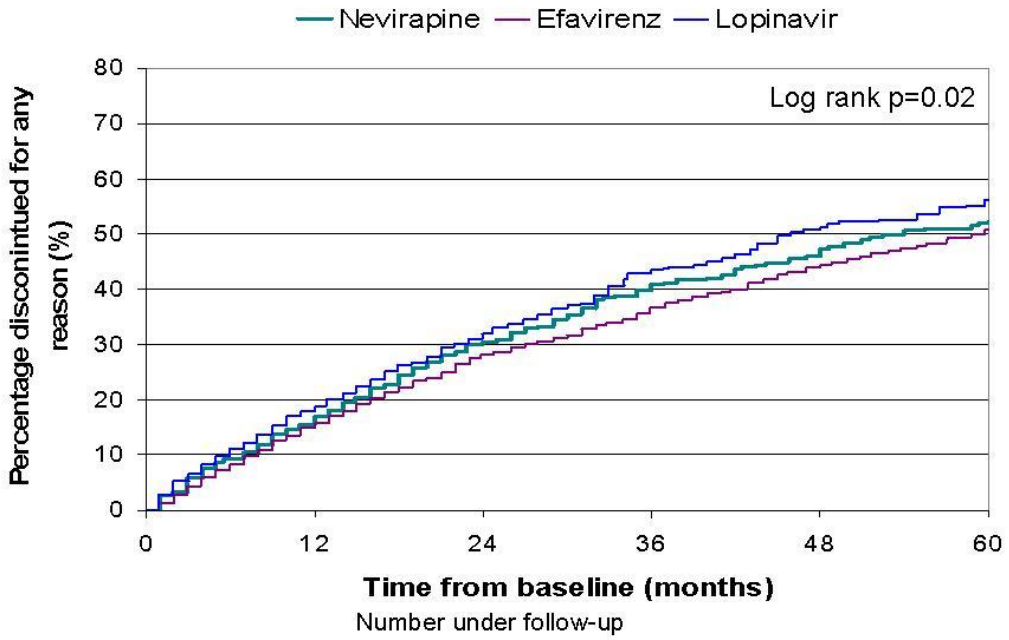
5.4.3 Discontinuation of treatment

5.4.3.1 All-cause discontinuation of third drug

A total of 1,417 (49%) patients discontinued nevirapine, efavirenz or lopinavir whilst under follow-up. 299 (50%) discontinued nevirapine, 748 (51%) patients discontinued efavirenz and 370 (45%) patients discontinued lopinavir. Figure 5.2 shows the Kaplan Meier estimation of the probability of all-cause discontinuation of these regimens. By 24 months after starting the regimen, 30.4% (95% CI 26.6-34.2) were estimated to have discontinued nevirapine, compared to 28.1% (95% CI 25.7-30.5) for efavirenz and 31.7% (95% CI 28.4-35.2) for lopinavir. The corresponding figures at 48 months were 47.2% (95%CI 42.9-51.5), 44.3% (95%CI 41.5-47.1), and 51.2% (95% CI 47.1-55.3) respectively (p=0.02).

In a multivariable Cox proportional hazards model (table 5.4), stratified by centre, compared to patients starting nevirapine there was no significant difference in the risk of discontinuation of efavirenz (Hazard ratio [HR] 1.06, 95%CI 0.91-1.23, p=0.43) or lopinavir (HR 1.14, 95%CI 0.96-1.36, p=0.13). There was a higher risk of discontinuation in female patients (HR 1.17, 95%CI 1.03-1.33, p=0.01), those with a lower nadir CD4 count (HR 1.07 per doubling, 95%CI 1.03-1.10, p<0.0001) and higher viral load (HR 1.07 per $\log_{10}$ increase, 95% CI1.03-1.11, p=0.0006). Older patients had lower odds of discontinuation (HR 0.94, 95%CI 0.89-1.00, p=0.05). Patients on a backbone of DDI / D4T had 39% higher risk of discontinuation of the third drug compared to patients with a backbone of AZT and 3TC (HR 1.39, 95% CI 1.14-1.69, P=0.0001).

Figure 5.2 Kaplan Meier risk of discontinuation of the third drug for any reason



Number under follow-up

Nevirapine	603	471	365	295	237	176
Efavirenz	1465	1157	900	704	531	383
Lopinavir	818	595	418	302	190	116

*baseline was defined as either the date of first virological suppression (defined as a single viral load < 500 copies/ml), or from 3 months after starting treatment, whichever occurred last

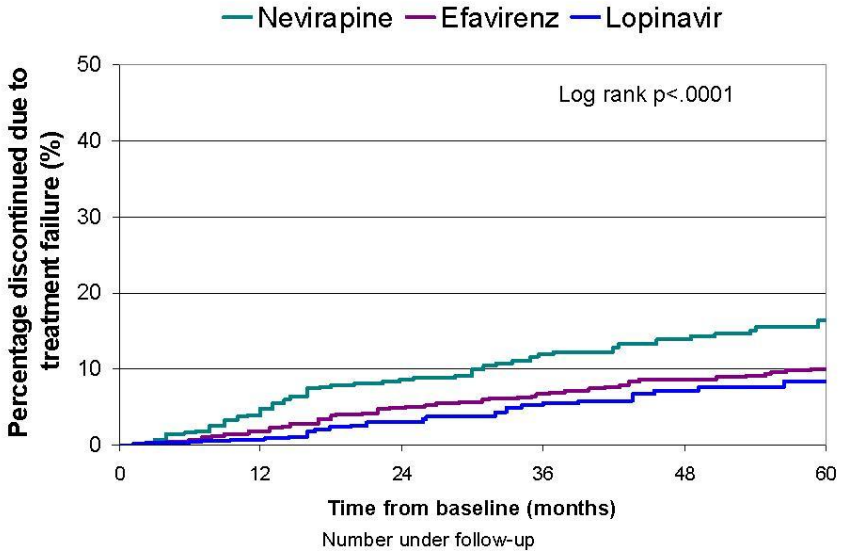
Table 5.4 Cox proportional hazards model investigating risk of all cause discontinuation of the third drug (both univariable and multivariable stratified by centre)

		Univariable			Multivariable		
		Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Regimen	Nevirapine	1.00	-	0.13			
	Efavirenz	1.09	0.94-1.27		1.06	0.91-1.23	0.43
	Lopinavir	1.19	1.00-1.41		1.14	0.96-1.36	0.13
Gender	Male	1.00	-	0.001	1.00		
	Female	1.23	1.09-1.39		1.17	1.03-1.33	0.01
HIV exposure group	Homosexual	1.00	-	0.23			
	IDU	1.14	0.97-1.34				
	Heterosexual	1.01	0.88-1.16				
	Other	0.90	0.71-1.13				
Ethnic Origin	White	1.00	-	0.76			
	Other	1.03	0.85-1.25				
Prior AIDS	No	1.00	-	0.23			
	Yes	0.93	0.82-1.05				
Hepatitis B status	Negative	1.00	-	0.82			
	Positive	1.08	0.84-1.39				
	Unknown	1.00	0.87-1.15				
Hepatitis C status	Negative	1.00	-	0.09	1.00	-	-
	Positive	1.18	1.01-1.67		1.15	0.99-1.34	0.07
	Unknown	0.91	0.77-1.09		0.90	0.75-1.07	0.23
Prior ARV	Naïve	1.00	-	0.41			
	Art	1.17	0.93-1.47				
	cART	1.04	0.90-1.20				
Age	per 10 years older	0.91	0.86-0.96	0.001	0.94	0.89-1.00	0.05
CD4 count	per 2 fold higher	1.02	0.97-1.06	0.47			
Nadir CD4	per 2 fold higher	1.06	1.03-1.10	<.0001	1.07	1.03-1.10	0.0001
Viral load	$\log_{10}$ copies/ml	1.09	1.05-1.13	<.0001	1.07	1.03-1.11	0.0006
Max viral load	$\log_{10}$ copies/ml	1.04	0.99-1.10	0.15			
Date started regimen	per more recent year	1.00	0.97-1.03	0.98			
NRTI backbone	AZT & 3TC	1.00	-	0.01	1.00	-	-
	DDI & D4T	1.38	1.13-1.68		1.39	1.14-1.69	0.0001
	D4T & 3TC	0.99	0.85-1.16		1.03	0.88-1.21	0.72
	Tenofovir + 1	1.08	0.89-1.32		1.06	0.86-1.29	0.59
	abacavir + 1	1.10	0.90-1.35		1.12	0.91-1.37	0.29
	Other	1.23	0.99-1.52		1.23	0.99-1.52	0.05

5.4.3.2 Discontinuation of the third drug due to treatment failure

74 (12%) patients discontinuing nevirapine, 101 (7%) patients discontinuing efavirenz and 33 (4%) patients discontinuing lopinavir did so because of reported treatment failure (virological, immunological or clinical). 155 (75%) patients discontinuing due to reported treatment failure (i.e. on patient follow-up forms) had a viral load >500 copies/ml measured in the 6 months prior to discontinuation when the viral load reported was assessed (62 [84%] nevirapine, 73 [72%] efavirenz, 20 [61%] lopinavir). Figure 5.3 shows the Kaplan Meier risk of discontinuation due to treatment failure. By 24 months after starting the regimen, 8.6% (95% CI 6.1-11.1) were estimated to have discontinued nevirapine , compared to 4.9% (95% CI 3.65-6.15) for efavirenz and 3.0% (95% CI 1.6-4.4) for lopinavir and after 48 months after starting the regimen, 13.9% (95% CI 10.6-17.2) were estimated to have discontinued nevirapine, compared to 8.6% (95% CI 6.8-10.4) for efavirenz and 7.2% (95% CI 4.6-9.8) for lopinavir (p<0.0001).

Figure 5.3 Kaplan Meier risk of discontinuation of the third drug due to treatment failure



Number under follow-up

Nevirapine	603	471	365	295	237	176
Efavirenz	1465	1157	900	704	531	383
Lopinavir	818	595	418	302	190	116

*baseline was defined as either the date of first virological suppression (defined as a single viral load < 500 copies/ml), or from 3 months after starting treatment, whichever occurred last

After adjustment for HIV exposure group, prior ARV treatment, viral load at starting regimen, date of starting regimen and NNRTI backbone, compared to patients starting nevirapine, patients starting efavirenz had a 48% lower risk of discontinuation due to treatment failure (HR 0.52, 95% CI 0.37-0.73, p=0.0002) and those starting lopinavir had a 63% lower risk of discontinuation due to treatment failure (HR 0.37, 95%CI 0.23-0.61, p<.0001) (table 5.4).

Furthermore, patients who had been exposed to cART previously had almost a 4-fold higher risk of discontinuation due to treatment failure (HR 3.83, 95% CI 2.28-6.45, p<.0001). A higher viral load at starting treatment was also associated with an increased risk of discontinuation due to treatment failure (HR 1.43 per log10 higher, 95%CI 1.27-1.61, p<.0001). In addition starting on the regimen more recently was associated with a 15% lower rate of discontinuation due to treatment failure per additional year (HR 0.85, 95% CI 0.77-0.95, p=0.005). A higher risk of discontinuation due to treatment failure was found in those not on a NRTI backbone of AZT and 3TC.

Table 5.5 Cox proportional hazards models to investigate the risk of discontinuation of the third drug due to treatment failure (both univariable and multivariable stratified by centre)

		Univariable			Multivariable		
		Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Regimen	Nevirapine	1.00	-	<.0001	1.00	-	-
	Efavirenz	0.56	0.40-0.78		0.52	0.37-0.73	0.0002
	Lopinavir	0.41	0.26-0.64		0.37	0.23-0.61	<.0001
Gender	Male	1.00	-	0.74			
	Female	0.95	0.97-1.33				
HIV exposure group	Homosexual	1.00	-	0.04	1.00	-	-
	IDU	0.62	0.40-0.96		0.66	0.42-1.04	0.07
	Heterosexual	0.87	0.62-1.22		0.91	0.64-1.29	0.57
	Other	0.42	0.19-0.93		0.45	0.19-1.05	0.06
Ethnic Origin	White	1.00	-	0.73			
	Other	0.91	0.51-1.61				
Prior AIDS	No	1.00	-	0.41			
	Yes	1.14	0.83-1.57				
Hepatitis B status	Negative	1.00	-	0.72			
	Positive	1.04	0.54-2.01				
	Unknown	0.86	0.69-1.25				
Hepatitis C status	Negative	1.00	-	0.79			
	Positive	0.90	0.91-1.34				
	Unknown	0.99	0.62-1.59				
Prior ARV	Naïve	1.00	-	0.0003	1.00	-	-
	ART	3.00	1.68-5.37		3.58	1.94-6.63	<.0001
	cART	2.19	1.41-3.40		3.83	2.28-6.45	<.0001
Age	per 10 years older	0.89	0.76-1.04	0.14			
CD4 count (per 2 fold higher)		1.03	0.92-1.15	0.65			
Nadir CD4 (per 2 fold higher)		1.02	0.95-1.11	0.54			
Viral load (log_{10} copies/ml)		1.16	1.05-1.28	0.003	1.43	1.27-1.61	<.0001
Max viral load (log_{10} copies/ml)		1.01	0.88-1.16	0.91			
Date started regimen (per year)		0.83	0.75-0.91	<.0001	0.85	0.77-0.95	0.005
NRTI backbone	AZT & 3TC	1.00	-	<.0001	1.00	-	-
	DDI & D4T	3.37	2.14-5.29		2.26	1.41-3.65	0.0007
	D4T & 3TC	1.50	0.97-2.31		1.28	0.82-2.01	0.27
	Tenofovir + 1	1.56	0.91-2.66		1.91	1.05-3.47	0.03
	abacavir + 1	2.63	1.59-4.33		2.31	1.37-3.89	0.001
	Other	2.34	1.36-4.05		2.42	1.39-4.23	0.001

5.4.3.3 Discontinuation of third drug due to toxicity or patient/physician choice (TOXPC)

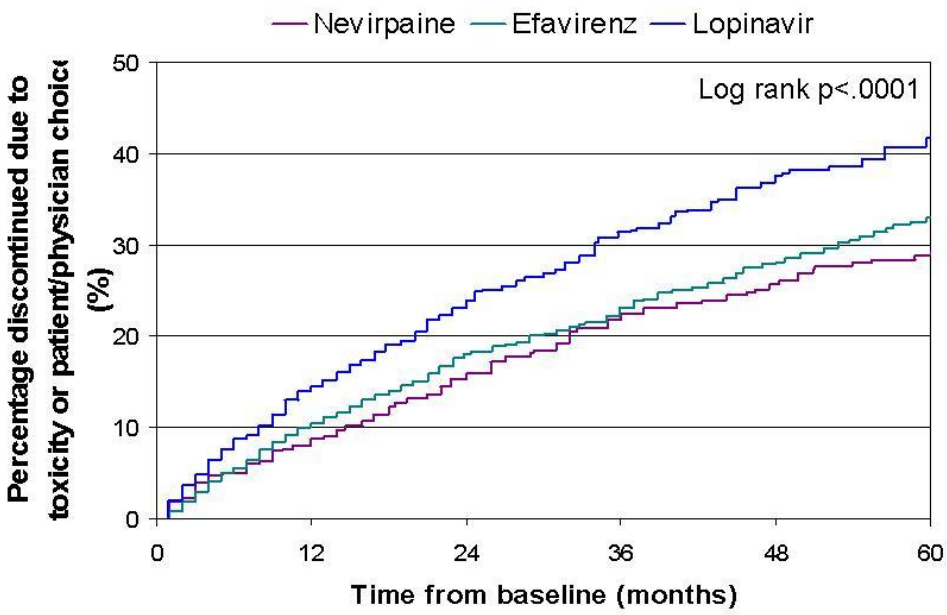
139 (23%) patients discontinuing nevirapine, 436 (30%) patients discontinuing efavirenz and 247 (30%) patients discontinuing lopinavir did so due to reported toxicity or patient/physician choice. As shown in table 5.6, the most commonly recorded toxicity for discontinuing nevirapine (21%) was associated with the GI tract, liver or pancreas; this was also the case for lopinavir (22%). Only 7% of patients discontinued efavirenz due to toxicities associated with the GI tract, liver or pancreas, the most common reported toxicities for efavirenz were associated with the central nervous system (26%).

Table 5.6 Toxicities reported as reason for discontinuing third drug

Reported toxicity	**nevirapine N(%)**	**efavirenz N(%)**	**lopinavir N(%)**
Total	**139 (100%)**	**436 (100%)**	**247 (100%)**
Abnormal fat redistribution	**7 (5%)**	**12 (3%)**	**25 (10%)**
Concern of cardiovascular disease	**1 (1%)**	**6 (1%)**	**7 (3%)**
Dyslipidemia	**4 (4%)**	**18 (4%)**	**26 (11%)**
Cardiovascular disease	**0 (0%)**	**1 (0%)**	**0 (%)**
Hypersensitivity reaction	**4 (3%)**	**2(0%)**	**0 (0%)**
Toxicity, from abdomen/GI tract	**11 (8%)**	**12 (3%)**	**19 (8%)**
Toxicity - GI tract	**2 (1%)**	**3 (1%)**	**30 (12%)**
Toxicity - Liver	**15 (11%)**	**11 (3%)**	**3 (1%)**
Toxicity - Pancreas	**1 (1%)**	**1 (0%)**	**2 (1%)**
Toxicity, from nervous system	**2 (1%)**	**112 (26%)**	**4 (2%)**
Toxicity, from kidneys	**0 (0%)**	**1 (0%)**	**2 (1%)**
Toxicity, from the endocrine system	**2 (1%)**	**1 (0%)**	**1 (0%)**
Diabetes	**0 (0%)**	**0 (0%)**	**0 (0%)**
Hematological toxicity	**1 (1%)**	**1 (0%)**	**1 (0%)**
Hyperlactataemia/lactic acidosis	**0 (0%)**	**3 (1%)**	**0 (0%)**
Toxicity, not specified above	**7 (5%)**	**26 (6%)**	**11 (4%)**
Patient's wish/decision	**48 (35%)**	**110 (25%)**	**62 (25%)**
Physician's decision	**34 (24%)**	**116 (27%)**	**54 (22%)**

The risk of discontinuation due to TOXPC 24 months after starting the regimen was estimated to be 15.9% (95% CI 12.7-19.1) for patients discontinuing nevirapine, compared to 18.1% (95% CI 16.0-20.2) for efavirenz and 24.0% (95% CI 20.8-27.2) for lopinavir (figure 5.4). By 48 months after starting the regimen, 25.7% (95% CI 21.6-29.8) were estimated to have discontinued nevirapine, compared to 28.1% (95% CI 25.4-30.4) for efavirenz and 37.5% (95% CI 33.3-37.5) for lopinavir p<0.0001).

Figure 5.4 Kaplan Meier risk of discontinuation of the third drug due to TOXPC



Number under follow-up

Nevirapine	603	471	365	295	237	176
Efavirenz	1465	1157	900	704	531	383
Lopinavir	818	595	418	302	190	116

*baseline was defined as either the date of first virological suppression (defined as a single viral load < 500 copies/ml), or from 3 months after starting treatment, whichever occurred later

After adjustment for gender, HIV exposure group, hepatitis C status, age, nadir Cd4 count, and viral load at starting regimen patients on efavirenz had 31% higher risk (HR 1.31, 95% CI 1.06-1.62, p=0.01) of discontinuation due to toxicities or patient/physician choice and patients on lopinavir had a 66% higher risk (HR 1.66, 1.31-2.10, p<.0001) of discontinuing due to toxicity or patient/physician choice, compared to those on nevirapine (Table 5.7). Additionally female patients had 22% higher risk of discontinuation due to TOXPC (HR 1.22, 95% CI 1.01-1.49, p=0.03) and higher nadir CD4 count was associated a higher risk of discontinuation (HR 1.08, 95%CI 1.04-1.13, p=0.0003).

Table 5.7 Cox proportional hazards models to investigate the risk of discontinuation of the third drug due to toxicity or patient choice (both univariable and multivariable stratified by centre)

		Univariable			Multivariable		
		Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Regimen	Nevirapine	1.00	-	<.0001	1.00		
	Efavirenz	1.32	1.07-1.63		1.31	1.06-1.62	0.01
	Lopinavir	1.67	1.33-2.10		1.66	1.31-2.10	<.0001
Gender	Male	1.00	-	0.02	1.00		
	Female	1.21	1.03-1.42		1.22	1.01-1.49	0.03
HIV exposure group	Homosexual	1.00	-	0.03	1.00		
	IDU	1.30	1.06-1.60		1.22	0.81-1.39	0.66
	Heterosexual	0.97	0.91-1.17		1.06	0.70-1.07	0.18
	Other	0.97	0.72-1.31		0.87	0.67-1.25	0.58
Ethnic Origin	White	1.00	-	0.39			
	Other	1.11	0.87-1.41				
Prior AIDS	No	1.00	-	0.13			
	Yes	0.88	0.74-1.04				
Hepatitis B status	Negative	1.00	-	0.57			
	Positive	0.98	0.81-1.61				
	Unknown	1.33	1.10-1.19				
Hepatitis C status	Negative	1.00	-	0.01	1.00		
	Positive	1.33	1.10-1.61		1.17	0.91-1.50	0.20
	Unknown	0.86	0.68-1.08		0.93	0.71-1.21	0.57
Prior ARV	Naïve	1.00	-	0.62			
	Art	1.02	0.74-1.41				
	cART	1.09	0.91-1.32				
Age (per 10 years)		0.93	0.86-1.00	0.05	0.96	0.89-1.04	0.32
CD4 count (per 2 fold higher)		1.02	0.96-1.08	0.51			
Nadir CD4 (per 2 fold higher)		1.08	1.03-1.12	0.0008	1.08	1.04-1.13	0.0003
Viral load (log_{10} copies/ml)		1.06	1.01-1.11	0.02	1.03	0.98-1.09	0.23
Max viral load (log_{10} copies/ml)		1.07	0.99-1.15	0.08			
Date started regimen (per year)		1.02	0.98-1.07	0.27			
NRTI backbone	AZT & 3TC	1.00	-	0.53			
	DDI & D4T	1.25	0.96-1.63				
	D4T & 3TC	1.05	0.86-1.28				
	Tenofovir + 1	1.11	0.86-1.44				
	abacavir + 1	1.01	0.77-1.33				
	Other	1.21	0.91-1.61				

5.4.3.4 Discontinuation of any drug

A secondary analysis investigated patients discontinuing any drug in the regimen (rather than nevirapine, efavirenz or lopinavir specifically). Where more than one of the drugs was discontinued, the first drug stopped was taken as the date of discontinuation. 2,143 patients (75%) discontinued at least one of the three/four drugs in the initial regimens. After adjustment for gender, hepatitis C, age, nadir CD4 count, viral load at starting regimen and NNRTI backbone, there was no significant difference in risk of discontinuation for any reason for patients on efavirenz (HR 0.91 95% CI 0.81-1.03,

p=0.15) or patients on lopinavir (HR 0.93, 95% CI 0.81-1.08, 0.35) compared to nevirapine (table 5.8). Of those patients who discontinued at least one drug in the regimen, 473 discontinued due to treatment failure; 74 (35%) on a nevirapine based regimen, 248 (20%) on efavirenz and 153 (23%) on lopinavir. After adjustment, there was a decreased risk of discontinuation due to treatment failure for patients on efavirenz (HR 0.49, 95%CI 0.35-0.69, p<.0001) and lopinavir (HR 0.40, 95% CI 0.25-0.64, p<.0001) compared to patients on nevirapine. 1110 patients discontinued due to TOXPC, 98 (46%) on nevirapine, 663 (53%) on efavirenz and 349 (52%) on lopinavir. After adjustment, there was an increased rate of discontinuation due to TOXPC for patients on lopinavir (HR 1.36, 95% CI 1.13-1.64, p=0.001) compared to nevirapine. There was no significant difference between patients on nevirapine and efavirenz (HR 1.12 95% CI 0.95-1.32, p=0.17).

Table 5.8 Multivariable analysis for discontinuation of any drug

Discontinuation due to	Regimen	HR	95% CI	P-value
[1]All cause	nevirapine	1.00	-	-
	efavirenz	0.91	0.81-1.03	0.15
	lopinavir	0.93	0.81-1.08	0.35
[2]Treatment failure	nevirapine	1.00	-	-
	efavirenz	0.49	0.35-0.69	<.0001
	lopinavir	0.40	0.25-0.64	<.0001
[3]Toxicity or patient/physician	nevirapine	1.00	-	-
	efavirenz	1.12	0.95-1.32	0.17
	lopinavir	1.36	1.13-1.64	0.001

1 also adjusted for gender, hepatitis C status, age, CD4 nadir, viral load, and NRTI backbone.
2 also adjusted for HIV exposure group, prior ARV treatment, viral load, date started regimen, and NRTI backbone.
3 also adjusted for gender, HIV exposure group, hepatitis C status, age, CD4 nadir, and viral load.

5.4.3.5 Competing risks for discontinuation of the third drug due to specific reasons

Competing risks analysis showed results consistent with those of the main analysis (table 5.9). After adjustment, compared to patients starting nevirapine, patients starting efavirenz had a 46% lower risk of discontinuation due to treatment failure (HR 0.54, 95% CI 0.39-0.76, p=0.0003) and those starting lopinavir had a 65% lower risk of discontinuation due to treatment failure (HR 0.35, 95%CI 0.22-0.56, p<.0001). Additionally, patients on efavirenz had 41% higher risk (HR 1.41, 95% CI 1.15-1.74, p=0.001) of discontinuation due to toxicities or patient/physician choice and patients on lopinavir had a 80% higher risk (HR 1.80, 1.43-2.27, p<.0001) of discontinuing due to toxicity or patient/physician choice, compared to those on nevirapine after adjustment (Table 5.9).

Table 5.9 Multivariable competing risks analysis for discontinuation of the third drug

Discontinuation due to	Regimen	HR	95% CI	P-value
[1]Treatment failure	nevirapine	1.00	-	-
	efavirenz	0.54	0.39-0.76	0.0003
	lopinavir	0.35	0.22-0.56	<.0001
[2]Toxicity or patient/physician	nevirapine	1.00	-	-
	efavirenz	1.41	1.15-1.74	0.001
	lopinavir	1.80	1.43-2.27	<.0001

1 also adjusted for HIV exposure group, prior ARV treatment, viral load, date started regimen, and NRTI backbone.
2 also adjusted for gender, HIV exposure group, hepatitis C status, age, CD4 nadir, and viral load.

5.4.3.6 Subgroup analysis of discontinuation of the third drug in Antiretroviral Naïve patients

1,036 patients were antiretroviral naïve at starting their regimen (166 [16%] on nevirapine, 561 [55%] on efavirenz and 309 [29%] on lopinavir). The test for interaction between treatment regimen and being naïve was significant for all-cause discontinuation of the third drug (p=0.0008). 412 patients discontinued either nevirapine (68, 41%), efavirenz (217, 39%) or lopinavir (127, 41%) for any reason whilst under follow-up. After adjustment for gender, age, nadir CD4 count, viral load at starting regimen and NNRTI backbone, patients on lopinavir had a marginally significantly higher rate of discontinuation for any reason (HR 1.39, 95%CI 0.96-2.01, p=0.07) than patients on nevirapine, there was no significant difference between patients on efavirenz and those on nevirapine (HR 0.92, 95%CI 0.67-1.28, p=0.62). Only 32 antiretroviral naïve patients discontinued due to treatment failure; 13 (8%) on nevirapine, 16 (3%) on efavirenz and 3 (1%) on lopinavir, limiting the ability to do further analyses. A higher number of patients discontinued due to toxicity or patient choice vs. failure; 34 (20%) discontinued nevirapine, 118 (21%) efavirenz and 84 (27%) lopinavir. Patients on lopinavir had a significantly higher rate of discontinuation due to toxicity or patient choice compared to patients on nevirapine (HR 1.69, 95% CI 1.06-2.76, p=0.02), the magnitude of effect is similar to the main analysis. There was no significant difference between patients on efavirenz and those on nevirapine (HR 0.98, 95%CI 0.64-1.48, p=0.91) after adjustment for nadir CD4 and hepatitis C status (table 5.10).

Table 5.10 Multivariable subgroup analysis in naïve patients

Discontinuation of third drug due to	Regimen	HR	95% CI	P-value
[1]All cause	nevirapine	1.00	-	-
	efavirenz	0.92	0.67-1.28	0.62
	lopinavir	1.39	0.96-2.01	0.07
[2]Treatment failure	nevirapine			
	efavirenz			
	lopinavir			
[3]Toxicity or patient/physician	nevirapine	1.00	-	-
	efavirenz	0.98	0.64-1.48	0.91
	lopinavir	1.69	1.06-2.76	0.02

1 also adjusted for gender, hepatitis C status, age, CD4 nadir, viral load, and NRTI backbone.
2 also adjusted for HIV exposure group, prior ARV treatment, viral load, date started regimen, and NRTI backbone.
3 also adjusted for gender, HIV exposure group, hepatitis C status, age, CD4 nadir, and viral load.

5.4.4 Incidence of serious non-AIDS related clinical events

Table 5.11 describes the number of serious non-AIDS related events developed after starting each regimen. Overall, there was a low rate of serious non-AIDS events. A total of 183 (6%) patients developed at least 1 non-AIDS related event whilst under follow-up; 49 (8%) on nevirapine, 81 (6%) on efavirenz and 53 (7%) on lopinavir.

Table 5.11 Number of patients experiencing a serious non-AIDS related event

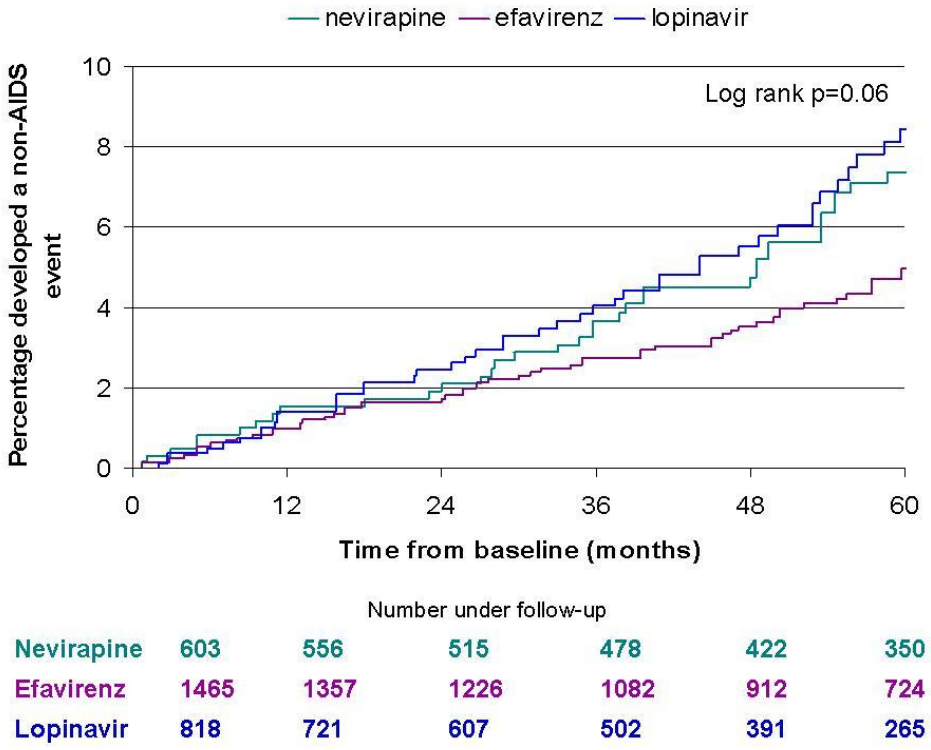
	Nevirapine	Efavirenz	Lopinavir
Total person years of follow-up	3238.6	7086.2	3134.3
Any clinical event (N,%)	49 (8.1)	81 (5.5)	53 (6.5)
NADM (N,%)	18 (3.0)	26 (1.8)	16 (2.0)
Liver related (N,%)	5 (0.8)	9 (0.6)	11 (1.3)
Pancreatitis (N,%)	3 (0.5)	3 (0.2)	5 (0.6)
End stage renal disease (N,%)	2 (0.3)	4 (0.3)	4 (0.5)
Stroke (N,%)	7 (1.2)	9 (0.6)	5 (0.6)
MI (N,%)	10 (1.7)	21 (1.4)	10 (1.2)
Other CVD events (N,%)	10 (1.7)	20 (1.4)	6 (0.7)

NADM=non AIDS-defining malignancies; MI=myocardial infarction
CVD=cardiovascular disease

Figure 5.5 shows the Kaplan Meier estimation of the probability of developing a serious non-AIDS event after baseline. The probability of developing a serious non-AIDS event was marginally lower on efavirenz than for the other 2 drugs (p=0.06). For example, by 24 months after starting the regimen, 1.5% (95% CI 0.5-2.4) of patients treated with nevirapine were estimated to have developed a serious non-AIDS event, compared to 1.0% (95% CI 0.5-1.5) for efavirenz and 1.4% (95% CI 0.6-2.2) for lopinavir. The corresponding figures at 48 months were 2.1% (95%CI 0.9-3.3), 1.7% (95%CI 1.1-2.3), and 2.5% (95% CI 1.4-3.6) respectively (log-rank p=0.06).

Overall, the incidence of serious non-AIDS related events was 1.5 per 100 PYFU (95% CI 1.1-1.9) for patients on a nevirapine regimen. Patients on efavirenz had a lower incidence of non-AIDS related events of 1.1 per 100 PYFU (95% CI 0.9-1.4), and on lopinavir had a higher incidence of 1.7 per 100 PYFU (95% CI 1.2-2.2). However, these differences were not significant, p=0.12 and p=0.57 respectively.

Figure 5.5 Kaplan Meier risk of developing a serious non-AIDS event



*baseline was defined as either the date of first virological suppression (defined as a single viral load < 500 copies/ml), or from 3 months after starting treatment, whichever occurred last

The results of the Poisson regression analysis are given in table 5.12. After adjustment for the factors shown in table 5.12, patient who had started an efavirenz based regimen had a non-significant lower incidence of non-AIDS related events compared to those who started a nevirapine regimen (IRR 0.75, 95% CI 0.51-1.09, p=0.13). There was no significant difference between who had started a lopinavir based regimen and those who started nevirapine (IRR 1.10, 95% CI 0.71-1.69, p=0.66). Female patients had a lower incidence of non-AIDS related events (IRR 0.51, 95%CI 0.32-0.83, p=0.007). Older patients (IRR 2.17 per 10 years older, 95%CI 1.84-2.56 p<.0001), those testing hepatitis C positive (IRR 2.21 vs. negative, 95%CI 1.31-3.75, p=0.003) and patients from north Europe compared to south Europe (IRR 1.92, 95%CI 1.26-2.91, p=0.002) had an increased incidence of non-AIDS related events.

Table 5.12 Poisson regression analysis: Risk of developing a non-AIDS clinical event

		Univariable			Multivarible		
		IRR	95% CI	P value	IRR	95% CI	P value
Treatment	Nevirapine	1.00	-	0.06	1.00	-	-
	Efavirenz	0.76	0.53-1.08		0.75	0.52-1.09	0.13
	Lopinavir	1.18	0.76-1.65		1.10	0.72-1.69	0.66
Gender	Male	1.00	-		1.00	-	-
	Female	0.39	0.25-0.61	<.0001	0.51	0.32-0.83	0.007
Ethnic origin	White	1.00	-		1.00	-	-
	Other	0.50	0.27-0.92	0.02	0.63	0.33-1.19	0.15
Exposure Group	Homosexual	1.00	-	0.001	1.00	-	-
	IDU	1.12	0.78-1.60		1.50	0.86-2.59	0.15
	Heterosexual	0.51	0.34-0.76		0.90	0.58-1.39	0.62
	Other	1.29	0.77-2.14		1.29	0.75-2.22	0.35
Region of Europe	South	1.00	-	0.0002	1.00	-	-
	West Central	1.35	0.89-2.05		1.50	0.94-2.39	0.09
	North	1.76	0.23-2.51		1.92	1.26-2.91	0.002
	East Central	0.71	0.42-1.20		1.22	0.69-2.15	0.49
	East	0.38	0.09-1.56		1.13	0.26-4.87	0.87
Hepatitis B	Negative	1.00	-	0.43			
	Positive	1.45	0.82-2.57				
	Unknown	0.96	0.68-1.35				
Hepatitis C	Negative	1.00	-	0.01	1.00	-	-
	Positive	1.70	1.19-2.42		2.21	1.31-3.75	0.003
	Unknown	0.74	0.50-1.09		0.68	0.40-1.16	0.15
Prior AIDS	No	1.00	-	0.08	1.00		
	Yes	1.31	0.96-1.78		1.12	0.80-1.56	0.52
Prior treatment	Naïve	1.00	-	<.0001	1.00	-	-
	ARV	2.29	1.23-4.27		1.67	0.84-3.33	0.14
	cART	2.31	1.55-3.46		1.05	0.64-1.72	0.85
Anaemia	No	1.00	-	0.006	1.00	-	-
	Yes	1.50	1.02-2.21		1.14	0.76-1.70	0.52
Diabetes	No	1.00	-	0.09	1.00	-	-
	Yes	1.78	0.94-3.37		0.90	0.46-1.75	0.75
Hypertension	No	1.00	-	<.0001	1.00	-	-
	Yes	2.19	1.47-3.25		1.22	0.80-1.49	0.34
Smoking status	No	1.00	-	0.03	1.00	-	-
	Yes	1.94	1.08-3.46		1.63	0.89-2.98	0.11
	Previous	1.21	0.60-2.46		0.88	0.43-1.82	0.74
Age	per 10 years	2.01	1.77-2.29	<.0001	2.17	1.84-2.56	<.0001
CD4 count (per 2 fold increase)		0.98	0.88-1.09	0.72			
Nadir CD4 (per 2 fold increase)		0.94	0.88-1.01	0.09	0.99	0.91-1.07	0.83
Viral load (per $\log_{10}$ higher)		0.87	0.89-0.95	0.003			
Peak viral load (per $\log_{10}$ higher)		0.80	0.70-0.90	0.0004	0.80	0.69-0.91	0.001
Date of starting regimen (per year)		0.90	0.83-0.98	0.01	0.88	0.79-0.99	0.03
NRTI backbone	AZT & 3TC	1.00	-	0.04	1.00	-	-
	DDI & D4T	1.41	0.87-2.28		1.23	0.73-2.05	0.44
	D4T & 3TC	1.12	0.75-1.66		0.90	0.59-1.36	0.60
	Tenofovir + 1	1.35	0.81-2.24		1.03	0.59-1.80	0.72
	abacavir + 1	1.93	1.24-3.02		1.53	0.96-2.44	0.07
	Other	0.72	0.36-1.45		0.62	0.31-1.26	0.19

IRR=incidence rate ratio

5.4.5 Deterioration of surrogate markers for clinical disease

As mentioned in the methods section, not all patients had information on surrogate markers such as, body weight or total cholesterol available for all analyses, therefore the number for patients included in each analysis focusing on each of these markers separately differed according to the availability of the data. In general, patients included and those excluded from each of the analysis were similar. However, a high proportion of patients in east Europe were excluded from the analysis focusing on haemoglobin measurements (93% of patients in east Europe did not have a measurement and were excluded), and HDL cholesterol measurements (90% of patients in east Europe were excluded). Table 5.13 summarises the number of patients included in each analysis, as well as the number of events observed during follow-up.

Table 5.13 Summary of patients included in each analysis

1.Developing or Worsening anaemia			
Regimen	N included	Baseline haemoglobin (mg/dl), median (IQR)	n events
Nevirapine	360 (59.7)	14.3 (13.2-15.3)	110 (30.6)
Efavirenz	721 (49.2)	14.4 (13.2-15.3)	222 (30.8)
Lopinavir	351 (42.9)	13.8 (12.8-14.8)	75 (21.4)
2.Losing >10% of body weight			
Regimen	N included	Baseline weight (Kg), median (IQR)	n events
Nevirapine	299 (49.6)	69 (61-77)	50 (16.7)
Efavirenz	755 (51.5)	71 (63-79)	134 (17.8)
Lopinavir	435 (53.2)	69 (61-78)	67 (15.4)
3.Total cholesterol >6.2mmol/l			
Regimen	N included	Baseline total cholesterol (mmol/l), median (IQR)	n events
Nevirapine	309 (51.2)	5.1 (4.3-6.0)	103 (33.3)
Efavirenz	699 (47.7)	5.1 (4.3-6.0)	228 (32.6)
Lopinavir	386 (47.2)	5.0 (4.1-6.0)	120 (31.9)
4.HDL cholesterol <0.9mmol/l			
Regimen	N included	Baseline HDL cholesterol (mmol/l), median (IQR)	n events
Nevirapine	194 (32.2)	1.2 (0.9-1.5)	46 (23.7)
Efavirenz	489 (33.4)	1.1 (0.9-1.4)	123 (25.2)
Lopinavir	256 (31.3)	1.1 (0.9-1.3)	81 (31.6)
5.AST >2 times upper limit of normal			
Regimen	N included	Baseline AST (IU/L), median (IQR)	n events
Nevirapine	296 (49.1)	25 (18-36)	38 (12.8)
Efavirenz	637 (43.5)	26 (19.39)	65 (10.2)
Lopinavir	454 (55.5)	23 (16-33)	48 (10.6)
6.ALT >2 times upper limit of normal			
Regimen	N included	Baseline ALT (IU/L), median (IQR)	n events
Nevirapine	310 (51.4)	30 (20-48)	74 (23.9)
Efavirenz	811 (55.4)	30 (19.52)	175 (21.6)
Lopinavir	491 (60.0)	26 (17.45)	77 (15.7)

Table 5.14 shows the incidence rates for deterioration of each marker of clinical disease by treatment regimen started at baseline. For patients who started on a nevirapine based regimen, the highest incidence rate was for total cholesterol increasing to >6.2 mmol/l, IR 7.9 per 100 PYFU (95%CI 6.4-9.5), this was also true for patients who started on efavirenz IR 8.9 per 100 PYFU (9%CI 7.7-10.0). The incidence rate of total cholesterol increasing to >6.2 mmol/l was also higher in the lopinavir group IR 10.6 per 100 PYFU (95%CI 8.1-12.5), but a higher incidence rate was observed for HDL cholesterol decreasing to <0.9mmol/l, IR 11.0 per 100 PYFU (8.6-13.4). This was almost double the incidence rate observed in the nevirapine and efavirenz groups. In all three groups there was a relatively low rate of AST increasing to >2 times the upper limit of normal (table 5.14).

Table 5.14 Crude incidence rates (IR) per 100 person years of follow-up (PYFU) for worsening of surrogate markers for clinical disease across the three treatment regimens

1.Developing or Worsening anaemia			
Regimen	N events	PYFU	IR per 100 PYFU (95% CI)
Nevirapine	110 (30.6)	1723.8	6.4 (5.2-7.6)
Efavirenz	222 (30.8)	2888.2	7.7 (6.7-8.7)
Lopinavir	75 (21.4)	1276.8	5.9 (4.5-7.2)
2.Losing >10% of body weight			
Regimen	n events	PYFU	IR per 100 PYFU (95% CI)
Nevirapine	50 (16.7)	1304.0	3.8 (2.8-4.9)
Efavirenz	134 (17.8)	3085.9	4.3 (3.6-5.1)
Lopinavir	67 (15.4)	1446.1	4.6 (3.5-5.7)
3.Total cholesterol >6.2mmol/l			
Regimen	n events	PYFU	IR per 100 PYFU (95% CI)
Nevirapine	103 (33.3)	1299.1	7.9 (6.4-9.5)
Efavirenz	228 (32.6)	2564.3	8.9 (7.7-10.0)
Lopinavir	120 (31.9)	1134.3	10.6 (8.4-12.5)
4.HDL cholesterol <0.9mmol/l			
Regimen	n events	PYFU	IR per 100 PYFU (95% CI)
Nevirapine	46 (23.7)	791.6	5.8 (4.1-7.5)
Efavirenz	123 (25.2)	1782.0	6.9 (5.7-8.1)
Lopinavir	81 (31.6)	735.4	11.0 (8.6-13.4)
5.AST >2 times upper limit of normal			
Regimen	n events	PYFU	IR per 100 PYFU (95% CI)
Nevirapine	38 (12.8)	1455.4	2.6 (1.8-3.4)
Efavirenz	65 (10.2)	2770.6	2.4 (1.8-2.9)
Lopinavir	48 (10.6)	1619.3	3.0 (2.1-3.8)
6.ALT >2 times upper limit of normal			
Regimen	n events	PYFU	IR per 100 PYFU (95% CI)
Nevirapine	74 (23.9)	1371.6	5.4 (4.2-6.6)
Efavirenz	175 (21.6)	3297.6	5.3 (4.5-6.1)
Lopinavir	77 (15.7)	1701.3	4.5 (3.5-5.5)

Table 5.15 Poisson regression analysis investigating the incidence of worsening of surrogate markers for clinical disease across the three treatment regimens

1.Developing or Worsening anaemia						
	Univariable			Mulitvariable		
Regimen	IRR	95% CI	Global p-value	IRR	95% CI	p-value
Nevirapine	1.00		0.07	1.00		
Efavirenz	1.20	0.96-1.51		1.16	0.91-1.46	0.23
Lopinavir	0.92	0.69-1.23		0.82	0.29-1.13	0.22
2.Losing >10% of body weight						
	Univariable			Mulitvariable		
Regimen	IRR	95% CI	Global p-value	IRR	95% CI	p-value
Nevirapine	1.00		0.58	1.00		
Efavirenz	1.13	0.82-1.57		1.13	0.81-1.56	0.46
Lopinavir	1.21	0.84-1.74		1.15	0.78-1.67	0.46
3.Total cholesterol >6.2mmol/l						
	Univariable			Mulitvariable		
Regimen	IRR	95% CI	Global p-value	IRR	95% CI	p-value
Nevirapine	1.00		0.09	1.00		
Efavirenz	1.12	0.89-1.42		1.04	0.58-1.32	0.72
Lopinavir	1.33	1.03-1.74		1.22	0.92-1.64	0.17
4.HDL cholesterol <0.9mmol/l						
	Univariable			Mulitvariable		
Regimen	IRR	95% CI	Global p-value	IRR	95% CI	p-value
Nevirapine	1.00		0.0006	1.00		
Efavirenz	1.19	0.85-1.67		1.16	0.82-1.65	0.39
Lopinavir	1.90	1.32-2.72		1.80	1.22-2.66	0.003
5.AST >2 times upper limit of normal						
	Univariable			Mulitvariable		
Regimen	IRR	95% CI	Global p-value	IRR	95% CI	p-value
Nevirapine	1.00		0.47	1.00		
Efavirenz	0.90	0.60-1.34		0.86	0.57-1.29	0.46
Lopinavir	1.14	0.74-1.74		1.14	0.72-1.80	0.59
6.ALT >2 times upper limit of normal						
	Univariable			Mulitvariable		
Regimen	IRR	95% CI	Global p-value	IRR	95% CI	p-value
Nevirapine	1.00		0.43	1.00		
Efavirenz	0.98	0.75-1.29		0.98	0.73-1.30	0.87
Lopinavir	0.84	0.61-1.15		0.84	0.60-1.18	0.30

Multivariable analysis:

1 adjusted for HIV exposure group, region of Europe, Hepatitis B and C status, NRTI backbone, prior treatment, maximum viral load, year of starting regimen and haemoglobin level at baseline

2 adjusted for HIV exposure group, hepatitis C status, CD4 count at starting treatment and weight at baseline.

3 adjusted for gender, ethnic origin, HIV exposure group, region of Europe, hepatitis B and C status, current BMI, age, date of starting regimen and total cholesterol at baseline

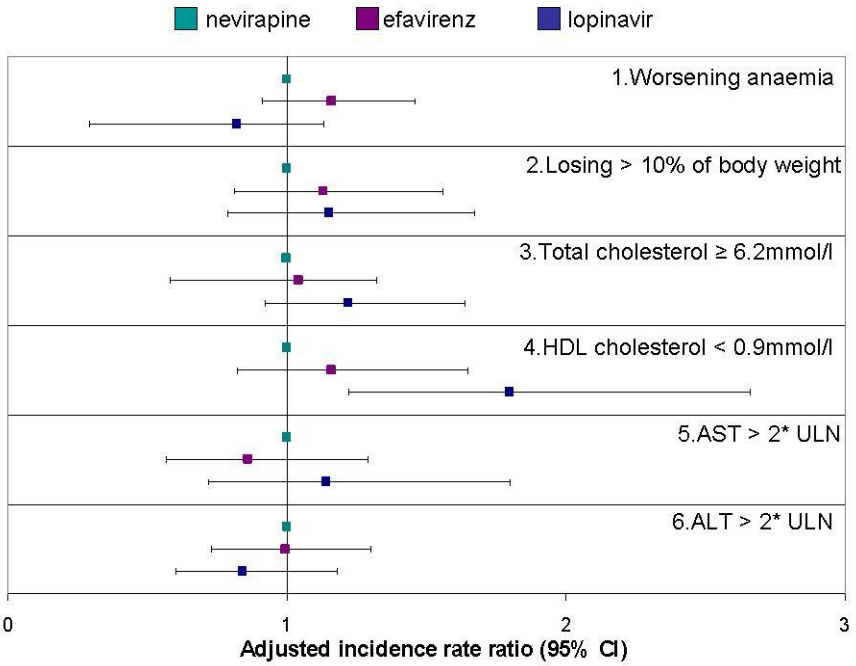
4 adjusted for gender, ethnic origin, HIV exposure group, region of Europe, hepatits C status, age, date of starting regimen and HDL cholesterol at baseline

5 adjusted for HIV exposure group, region of Europe, hepatitis C status, age, viral load, date of starting regimen and baseline AST level

6 adjusted for gender, HIV exposure group, region of Europe, Hepatitis B and C status, prior AIDS diagnosis, NRTI backbone, age and ALT level at baseline

The results of the univariable and multivariable Poisson regression analysis of each event are shown in table 5.15. After adjustment for gender, ethnic origin, HIV exposure group, region of Europe, hepatitis C status, age, date of starting regimen and HDL cholesterol at baseline there was a higher rate for decreasing HDL cholesterol to < 0.9 mmol/l (IRR 1.80, 95%CI 1.22-2.66, p=0.003) in those who had started on a lopinavir based regimen compared to those who had started on nevirapine. There was no significant difference between efavirenz (IRR 1.16, 95%CI 0.82-1.65, p=0.39) and nevirapine. In univariable analysis a higher incidence rate of total cholesterol increasing to >6.2mmol/l was also observed in patients who had started on lopinavir based cART (IRR 1.33, 95%CI 1.03-1.74), compared to nevirapine. A non-significant increased rate was observed after adjustment for gender, ethnic origin, HIV exposure group, region of Europe, hepatitis B and C status, current BMI, age, date of starting regimen and total cholesterol at baseline, (IRR 1.22, 95%CI 0.92-1.64, p=0.17) this may be due to lack of power. As shown in figure 5.6, no other significant differences in the incidence rates of deterioration of the other surrogate markers for clinical disease was observed between nevirapine and efavirenz and lopinavir, after each model was adjusted for the factors listed below table 5.15.

Figure 5.6 Poisson regression analysis models investigate the incidence of worsening of surrogate markers for clinical disease across the three treatment regimens



5.4.6 Incidence of all-cause mortality

23 patients on nevirapine died with an incidence rate of 0.68 per 100 PYFU (95% CI 0.40-0.96), 61 patients on efavirenz died with an incidence on 0.84 per 100 PYFU (95% CI 0.63-1.05) and 50 patients on lopinavir with an incidence rate of 1.55 per 100 PFYU (95% CI 1.12-1.98). Patients on lopinavir had a significantly higher crude rate of death than those on nevirapine (IRR 2.27, 95%CI 1.39-3.72, p=0.001). In contrast, there was no significant difference in crude mortality rates between patients on efavirenz and nevirapine (IRR 1.23, 95% CI 0.76-1.99, p=0.39).

Table 5.9 shows the cause of death reported for each patient who died. In patients on nevirapine only 1 death was due to AIDS related causes, compared to 14 on efavirenz and 10 on lopinavir. After AIDS related causes, hepatitis related deaths were the second biggest cause of death accounting for 17% of the deaths. Of note, liver cancer was included in hepatitis related causes. All other non-AIDS malignancies (NADM) were grouped together and 12% of the deaths were due to these cancers.

Table 5.16 Cause of death by treatment group

	All	Nevirapine	Efavirenz	Lopinavir	P-value
All-cause	**134 (4.6)**	**23 (3.8)**	**61 (4.2)**	**50 (6.1)**	**0.05**
AIDS related death	25 (18.7)	1 (4.3)	14 (23.0)	10 (20.0)	0.09
Non-AIDS infection	11 (8.2)	2 (13.0)	5 (8.2)	3 (6.0)	0.86
Hepatitis related	23 (17.2)	4 (17.4)	8 (13.1)	11 (22.0)	0.11
NADM	16 (11.9)	4 (17.4)	8 (13.1)	4 (8.0)	0.90
CVD	13 (9.7)	1 (4.4)	8 (13.1)	4 (8.0)	0.49
Violent	13 (9.7)	4 (17.4)	6 (9.8)	3 (6.0)	0.67
Other	7 (5.2)	1 (4.4)	3 (4.9)	3 (6.0)	0.68
Unknown	26 (19.4)	5 (21.7)	9 (14.8)	12 (24.0)	0.11

After adjustment for HIV exposure group, hepatitis C status, prior antiretroviral treatment, age, CD4 count at starting regimen, anaemia, hypertension, smoking status and NRTI backbone, patients on lopinavir had a marginally significant increased risk of death (IRR 1.66, 95%CI 0.99-2.78, p=0.05) compared to patients on nevirapine. There was no significant difference in the risk of death between patients on efavirenz and those on lopinavir (IRR 1.19, 95%CI 0.73-1.94, p=0.48).

5.5 Discussion

The analyses presented in this chapter aimed at comparing the long term durability of nevirapine, efavirenz and lopinavir based cART regimens. As the focus was on long term durability, patients were only included once virologic suppression had been achieved, and after at least 3 months exposure to the drug to exclude discontinuations due to early-onset potentially treatment limiting toxicities. No significant difference was found in the rate of discontinuation for any reason between the three treatment regimens, although differences were found in the rate of discontinuation for specific reasons. In particular, patients starting nevirapine had a higher rate of discontinuation due to reported treatment failure and a lower rate of discontinuation due to toxicity or patient/physician choice compared to those on efavirenz and lopinavir. There was no significant difference in the development of any non-AIDS clinical events. However, the number of non-AIDS clinical events was quite small and this may have been due to lack of power. Additionally, the incidence rate of worsening in anaemia, severe weight loss, or increasing in ALT or AST levels was not significantly different between those who had started a nevirapine based cART regimen and those who started efavirenz or lopinavir. Patients on lopinavir had a higher rate of decline of HDL cholesterol compared to patients on nevirapine, while there was no difference in low HDL cholesterol between patients on efavirenz and nevirapine. Furthermore, patients on lopinavir had a marginally higher mortality rate compared to nevirapine but there was no significant difference between efavirenz and nevirapine, again perhaps due to lack of power.

Randomised controlled trials have found that nevirapine and efavirenz have similar rates of treatment failure[300;600]. The definition of treatment failure in the 2NN clinical trial[300] was defined as a combined endpoint of virological failure, disease progression or therapy change and the main reason given for treatment failure was a change in therapy. Annan et al.[600] defined treatment failure as either virological failure or discontinuation of therapy. In addition, the ARTEN study[601] demonstrated non-inferiority between nevirapine and atazanavir, a ritonavir boosted PI, in a population of antiretroviral naïve patients where the primary endpoint was two consecutive viral loads <50copies/ml measured prior to week 48 from treatment initiation.

The analyses reported in this chapter were based on a reported reason for discontinuation of treatment rather than treatment failure defined through virologic or immunologic measurements, in patients who had initially tolerated and responded to treatment. This definition is closer to the more recent study definition of treatment failure and the results presented here are consistent with their findings.

Previous analyses of patients enrolled in cohort studies[299;567;568] have found that in both antiretroviral naïve and experienced patients[298], efavirenz had a significantly lower rate of treatment failure compared to nevirapine; the outcome in these analyses made use of the actual viral loads instead of the reason for stopping a drug given by the treating clinicians. When the analysis on this chapter was restricted to discontinuations due to reported treatment failure, rather than all cause discontinutations, our findings were similar to these observational studies in that a decreased rate of discontinuation due to reported treatment failure was found in those on efavirenz compared to nevirapine. This difference between cohort studies and RCT's is likely due to the fact that many people starting nevirapine and efavirenz in a routine clinical setting differ in a number of ways, many of which are not measured in the observational setting, e.g. people likely to have central nervous system (CNS) toxicity are put on nevirapine, and CNS abnormality may have an impact of some other factors related to virologic failure, such as adherence.

It has previously been reported that the choice of nucleoside reverse transcript inhibitor (NRTI) backbone is a significant predictor of virological success and treatment failure[600], and therefore the pair of nucleosides is a potential confounding factor for the comparison of interest. However, even after adjustment for NRTI backbone, significant differences remained in the risk of discontinuation due to reported treatment failure. A systematic review by Bartlett et al.[602] found that 4%-16% of patients on efavirenz discontinued treatment due to adverse events, and that discontinuations appeared to be higher in patients on an NRTI backbone of lamivudine plus zidovudine or abacavir, and lower in regimens with a backbone of tenofovir and emtricitabine, tenofovir and lamivudine, stavudine and lamivudine, and didanosine and emtricitabine, although no statistical tests for differences were performed. In contrast to these findings, the analysis in this thesis found that patients with a NRTI backbone of stavudine and didanosine had a higher risk of discontinuation for any reason compared to those with a backbone of zidovudine and lamivudine.

Further, when looking at discontinuation due to reported treatment failure a higher risk of discontinuation were found in patients on a different backbone to zidovudine and lamivudine. In patients with extensive resistance to other drug classes nevirapine has been found to be associated with an inferior virological outcome compared to patients on efavirenz[565]. However, the same analysis showed that after accounting for resistance there was still a difference in virological outcomes between nevirapine and efavirenz[565].

In the analysis reported here, very few discontinuations in ART-naïve patients, in any group, were due to reported treatment failure. Therefore in treatment naïve patients, the results suggest that if the regimen can be successfully tolerated in the first few months and viral suppression achieved nevirapine is a durable treatment strategy, in terms of discontinuation due to treatment failure, compared to efavirenz and lopinavir

Patients on lopinavir and efavirenz had a higher rate of discontinuation due to toxicities or patient/physician choice. Other studies have found that nevirapine was associated with a higher rate of toxicities when compared to efavirenz[300;345], and the ARTEN study[603] found that discontinuation was higher in those on nevirapine compared to atazanavir. However, most of the discontinuations due to toxicity in nevirapine have been reported in the first few months on therapy[299;400;603]. The principle adverse event associated with nevirapine is hypersensitivity[289;290;299;604]. Additionally ,life threatening hepatotoxicity[605] and hepatitis have also been reported in patients on nevirapine. These side effects mainly occur in the first 6 weeks of therapy[223]. As mentioned previously, this analysis focused on patients who had tolerated the first 3 months of therapy. Thus short term toxicities, such as hypersensitivity, leading to early discontinuation would have been excluded. Lodwick et al[606] found that, compared to patients on efavirenz, there was no significant difference in the rate of treatment change due to toxicities in patients on nevirapine, but a significantly increased rate of changes due to toxicity in patients on lopinavir. This study used similar inclusion criteria to this analysis but only included antiretroviral naïve patients, consistent with our sensitivity analysis in antiretroviral-naïve patients.

Non-AIDS related malignancies and cardiovascular related events were the most commonly observed non-AIDS related events. A lower incidence of non-AIDS related events was found in to those who started an efavirenz based cART regimen compared to nevirapine. Although this difference was non-significant, it may be due to lack of power as a small number of events were observed.

Further, the lower limit of the 95% confidence interval was 0.52 indicating that there could be up to a 50% lower incidence of non-AIDS events in those who started on efavirenz. No significant difference was also observed in the incidence rate of non-AIDS events in the lopinavir group compared to nevirapine.

Older patients were found to be at a significantly increased risk of non-AIDS related events and so were patients in north Europe compared to patients in south Europe. Previous studies have reported that increasing age is an important risk factor for a number of non-AIDS events such as cancer, liver and cardiovascular disease[377;586]. The higher incidence in north Europe is surprising but may be partly due to better surveillance and reporting of events in this region, or higher underlying event rates in the general population, due to diet and other environmental factors. These differences are discussed in more detail in chapter 7 comparing differences in mortality rates across Europe.

To investigate the long term durability of the regimens investigated, in addition to clinical events, changes in laboratory values were also used, as they may give an indication of early problems before a serious event occurs. No significant differences between the regimens was found in the risk of developing or worsening anaemia, severe weight loss, or increasing AST or ALT levels committee, although this may in part be due to low power, particularly for developing or worsening anaemia. There may also be problems with selection bias in these analyses as those patients with measurements available may be different to those without measurements and there may have been a difference in those without measurements. Patients on lopinavir had a higher incidence of developing HDL cholesterol < 0.9 compared to patients on nevirapine. Nevirapine and efavirenz have both been found to increase HDL cholesterol [607;608]. There also appears to be some evidence of an increase in total cholesterol in there how started on lopinavir compared to nevirapine, all the difference was non-significant. The ARTEN study also found that nevirapine had a more favourable lipid profile to atazanavir[603]. In this study there was no significant difference in the incidence of developing a high total cholesterol or low HDL cholesterol between patients on efavirenz and nevirapine, however the 2NN study[609] found significantly larger increased in HDL cholesterol on nevirapine compared to efavirenz.

A marginally higher mortality rate was found in patients on a lopinavir based regimen compared to nevirapine. A higher proportion of intravenous drug users and patients who had tested positive for hepatitis C at baseline were on lopinavir compared to nevirapine.

This is probably due to concerns over poor adherence from intravenous drug users[330], and the related high risk of the development of resistance associated with NNRTIs[224], as well as the increased risk of liver related toxicities associated with nevirapine[605]. Therefore, it is possible that individuals on lopinavir had a higher underlying risk of death, as there was a high rate of hepatitis/liver related death in the lopinavir group (22% of all deaths). Typically those on lopinavir also have higher viral loads. However, the multivariable estimates were adjusted for hepatitis C status and HIV exposure group. Although current IDU status could not be adjusted for as this information has only very recently been added to the EuroSIDA follow-up forms (Appendix I).

There are a number of limitations to this analysis which should be noted. Data were collected as part of an observational cohort study, and although many imbalances between people starting different regimens can be accounted for in the adjusted analysis, there may still be unmeasured confounders that we did not account for that are either unmeasured or unknown. Cohort studies are not randomised and bias due to confounding by indication or some other unknown factors is difficult to exclude. Additionally, some selection bias may have been introduced as a higher proportion of patients on nevirapine were excluded due to having been exposed to prior treatment of one of the 3 drugs. Furthermore, there was a very high rate of patients excluded from this analysis from Eastern Europe, mainly due to having no CD4 count or viral load available at baseline, and even more exclusions in the analysis involving laboratory markers such as haemoglobin levels or cholesterol. These limitations are discussed in more detail in the Conclusion chapter of this thesis (Chapter 8).

This analysis differs from previous analyses comparing nevirapine based cART regimens with efavirenz or boosted PI regimens in that a significant number of both treatment naïve and treatment experienced patients were included. This analysis also looked at time to discontinuation of treatment rather than virological end points and addresses a different study population (only patients who had achieved an initial response to the regimen were included).

In conclusion, based on data collected as part of routine clinical practice across Europe in patients who had achieved an initial response and tolerated the first three months of their regimen, based on risk of all-cause discontinuation and development of serious clinical events nevirapine based cART regimens have similar durability to that of efavirenz and lopinavir. However, patients on nevirapine had a higher rate of discontinuation due to reported treatment failure and those on efavirenz or lopinavir which was compensated by a lower rate of discontinuation due to toxicity or patient physician choice compared to lopinavir. Analysis restricted to people starting these drugs when they were ART-naïve showed a very low incidence of discontinuations, regardless of the drug started due to reported treatment failure. The rate of discontinuation due to toxicity or patient/physician choice remained significantly increased in patients on lopinavir compared to nevirapine, and the effect was of similar magnitude.

Results are consistent with those of clinical trials and current guidelines indicating NNRTI and PI/r as recommended choices for first line regimens. Clinical trials typically have short follow-up, so these results are important because they extend the comparisons to events occurring long after the first weeks of treatment. The median follow-up time for the main analysis, discontinuation of the third drug for any reason, was 2.6 years. Although, it is also worth noting that treatment for HIV is currently expected to be lifelong and this still may not be long enough to assess the long term durability of cART. It is possible that there will be longer term differences in outcomes for those who discontinued treatment due to treatment failure and those who discontinue due to toxicities or patient/physician choice due to the rate at which available alternative regimens are used and new drugs become available. This is partly addressed by the intent to treat analysis looking at development of clinical events, deterioration of lab markers and death rates.

A manuscript of this analysis was published in HIV Medicine in May 2011 and can be found in Appendix VI.

Chapter 6 The relationship between current level of immunodeficiency and non-AIDS defining malignancies

6.1 Introduction

Three different types of cancer have been classified as AIDS defining malignancies (ADM): Kaposi's sarcoma (KS), Non-Hodgkin's lymphoma (NHL) and, more recently, cervical carcinoma[136]. These three cancers all occur at significantly higher rates in HIV-positive patients than in the general population[404]

KS is rare in the general population and is mainly found in immune compromised patients after an organ transplant, or in patients over the age of 60[610]. Studies have found that people with HIV are 100 to 300 times more likely to develop KS[20]. However, the incidence of KS in HIV-positive patients has declined dramatically since the introduction of cART[611-615]. The EuroSIDA study has previously reported that the incidence of KS in 2003 among patients with HIV was less than 10% of the incidence reported in 1994[612]. KS in HIV-positive patients is associated with the human herpesvirus 8 (HHV-8), also called Kaposi's sarcoma herpes virus (KSHV)[419;616;617], the transmission of which is thought to be sexual[610;618]. Although there is no evidence that HHV-8 directly causes KS[20] in either HIV-positive or HIV-negative patients, risk of KS increases relative to increasing levels of HHV-8[619]. Additionally, some studies have found a link between decreasing immunosuppression and increasing risk of KS[421].

There are several different types of NHL including primary central nervous system (CNS), primary effusion lymphoma and Burkitt's lymphoma[610;620]. Like KS, patients infected with HIV are at a higher risk of developing NHL with studies reporting a 100-200 times higher risk of NHL in the HIV-positive population[610;621]. There has been a reported decrease in the incidence of NHL since the introduction of cART[615;622;623], although EuroSIDA reported that the decreasing incidence of NHL was not as great as for other AIDS defining events. This means that in more recent times a higher proportion of AIDS defining events are due to NHL[159].

Differences in the rate of decline have been observed depending on the type of NHL[622], CNS lymphomas have shown the most significant decline[407;624] whereas HIV does not appear to be directly responsible for Burkitts NHL[610] and Engels et al. reported no significant decline in the incidence of Burkitts NHL[407]. The Epstein - Barr virus (EBV) has been found to be associated with the two most common NHL subtypes found in HIV-positive patients[419;625]. EBV is present in two-thirds of AIDS-related lymphomas[626]. However, the proportion of tumours containing the EBV genome varies according to the type of NHL: almost all CNS lymphomas, about 90% immunoblastic lymphomas and less than 50% of Burkitt's[626;627].

In 1993 cervical carcinoma was added to the CDC list of AIDS defining illnesses[136]. It is the second most common cancer among women[628]. The principal risk factor is infection with the human papillomavirus (HPV)[628-630] which has been detected in all cases of cervical cancers; it is generally accepted that the virus is required for the development of cancer[628]. Studies have found that the introduction of cART has not resulted in a decrease in the incidence of cervical cancer in HIV-positive patients[407;614;631] and no correlation has been found between immunosuppression and risk of developing cervical carcinoma[421].

In the context of HIV infection, all other cancers are classified as non-AIDS defining malignancies (NADM). In developed countries, where the use of cART has been followed by a reduction in many ADM[405], NADM are now more common than ADM[406-408]. An urban cohort study reported that between 1996 and 2005 NADM rates increased from 3.9 to 7.1 cases/1000 person-years[405]. Worryingly, as with ADM, many NADM occur at a higher rate in the HIV-positive population than the general population[407;409;410;632]. The most recent and large studies have shown a 1.7-3-fold increased risk of developing non-AIDS malignancies in HIV-positive patients as compared with the general population[633]. The incidence of Hodgkin's lymphoma, malignancy of liver, lung, anus, and oral cavity/pharynx have all been found to be higher among the HIV-positive population than in the general population[407;410], and a similar finding has been reported for melanoma, leukemia, vaginal, colon, rectal, and kidney cancer in some studies [410]. The increasing incidence of some NADM and the decrease in ADM in the cART era has meant that NADM now account for a greater proportion of morbidity and mortality in HIV-positive patients than ADM [409].

At least part of the increased risk of NADM in HIV-positive patients is explained by the higher prevalence of associated risk factors. Smoking has been found at an increased level in HIV-positive patients[18;372], and some studies have attributed the increased incidence of lung cancer to high rates of cigarette smoking[414;415]. Furthermore, excessive alcohol use has been shown to be significant in predicting the risk of developing cancer [416] and has been found at an increased level in the HIV-positive population compared to the general population[18;411]. Additionally, co-infection with oncogenic viruses (viruses capable of causing tumours) could account for some of the increased risk[418;620;629;634]. The proportion of cancers in the general population that are caused by infectious agents is estimated to be around 20%[419]. This is true for the three ADM[625;627;630] and for many NADM that occur at an increased rate in HIV-positive patients[418;620;629;634].

Hodgkin's lymphoma (HL) has been found at a higher rate in HIV-positive persons compared to the general population with between 5 to 15 fold increased risk[410;635-638]. Similarly to NHL, HL has been linked to the EBV virus[419;620;634] but, as the prevalence of EBV in the general population is very high[419], it is not thought that co-infection can account for all of the increased risk[255]. There is also some evidence of an association of EBV with nasopharyngeal carcinoma and around 10% of gastric carcinomas[639], although some types of gastric cancer are associated with the bacterium Helicobacter pylori[640].

Rates of anal cancer have been found to be around 40 times higher in the HIV-positive population compared to the general population[410]. Multiple risk factors are thought to contribute to the development of anal cancer including HPV infection; Frisch et al. reported that 95% and 83% of anal cancers in woman and men respectively were positive for HPV[641]. Additional risk factors include anoreceptive intercourse, cigarette smoking, number of previous sexual partners and immunnosuppression[629;642;643]. HIV-positive patients tend to develop more aggressive anal cancer and have a poorer prognosis than patients of the same age who are HIV-negative[629]. The HPV virus has also been linked to cancer of the vulva, vagina, penis, oral cavity, oropharynx and tonsil with some limited evidence of an association with cancer of the larynx[419;628]. Although HPV is accepted as a risk factor for oral and pharyngeal cancer the major risk factors are tobacco and alcohol[644]

Some studies have reported that the incidence of liver cancer in HIV-positive patients is increasing over time[407;410]. Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide[645]. It is associated with infection with hepatitis B and C viruses[417-419;646] that are reported to infect over 300 million and 170 million people respectively worldwide[419]. HIV-positive patients have a greater incidence of infection with hepatitis B and C than the general population, with this being especially true for injecting drug users[647]. Liver disease is now a leading cause of death among HIV-positive patients[361] with a high proportion attributable to HCC[380]. Almost all reported cases of HCC in HIV-positive patients are in individuals co-infected with either hepatitis B or C[413]. As well as infection with hepatitis, heavy alcohol use is also related to an increased risk of HCC[417], and this could explain some of the increased risk in HIV-positive patients.

Many of these viruses (EBV, HPV, and hepatitis) however, are common in the general adult population but the development of tumours occurs in only a small fraction of those infected[620]. A recent study by Silverberg et al.[255] found that 70% of all cancers in HIV-positive persons were infection related, compared with only 12% in HIV-negative patients of a similar age and sex. However, HIV-positive patients have also been found to have a 30% increased risk of non-infection NADM compared to HIV-negative patients[255]. Thus, although some of the increased risk can be accounted for by a higher prevalence of related risk factors it does not account for all of the increased risk.

It is thought that impaired immune function may result in the general reduced immune surveillance for malignant cells or it may impair the ability to suppress oncogenic viruses that may result in a higher risk of these cancers[255]. There is growing interest in the correlation between immunodeficiency and NADM, with some studies supporting the theory that NADM become more frequent in immunodeficient patients with or without HIV infection[420-422]. As HIV-positive individuals are surviving longer on cART and the population is aging, more are at risk of developing a serious non-AIDS clinical event such as NADM. It is important that any risk factors, particularly those that are related to HIV infection, are identified and understood so that any excess risk can potentially be reduced.

6.2 Aim

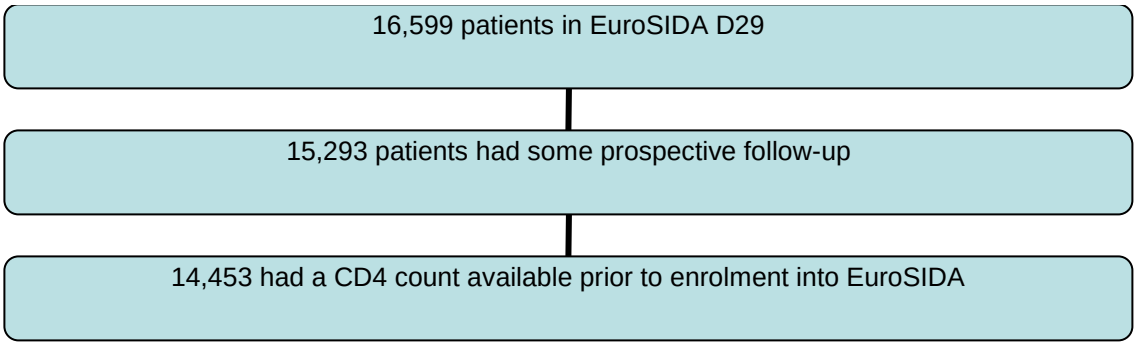
The aim of this chapter was to investigate the incidence of NADM across Europe in HIV-positive patients and to identify the risk factors associated with the development of NADM. In particular we sought to investigate the link with immunodeficiency to determine whether current CD4 count was independently associated with risk of NADM after accounting for associated risk factors measured in EuroSIDA. Finally, in those that developed a NADM, we investigated the risk of death and patterns of survival.

6.3 Methods

6.3.1 Patient selection

As mentioned in earlier chapters 16,599 patients were included in the D29 EuroSIDA. Figure 6.1 shows how patients were selected for inclusion into this analysis. All patients who had some prospective follow-up and a CD4 count measured prior to enrolment into EuroSIDA were included in the analysis, giving a total of 14,453 patients.

Figure 6.1 Patient selection



6.3.2 Statistical analysis

Baseline was defined as the date of enrolment into EuroSIDA. Patients were followed until either death or their last recorded visit in EuroSIDA, if they were not known to have died. Therefore patients could develop more than one distinct NADM whilst under follow-up. Any NADM diagnosed before enrolment into EuroSIDA was classed as a prior NADM and recurrences of the same NADM were excluded.

NADM were classified using the ICD-10 code classification system[648]. The incidence of NADM per 1000 person years of follow-up (PYFU) was calculated, and stratified by current (time-updated) CD4 count. PYFU accrued within current CD4 count strata, and PYFU were censored when the most recent CD4 count was more than 6 months old and started again when a new CD4 count became available. Poisson regression analysis was used to determine the factors associated with the development of NADM. Factors investigated included gender, age, race, exposure group, region of Europe, and nadir CD4. In addition CD4 count, viral load, year of follow-up, smoking status, anaemia, hepatitis B and C status, starting cART and diagnosis of any NADM, ADM or other AIDS defining event were included as time updated covariates. cART was defined as any regimen including three or more ARVs. Patients were classed as Hepatitis B positive if they had a positive HBV surface antigen test recorded and Hepatitis C positive if they had a positive HCV antibody test. Anaemia was defined as a haemoglobin level ≤ 12 or ≤14 (mg/dl) for females and males respectively[598]. Factors that were significant in univariable analysis ($p<0.1$) were included in multivariable analyses.

NADM were categorized into 3 categories: 'virus-related'; 'non-virus-related epithelial'; and 'other' cancers (Table 6.3). These groups were defined *a priori* before starting analyses and after review by several study group clinicians. Several viral infections are considered to be the causative organism of certain cancers in humans, with nearly 15% of all cancers thought to have an infectious agent in their etiology[610]. As previous studies have found a link between virus related cancers such as HL[635], HCC[647], and anal cancer[636] with immunodeficiency determined by time to AIDS diagnosis[635;636] or CD4 count within 1 year of diagnosis [647], it was decided to group these 'virus related' NADM together.

The most common non-virus related epithelial cancers (e.g. lung and prostate) remain difficult to treat, and the majority remain incurable with very little improvement in survival over the past decade[649]. In addition, based on onco-epidemiologic studies, risk of epithelial cancers starts to increase from the age of 30[650].

Therefore, all non-virus-related epithelial cancers were grouped together. Although stomach cancer has been linked to EBV, only 5-10% of carcinomas worldwide are thought to be due to EBV[419;639], and therefore stomach and gastric cancers were included in the 'non-virus related epithelial' group. Similarly, cancers of the nasopharynx have been reported to differ histologically and aetiologically compared to other oral cancers that are HPV related[628]. The final group ('other') consisted of NADM which were not easily categorised into the other two groups including leukaemia, myeloma, brain tumours and melanoma, as well as any cancers that were not well defined within the database. Non-melanoma skin cancer was excluded as it was not systematically ascertained and not thought to cause significant morbidity or mortality[405].

The analysis was performed on all NADM combined, and separately for each NADM group. Where there was a sufficient number of events (>30) for a specific NADM, the analysis was repeated for that NADM alone. In addition, previous studies have shown that rates of breast, prostate and colorectal cancers are not raised in people with HIV[420]; therefore an additional sub-group analysis focused on these 3 cancers combined.

In patients diagnosed with NADM, Kaplan Meier survival analysis was used to estimate a patient's risk of dying after diagnosis and to compare the risk of death across the three different cancer groups. Cox proportional hazards models, stratified by centre, were used to investigate the factors that were significantly associated with a patient's risk of death after NADM diagnosis. Factors investigated included gender, age, race, exposure group, region of Europe, and nadir CD4. In addition, baseline CD4 count, viral load, year of follow-up, smoking status, anaemia, hepatitis B and C status, starting cART and diagnosis of any NADM, ADM or other AIDS defining event were also considered. For this analysis, nadir CD4 was defined as the lowest CD4 count measured prior to baseline. Factors that were significant in univariable analysis ($p<0.1$) were included in multivariable analyses.

The data collected on NADM only became part of the routine data collection and the EuroSIDA quality assurance exercise in 2003. Therefore a sensitivity analysis in which follow-up was left censored at 1st January 2003 was performed.

6.4 Results

6.4.1 Patient characteristics

There were 14,453 patients included in the analysis. Table 6.1 gives the baseline characteristics for patients included in the study stratified by those who did and did not develop an NADM.

Patients were mainly male (75.3%), of white ethnic origin (88.7%), with a median age of 36 (interquartile range [IQR] 31-44 years). 9,772 (67.6%) patients were on cART when they were enrolled into EuroSIDA, only 10.9% were treatment naïve. 3,486 (24.1%) had experienced at least one AIDS defining illness (not including ADM) prior to baseline and 680 (4.7%) had previously been diagnosed with a malignancy (either ADM or NADM). Median CD4 count at enrolment was 300/mm^3 (IQR 150-453) and median viral load in patients with data available was 2.70 per $\log_{10}$ copies/ml. As mentioned in the introduction, possible risk factors for the development on certain cancers include smoking and co-infection with other viruses: 645 (4.5%) patients were hepatitis B positive and 2,782 (919.3%) were hepatitis C positive. 2,809 (19.4%) patients were recorded as current smokers and an additional 890 (6.2%) had reported having smoked previously.

Table 6.1: Patient characteristics at baseline*

		All Patients		Did not develop NADM		Developed NADM	
		N	%	N	%	N	%
All patients		14453	100	14106	97.6	347	2.4
Gender	Male	10879	75.3	10590	75.1	289	83.3
Ethnic origin	White	12822	88.7	12497	88.6	325	93.7
Exposure Group	Homosexual	5965	41.3	5773	40.9	192	55.3
	IDU	3488	24.1	3424	24.3	64	18.4
	Heterosexual	4017	27.8	3951	28.0	66	19.0
	Other	983	6.8	733	5.2	25	7.2
Region of Europe	South	4192	29.0	4098	29.1	94	27.1
	Central	3324	23.3	3268	23.2	106	30.5
	North	3724	25.8	3598	25.5	126	36.3
	East	1455	10.1	1442	10.2	13	3.7
	East Central	1329	9.2	1324	9.4	5	1.4
	East	379	2.6	376	2.7	3	0.9
Hepatitis B status	Negative	9194	63.6	8983	63.7	211	60.8
	Positive	645	4.5	616	4.4	29	8.4
	Unknown	4614	31.9	4507	32.0	107	30.8
Hepatitis C status	Negative	6263	43.3	6121	43.4	141	40.6
	Positive	2782	19.3	2731	19.4	51	14.7
	Unknown	5409	37.4	5254	37.2	155	44.7
Treatment started at or prior to baseline	Naive	1569	10.9	1539	10.9	30	8.6
	ART	3112	21.5	2971	21.1	141	40.6
	cART	9772	67.6	9596	68.0	176	50.7
Prior diagnosis of AIDS or malignancy	None	10287	71.2	10047	71.2	240	69.2
	Prior AIDS (not ADM)	3486	24.1	3403	24.1	83	23.9
	Prior ADM	573	4.0	551	3.9	22	6.3
	Prior NADM	107	0.7	105	0.7	2	0.6
Smoking status	Current	2809	19.4	1492	10.6	18	5.2
	Previous	890	6.2	2758	19.6	51	14.7
	Never	1510	10.4	876	6.2	14	4.0
	Unknown	9244	64.0	8980	63.7	264	76.1
Viral load^{+}	≤500 copies/ml	4776	50.9	4663	50.8	123	60.1
	>500 copies/ml	4613	49.1	4525	49.2	79	39.1

Patient characteristics at baseline* (continued)

		All Patients		Did not developed NADM		Developed NADM	
		Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**
Age	Years	36	31-44	36	31-44	42	34-50
CD4 count	/mm^3	300	153-453	300	154-456	243	133-366
CD4 nadir	/mm^3	177	64-302	179	64-304	144	67-242
Viral load$^+$	$\log_{10}$ copies/ml	2.70	1.70-4.23	2.79	1.69-4.22	3.03	2.30-4.36
Recruitment	month/year	Jam 99	Jan96-Jan 04	Feb 99	Jan 96-Feb 04	March 97	June 94-March 99

*baseline was defined as date of enrolment into EuroSIDA, $^+$5064 (35.0%) patients had no viral load data available at baseline, IDU: Intravenous Drug User, ADM: AIDS defining malignancy, NADM: non-AIDS defining malignancy, IQR: inter quartile range

6.4.2 Non-AIDS defining malignancies

338 patients developed one NADM whilst under follow-up and 9 patients developed 2 different NADM during 83,398 person years of follow-up [PYFU] to give an incidence rate [IR] of 4.3 per 1000 PYFU (95% confidence interval [CI] 3.8-4.7). The characteristics of patients at the time of their first NADM diagnosis whilst under follow-up in EuroSIDA are shown in table 6.2. The median age of patients at the time of diagnosis was 47 years, around 10 years older than the median age of all patients at enrolment into the study. Median CD4 count at the time of diagnosis was 315 cells/mm^3 (IQR 190-532) and median viral load was 1.90 per $\log_{10}$ copies/ml (IQR 1.70-3.30). The majority of patients were on cART (92.8%); 14.4% were hepatitis B positive, 19.6% were hepatitis C positive and 51.3% were either current or previous smokers.

Table 6.2: Characteristics of patients at time of first NADM diagnosis whilst under follow-up in EuroSIDA

		All Patients	
		N	**%**
All patients		347	100
Gender	Male	289	83.3
Ethnic origin	White	325	93.6
Exposure Group	Homosexual	192	55.3
	IDU	64	18.4
	Heterosexual	66	19.0
	Other	25	7.3
Region of Europe	South/Argentina	97	27.9
	Central	106	30.6
	North	126	36.5
	East	17	5.2
Hepatitis B status	Negative	255	73.5
	Positive	50	14.4
	Unknown	42	12.1
Hepatitis C status	Negative	216	62.3
	Positive	68	19.6
	Unknown	63	18.2
Treatment started at or prior to diagnosis	Naive	4	1.2
	ART	21	6.0
	cART	322	92.8
Prior diagnosis of AIDS or malignancy	None	185	53.3
	Prior AIDS (not ADM)	120	34.6
	Prior ADM	35	10.1
	Prior NADM	7	2.0
Smoking status	Current	51	14.7
	Previous	127	36.6
	Never	72	20.8
	Unknown	97	27.9
Viral load[+]	≤500 copies/ml	212	66.0
	>500 copies/ml	109	34.0
		Median	**IQR**
Age		48	41-56
CD4 count	/mm^3	315	190-532
CD4 nadir	/mm^3	90	29-188
Viral load*	log_{10} copies/ml	1.90	1.70-3.30
Maximum viral load*	log_{10} copies/ml	4.92	3.97-5.45
Date of diagnosis	month/year	05/03	01/00-06/06

* 26 patients had no viral load measurement available

172 NADM developed while under follow up in EuroSIDA were classed as 'virus-related', 135 were classed as 'non-virus-related epithelial' and 49 'other'. The specific cancers included in each category are shown in table 6.3. The most commonly observed cancer was anal cancer (n=69), followed by Hodgkin's lymphoma (n=69) and lung cancer (n=31).

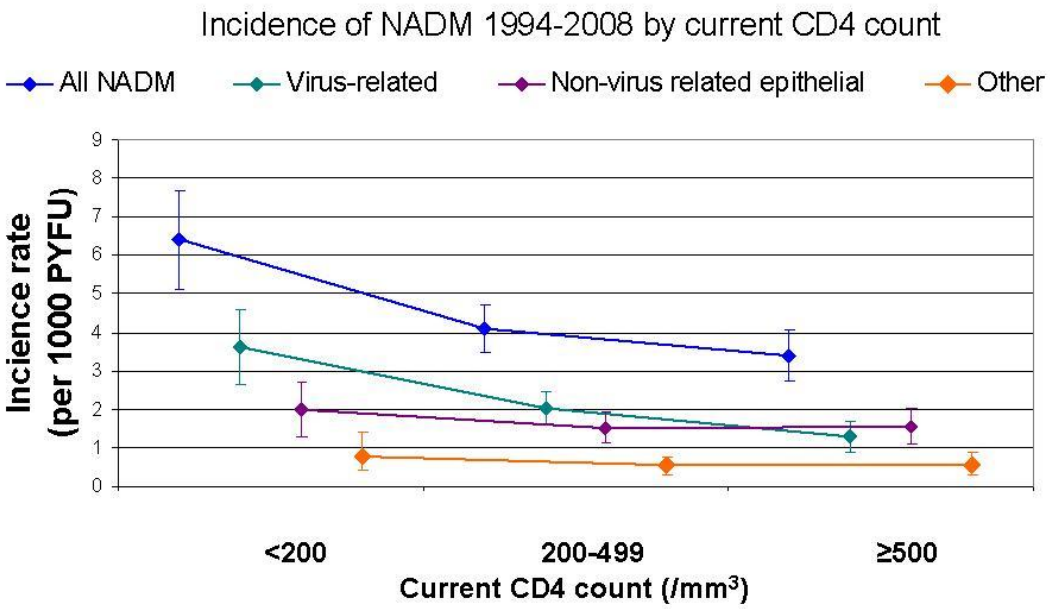
Table 6.3: Cancers included in each category

Virus related (N=172)	Non-virus related epithelial (N=135)	Other (N=49)
HPV related	Lung (31)	Unspecified (20)
Anal (69)	Breast (17)	Melanoma (13)
Oral (12)	Prostate (15)	Brain (4)
Larynx (8)	Rectal (12)	Multiple Myeloma (4)
Vulval (5)	Colon (11)	Myeloid Leukaemia (3)
Pharynx (2)	Pancreas (7)	Leukaemia (3)
Penile (2)	Stomach (7)	Liposarcoma (1)
EBV related	Oesophagus (6)	Lymphoid Leukaemia (1)
Hodgkin (52)	Kidney (6)	
HBV and HCV related	Bladder (6)	
Liver (22)	Testicular (4)	
	Lip (2)	
	Thyroid (2)	
	Uterine (2)	
	Ovarian (2)	
	Urinary unspecified (2)	
	Nasopharynx (1)	
	Gall bladder (1)	
	Gastric (1)	

6.4.3 Association with immunodeficiency

There was a decreasing incidence of all NADM combined with increasing current CD4 count (figure 6.2). For example, at a current CD4 count ≤200/mm^3, the incidence was 6.4 per 1000 PYFU (95% CI 5.1-7.7) compared to 3.4 per 1000 PYFU (95% CI 2.7-4.1) at a current CD4 count >500/mm^3, p<.0001.

Figure 6.2 Incidence of NADM 1994-2008 by current CD4 count



Current CD4 count	<200	200-499	≥500
Number of events			
All NADM	99	159	98
Virus-related	56	79	37
Non-virus related epithelial	31	59	45
Other	12	21	16

Figure 6.2 also shows that there is a clear decrease in incidence of 'virus-related' NADM with increasing CD4 count, from 3.6 at CD4 counts ≤200/mm^3 (95% CI 2.7-4.6) to 1.3 at CD4 count >500/mm^3 (95% CI 0.9-1.7) p<.0001. The incidence of 'non-virus-related epithelial' cancer showed a small but marginally significant decrease as current CD4 count increased (p=0.06), while no significant difference in the incidence of 'other' cancers as found (p=0.35), which may be due to lack of power in this group.

The results of the multivariable Poisson regression analysis are shown in table 6.4. For any NADM, after adjustment, a higher current CD4 count was associated with a lower incidence of NADM (incidence rate ratio [IRR] 0.87 per doubling, 95% CI 0.82-0.94, p<.0001). Nadir CD4 count was not found to be significant after adjustment (IRR 1.03, 95%CI 0.98-1.09, p=0.26). Hepatitis B antigen positive patients had double the incidence rate of NADM compared to those who were hepatitis B antigen negative (IRR 2.14, 1.58-2.90, p<.0001). A previous diagnosis of AIDS (excluding malignancies) increased the incidence of NADM by 39% (IRR 1.39, 1.09-1.78, p=0.007), and a prior malignancy (either AIDS or non-AIDS) almost doubled the incidence of NADM (IRR 1.84, 1.30-2.59, p=0.0005) compared to no prior diagnosis. Age and ethnicity were also associated with the incidence of NADM; non-white patients had a 51% lower incidence of NADM (IRR 0.49, 0.32-0.77, p=0.002) and older patients had a 51% increased incidence per 10 year increment (IRR 1.51, 1.37-1.67, p<0.0001).

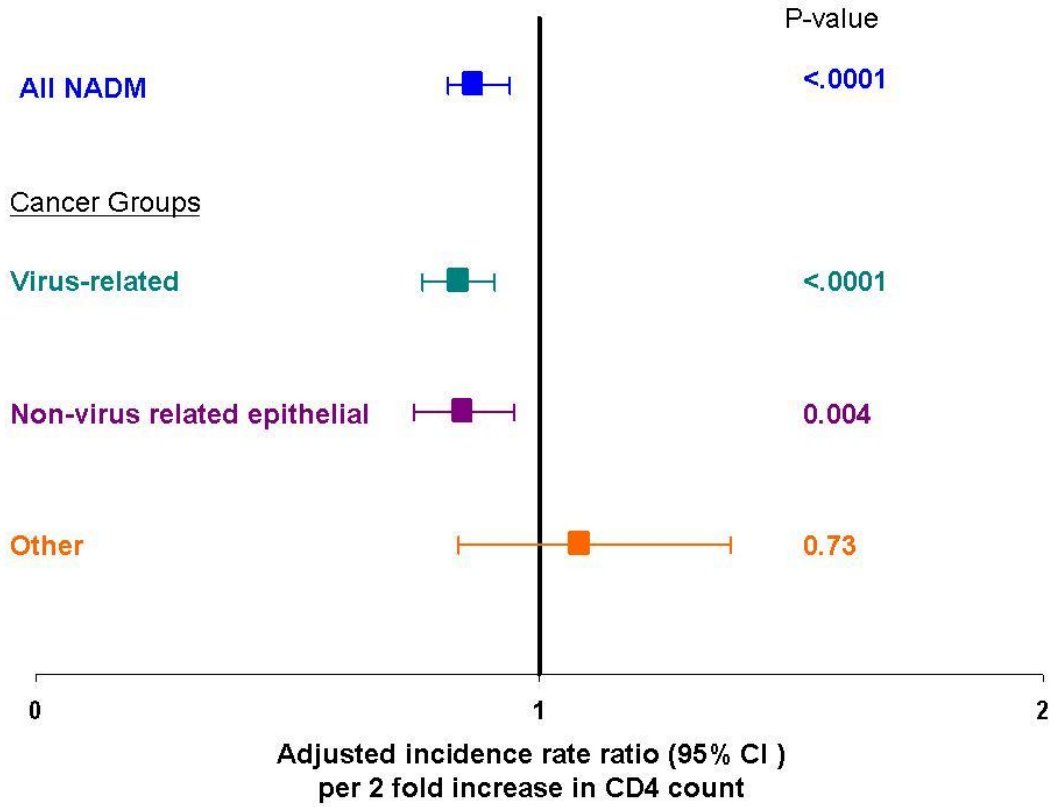
In the 'virus-related' group, lower incidence of NADM was associated with higher current CD4 counts (IRR per doubling 0.84, 95% CI 0.77-0.91, p<.0001). Higher current CD4 count was also associated with a lower incidence of 'non-virus-related epithelial' cancers (IRR 0.84, 95% CI 0.75-0.95, p=0.004). However, current CD4 count was not a significant factor in predicting the incidence in the 'other' NADM group (IRR 1.04, 0.83-1.31, p=0.73). Nadir CD4 count was not associated with development of any of the cancer groupings after adjustment for current CD4 count. A prior malignancy diagnosis (IRR 2.45, 1.56-3.84, p<.0001) and a hepatitis B positive diagnosis (IRR 2.53, 1.69-3.77, p<.0001) were associated with an increased incidence of 'virus-related' cancers. Older age was associated with a higher incidence of 'non-virus-related epithelial' (IRR 2.02, 1.77-2.36, p<.0001) and 'other' (IRR 1.35, 1.05-1.73, p=0.02) cancers. Year of follow-up was also associated with an increased rate of 'non-virus related epithelial' cancer (IRR 1.06, 1.01-1.11, p=0.01).

Table 6.4 Multivariable Poisson regression analysis

		All NADM			Virus-related			Non-virus related epithelial			Other		
		IRR	95% CI	p-value	IRR	95% CI	p-value	IRR	95% CI	p-value	IRR	95% CI	p-value
Gender	Male	1.00	-	-	1.00	-	-				1.00	-	-
	Female	1.08	0.78-1.50	0.64	0.87	0.52-1.46					0.18	0.04-0.77	0.02
Ethnic origin	White	1.00	-	-	1.00	-	-	1.00	-	-			
	Other	0.49	0.32-0.77	0.002	0.43	0.21-0.86	0.02	0.65	0.34-1.23	0.18			
Exposure group	Homosexual	1.00	-	-	1.00	-	-				1.00	-	-
	IDU	1.01	0.69-1.50	0.94	1.12	0.75-1.65	0.58				0.55	0.12-2.44	0.43
	Heterosexual	0.74	0.54-1.03	0.07	1.51	0.29-0.88	0.02				1.34	0.66-2.75	0.42
	Other	0.80	0.53-1.22	0.30	0.47	0.22-1.02	0.06				1.40	0.53-3.69	0.50
Region of Europe	South/Argentina	1.00			1.00	-	-	1.00	-	-	1.00	-	-
	Central	1.22	0.94-1.62	0.14	1.40	0.96-2.04	0.08	0.91	0.58-1.43	0.67	2.36	0.91-6.11	0.08
	North	1.11	0.84-1.45	0.47	1.04	0.71-1.53	0.85	1.07	0.76-1.63	0.76	2.38	0.95-5.96	0.06
	East	0.59	0.36-0.97	0.04	0.30	0.12-0.76	0.01	0.53	0.25-1.15	0.11	3.13	0.92-10.62	0.07
Hepatitis B status	Negative	1.00	-	-	1.00	-	-	1.00	-	-			
	Positive	2.14	1.58-2.90	<.0001	2.53	1.69-3.77	<.0001	1.77	1.03-3.04	0.04			
	Unknown	0.71	0.50-1.03	0.07	0.68	0.42-1.12	0.13	0.75	0.44-1.28	0.29			
Hepatitis C status	Negative	1.00	-	-							1.00	-	-
	Positive	0.95	0.66-1.37	0.79							0.59	0.18-1.94	0.39
	Unknown	1.03	0.67-1.59	0.88							1.62	0.42-6.30	0.49
Ever smoked	Never	1.00	-	-							1.00	-	-
	Current	1.37	0.99-1.90	0.06							5.01	1.15-21.87	0.03
	Previous	1.39	0.97-1.98	0.07							4.32	0.93-20.01	0.06
	Unknown	1.75	1.23-2.48	0.002							12.31	2.81-53.82	0.0009
Prior diagnosis	None	1.00	-	-	1.00	-	-				1.00	-	-
	AIDS*	1.39	1.09-1.78	0.007	1.40	0.97-2.01	0.07				1.79	0.94-3.40	0.07
	Malignancy	1.84	1.30-2.59	0.0005	2.45	1.56-3.84	<.0001				1.78	0.64-4.95	0.27
Age	per 10 yr	1.51	1.37-1.67	<.0001	1.13			2.02	1.77-2.36	<.0001	1.35	1.05-1.73	0.02
CD4	doubling	0.87	0.82-0.94	<.0001	0.84	0.78-0.91	<.0001	0.84	0.75-0.95	0.004	1.04	0.83-1.31	0.73
CD4 nadir		1.03	0.98-1.09	0.26	1.01	0.95-1.09	0.65				0.97	0.84-1.12	0.65
Year of follow-up								1.06	1.01-1.11	0.01			
Ever started cART		1.52	1.10-2.11	0.01	1.48	0.93-2.33	0.09				2.26	0.81-6.28	0.12

*AIDS did not include any AIDS related malignancies; all malignancies both ADM and NADM were included in the malignancy category.

Figure 6.3: Adjusted incidence of NADM by current CD4 count



All NADM also adjusted for gender, ethnic origin, HIV exposure group, region of Europe, hepatitis B and C status, ever smoked, prior diagnosis of AIDS or malignancy, age, CD4 nadir, and having started cART; Virus-related also adjusted gender, ethnic origin, HIV exposure group, region of Europe, hepatitis B, prior diagnosis of AIDS or malignancy, age, CD4 nadir, and having started cART, Non-virus related epithelial also adjusted for ethnic origin, region of Europe, age, and year of follow-up, Other also adjusted for gender, HIV exposure group, region of Europe, hepatitis C status, ever smoked, prior diagnosis of AIDS or malignancy, age, CD4 nadir, and having started cART

Figure 6.3 shows the incidence rate ratio per doubling in current CD4 count, after adjustment, for each of NADM groupings.

6.4.4 Specific NADM

The three most frequently observed cancers were anal cancer (n=69), HL (n=52), and lung cancer (n=31). Each of these cancers was investigated separately. In addition, as mentioned in the methods, previous studies have shown that rates of breast, prostate and colorectal cancers are not raised in people with HIV[420]; therefore an additional sub-group analysis focused on these 3 cancers combined.

6.4.4.1 Anal cancer

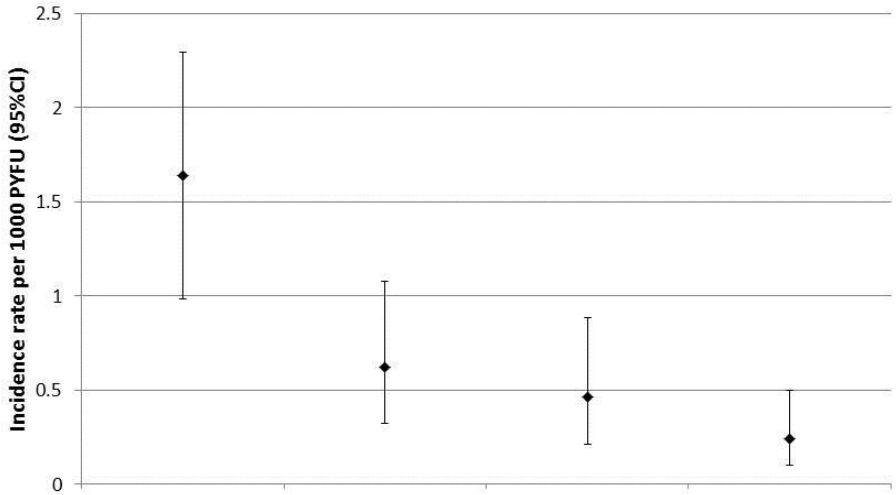
For anal cancer, after adjustment, there was a significantly decreased incidence with increasing current CD4 count (IRR 0.86 per doubling, 95% CI 0.75-0.99, p=0.03), nadir CD4 count was also found to be marginally significant (IRR 0.93 per doubling, 95% CI 0.84-1.01, p=0.09). Homosexual transmission group had a higher incidence of anal cancer compared to patients infected via heterosexual transmission (IRR 2.96, 95% CI 1.40-6.24, p=0.04). Patients with a prior malignancy diagnosis (IRR 3.25, 95% CI 2.71-6.16, p=0.0003), those who were hepatitis B antigen positive (IRR 2.29, 95% CI 1.25-4.18, p=0.007) and those with a later year of follow-up (IRR 1.10, 1.02-1.18, p=0.01) also had a higher incidence of anal cancer (table 6.5).

6.4.4.2 Hodgkin's lymphoma (HL)

As there is some uncertainty about the association of HL at intermediate levels of immunodeficiency and debate over whether there is a linear relationship with CD4 count and the risk of developing HL, the association of current CD4 count was initially investigated in different CD4 count strata. Figure 6.4 shows the crude incidence rate of HL by current CD4 count strata, indicating a linear relationship.

In univariable analysis, compared to patients with a current CD4 count ≥500/mm^3, patients with a current CD4 count <200/mm^3 had a 6.4 times higher incidence of HL (IRR 6.40, 95%CI 2.76-14.84, p<.0001) and those with a CD4 count between 200-349/mm^3 had 2.5 times the incidence of HL (IRR 2.55, 1.00-6.40, p=0.0.04), and those with a CD4 count between 350-499 had double incidence of HL (IRR 1.92, 0.71-5.14, p=0.19)

Figure 6.4 Crude incidence rate of Hodgkin's Lymphoma by CD4 count strata



N events	24	12	9	7
PYFU	14660	19426	19360	28869

Therefore in adjusted analysis current CD4 count was included as a continuous variable. Table 6.5 shows the results of the adjusted analysis. A Higher current CD4 count was associated with a lower incidence of HL (IRR 0.83 per 2 fold higher, 95%CI 0.73-0.95, p=0.005), while a prior AIDS diagnosis (IRR 1.78, 95% CI 0.94-3.37, p=0.08) was marginally associated with a higher incidence of HL.

6.4.4.3 Lung cancer

Table 6.5 shows that after adjustment for smoking status, prior AIDS diagnosis and age, the incidence of lung cancer was significantly decreased with a higher current CD4 count (IRR 0.76, 95%CI 0.64-0.90, p=0.0002). Additionally, being a current smoker compared to having never smoked (IRR 16.80, 95%CI 2.23-126.2, p=0.0006) and older age (IRR 2.79 per 10 years older, 95% CI 2.22-3.50, p<.0001) were also significantly associated with an increased the rate of lung cancer.

6.4.4.4 Combined analysis of breast, colon and prostate cancer

There were 55 diagnoses of breast, colon or prostate cancer (table 6.5). There was no significant relationship with the incidence of these cancers combined and current CD4 count (IRR 1.01 per 2 fold higher, 95% CI 0.79-1.30, p=0.92), after adjustment for HIV exposure group, region of Europe, hepatitis B and C status, smoking status, age, and year of follow-up (table 7.5) . Older age (IRR 1.87 per 10 years older, 95%CI 1.48-2.35, p<.0001) and increasing year of follow-up (IRR 1.15 per year, 95%CI 1.06-1.25, p=0.0005) were associated with an increased rate of these combined cancers.

Table 6.5 Multivariable Poisson regression analysis for specific NADM

		Anal cancer			Hodgkin's Lymphoma			Lung cancer			Breast, colon and prostate cancer combined		
		IRR	95% CI	p-value	IRR	95% CI	p-value	IRR	95% CI	p-value	IRR	95% CI	p-value
Gender	Male	1.00	-	-	1.00	-	-						
	Female	0.78	0.24-2.55	0.68	0.72	0.29-1.77	0.47						
Ethnic origin	White	1.00											
	Other	0.23	0.06-0.95	0.04									
Exposure group	Other	1.00			1.00						1.00		
	Homosexual	2.96	1.40-6.25	0.004	1.67	0.89-3.12	0.11				1.57	0.91-2.74	0.10
Region of Europe	South	1.00			1.00						1.00		
	Central	1.62	0.83-3.14	0.15	1.54	0.84-2.83	0.16				1.70	0.81-3.55	0.15
	North	1.52	0.81-2.86	0.19	0.45	0.19-1.03	0.05				1.40	0.67-2.91	0.37
	East	0.18	0.02-1.45	0.10	0.72	0.19-2.70	0.62				0.68	0.19-2.43	0.55
Hepatitis B status	Negative	1.00									1.00		
	Positive	2.29	1.25-4.20	0.007							2.83	1.40-5.71	0.003
	Unknown	0.53	0.21-1.30	0.16							0.32	0.08-1.30	0.11
Hepatitis C status	Negative	1.00									1.00		
	Positive	0.46	0.17-1.25	0.12							1.14	0.46-2.79	0.78
	Unknown	2.20	0.71-6.83	0.17							1.37	0.41-4.56	0.60
Ever smoked	Never							1.00			1.00		
	Current							16.80	2.24-126.21	0.006	0.43	0.18-1.04	0.06
	Previous							4.68	0.53-41.41	0.16	1.05	0.51-2.15	0.90
	Unknown							6.60	0.81-53.82	0.07	1.82	0.87-3.80	0.11
Prior diagnosis	None	1.00			1.00			1.00					
	AIDS*	1.44	0.79-2.63	0.23	1.78	0.94-3.37	0.07	1.53	0.73-3.20	0.26			
	Malignancy	3.25	1.71-6.16	0.0003	1.94	0.77-4.88	0.16	2.32	0.82-6.59	0.11			
Age	per 10 yr							2.79	2.22-3.50	<.0001	1.87	1.48-2.35	<0.0001
CD4	doubling	0.86	0.75-0.99	0.03	0.83	0.73-0.95	0.005	0.76	0.64-0.90	0.001	1.01	0.79-1.30	0.91
CD4 nadir		0.93	0.85-1.01	0.09									
Year of follow-up		1.10	1.02-1.18	0.01	0.96	0.89-1.04	0.28				1.15	1.06-1.25	0.0005

6.4.5 Survival after NADM diagnosis

Individuals who were diagnosed with a NADM were followed from their first diagnosis of NADM until either their last recorded visit in EuroSIDA or death. The median follow-up time was 1.7 years after diagnosis.

Table 6.6 Characteristics of individuals at the time of NADM diagnosis who died

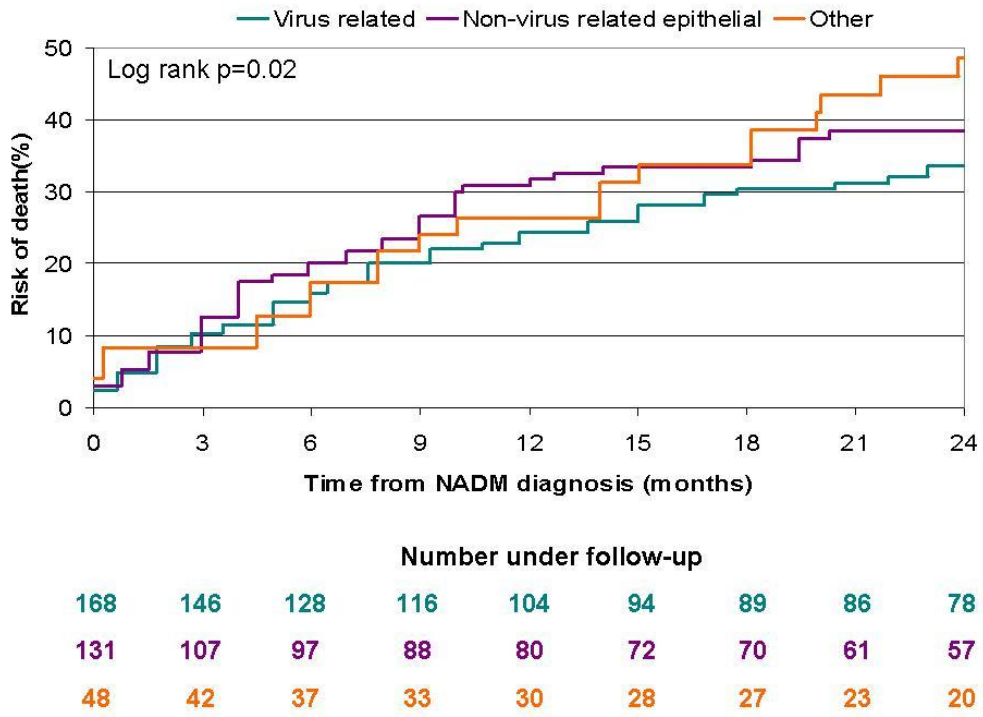
		Patients	
		N	**%**
All patients		145	100
Cancer group	Virus related	58	40
	Non-virus related epithelial	64	44.1
	Other	23	15.9
Gender	Male	126	86.9
Ethnic origin	White	135	93.1
Exposure Group	Homosexual	80	55.2
	IDU	33	22.8
	Heterosexual	24	16.6
	Other	8	5.5
Region of Europe	South/Argentina	37	25.5
	Central	41	28.3
	North	57	39.3
	East	10	6.9
Hepatitis B status	Negative	101	69.7
	Positive	23	15.9
	Unknown	21	14.5
Hepatitis C status	Negative	83	57.2
	Positive	29	20.0
	Unknown	33	22.8
Treatment started at or prior to diagnosis	Naive	1	0.7
	ART	9	6.2
	cART	135	93.1
Prior diagnosis of AIDS or malignancy	None	59	40.7
	Prior AIDS (not ADM)	69	47.6
	Prior ADM	15	10.3
	Prior NADM	2	1.4
Smoking status	Current	12	8.3
	Previous	45	31.0
	Never	31	21.4
	Unknown	57	39.3
		Median	**IQR**
Age		47	41-56
CD4 count	/mm^3	233	121-398
CD4 nadir	/mm^3	62	14-162
Viral load*	$\log_{10}$ copies/ml	2.30	1.70-3.50
Maximum viral load*	$\log_{10}$ copies/ml	4.96	3.32-5.51
Date of diagnosis	month/year	03/02	11/98-12/03

145 patients died after a NADM diagnosis whilst under follow-up in EuroSIDA. Table 6.6 gives the characteristics at the time of diagnosis in those who died.

The cause of death was reported for 119 (82.1%) of these patients. Of those with a known cause of death, 74 patients (62.2%) died from their NADM; 26 (21.8%) were classed as HIV related, 9 (7.6%) were liver or hepatitis related complications (excluding HCC), 3 (2.5%) were from an MI, 2 (1.7%) due to pneumonia, 1 pancreatitis, 1 diabetes related, 1 sepsis, 1 septic shock and 1 peritonitis.

The Kaplan Meier risk of death in the 6 months after a NADM diagnosis was 17.6% (95% CI 13.0-22.2) and 27.4 % (95% 22.5-32.3) in the first year after diagnosis. Figure 6.5 shows the risk of death stratified by different cancer groupings. Patients developing a virus related NADM appear to have the lowest rate of death.

Figure 6.5 Kaplan Meier risk of death after a NAMD diagnosis



In adjusted models (table 6.7), patients diagnosed with either non-virus related epithelial cancer had a 1.8 times higher risk of death compared to patients diagnosed with virus related cancers. Patients with a higher CD4 count had a 32% lower risk of death per doubling in CD4 count. Patients who had had a prior AIDS diagnosis or who had been infected via IDU transmission also had a higher risk of death.

Table 6.7 Hazard rate of death after diagnosis with a NADM

		Univariable			Multivariable		
		HR	95% CI	p-value	HR	95% CI	p-value
Cancer group	Virus related	1.00		0.03	1.00		
	Non-Virus epithelial	1.59	1.02-3.48		1.85	1.1-3.08	0.01
	Other	1.90	1.05-3.41		1.82	0.92-3.61	0.08
Gender	Male	1.00		0.09	1.00		
	Female	0.62	0.36-1.08		0.68	0.36-1.30	0.24
Ethnic origin	White	1.00		0.81			
	Other	1.12	0.44-2.87				
Exposure group	IDU	1.00		0.04	1.00		
	Non-IDU	0.57	0.32-1.00		0.48	0.25-0.92	0.02
Hepatitis B status	Negative	1.00		0.15	1.00		
	Positive	1.51	0.91-2.50		2.31	1.29-4.13	0.004
	Unknown	1.54	0.85-2.79		0.94	0.46-1.92	0.86
Hepatitis C status	Negative	1.00		0.50			
	Positive	1.41	0.79-2.51				
	Unknown	0.79	0.40-1.55				
Ever smoked	Never	1.00		<.0001	1.00		
	Current/previous	1.98	0.91-4.29		1.63	0.70-3.80	0.26
	Unknown	5.01	2.24-11.20		4.28	1.73-10.59	0.001
Prior diagnosis	None	1.00		0.001	1.00		
	AIDS*	2.21	1.44-3.39		2.00	1.2-3.30	0.006
	Malignancy	1.23	0.63-2.41		1.34	0.64-2.82	0.43
Anaemia	No	1.00		<.0001	1.00		
	Yes	3.10	1.80-5.32		1.74	0.95-3.19	0.07
	Unknown	1.46	0.81-2.65		0.82	0.34-2.00	0.66
Age	per 10 yr	1.07	0.88-1.31	0.48			
CD4	doubling	0.67	0.59-0.76	<.0001	0.68	0.58-0.80	<.0001
CD4 nadir		0.84	0.77-0.92	<.0001			
Viral load	$\log_{10}$ higher	1.04	0.89-1.23	0.60			
Peak viral load	$\log_{10}$ higher	0.96	0.85-1.08	0.47			
Year of follow-up		0.92	0.87-0.98	0.006	1.10	0.98-3.19	0.07
Ever started cART		1.62	0.71-3.70	0.25			

6.4.6 Sensitivity analysis

In the main analysis, all patients in EuroSIDA were included from the time of first enrolment into EuroSIDA. However, in the early period of data collection there was very little interest in non-AIDS defining events. As a consequence of this, the data collected on NADM only became part of the routine data collection and the EuroSIDA quality assurance exercise in 2003. Therefore, a sensitivity analysis in which follow-up was left censored at 1st January 2003 was performed. A total of 11,106 patients were included in the analysis and were followed for a total of 42,385 PYFU. During this time a total of 187 NADM occurred.

Table 6.8 Multivariable Poisson regression analysis with follow-up left censored at 1/1/2003

Cancer type	N events	Adjusted IRR per doubling of current CD4 count	95 %CI	P-value
All NADM	187	0.81	0.74-0.88	<.0001
Virus related	86	0.76	0.70-0.83	<.0001
Non-virus related epithelial	79	0.92	0.18-1.08	0.29
Other	22	0.79	0.56-1.11	0.17

All NADM also adjusted for gender, ethnic origin, HIV exposure group, region of Europe, hepatitis B and C status, ever smoked, prior diagnosis of AIDS or malignancy, age, CD4 nadir, and having started cART;
Virus-related also adjusted gender, ethnic origin, HIV exposure group, region of Europe, hepatitis B, prior diagnosis of AIDS or malignancy, age, CD4 nadir, and having started cART,
Non-virus related epithelial also adjusted for ethnic origin, region of Europe, age, and year of follow-up,
Other also adjusted for gender, HIV exposure group, region of Europe, hepatitis C status, ever smoked, prior diagnosis of AIDS or malignancy, age, CD4 nadir, and having started cART

The results from the multivariable Poisson regression analysis are consistent with the main analysis. A higher current CD4 count associated with a 19% lower rate of NADM diagnosis (IRR 0.81, 95% 0.74-0.88, p<.0001). Similarly, a higher current CD4 count was associated with a lower rate of 'virus related', 'non-virus related epithelial' and other cancers, although the associations were not significant for 'non-virus related epithelial' and other cancers (table 6.8).

Further as a number of patients had missing data relating to their hepatitis B and C status and also their smoking history a sensitivity analysis was performed only including individuals with complete data to check that the estimates for the association between current CD4 count development of an NADM were robust. A total of 9138 individuals were included in this analysis, and 218 NADM were observed during follow-up. The results were consistent with the main analysis (table 6.9).

Table 6.9 Multivariable Poisson regression analysis including only those with complete data

Cancer type	N events	Adjusted IRR per doubling of current CD4 count	95 %CI	P-value
All NADM	218	0.81	0.75-0.88	<.0001
Virus related	113	0.78	0.72-0.85	<.0001
Non-virus related epithelial	83	0.85	0.72-1.00	0.05
Other	22	0.82	0.57-1.18	0.29

6.5 Discussion

The incidence of NADM in EuroSIDA from 1994-2007 was 4.3 per 1000 PYFU. After adjustment, a higher current CD4 count was independently associated with a decreased incidence of NADM. In addition, an increased rate of 'virus-related' cancers and 'non-virus related epithelial' cancers was found in immunodeficient patients. Hodgkin's lymphoma, anal and lung cancers were all found at a higher rate in patients with lower current CD4 counts after adjustment for other demographic and traditional risk factors.

The findings in this chapter confirm results from other studies that have found a link between immunodeficiency and certain NADM[420;422;586;635;636;647;651] although others have not found an association[415;421;652]. However, only a few of these studies focused on current CD4 count as a measure of immunodeficiency[586;647;651]. It is important to distinguish between CD4 count nadir and current CD4 count; the former measures the lowest point the CD4 count has reached and will address the long term risk of NADM. In contrast, the current CD4 count measures the short term risk of NADM, based on the latest information, and takes account of increases in CD4 count which are associated with starting cART. In the analysis presented here, current CD4 count and not CD4 nadir was found to be important for the development of NADM, a finding also found by the Swiss HIV Cohort Study (SHCS) where the latest CD4 count was associated with the risk of HCC but that CD4 nadir was insensitive in predicting the risk of HCC[647].

Patients with HIV are now living longer[10] and aging, which may allow other comorbidities such as NADM to develop. Older age was a strong predictor of the development of any NADM. This is not surprising given that around 77% of all cancers are diagnosed in patients over the age of 55[416;653]. Individuals in East Europe had a lower risk of developing an NADM. This may, in part, be due to competing risks from other events as individuals in East Europe have been found to have a higher rate of AIDS events and increased mortality[424;654].

An increased risk of developing 'virus-related' cancers was found in immunodeficeint patients. Similar findings were reported in a meta-analysis looking at 'virus related' cancer in immunodeficient patients, irrespective of the cause of immundeficiency[420]. Additionally, prior malignancy diagnosis was associated with an increased incidence, the majority of which were ADM (87%) which are caused by oncogenic viruses[627;630].

This may be an indicator that if you lose immunosurveillance that otherwise protects you against oncogenic viruses, you are at an increased risk of all types of cancer caused by oncogenic viruses. HIV transmission group was also associated with an increased incidence of virus-related cancers, particularly when focusing on anal cancers.

Studies prior to the HIV epidemic found an increased risk of HPV related anal cancer in homosexual men[655;656]. This increased risk has been found to be even higher in those infected with HIV[630;656;657]. Homosexual HIV transmission was found to be associated with almost a 3 times higher risk of anal cancer. Immunodeficiency was also significantly associated with an increased risk as also found in other studies[636;642;657-659], measured by either time to AIDS diagnosis[636] or low CD4 counts[657;658]. Guiguet et al.[660] looked at the effect of immunodeficiency, HIV viral load and antiretroviral therapy on the risk of malignancy (Kaposi's sarcoma, non-Hodgkin lymphoma, cervical cancer, Hodgkin's lymphoma, lung cancer, liver cancer, and anal cancer), and found that current CD4 count was the most predictive risk factor for all malignancies apart from anal cancer. They found that anal cancer was significantly associated with the duration of immunosuppression defined as CD4 count < 200 cells/mm^3.

The development of HL was found to be associated with immunodeficiency. A number of studies support this finding[420;661;662]. The incidence of HL has also been linked to immunodeficiency determined by time to AIDS diagnosis[635]. Other studies, however, have found no association[421]. Biggar et al. found that the incidence of HL is highest at moderate CD4 counts (150-199 cells/mm^3)[661], and another study[663] found that the risk of HL increased for CD4 counts less than 350cells/mm^3 and peaked at 50-99cells/mm^3. This was investigated in this chapter, and evidence of a linear relationship between decreasing CD4 count and increasing incidence of HL was found. CD4 count was split into four categories (<200, 200-349, 350-499, $\geq$500 cells/mm^3). It may be that if there had been sufficient events to split the CD4 count into more categories, particularly in the <200 category, a non-linear relationship may have been observed.

Although there were insufficient numbers of liver related cancer (including HCC) to analyse independently of other virus related cancers, an association between immunodeficiency and increasing risk of HCC has been observed previously[647]. A current CD4 count <200cells/mm^3 was found to be associated with a 7-fold increased rate of liver cancer compared to patients with CD4 count >500cells/mm^3 [647].

However, another study found that the incidence of HCC after adjustment for HCV infection and alcohol use was not found to be significantly associated with HIV infection[417]. EuroSIDA does not currently collect data on alcohol use, so no adjustment could be made for this.

Non-virus-related epithelial cancers were also found at an increased rate in immunodeficient patients. A meta-analysis by Grulich et al [420] comparing rates of cancer in HIV-positive patients and transplant patients found little evidence of an increased risk of epithelial cancers in either group compared to the general population. Rates of prostate cancer have actually been reported to be lower than in the general population[410;420;614]. The combined analysis, reported in this chapter, looking at prostate, breast, rectum and colon cancer found no relationship between immune deficiency and the development of these cancers, which supports findings that these cancers have not been found at an increased rate in HIV-positive patients[420].

HIV-positive patients have been found to have a 7-fold increased rate of lung cancer compared to the general population[664-666]. It is speculated that this increased risk is due to unmeasured confounding, and the higher rates of smoking in HIV-positive patients[18;372;667]. However, some studies have found that the increased risk remains even after adjustment for smoking[668-670]. Lung cancer has been linked to immunosuppression through studies into HIV-positive patients with AIDS[407;635;668]. However, other studies have reported no association with severe immunodeficiency[665]. Grulich et al[420] reported an increase in rates of lung cancer. Lung cancer was the most common cancer in the epithelial group in our study and was found to be associated with current CD4 count. This may explain the association found with immunodeficiency and 'non-virus related epithelial cancer'. Additionally, being a current smoker, one of the main risks for lung cancer[671], and older age were associated with an increased risk of lung cancer status.

No association was found with immunodeficiency in the 'other' group, which was predominately malignant melanomas and cancers at an unspecified location. Older age and smoking status were significantly associated with an increase risk in this group. This is supported by other studies that have reported that the most common non-AIDS defining skin cancers, including basal cell carcinoma, squamous cell carcinoma and malignant melanoma among HIV-positive patients have not been found to be significantly associated with immune function, but rather are related to traditional factors such as aging and skin colour[672].

Malignancies are the most frequent cause of death in HIV-positive patients accounting for around one third of all deaths[633], and one of the most frequent causes of hospitalization[673]. The results from this chapter suggest that patients with virus-related cancers have better survival rates than the two other cancer groups. A study in Brazil found that patients infected with HIV who developed Hodgkin's lymphoma had high rates of remission, and overall survival rates were comparable to those in patients without HIV infection[674]. Studies have also found the survival rate after a diagnosis of anal cancer is similar to that of the general population[675-677]. One study reported that the two year survival rate after a diagnosis of anal cancer was 77% in HIV-positive and 75% HIV-negative patients, and HIV infection was not found to be significantly associated with outcome [675]. Another study into treatment of anal cancer found that the 5 year overall survival was 65% in HIV-negative patients and 61% in HIV positive patients, again these differences were not significant[676]. Liver disease including HCC is now a leading cause of death among HIV-HCV co-infected patients and is becoming an important cause of death among HIV-HBV co-infected patients[360]. Brau et al[413] reported that HCC developed faster in patients co-infected with HIV and HCV compared to patients infected with HCV alone. However median survival times were similar at 6.9 months and 7.5 months respectively (p=0.44).

The highest number of deaths due to NADM were lung cancer related. Studies have reported that the prognosis after a diagnosis of lung cancer is poor in HIV-positive patients because it is often diagnosed later [678;679] and the response to therapy is poor [665]. Surgery is the treatment of choice for some lung cancer cases, but prognosis remains poor[665;666]. However, studies have reported that surgery improves outcome in much the same way as in the general population[665]. A study in Maryland, of patients diagnosed with lung cancer between 1996 and 2003, found that HIV-positive patients were able to receive standard treatment regimens and the overall survival was 5.2 months, which was comparable to HIV-negative patients[680]. Other non-virus related epithelial cancers appear to have similar survival rates as the general population. One study reported that the 5 year survival rate after a diagnosis of breast cancer was 80%, which was similar to the control group of patients with undetermined HIV status[681], and no association has been found between HIV status and outcome after a diagnosis of prostate cancer[682]. This analysis looking at death rates was very exploratory, as there was no information available on the treatment that each individual received or how advanced the cancer was at diagnoses; thus it is impossible to speculate the impact that this may have had on the results.

The patients enrolled in the EuroSIDA study are a very diverse and heterogeneous group which provide an ideal population for considering the development of NADM over many years of follow-up. However, there are a number of limitations of our work. Excessive alcohol use has been shown to be significant in predicting the risk of developing cancer[416], as has smoking, both of which have been found at an increased level in the HIV-positive population compared to the general population[18]. We were able to adjust for smoking status in patients with this data recorded although we do not have information on the amount smoked (e.g. pack years). Also, we do not currently collect data on alcohol intake. Further, anaemia was included in the multivariate model as a time-updated covariate under the assumption that anaemia may be predictive of the development of an NADM, but the exact date of the development of the NADM cannot be established, only the date of diagnosis, and it may be the case that the NADM was the reason for the individual developing anaemia. The interpretation of the role of anaemia in the development of NADM should therefore be interpreted with caution. In addition, the centres included in EuroSIDA may not be representative of all clinics in Europe, as patients in EuroSIDA are those attending clinics for routine outpatient appointments, they have good access to care and may be monitored more frequently than other HIV-positive patients. This may have led higher rates of malignancies being reported and perhaps earlier detection. The NADM were grouped into 3 categories *a priori,* as there was insufficient power to consider them all individually. This has the advantage that we can look in detail in three broad categories which have many features in common, but one of the main disadvantages is that the groupings are heterogeneous, particularly in the 'other' group.

Centres participating in the study may have become more aware of the importance of non-AIDS diagnoses and have recorded more NADM in recent years, although EuroSIDA has an extensive quality assurance program and the data on NADM has been part of the quality assurance exercise since 2003. However, a sensitivity analysis in which follow-up was left censored at 1st January 2003 showed consistent results. Furthermore, the death rates analysis after a NADM diagnosis should be interpreted with caution. The one important limitation to this analysis is that no information was available on the type of treatment patients may have received for their NADM diagnosis. Additionally, the cause of death was not known for all patients.

Currently there is debate over whether starting cART earlier provides any beneficial effect for NADM[683] and there have been conflicting reports from other studies on trends of cancer

incidence over time[406;614;684]. For example, one study reported that there had been an increase in the incidence of anal cancer in the cART era, but a decrease in the incidence of HCC[685]. Other studies have shown that the use of cART does not appear to affect the incidence or overall survival of anal cancer[686;687].

Additionally, some studies have actually demonstrated an increased risk of HL in the cART era[407;410;614;684], with one study reporting HL incidence was higher in HIV-positive patients using cART than those not[415]. Furthermore, the incidence of HIV-related lung cancer was reported to have increased from 0.008 per 1,000 PFYU in the pre-HAART era to 0.7 per patient-years follow-up in the post-HAART era[688]. In our study we observed a significant increase in non-virus related epithelial cancer, anal cancer and breast, colon, and prostate combined with increasing calendar year of follow-up. No significant difference was observed over time for the other groups after adjustment for other factors. Some of the increase in rate may in part be due to competing risk, in that individuals in the cART era are not dying from AIDS and are thus surviving longer, and therefore have a higher chance of developing a NADM or another illness.

Whilst our study is not based on data from a strategic trial, and cannot establish that increasing the CD4 counts after starting cART would cause a decrease in NADM, it does suggest that earlier treatment may be beneficial for reducing the incidence of NADM by reducing the risk of severe immunosuppression. Since the introduction of cART, overall survival of HL has improved, with studies reporting a 2 year overall survival rate of 45% in the pre-cART era and 62% in the post cART era[689]. Furthermore, a recent study looking at cancer incidence in transplant patients found that the effects of immunosuppression were reversible for some cancer types[690]. The risk of developing infection related cancers was reversed with the reversal of immunosuppression, whereas other cancer types remained significantly increased after a reduction in immunosuppression[690]. In support of this, some HL tumours have reportedly undergone spontaneous remission when immunosuppressive therapy was discontinued[662]. The START (Strategic Timing of Antiretroviral Therapy) trial to determine whether starting cART early (before CD4 drops to less than 500 cells/mm^3), rather than waiting until CD4 drops to less than 350 cells/mm^3 as current guidelines recommend, reduces the occurrence of serious morbidity and mortality will also explore whether cART protects against NADM[691].

Our study has shown a link with immunodeficiency and the development of certain NADM, particularly those that are virus-related. Starting cART earlier to reduce the proportion of

patients with a low CD4 count may decrease the rate of developing many common non-AIDS related malignancies, although the potential harms for longer exposure to drugs and risks of toxicities would have to be balanced against the potential benefits. Cohorts and clinical trials need to collect data on both AIDS and non-AIDS related illnesses to obtain a better understanding of the incidence and risk factors for the development of non-AIDS illnesses including cancer. Greater understanding of how these cancers develop and their risk factors may help in the prevention and treatment of malignancies in both the HIV-positive and HIV-negative populations.

A manuscript of this analysis was published in Cancer in November 2010 and can be found in Appendix VII.

Chapter 7 Regional differences in the incidence of AIDS and non-AIDS related mortality in HIV-positive patients across Europe

7.1 Introduction

This thesis has so far looked at the long term outcomes of HIV-positive patients across Europe and Argentina. A number of surrogate markers have been used to measure this, such as viral load, CD4 count, and the development of serious clinical events including both AIDS and non-AIDS related, with the last chapter having a particular focus on non-AIDS defining malignancies. However, the overall aim of a long term durable cART regimen is to reduce the patient's risk of mortality.

7.1.1 All-cause mortality

Mortality rates in the USA and Europe peaked in the mid90s[540;692;693], just prior to the introduction of cART when a dramatic decline in mortality rates was seen[156;157;234;272;692]. Figure 7.1 shows the decrease in the rate of AIDS or death by calendar year within EuroSIDA. The risk of death for an HIV-positive patient in the cART era has been estimated to be >85% lower than in the pre-cART era[272]. Further, reports from studies in the more recent cART era show that mortality rates continue to decrease[694-696], due to improvements in cART, both in terms of efficacy and less toxicity[6], and its availability. Table 7.1 summarises the mortality rates observed in different studies of HIV positive patients, from around the time when cART was first available (1996) to the current treatment era. In the cART era all cause-mortality rates have been estimated to range from 9.4 to 24 per 1,000 PYFU.

Studies conducted in the current treatment era have found the patients successfully treated with cART have a moderate excess mortality rate compared to the general population that is comparable with patients having other chronic conditions, such as diabtetes[538]. Further, Sighem et al.[697] reported that the life expectancy of asymptomatic HIV-positive patients, who are still treatment naïve and have not experienced any CDC stage B or C events 24 weeks after diagnoses, approaches that of non-infected individuals. Using stochastic computer simulation, it has been estimated in homosexual men in the UK, 7.0 years of life are lost due to HIV[698].

Figure 7.1 Incidence rate of AIDS or death per 100 person years of follow-up in EuroSIDA by calendar year

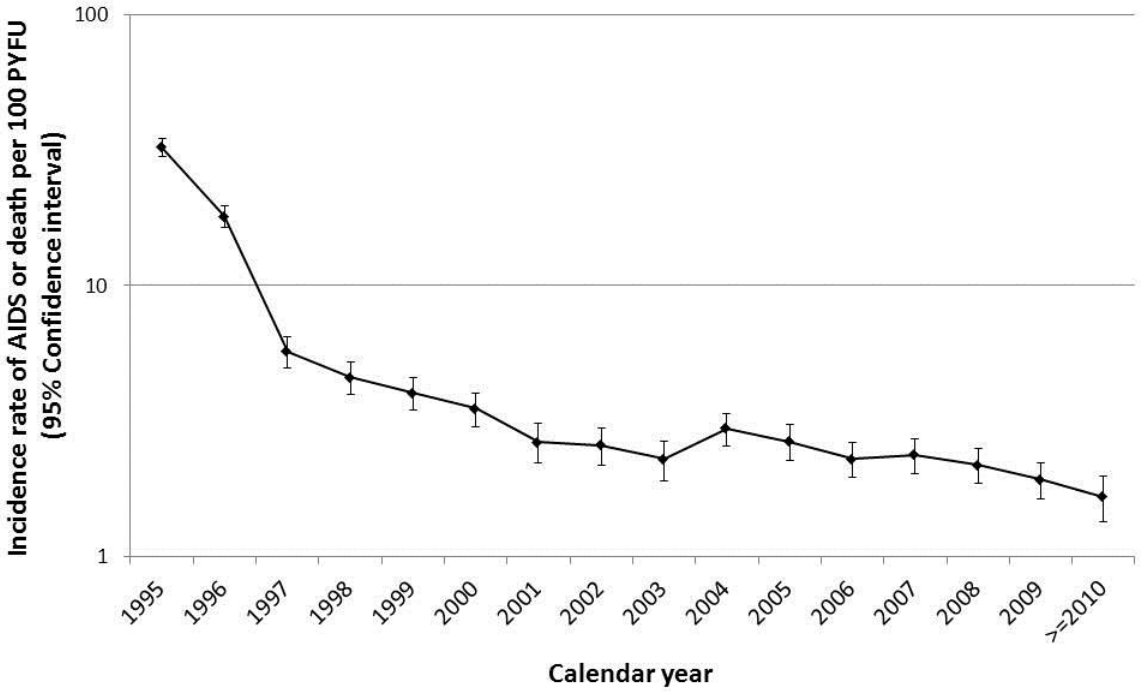


Table 7.1 Mortality rates in developed countries in the cART era

Study	Time Period	Geographical region	Mortality rate per 1000 PYFU	Percentage of deaths attributed to AIDS related causes
ARTCC[699]	1996-2006	Europe and USA	12.1	49.6%
Weber[361]	1999-2004	Europe, USA, Australia	16.0	31.1%
Petoumenos[700]	1999-2004	Australia	15.8	40%
Martinez[701]	1997-2004	Spain	45 (1997) 14 (2004)	40%
Pallella[695]	1996-2004	USA	70 (1996) 13.0 (2004)	54.2% (1996) 25.0% (2004)
Sabin[702]	1998-2003	UK	19.4 (1998-2000) 9.4 (2001-2003)	53.3% (1998) 43.8% (2003)
Krentz[703]	1984-2003	Canada	117 (1984-1996) 24 (1997-2003)	90% (1984-1996) 67% (1997-2003)

Other studies have also identified subgroups of HIV-positive individuals that have mortality rates approaching that of the general population[538;697;704;705]. In particular, the Swiss HIV cohort compared mortality rates in their cohort to those of the Swiss general population from 1997-2001. They found that those who were successfully treated with cART and who were not co-infected with hepatitis C had an excess death rate that was < 6 per 1,000 per year, similar to those with successfully treated cancer[539]. Zwahlen et al.[538] also identified a subgroup of homosexuals who were AIDS free at starting cART, and had a CD4 count $\geq$ 350 cells/mm^3 and a viral load ≤500 copies/ml 6 months after starting cART, where the mortality rate was estimated to be 5% higher than the corresponding gender and age matched general population[538]. Similarly, a recent study by Obel et al.[704] (figure 7.2), reported that HIV-positive patients on cART did have a substantially increased risk of death compared to the general population, but that this increased risk was associated with well known HIV and non-HIV related risk factors that were identifiable prior to or in the first year after cART initiation. These risk factors included detectable viral load (>49copies/ml), CD4 count <200cells/mm^3, AIDS defining disease, comorbidities, drug or alcohol abuse. Obel et al. also identified a sub-group of HIV-positive individuals, who had no identifiable risk factors, that had an almost identical risk of death to the general population[704].

Figure 7.2 Cumulative survival for HIV-positive patients starting HAART and persons from the general population[704]

Figure not available due to copyright restrictions

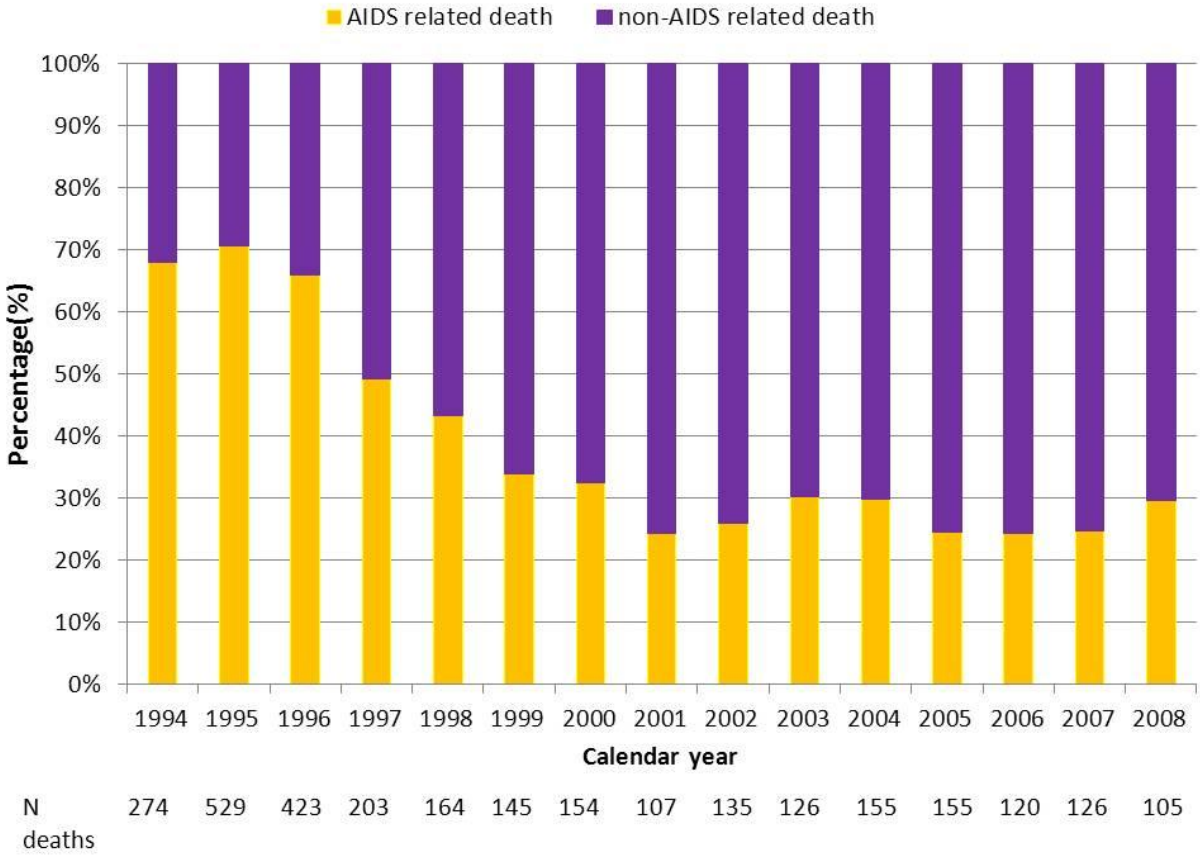
One of the main problems with comparing mortality rates in HIV-positive patients to that of those in the general population is finding a suitable comparison group. Various non-HIV specific factors have been identified as being associated with a risk of all-cause mortality including Hepatitis C co-infection[703;706], older age[696], IDU transmission route[707], poor socio-economic status[708], low haemoglobin and creatinine levels[708], low vitamin D levels[709], smoking and alcohol abuse[696]. Many of these factors occur at higher rates in the HIV-positive population[372;417;667] than in the general population, so a direct comparison may be biased. Additionally, HIV associated risk factors such as lower CD4 cell counts and higher plasma viral load at cART initiation[701] have been found to be associated with an increased risk of all-cause mortality.

7.1.2 AIDS related mortality

The main reason for the decline in all-cause mortality in HIV-positive patients, is that deaths due to AIDS-related causes have declined significantly in the cART era, both in absolute terms and in the proportion of deaths attributed to AIDS related causes[234;272;692]. The percentage of deaths attributed to AIDS was estimated to be as high as 90% in the pre-cART era[703], to as low as 25% in the current treatment era[695]. In 2009, UNAIDS estimated that there was approximately 1.6 million deaths due to AIDS related causes worldwide[710].

Figure 7.3 shows the change in the proportion of deaths attributed to AIDS and non-AIDS related causes in the EuroSIDA study over time. At the start of the study 70% of the deaths were due to AIDS related causes whereas in the current treatment era just over 20% of the deaths are attributed to AIDS related causes. AIDS related death is particularly prevalent among those with the lowest CD4 counts[696;711].

Figure 7.3 Proportion of death due to AIDS and non-AIDS related causes in the EuroSIDA study by calendar year[712]



One study reported the median CD4 count of those dying from AIDS to be 15 cells/mm^3 (IQR 6-42), with little difference between the pre and current cART periods[703]. Additionally, age, infection via intravenous drug use, previous AIDS diagnosis, CD4 count at cART initiation and a viral load at cART initiation >100,000 copies/ml have been found to be strongly associated with progression to AIDS and death[538;711;713]. Further, not all AIDS events have been found to be associated with the same risk of death[714;715]. The risk of death from non-Hodgkin's lymphoma has been found to be particularly high[714;716], and previous EuroSIDA analysis has reported that the decreasing incidence of NHL was not as great as for other AIDS defining events after the introduction of cART[622]. However, the survival rate has improved in recent years due to more intensive chemotherapy regimens, increased complete remission rates and the use of effective cART[689]. In the current treatment era (2000-2005), the proportion of deaths attributed to non-Hodgkin's lymphoma, Kaposi's sarcoma or cervical carcinoma remained stable between 2000 and 2005[717].

The overall continued decrease in AIDS related deaths is due to improvements in the effectiveness, tolerability and availability of cART regimens over time[155;718]. The clinical benefit of cART is largely a result of its ability of produce sustained suppression of viral replication[466], with the proportion of deaths classed as AIDS related decreasing with increasing duration on cART[699].

In studies where specific causes of death have been investigated, AIDS-related deaths still account for the highest proportion of death, followed by non-AIDS defining infections, non-AIDS defining malignancies, liver related causes, cardiovascular disease, violence/drug related cause[359;363;694-696;699;703;717]. The role of non-AIDS related deaths is discussed in more detail in the following section.

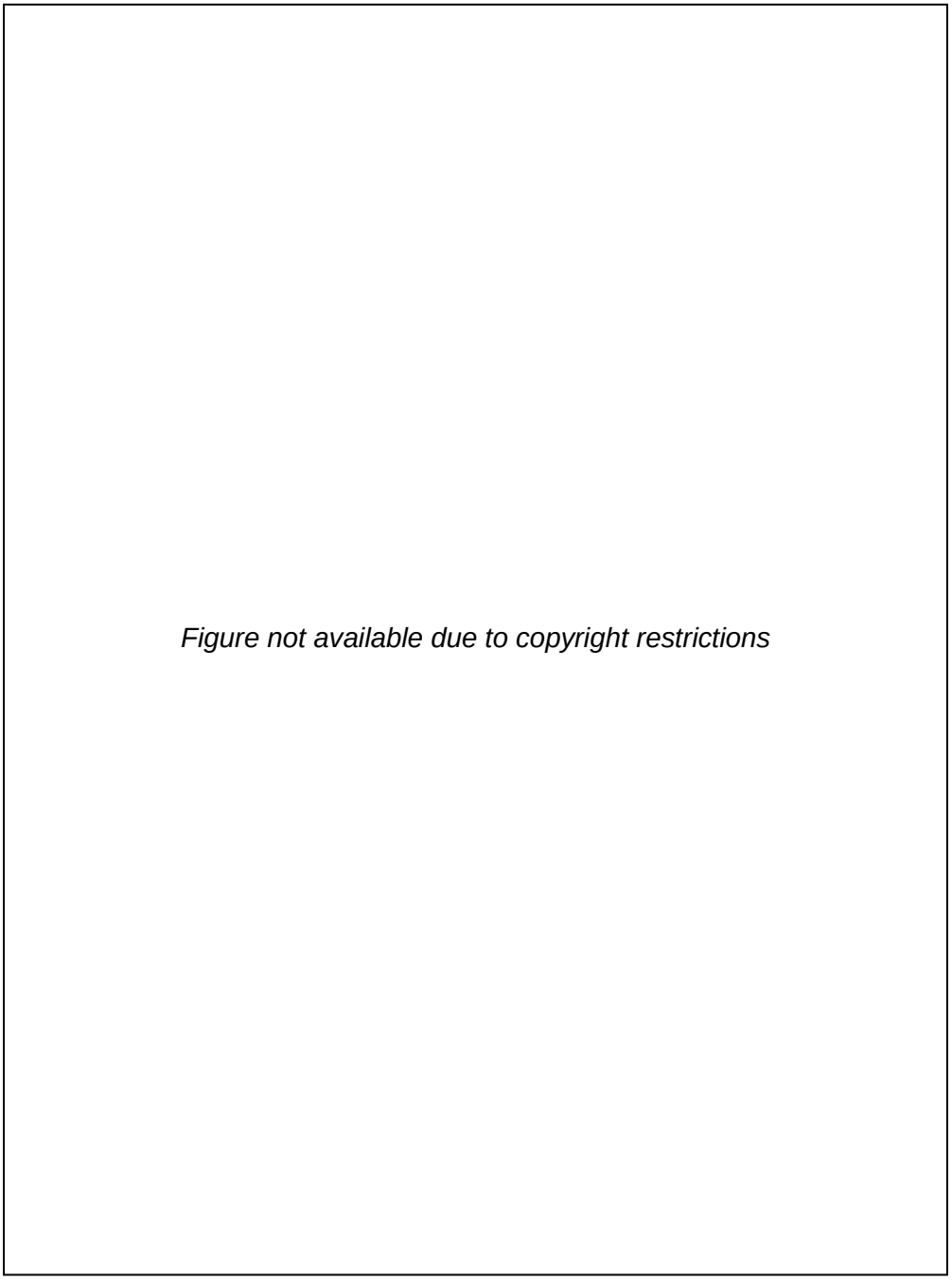
7.1.3 Non-AIDS related deaths

As shown in figure 7.3, a high proportion of the mortality observed in HIV-positive individuals is now due to non-AIDS related causes[354;359;695]. It has been reported that deaths due to non-AIDS related causes now account for between 50-66% of all deaths that occur in HIV-positive patients[363;364;719]. The most common non-AIDS related deaths have been reported to be cardiovascular disease, liver related, non-AIDS defining infections, non-AIDS defining malignancies and deaths due to violence/substance abuse[359;363;694-696;699;703;711;717]. Some studies have shown that the incidence of non-AIDS related mortality, from causes specific such as cardiovascular disease (CVD), liver disease, and cancer, has also declined in the cART era [354;720].

Figure 7.4 shows the results from the D:A:D study looking at the association between calendar time and rate of death[696]. They found that for many of the specific causes the rate of death decreased over time. Rates of death also fell for chronic viral hepatitis, liver failure, myocardial infarction, suicide and drug overdose. They reported no other obvious trends observed in relation to specific causes of death, with the exception of deaths in the 'unknown' category, which increased from 0.34 per 1000 person years in 1999/2000 to 1.05 in 2007/2008, which is likely due to reporting delays. However, a study of individuals living in Southern Alberta in Canada between 1984 to 2003 reported that deaths from non-AIDS related conditions had increased both as an absolute number of deaths and as a proportion of all deaths in HIV-positive patients[703]. It has also been reported that death due to liver disease and non-AIDS defining infections have been found to be significantly increasing over time[397;694;701;721]. The Mortalité study also reported that, adjusted for age and gender, the proportion of death attributable to non-AIDS related malignancies increased significantly from 2000 to 2005[717]

Many non-HIV related risk factors are associated with an increased risk of non-AIDS related mortality, such as age[696;711], co-nifections[255;628;706] and other lifestyle factors such as smoking, alcohol and drug misuse. Smoking has been found to be associated with an increased risk of death, particularly from CVD and non-AIDS malignancies[665;696]. Additionally, studies have reported a significant association between alcohol abuse, which is high in the HIV-positive population[411], and the development of some non-AIDS defining mailgnancies[417;722], and as a consequence mortality[364;385].

Figure 7.4. Rate of death in the D:A:D study according to calendar year and specific cause of death. (a) AIDS-related, Liver-related, CVD-related, and non-AIDS malignancy deaths. (b) Other causes of death.[696]



Further, individuals infected via IDU have been found to have higher rates of non-AIDS related mortality[711], with particularly high rates of liver-related and violent death[699]. The increased rate of liver related mortality is thought to be in large part due to co-infection with hepatitis B and C[364;706;723]. However, survival of IDUs with and without HCV co-infection has been shown to be similar[721]. Co-infection with other viruses such as the human papilloma virus or the Epstein Barr virus may also increase the risk of developing a non-AIDS defining event and in turn, a higher risk of death[255;628;629].

Other HIV-associated factors have also been identified for many of specific cause of non-AIDS related death, including immunodeficiency and inflammation attributable to uncontrolled viremia[724;725]. Immunodeficiency has been found to be associated with most non-AIDS related deaths, in particular non-AIDS malignancies, liver disease and renal failure[361;699;711]. The only exception appears to be cardiovascular disease[353]. Additionally, some evidence has found that HIV-infection may increase the risk of some serious non-AIDS related events such as cardiovascular, renal, hepatic and malignancies[353] , even in individuals with high CD4 counts[356;726]. HIV has been found to be an independent risk factor for cardiovascular disease[720;727], and deaths due to cardiovascular disease have been found to be associated with viral replicaion[363].

Little variation in the rates of non-AIDS related deaths and the time on cART have been observed[699;728]. Individuals dying from non-AIDS related causes tend to have initiated cART at higher CD4 counts, have more cART experience and been receiving cART close to the time of death[695]. Martinez et al.[701] reported that almost one third of patients in their study, in the cART era, died with optimal virologic suppression. The causes of death in these patients were commonly non-infectious diseases.

7.1.4 Differences in prevalence, access to cART and mortality rates across Europe

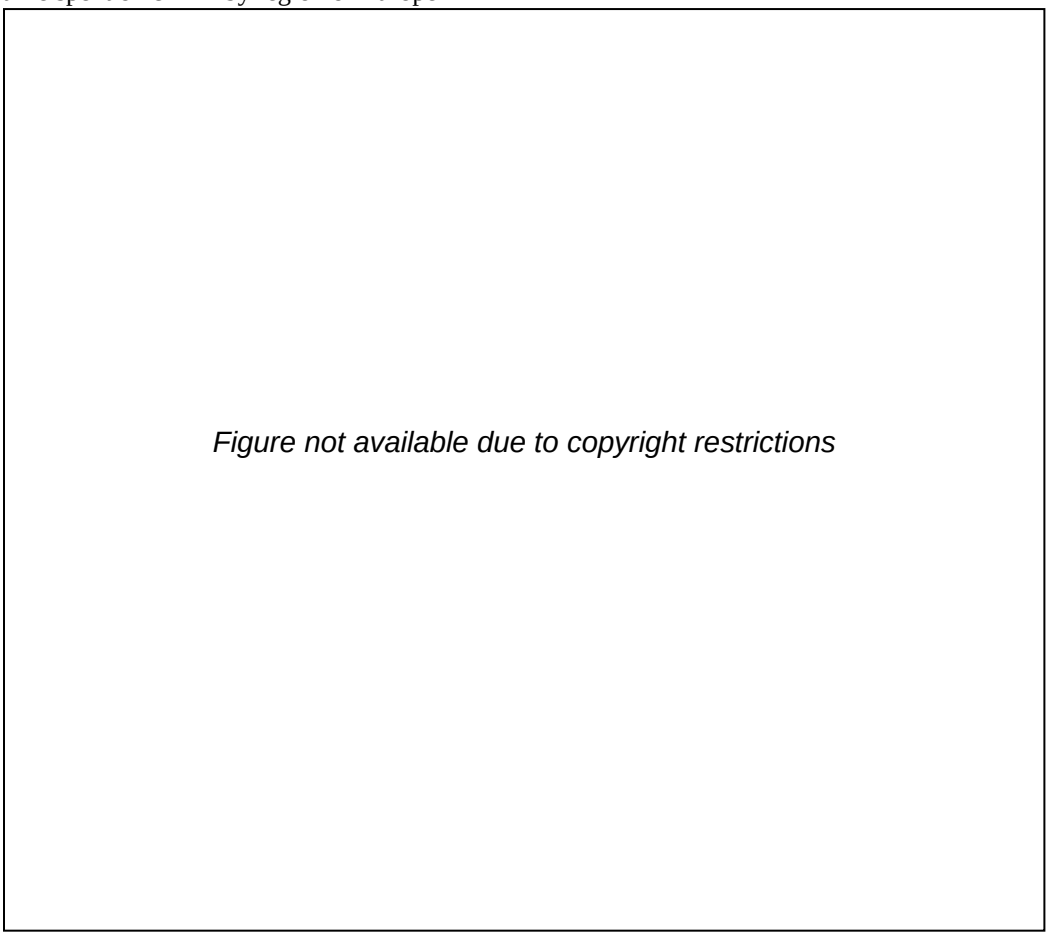
In 1999, shortly after the introduction of cART, the EuroSIDA study reported that the mortality rate in West Central Europe was significantly lower than in North and South Europe[729]. The epidemic in North, South, and West Central Europe began in the late 1980s[730], and although the number of people living with HIV has risen by around 30% from 2000 to 2.3 million in 2009, the number of new infections has remained relatively stable at around 100,000 new infections each year[731]. Mortality rates in this region of Europe peaked in the mid 90s[693], and since then widespread access to antiretroviral therapy has led to considerable drops in mortality[732]. UNAIDS reported that in North America and Western Europe (grouped together in UNAIDS reports), the number of deaths due to AIDS related causes has remained relatively stable in the current treatment era, with approximately 35,000 AIDS related deaths in 2009 compared to 37,000 in 2001[731].

The epidemic in East Europe is more recent; the first outbreaks were reported among injection drug users in southern Ukraine in 1995, which were rapidly followed by other drug related outbreaks in Russia in 1996[733]. From then on, there has been a rapid increase in HIV infections in East Europe[8;150], and this region now has one of the fastest growing HIV epidemics worldwide[8]. In East Europe and Central Asia (grouped together in UNAIDS reports), the number of people living with HIV almost tripled between 2000 and 2009 and is now estimated to be around 1.4 million[734], with Russia and the Ukraine accounting for nearly 90% of all new infections[734]. Additionally, a 4-fold rise has been reported in AIDS related deaths from an estimated 18,000 in 2001 to 76,000 in 2010[734].

The differences in mortality rates observed in the previous EuroSIDA analysis were attributed to different treatment policies and drug availability in these regions[729]. The uptake of cART in East Europe remains low, and previous studies have reported substantial differences in access to care and treatment compared to the rest of Europe[424;735]. A recent UNAIDS report estimated that in 2009, 19% (15%-21%) of those eligible for treatment in East Europe were accessing it, and although low, this proportion was estimated to have increased by 34%[733].

More recent analysis within EuroSIDA has sought to develop a concise set of indicators to assess and compare the delivery of healthcare in the large, and heterogeneous, population of HIV-positive patients across Europe, in countries with highly varying levels of resources. These health care indicators looked at adherence to treatment guidelines and ability to achieve a clinical response of cART initiation. Only a few, and generally minor, differences were seen between South, North, and West Central Europe in most healthcare indicators. However, East Europe, Argentina and to some extent East Central Europe differed significantly from the rest of the regions[735]. The figure below, using the same variable described in chapter 3 (figure 3.2), shows that compared to North Europe, individuals form South (SE), East Central (ECE) and East (EE) Europe had significantly lower odd of being suppressed (HIC-RNA <500 copies/ml) >90% of the time they were on cART.

Figure 7.5 Odds Ratio (OR) of having suppressed HIV-RNA (<500 copies/ml) during more than 90% of time spent on cART by region of Europe[735]



Previous analysis within this thesis has also identified differences in various outcomes across the regions. In chapter 5, assessing the long term durability of nevirapine, efavirenz and lopinavir based cART regimens, a higher rate on non-AIDS events was observed in North Europe compared to South Europe. Additionally, the last chapter found that those in East Europe had a lower risk of developing an NADM. One theory was that this may, in part, be due to competing risks from AIDS related events and mortality.

The EuroSIDA study has grown substantially since the last reports on mortality rates[10;354], particularly in Eastern Europe where few cohort studies are established. The study therefore provides a unique opportunity to establish whether, in the current treatment era, regional differences in the mortality rate of HIV-positive individuals remain, to try and understand the factors underlying any such differences and whether they are changing overtime.

7.2 Aim

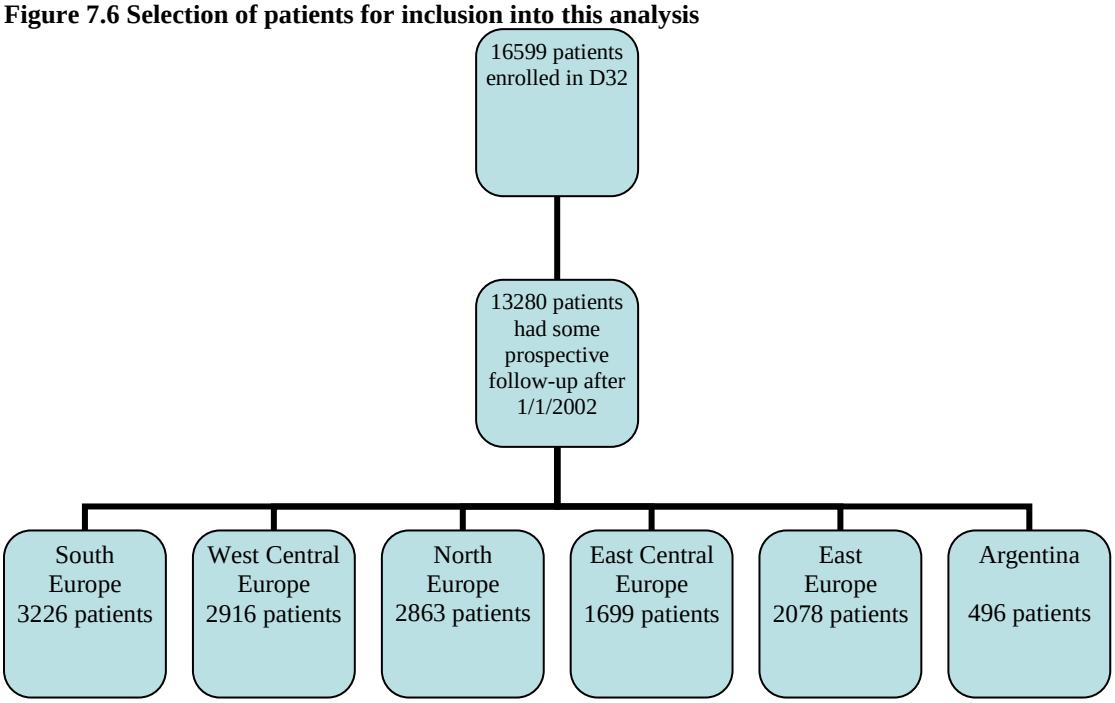
The aim of this chapter was therefore to report rates of all-cause, AIDS and non-AIDS related mortality, in the current treatment era, across different regions of Europe and Argentina and to investigate trends over time. Furthermore we aimed to try and understand the factors attributable to any differences observed across the regions.

7.3 Methods

7.3.1 Patient selection

There were 16,599 patients included in the D32 update. To investigate mortality rates across the different regions of Europe, patients were included who had some prospective follow-up after 1st January 2002, as this was the median date of first visit for patient enrolled in Cohort V. Cohort V was the first cohort to enrol a significant number of patients from Eastern Europe into EuroSIDA. Therefore, patients were included from baseline, which was defined as either 1st January 2002 or enrolment into EuroSIDA, whichever occurred later. Figure 7.6 shows the number of patients selected for inclusion into this analysis by region.

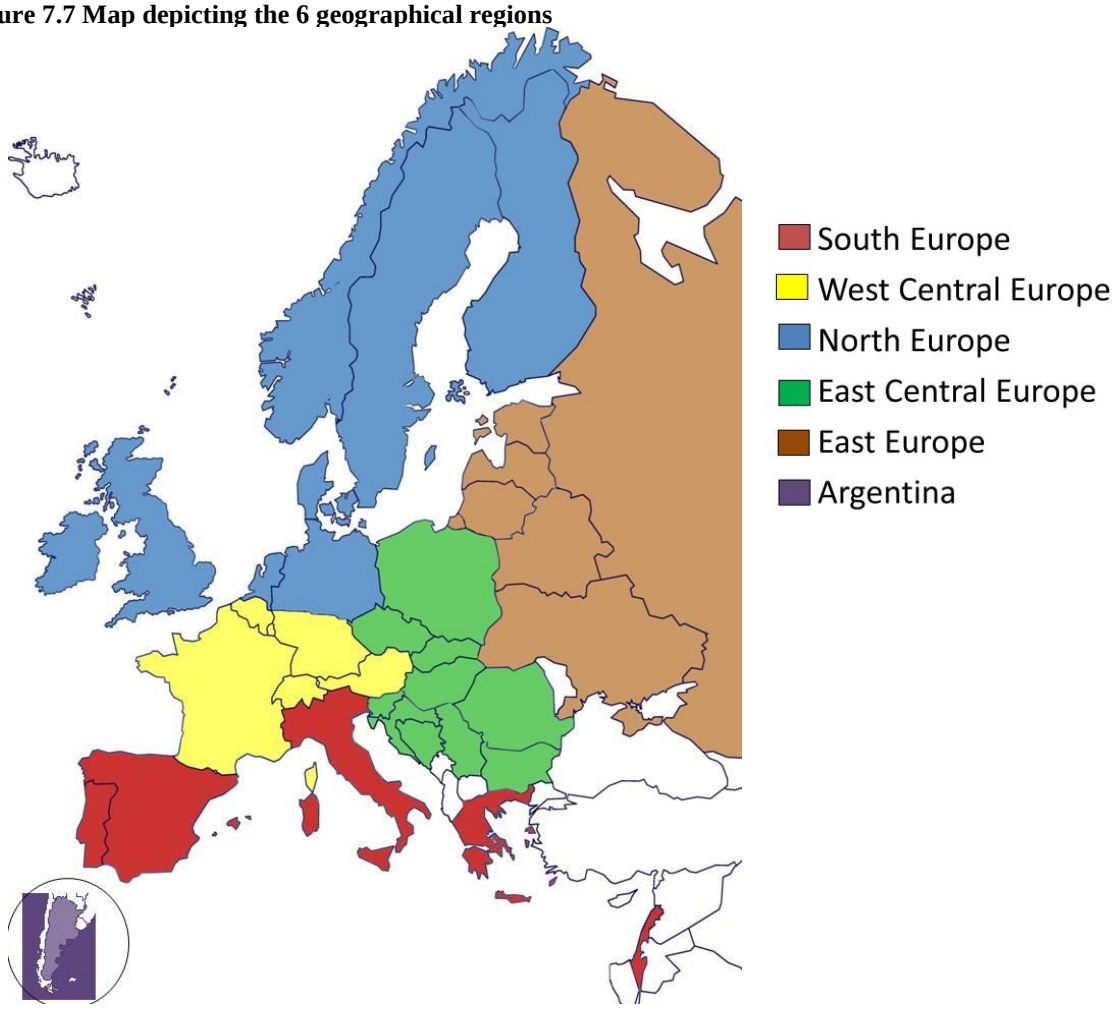
Figure 7.6 Selection of patients for inclusion into this analysis



As discussed in Chapter 2, EuroSIDA individuals were divided into 6 regions (figure 7.7) according to country of residence as follows:

- **South Europe**: Greece, Israel, Italy, Portugal, Spain.
- **West Central Europe**: Austria, Belgium, France, Germany, Luxembourg, Switzerland.
- **North Europe**: Denmark, Finland, Ireland, the Netherlands, Norway, Sweden, United Kingdom
- **East Central Europe**: Bulgaria, Croatia, the Czech Republic, Hungary, Poland, Romania, Serbia, Slovakia
- **Eastern Europe**: Belarus, Estonia, Latvia, Lithuania, the Russian Federation, Ukraine
- **Argentina**

Figure 7.7 Map depicting the 6 geographical regions



Patients were followed until their death, or 6 months after their last recorded visit reported on EuroSIDA follow-up forms, whichever occurred first. Some centres may be linked to death registries, and therefore following all individuals until death, including those who had been lost to follow-up, may introduce differential censoring between those who did and did not die.

7.3.2 Coding causes of death

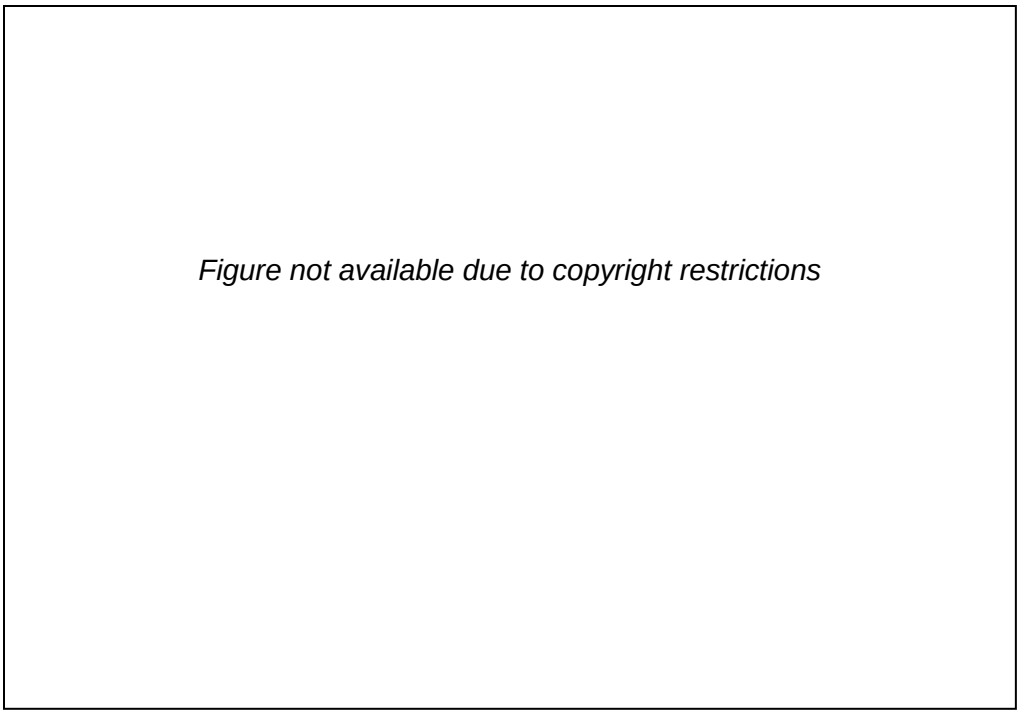
For patients enrolled in the EuroSIDA cohort who died, date and cause of death are reported by the site investigator on the EuroSIDA follow-up forms and, since 2004, a Coding of Death in HIV (CoDe) case report form is additionally completed for each fatal case[736]. For this analysis, deaths were classified into AIDS-related deaths or non-AIDS related deaths using a three step hierarchical process[737].

The Coding of Death in HIV (CoDe) project started in 2004, is a uniform coding system applied to studies of individuals with HIV infection (full details available at **www.cphiv.dk**). It consists of a form providing detailed data on the causes of death and contributing factors, and a centralised review process of the data collected (see Appendix III). The underlying cause of death was defined as the disease or injury that initiated the morbid trend of events. If a CoDe form was available, deaths attributed to AIDS infections (CoDe 1.11) or AIDS malignancy (CoDe 1.12) were classed as AIDS related and all other deaths non-AIDS related.

If no CoDe form was available, then EuroSIDA follow-up forms were assessed (see Appendix I). If the illness causing death reported on the EuroSIDA follow-up forms was HIV related, an AIDS defining event or an invasive bacterial infection then the death was classed as AIDS related and all other deaths non-AIDS related.

Finally, to allow for patients with missing information on the cause of death to be included in the analysis, if the cause of death could not be determined using either CoDe or EuroSIDA follow-up forms then a computer based algorithm was used to determine if the death was AIDS related or non AIDS-related[738], as shown in figure 7.8.

Figure 7.8 Computer based algorithm for assigning cause of death as AIDS or non-AIDS related[738]



In this case, if the patient had experienced an AIDS defining event within the upper limit of survival time for the preceding AIDS defining event (table 7.2), or within 17 months prior to death (if the survival time was not known) then the death was classified as AIDS related causes[739;740]. If the patient had never experienced an AIDS defining event, or the most recent AIDS defining event was out with the upper limit of survival (or 17 months), then the patient was classed as dying from non-AIDS related causes.

Deaths classed as non-AIDS related were further classified into non-AIDS related infection, liver related (including deaths due to hepatitis B or C, liver failure or cirrhosis and liver cancer), non-AIDS defining malignancies (including all malignancies excluding AIDS defining cancers [Kaposi's Sarcoma, Non-Hodgkin's Lymphoma and cervical carcinoma} and liver cancer), cardiovascular disease (including a stroke, Myocardial Infarction [MI], heart or vascular disease), violent (including an accident or violence, suicide, euthanasia, substance abuse or overdose), other (causes associated with < 20 deaths) or unknown (deaths with insufficient information to determine cause of death and defined as non-AIDS related using the computer based algorithm).

Table 7.2 Median survival time (months) for each AIDS-defining event[739]

7.3.3 Statistical methods

The incidence of all-cause, AIDS and non-AIDS related mortality was calculated per 1000 person years of follow-up (PYFU), and stratified by region and calendar year.

Poisson regression analyses were used to investigate factors associated with the incidence of mortality from all-cause, AIDS and non-AIDS related causes. Variables investigated were age, gender, ethnic origin, smoking status, anaemia, hypertension, diabetes, hepatitis B and C status, and year of follow-up. Age and year of follow-up were treated as continuous variables. Factors that were significant in univariable analysis ($p<0.1$) were included in the multivariable model.

Individuals were classed as Hepatitis B positive if they had a positive HBV surface antigen test recorded and Hepatitis C positive if they had a positive HCV antibody test. Hypertension was defined as a diastolic blood pressure ≥ 90 or a systolic blood pressure ≥ 140 mmHg or receiving anti-hypertensive medication. If a diagnosis of insulin-dependent diabetes was reported by the investigating centre, or if an individual was receiving anti-diabetic medication they were classed as diabetic. Anaemia was defined as a haemoglobin level ≤ 12 or ≤14 mg/dl for females and males respectively[598]. In addition, variables related to HIV infection were also investigated including HIV transmission group, any previous AIDS defining illnesses, CD4 count, on cART viral load and Hepatitis C status.

7.3.3.1 Categorisation of CD4 count and viral load

In chapter 3, the median time between successive CD4 and viral load measurements was 3 months (IQR 3–4 months) in patients who were maintained on a stable and fully suppressed cART regimen for at least 1 year. However, in that analysis a high proportion of patients in East Europe were excluded, due to missing values. Current guidelines recommend clinical and laboratory evaluation of HIV-disease status at least every half year before initiation of cART and on a 6-12 month basis in clinically stable patients on cART with suppressed HIV-RNA[214;223;498]. Adequate monitoring of untreated patients is important in terms of the timely initiation of cART and the prevention of AIDS and, ultimately, death[735].

Table 7.3 below shows the differences in the frequency of CD4 count and viral load monitoring across the 6 different regions. A substantial number of patients in East Europe have missing CD4 counts and viral loads for a significant proportion of their follow-up. This is particularly true for viral load measurements in patients who have not yet started cART.

Table 7.3 Time between CD4 count and viral load measurements by region

	Time in months between CD4 measurements Median (IQR)	Time in months between viral load measurements Median (IQR)
South Europe	3.7 (3.0-4.1)	3.8 (3.0-4.2)
West Central Europe	3.0 (2.6-3.4)	3.0 (2.7-3.4)
North Europe	3.0 (3.0-3.7)	3.0 (3.0-3.6)
East Central Europe	4.1 (3.2-5.6)	5.3 (3.9-6.3)
East Europe	5.2 (3.7-7.3)	6.1 (3.9-9.2)
Argentina	6.5 (4.7-9.0)	6.7 (4.6-8.9)

Therefore, in this analysis CD4 count was included as a categorical variable <200, 200-349, 350-500, ≥500 cell/mm^3, and missing (no CD4 count measured in the previous 6 months), to allow for patients with missing values to remain in the analysis. Further, treatment for HIV was split into 4 categories, not on cART, on cART with a viral load <500 copies/ml, on cART with a viral load ≥500 copies/ml, or on cART with no viral load measurement available in the previous 6 months. cART was defined as receiving ≥ 3 antiretrovirals. Both current and baseline values were investigated for variables that could change over time, such as age, and CD4 count

7.4 Results

7.4.1 Baseline characteristics

13,280 HIV-positive individuals were included in the analysis. There were clear differences in demographics across the regions at baseline (table 7.4). In East Europe, individuals were more likely to be younger (median age 30), female (43%) and infected via intravenous drug use [IDU] (48%) or heterosexual sex (40%). In contrast, the majority of the individuals in West Central (45%), and North Europe (59%) were infected through homosexual sex. Similar baseline CD4 counts were observed across the regions.

3,666 patients had an AIDS diagnosis prior to baseline. Table 7.5 shows the five most common AIDS diagnosis overall and the proportion of each observed in each region. The most commonly observed prior AIDS diagnosis was Oesophageal candidiasis (16.6%), it was the most commonly reported AIDS diagnosis in West Central (19.8%), East Central (20.0%) and East Europe (24.4%) and the second most common in North Europe (16.9%). The most commonly reported AIDS diagnosis in South, and North Europe and Argentina was *Pneumocystis jiroveci pneumonia* (PCP), which was the second most common overall. A higher proportion of prior AIDS was due to Pulmonary Mycobacterium Tuberculosis (TB) and HIV wasting syndrome (14%) in East Europe (18%) compared to the other regions.

10,263 (77.3%) patients had started cART prior to baseline, as shown in table 7.6, In South, West Central, North, East Central Europe and Argentina, around 80% had started cART prior to baseline, compared to only 31% in East Europe. Median CD4 count and viral load in those starting cART were similar across the regions. However, 7% of those who had started cART in East Europe did not have a viral load measurement available at the time of starting. In South, West Central, North and East Central Europe over 50% of patients starting cART started on a PI based regimen whereas in East Europe and Argentina a higher proportion started on an NNRTI based regimen.

Table 7.4 Baseline characteristics (baseline was defined as either 1st January 2002 or enrolment into EuroSIDA which ever occurred later)

		South Europe	West Central Europe	North Europe	East Central Europe	East Europe	Argentina
Total		3226	2916	2865	1699	2078	496
Gender (N.%)	Male	2379 (73.7)	2244 (77.0)	2330 (81.3)	1220 (71.8)	1170 (56.3)	308 (62.1)
Race (N.%)	White	2956 (92.4)	2098 (75.2)	2423 (85.5)	1667 (99.6)	2069 (99.9)	48 (98.6)8
Exposure Group (N.%)	Homosexual	1099 (34.1)	1306 (44.8)	1692 (59.1)	626 (36.9)	154 (7.4)	126 (25.4)
	Injection Drug User	946 (29.3)	404 (13.9)	317 (11.1)	447 (26.3)	995 (47.9)	62 (12.5)
	Heterosexual	978 (30.3)	848 (29.1)	684 (23.9)	489 (28.8)	827 (39.8)	292 (58.9)
Hepatitis B (N.%)	Negative	2171 (67.3)	2308 (79.2)	2337 (81.6)	1405 (82.7)	1647 (79.3)	357 (72.0)
	Positive	160 (5.0)	201 (6.9)	207 (7.2)	81 (4.8)	114 (5.5)	26 (5.2)
	Unknown	895 (27.7)	407 (13.9)	321 (11.2)	213 (12.5)	317 (15.2)	113 (22.8)
Hepatitis C (N.%)	Negative	1586 (49.2)	1984 (68.0)	1839 (64.2)	996 (58.6)	698 (33.6)	294 (59.3)
	Positive	751 (23.3)	457 (15.7)	327 (11.4)	478 (28.1)	1011 (48.7)	94 (19.0)
	Unknown	889 (27.6)	475 (16.3)	699 (24.4)	225 (13.2)	369 (17.8)	108 (21.8)
Hypertension	No	197 (6.1)	179 (6.1)	170 (5.9)	249 (14.7)	438 (21.1)	57 (11.5)
	Yes	130 (4.0)	265 (9.1)	221 (7.7)	153 (9.0)	54 (2.6(	26 (5.2)
	Unknown	2899 (89.9)	2772 (84.8)	2474 (86.4)	1297 (76.3_	1586 (76.3)	413 (83.3)
Diabetes	No	2898 (89.8)	202 (69.3)	2623 (91.6)	1610 (94.8)	2060 (99.1)	490 (98.8)
	Yes	177 (5.5)	149 (5.1)	92 (3.2)	43 (2.5)	5 (0.2)	4 (0.8)
	Unknown	152 (4.7)	747 (25.6)	148 (5.2)	46 (2.7)	13 (0.6)	2 (0.4)
Anaemia	No	2180 (67.6)	1899 (65.1)	1622 (56.6)	773 (45.5)	183 (8.8)	187 (37.7)
	Yes	755 (24.0)	747 (25.6)	910 (31.8)	296 (17.4)	101 (4.9)	204 (41.1)
	Unknown	271 (8.4)	270 (9.3)	333 (11.6)	630 (37.1)	1794 (86.3)	105 (21.2)
Smoking	Never	698 (21.6)	755 (25.9)	567 (19.8)	417 (24.5)	387 (18.6)	153 (30.9)
	Current	1469 (45.5)	1196 (41.0)	11554 (40.3)	868 (51.1)	1120 (53.9)	163 (32.9)
	Previous	625 (19.4)	648 (21.8)	624 (21.8)	291 (17.1)	377 (18.1)	143 (28.8)
	Unknown	434 (13.5)	317 (10.9)	520 (18.2)	123 (7.2)	194 (9.3)	37 (7.5)
Age (median, IQR)		40 (35-45)	42 (37-49)	43 (37-50)	35 (30-42)	30 (25-36)	37 (31-43)
CD4 count (median, IQR)		450 (292-651)	432 (286-613)	420 (279-597)	373 242-543)	408 (261-575)	342 (201-510)
Missing CD4 count (N, %)		17 (0.5)	11 (0.4)	15 (0.5)	6 (0.4)	110 (5.3)	5 (1.0)
Baseline date (median, IQR)		1/02 (1/02-12/03)	1/02 (1/02-12/05)	1/02 (1/02-11/03)	1/04 (1/02-1/08)	2/06 (4/04-6/08)	2/06 (11/03-6/06)

IQR: Interquartile range

Table 7.5 AIDS diagnosis prior to baseline by region

	South Europe	West Central Europe	North Europe	East Central Europe	East Europe	Argentina
Prior AIDS diagnosis (N,% of total)	827 (25.6)	951 (32.6)	907 (31.7)	420 (24.7)	401 (19.3)	160 (32.3)
Oesophageal candidiasis (N,%)	76 (9.2)	188 (19.8)	153 (16.9)	84 (20.0)	98 (24.4)	9 (5.6)
Pneumocystis jiroveci pneumonia (PCP) (N,%)	148 (17.9)	131 (13.8)	190 (20.9)	63 (15.0)	33 (8.2)	36 (22.5)
Pulmonary Mycobacterium Tuberculosis (TB) (N,%)	130 (15.7)	54 (5.7)	53 (5.8)	54 (12.9)	72 (18.0)	20 (12.5)
Kaposi's' Sarcoma (N,%)	102 (12.3)	95 (10.0)	124 (13.7)	17 (4.0)	8 (2.0)	14 (8.8)
HIV wasting syndrome (N,%)	38 (4.6)	49 (5.2)	24 (2.6)	54 (12.9)	56 (14.0)	9 (5.6)

Table 7.6 Characteristics of patients who had started cART prior to baseline by region

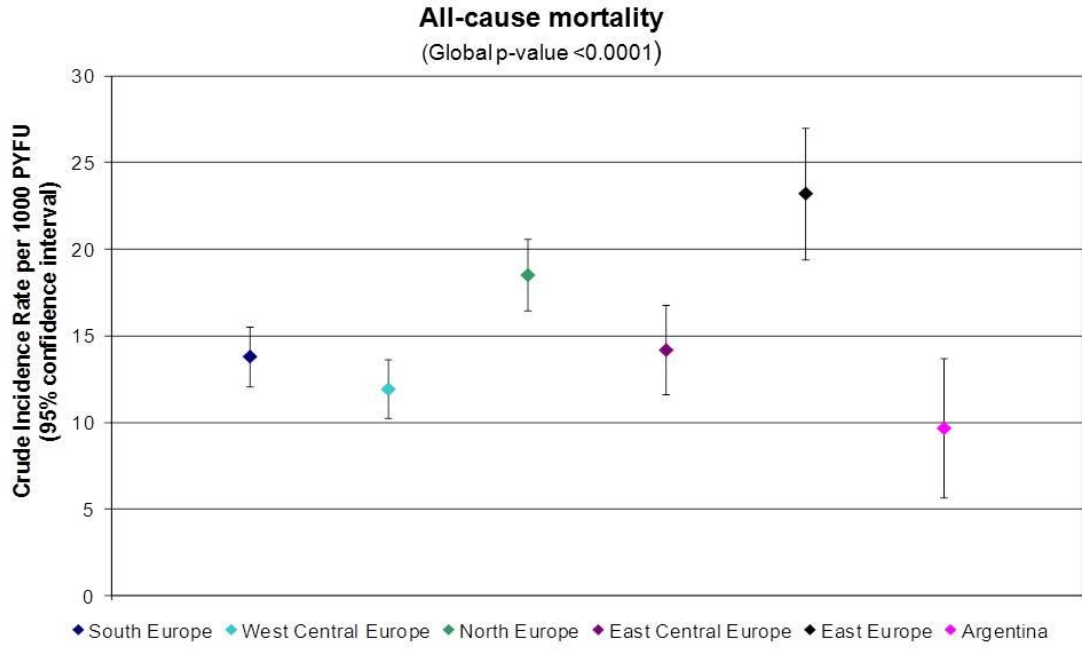
	South Europe	West Central Europe	North Europe	East Central Europe	East Europe	Argentina
N started cART (N,%)	2776 (86.1)	2577 (88.4)	2523 (88.1)	1336 (78.6)	654 (31.5)	397 (80.0)
CD4 count at starting cART (median, IQR)	250 (130-420)	218 (98-364)	201 (92-320)	198 (80-327)	191 (106-319)	169 (65-282)
Missing CD4 count (N, %)	0	0	0	0	2 (0.3)	1 (0.3)
Viral load at starting cART (median, IQR)	3.87 (2.60-4.80)	4.03 (2.66-4.97)	4.28 (2.70-5.11)	4.44 (2.70-5.22)	4.21 (2.60-5.26)	4.47 (1.99-5.23)
Missing Viral load (N,%)	0	0	0	11 (0.8)	46 (7.0)	6 (1.5)
Initial cART regimen started (N, %)						
NNRTI based cART	488 (17.6)	501 (19.4)	524 (20.8)	398 (29.8)	355 (54.3)	199 (50.1)
PI based cART	1592 (57.4)	1404(54.5)	1398 (55.4)	734 (54.9)	258 (39.5)	157 (39.6)
Other	696 (25.1)	672 (26.1)	601 (23.8)	204 (15.3)	41 (6.3)	41 (10.3)
Years on cART prior to baseline (median, IQR)	4.28 (2.66-4.96)	4.91 (3.47-5.75)	4.63 (2.97-5.30)	2.00 (0.77-3.63)	1.17 (0.49-2.32)	2.32 (0.88-4.43)

IQR:Interquartile range, NNRTI: Non-nucleoside reverse transcript inhibitor, PI: Protease Inhibitor

7.4.2 All-cause mortality

From 2002 to 2010, individuals were followed for a total of 67,905 PYFU. During this time 1,038 patients died; the crude mortality rate was 15·3 per 1,000 PYFU (95% confidence interval [CI] 14·4-16·2). Significant differences in mortality rates were seen across the 6 different regions (global p<0·0001). Figure 7.9 shows that higher all-cause mortality rates were observed in North (IR 18.5 per 1,000 PYFU, 95% CI 16.4-20.6) and East (IR 23.23 per 1,000 PYFU, 95% CI 19.4-27.0) Europe compared to the other regions.

Figure 7.9: Crude all-cause mortality rate by region



	South	West Central	North	East Central	East	Argentina
N deaths	246	195	314	118	143	22
Person years follow-up (PYFU)	17828	16348	16967	8317	6168	2276
Crude IR per 1000 PYFU	13.8	11.9	18.5	14.2	23.2	3.7

Compared to South Europe, before adjustment, individuals in North Europe had a 1.35 times higher rate of all-cause mortality and individuals in East Europe had a 1.59 times higher rate (table 7.7). There was no significant difference in the risk of all-cause mortality between South Europe and West Central, East Central or Argentina. After adjustment for fixed variables (gender and HIV exposure group), and baseline variables (age, CD4 count, treatment, hepatitis B and C status, hypertension, diabetes, anaemia, smoking status and prior AIDS diagnosis) there remained a significantly increased risk of all cause-mortality in North and East Europe compared to South Europe.

Table 7.7 All-cause mortality rate by region: results from univariable and multivariable Poisson regression analysis

Region	Univariable			Multivariable[a] (baseline)			Multivariable[b] (time -updated)		
	IRR	95% CI	p-value	IRR	95% CI	p-value	IRR	95% CI	p-value
South	1.00			1.00			1.00		
West Central	0.86	0.72-1.04	0.12	0.97	0.80-1.19	0.80	0.93	0.76-1.14	0.48
North	1.35	1.14-1.60	0.0004	1.48	1.24-1.78	<.0001	1.40	1.18-1.68	0.0001
East Central	1.02	0.82-1.27	0.87	1.39	1.10-1.75	0.005	1.35	1.09-1.69	0.007
East	1.59	1.29-1.95	<.0001	2.07	1.53-2.80	<.0001	1.76	1.39-2.22	<.0001
Argentina	0.69	0.45-1.07	0.095	0.94	0.60-1.48	0.79	0.75	0.48-1.18	0.21

[a]adjusted for baseline variables gender, age, HIV exposure group, hepatitis B and C status, prior AIDS diagnosis, hypertension, diabetes, anaemia, smoking status, CD4 count, baseline date and on cART viral load

[b]adjusted as in a but age, hepatitis B and C status, hypertension, diabetes, anaemia, smoking status, CD4 count, year of follow-up and on cART viral load were included as time-updated covariates

Additionally, after adjustment for these baseline variables a higher rate of mortality was also observed in East Central Europe compared to South. The higher risk of all-cause mortality observed in North, East Central and East Europe compared to South Europe remained after adjustment for time-updated variables rather than those measured at baseline (table 7.6).

Other factors found to be significantly associated with the risk of all-cause mortality are shown in table 7.8. Having been infected with HIV via IDU was associated with a high risk of mortality compared to homosexual transmission. Older age, being hepatitis B or C positive, diabetic, anaemic, a current smoker compared to having never smoked, or having a prior AIDS diagnosis were also associated with an increased all-cause mortality rate. Calendar year of follow-up was associated with a lower risk of all-cause mortality

A lower CD4 count was associated with an increased risk of all-cause mortality. Individuals with a current CD4 count < 200 copies/ml had over a 7 times higher risk of mortality compared to those with a CD4 count $\geq$ 500 copies/ml. Individuals not on cART had an almost three times higher risk of mortality compared to those on cART with a suppressed viral load. Those on cART with uncontrolled viral replication had a borderline significantly increased risk of mortality; although it was lower than those not on cART.

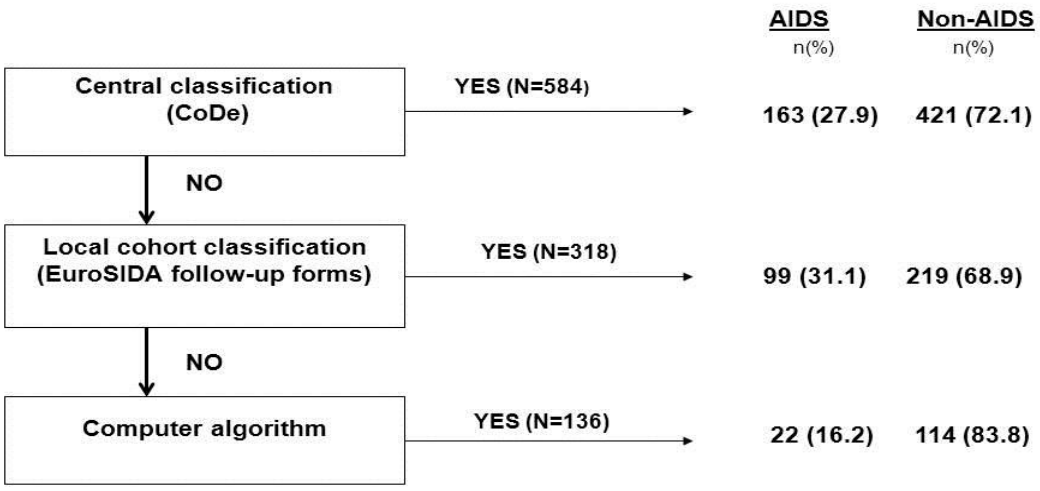
Table 7.8 Factors associated with risk of all-cause mortality – results from Poisson regression analysis

		Univariable			Multivariable* (baseline)			Multivariable* (time -updated)		
		IRR	95% CI	Global p-value	IRR	95% CI	p-value	IRR	95% CI	p-value
Gender (male vs. female)		0.68	0.58-0.79	<.0001	0.85	0.72-1.01	0.07	0.82	0.69-0.98	0.02
Race (white vs. other)		0.76	0.62-0.94	0.01						
Age	10 year older	1.42	1.35-1.50	<.0001	1.63	1.53-1.75	<.0001	1.64	1.54-1.75	<.0001
HIV exposure group	Homosexual	1.00		<.0001	1.00			1.00		
	IDU	2.03	1.76-2.35		2.06	1.65-2.56	<.0001	1.78	1.42-2.24	<.0001
	Heterosexual	0.87	0.74-1.03		1.07	0.89-1.30	0.46	1.04	0.86-1.25	0.69
	Other	1.27	0.99-1.62		1.19	0.92-1.53	0.18	1.10	0.86-1.42	0.44
CD4 count	≥500	1.00		<.0001						
	350-499	1.34	1.10-1.63		1.14	0.94-1.40	0.19	1.32	1.03-1.71	0.03
	200-349	1.95	1.62-2.34		1.36	1.13-1.64	0.001	2.25	1.78-2.83	<.0001
	<200	4.71	3.98-5.58		2.65	2.21-3.19	<.0001	7.38	5.97-9.14	<.0001
	Missing	22.46	16.4-30.6		8.66	5.97-12.56	<.0001	3.82	3.00-4.86	<.0001
Treatment	On cART VL ≤500	1.00		<.00001	1.00			1.00		
	Not on cART	1.85	1.60-2.14		1.96	1.67-2.30	<.0001	2.82	2.40-3.31	<.0001
	On cART VL >500	2.43	2.09-2.83		1.98	16.9-2.32	<.0001	1.21	0.99-1.49	0.06
	On cart missing VL	13.24	8.97-19.55		5.13	3.24-8.12	<.0001	1.40	1.12-1.75	0.002
Hepatitis B (negative vs. positive)		1.55	1.25-1.92	0.0008	1.43	1.15-1.77	0.001	1.43	1.18-1.73	0.0003
Hepatitis C (negative vs. positive)		2.04	1.78-2.35	<.0001	1.38	1.13-1.69	0.001	1.36	1.10-1.68	0.005
Hypertensive (no vs. yes)		1.77	1.16-2.71	0.01	0.89	0.55-1.44	0.62	0.76	0.58-1.01	0.05
Diabetic (no vs. yes)		2.18	1.74-2.73	<.0001	1.86	1.47-2.37	<.0001	1.44	1.16-1.79	0.001
Anaemic (no vs. yes)		2.37	2.07-2.71	<.0001	1.64	1.42-1.89	<.0001	3.57	2.94-4.33	<.0001
Smoking status	Never	1.00		<.0001	1.00			1.00		
	Current	1.64	1.37-1.96		1.27	1.05-1.53	0.01	1.30	1.07-1.57	0.007
	Previous	1.27	1.03-1.56		1.11	0.90-1.37	0.31	1.13	0.92-1.38	0.25
Prior AIDS diagnosis (no vs. yes)		1.96	1.74-2.22	<.0001	1.54	1.36-1.76	<.0001	1.53	1.34-1.74	<.0001
Calendar year		0.89	0.86-0.93	<.0001	0.83	0.78-0.83	<.0001	0.88	0.85-0.91	<.0001

*Also adjusted for region, IDU: Injection drug user

Figure 7.10 shows how many causes of death were determined to be AIDS or non-AIDS related at each stage in the hierarchical process for coding causes of death. The majority of the deaths (56%) were coded using CoDe. The computer algorithm was used to code the causes of death in 13.1% of the cases.

Figure 7.10 The 3-step algorithm for assigning death as AIDS or non-AIDS related in the EuroSIDA study[736]



The proportions of deaths classified at each stage are shown in table 7.9, stratified by region. A marginally higher proportion of deaths in East Europe, East and Argentina were classified using CoDe. This is probably due to more of the deaths in these regions being in the more recent time period when CoDe was available (as shown in table 7.9).

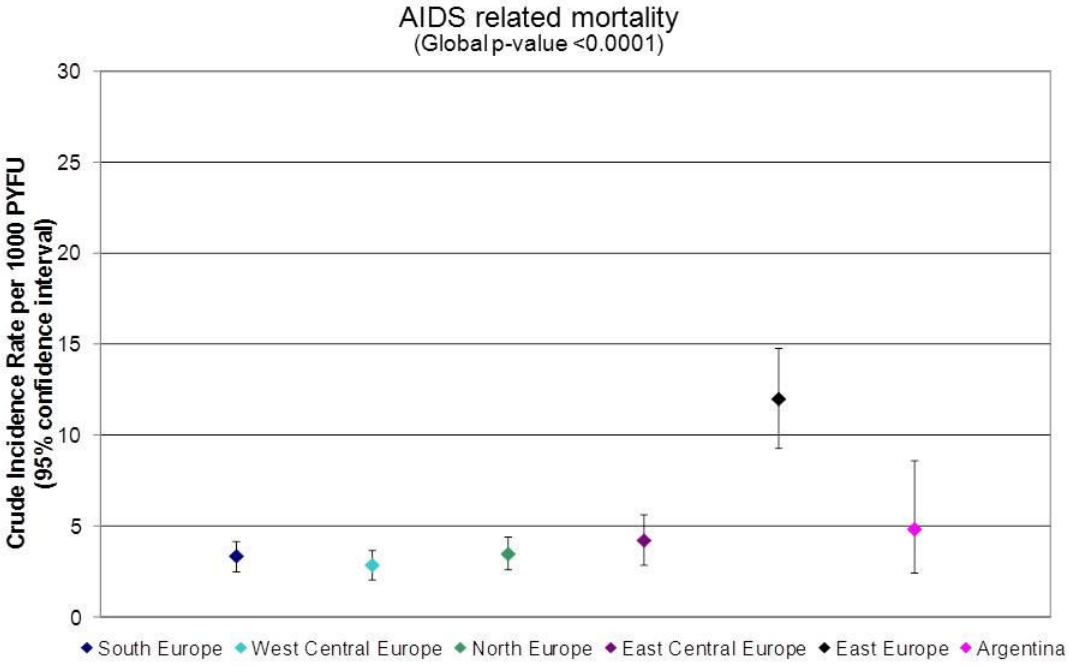
Table 7.9 Number of death classified at each stage by region

	Region (N, %)					
Classification stage	**South**	**West Central**	**North**	**East Central**	**East**	**Argentina**
Total	246	195	314	118	143	22
CoDe	136 (55.3)	96 (49.2)	169 (53.8)	80 (67.8)	84 (58.7)	19 (86.4)
EuroSIDA forms	76 (30.9)	63 (32.3)	100 (31.9)	28 (34.3)	49 (34.3)	2 (9.0)
Computer algorithm	34 (13.8)	36 (18.5)	45 (14.3)	10 (8.5)	10 (7.0)	1 (4.6)
Median date of death	7/05	1/05	3/05	10/06	7/07	3/06

7.4.3 AIDS related mortality

Of the 1,038 patients who died, AIDS was attributed as the cause of death in 284 (27%) patients. The crude rate of death due to AIDS related causes from 2002-2010 was 4·2 AIDS related deaths per 1,000 PYFU (95% CI 3·7-4·7). Figure 7.11 shows the crude mortality rate for AIDS related causes by region. East Europe had the highest rate of AIDS related mortality (IR 12.0 per 1,000 PYFU, 95% CI 9.3-14.7). The crude mortality rate due to AIDS related causes in East Europe was over three times higher than in South Europe (incidence rate ratio [IRR] 3.63, 95%CI 2.57-5.10, p<·0001). A similar mortality rate was observed in the other five regions, ranging from 2.8 per 1000 PYFU (95%CI 2.0-3.6) in West Central Europe to 4.8 per 1,000 PYFU (95%CI 2.0-3.6) in Argentina.

Figure 7.11 Crude incidence rate of AIDS related mortality by region



	South	West Central	North	East Central	East	Argentina
N deaths	59	46	59	35	74	11
Person years follow-up (PYFU)	17828	16348	16967	8317	6168	2276
Crude IR per 1000 PYFU	3.3	2.8	3.5	4.2	12.0	4.8

Table 7.10 Patient characteristics at the time of AIDS related death

	South	West Central	North	East Central	East	Argentina	p-value
Total (N,% of total)	59 (20.8)	46 (16.2)	59 (20.8)	35 (12.3)	74 (26.1)	11 (3.9)	
Age (median, IQR)	45 (41-50)	48 (39-58)	48 (42-57)	40 (34-53)	35 (30-39)	36 (34-47)	<.0001
CD4 count within 6 months (median, IQR)	43 (18-150)	73 (17-240)	66 (10-153)	91 (28-290)	117 (32-231)	76 (44-136)	0.20
Missing CD4 (N,%)	16 (27.1)	8 (17.4)	16 (27.1)	4 (11.4)	30 (40.5)	5(45.5)	
Date of death (median, IQR)	4/05 (10/05-1/07)	2/05 (1/04-11/06)	8/04 6/03-4/06)	7/06 (12/03-11/08)	4/07 (8/04-1/09)	5/07 (2/05-10/07)	<.0001
Started cART (N,%)	55 (93.2)	43 (93.5)	58 (98.3)	32 (91.4)	37 (50.0)	9 (81.8)	<.0001
Most recent AIDS diagnosis (N, %)							
Non-Hodgkin's lymphoma	17 (28.8)	9 (19.6)	14 (19.6)	7 (20.0)	1 (1.4)	1 (9.1)	<.0001
Oesophageal candidiasis	3 (5.1)	4 (8.7)	5 (8.5)	0 (0)	16 (21.6)	1 (9.1)	
HIV wasting syndrome	2 (3.4)	5 (10.9)	6 (10.2)	2 (5.7)	8 (10.8)	1 (9.1)	
Years since last AIDS diagnosis (median, IQR)	0.65 (0.10-3.17)	0.59 (0.16-1.71)	0.64 (0.15-2.42)	0.76 (0.35-1.76)	0.22 (0.07-0.87)	0.08 (0.01-1.22)	0.007
Patients reporting ≥1 non-AIDS event	14 (23.7)	7 (15.2)	7 (11.9)	6 (17.1%)	14 (18.9%)	1 (9.1%)	0.58
Non-AIDS defining malignancy	8 (13.6)	2 (4.3)	1 (1.7)	1 (2.9)	1 (1.4)	1 (9.1)	
Cardiovascular disease	4 (6.8)	3 (6.5)	4 (6.8)	3 (8.6)	1 (1.4)	0 (0)	
Liver disease	1 (1.7)	1 (2.2)	1 (1.7)	0 (0)	5 (6.8)	0 (0)	
End stage renal disease	0 (0)	1 (2.2)	1 (1.7)	0 (0)	2 (2.7)	0 (0)	
Pancreatitis	1 (1.7)	1 (2.2)	0 (0)	2 (5.7)	6 (8.1)	0 (0)	

Table 7.11 AIDS related mortality rate by region: results from univariable and multivariable Poisson regression analysis

Region	Univariable			Multivariable[a] (baseline)			Multivariable[b] (time-updated)		
	IRR	95% CI	p-value	IRR	95% CI	p-value	IRR	95% CI	p-value
South	1.00	-	-	1.00	-	-	1.00	-	-
West Central	0.85	0.58-1.25	0.41	0.90	0.61-1.34	0.60	0.82	0.56-1.20	0.29
North	1.06	0.74-1.52	0.76	1.13	0.77-1.66	0.52	1.04	0.72-1.48	0.84
East Central	1.26	0.83-1.91	0.27	1.42	0.92-2.21	0.11	1.54	1.01-2.36	0.04
East	3.43	2.44-2.44	<.0001	2.44	1.46-4.08	0.0006	3.26	2.19-4.84	<.0001
Argentina	1.44	0.76-2.74	0.26	1.00	0.51-1.97	0.99	1.01	0.52-1.95	0.98

[a]adjusted for baseline variables gender, age, HIV exposure group, hepatitis B and C status, AIDS diagnosis prior to baseline, hypertension, anaemia, CD4 count, baseline date and on cART viral load. [b]adjusted as in c but age, hepatitis B and C status, hypertension, anaemia, CD4 count, year of follow-up and on cART viral load were included as time-updated covariates

Patients dying from AIDS related causes in East Europe, and Argentina were younger than those in other regions (table 7.10). A high proportion of the most recent AIDS diagnosis prior to baseline was Non-Hodgkin's lymphoma in South, West Central, North and East Central Europe. In East Europe the most common recent AIDS related diagnosis prior to death was oesophageal candidiasis.

Table 7.11 gives the results of the Poisson regression analysis and presents the incidence rate ratios for AIDS related mortality. Adjusting for gender, age, HIV exposure group, AIDS diagnosis prior to baseline, hypertension, anaemia, baseline CD4 count, baseline date and baseline treatment accounted for some of the higher rate of AIDS-related mortality observed in East Europe. However, the rate of AIDS-related mortality was still more than double the rate in South Europe (IRR 2·40, 95%CI 1·44-4·02, p=0·0008). The increased incidence of AIDS related mortality in East Europe remained after modelling age, hypertension, anaemia, CD4 count, year of follow-up and treatment as time updated covariates.

Table 7.12 shows the other factors that were found to be associated with risk of AIDS related mortality. As with all-cause mortality older age, anaemia, and a prior AIDS diagnoses were associated with an increased risk of death from AIDS related causes. Similarly, increasing calendar year of follow-up was associated with a decreased risk of AIDS related mortality

A lower CD4 count was associated with an increased risk of AIDS related mortality. Not being on cART was associated with a 6.3 times higher rate (95% CI 4.38-8.97), and being on cART with a uncontrolled viral replication was associated with a 1.75 time higher rate (95% CI 1.16-2.64, p=0.0007) of AIDS related mortality compared to being on cART with a suppressed viral load.

Table 7.12 Poisson regression analysis investigative factors associated with AIDS related mortality

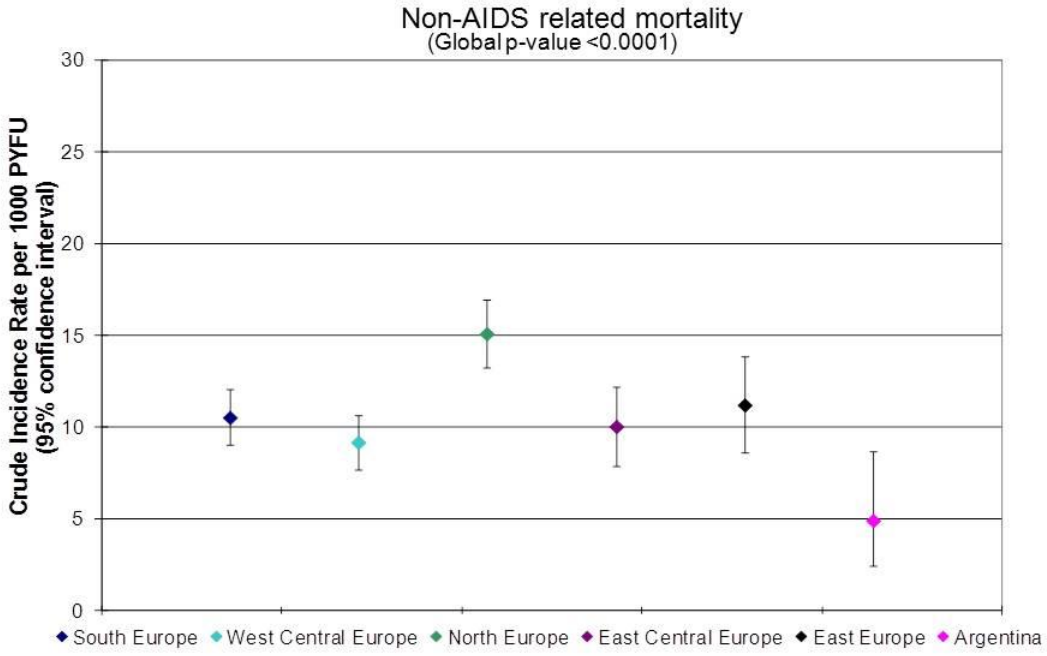
		Univariable			Multivariable* (baseline)			Multivariable* (time -updated)		
		IRR	95% CI	Global p-value	IRR	95% CI	p-value	IRR	95% CI	p-value
Gender (male vs. female)		0.75	0.56-1.00	0.04	0.72	0.52-1.00	0.05	0.70	0.51-0.97	0.03
Race (white vs. other)		0.80	0.54-1.17	0.25						
Age	10 year older	1.06	0.95-1.19	0.30	1.33	1.17-1.50	<.0001	1.28	1.12-1.46	0.0003
HIV exposure group	Homosexual	1.00		0.0003	1.00			1.00		
	IDU	1.94	1.45-2.60		1.55	1.02-.35	0.03	1.26	0.92-1.73	0.14
	Heterosexual	1.35	1.01-1.82		1.39	0.98-1.96	0.06	1.37	0.98-1.91	0.06
	Other	1.17	0.71-1.95		1.05	0.63-1.77	0.84	1.05	0.63-1.76	0.84
CD4 count	≥500	1.00		<.0001	1.00					
	350-499	1.73	1.08-2.79		1.45	0.90-2.34	0.12	1.72	0.79-3.72	0.17
	200-349	2.64	1.70-4.09		1.79	1.15-2.79	0.01	3.33	1.67-6.66	0.0007
	<200	12.99	8.90-18.95		5.90	3.95-8.80	<.0001	27.0	14.80-50.02	<.0001
	missing	54.0	30.40-95.89		11.96	6.34-22.56	<.0001	8.01	4.27-15.04	<.0001
Treatment	on cART VL ≤500	1.00		<.0001	1.00					
	Not on cART	5.78	4.15-8.05		5.06	3.54-7.25	<.0001	6.27	4.38-8.97	<.0001
	on cART VL >500	7.51	5.35-10.55		4.35	3.06-6.19	<.0001	1.75	1.16-2.64	0.007
	on cart missing VL	36.66	18.01-74.62		9.60	4.44-20.78	<.0001	2.08	1.35-3.20	0.0009
Hepatitis B (negative vs. positive)		1.66	1.11-2.50	0.02	1.63	1.08-2.47	0.02	1.64	1.14-2.34	0.007
Hepatitis C (negative vs. positive)		1.48	1.12-1.95	0.02	1.21	0.82-1.78	0.33	0.92	0.64-1.32	0.66
Hypertensive (no vs. yes)		0.21	0.08-0.52	0.001	0.23	0.08-0.67	0.007	0.71	0.41-1.26	0.24
Diabetic (no vs. yes)		0.81	0.42-1.58	0.24						
Anaemic (no vs. yes)		3.12	2.35-4.15	<.0001	1.80	1.34-2.42	<.0001	6.11	3.71-10.05	<.0001
Smoking status	Never	1.00		0.24						
	Current	1.13	0.82-1.56							
	Previous	1.27	0.89-1.81							
Prior AIDS diagnosis (no vs. yes)		2.85	2.36-3.60	<.0001	2.23	1.73-2.88	<.0001	2.11	1.62-2.74	<.0001
Calendar year		1.03	0.97-1.09	0.36	0.87	0.79-0.95	0.003	0.89	0.84-0.94	<.0001

*also adjusted for region

7.4.4 Non-AIDS related mortality

The majority (754, 73%) of the observed deaths during follow-up were attributed to non-AIDS related causes, the crude mortality rate was 11·1 non-AIDS related deaths per 1,000 PYFU (95% CI 10·3-11·9). From figure 7.12, the region with the highest rate of non-AIDS related mortality was in North Europe (IR 15.0 per 1,000 PYFU, 95% 13.2-16.8). Compared to South Europe, the rate of non-AIDS related mortality in North Europe was 1.4 times higher (IRR 1·44, 95% CI 1·19-1·73, p=0·0001). Argentina had the lowest crude rate on non-AIDS related mortality (IR 4.8 per 1,000 PYFU, 95% CI 2.4-8.6) which was significantly lower than South Europe (IRR 0.45, 95%CI 0.25-0.83, p=0.01)

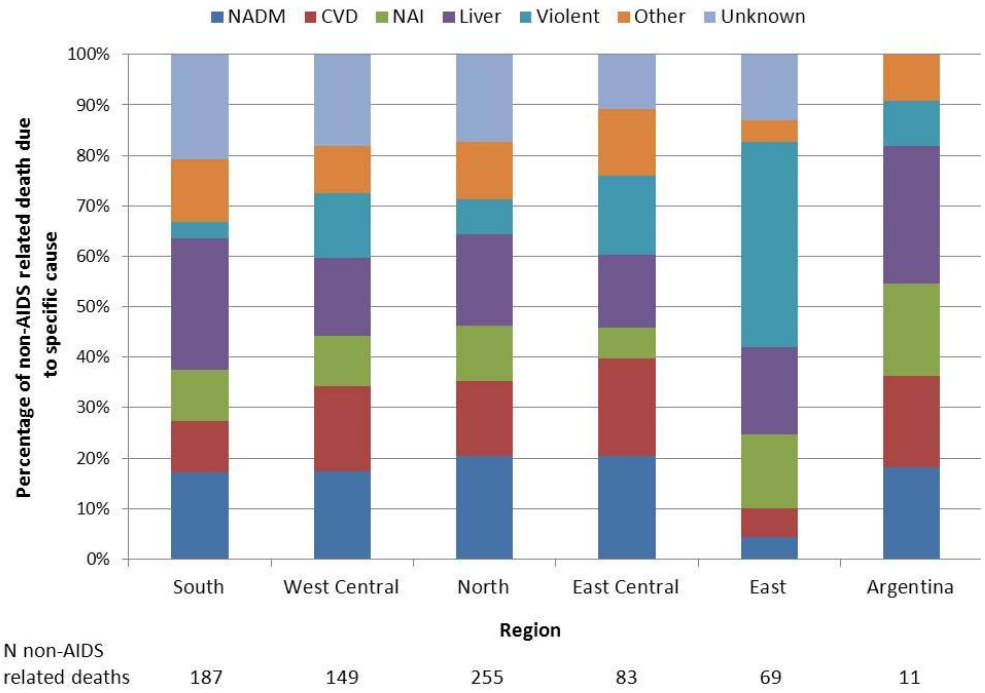
Figure 7.12 Crude rate of death due to non-AIDS related causes by region



	South	West Central	North	East Central	East	Argentina
N deaths	187	149	255	83	69	11
Person years follow-up (PYFU)	17828	16348	16967	8317	6168	2276
Crude IR per 1000 PYFU	10.5	9.1	15.0	10.0	11.2	4.8

Figure 7.13 shows the percentage of non-AIDS related deaths attributed to each specific cause by region. In South, West Central and North Europe the three most common causes of non-AIDS related death were due to NADM, CVD and liver related causes. A similar pattern was also seen in East Central Europe. In East Europe deaths due to violent causes were the most common cause of non-AIDS related death, they accounted for 40.1% of the non-AIDS related deaths in this region, death due to liver related causes was the next most common accounting for 17.4%.

Figure 7.13 Proportion of specific non-AIDS related causes of death by region



NADM: non-AIDS defining malignancies excluding liver cancer, CVD: cardiovascular disease, NAI: non-AIDS related infection, Liver: liver related including deaths due to hepatitis B or C, liver failure or cirrhosis and liver cancer, violent: deaths due to unnatural causes including an accident or violence, suicide, euthanasia, substance abuse or overdose), other: causes associated with < 20 deaths, unknown: deaths with insufficient information to determine cause of death.

Table 7.13 shows the patient characteristics at the time of non-AIDS related death. Individuals in East Europe dying from non-AIDS related causes were younger than those dying in the 5 other regions. There was also a higher percentage with no CD4 count measured in the 6 months prior to death in East Europe. Further, a lower percentage of prior non-AIDS events had been reported for patients in East Europe how died from a non-AIDS related cause.

Table 7.13 Patient characteristics at non-AIDS related death

	Region						
	South	West Central	North	East Central	East	Argentina	p-value
Total (N,% of total)	187	149	255	83	69	11	
Age (median, IQR)	47 (42-59)	48 (42-59)	50 (43-59)	44 (37-54)	34 (30-41)	41 (32-48)	<.0001
CD4 count within 6 months (median, IQR)	232 (113-408)	275 (178-479)	315 (162-476)	260 (141-420)	261 (130-355)	130 (19-415)	0.10
Missing CD4 (N,%)	32 (17.1)	28 (18.8)	54 (21.2)	16 (19.3)	40 (58.0)	5 (45.5)	
Year of death (median, IQR)	7/05 (9/03-5/07)	12/04 8/03-1/07)	5/05 (9/03-4/07)	10/06 (10/04-7/08)	6/07 (8/05-12/08)	8/05 (11/03-7/07)	<.0001
Started cART (N,%)	179 (95.7)	142 (95.3)	244 (95.7)	72 (86.8)	27 (39.1)	11 (100)	
Prior AIDS diagnosis (N,%)	87 (46.5%)	77 (51.7%)	109 (42.8%)	38 (45.8%)	29 (42.0%)	5 (45.5%)	0.62
Years since last AIDS diagnosis (median, IQR)	5.67 (2.09-9.17)	6.92 (2.24-10.56)	7.70 (8.00-10.16)	5.42 (3.12-8.27)	1.06 (0.29-2.53)	2.05 (2.00-2.22)	<.0001
Patients reporting ≥1 non-AIDS event	83 (44.4)	51 (34.2)	88 (34.5)	34 (40.1)	14 (20.0)	5(45.5)	0.01
Non-AIDS defining malignancy	33 (17.6)	19 (12.8)	37 (14.5)	14 (16.9)	2 (2.9)	2 (18.2)	
Cardiovascular disease	25 (13.3)	24 (16.1)	42 (16.5)	14 (16.9)	1 (1.4)	1 (9.1)	
Liver disease	22 (11.8)	8 (5.4)	10 (3.9)	9 (10.8)	9 (13.0)	2 (18.2)	
End stage renal disease	9 (4.8)	5 (3.3)	7 (2.7)	1 (1.2)	4 (5.7)	0 (0)	
Pancreatitis	8 (4.2)	5 (3.3)	3 (1.2)	0 (0)	1 (1.4)	0 (0)	

Table 7.14 Poisson regression analysis incidence rate ratio of non-AIDS related mortality by regions

	Univariable			Multivariable[a] (baseline)			Multivariable[b] (time updated)		
	IRR	95% CI	p-value	IRR	95% CI	p-value	IRR	95% CI	p-value
South	1.00			1.00			1.00		
West Central	0.87	0.70-1.08	0.20	0.98	0.78-1.23	0.83	0.89	0.71-1.13	0.34
North	1.44	1.19-1.74	0.0001	1.59	1.30-1.96	<.0001	1.43	1.17-1.73	0.0004
East Central	0.94	0.73-1.22	0.65	1.37	1.04-1.80	0.02	1.22	0.94-1.58	0.12
East	1.01	0.77-1.33	0.95	1.71	1.16-2.52	0.007	1.24	0.91-1.68	0.16
Argentina	0.45	0.25-0.83	0.01	0.81	0.43-1.51	0.50	0.55	0.29-1.04	0.06

[a]also adjusted for variable in table 8: gender, age, HIV exposure group, hepatitis B and C status, prior AIDS diagnosis, hypertension, diabetes, anaemia, smoking status, CD4 count, baseline date and treatment

[b]adjusted as in a but age, hepatitis B and C status, hypertension, diabetes, anaemia, smoking status, CD4 count, treatment and calendar year of follow-up included as time-updated variables.

Table 7.14 shows that a higher incidence of non-AIDS related mortality was observed, after adjusting for gender, age, HIV exposure group, hepatitis B and C status, prior AIDS diagnosis, hypertension, diabetes, anaemia, smoking status, CD4 count, baseline date and treatment in North Europe (IRR 1·58, 95%CI 1·29-1·94, p<·0001), East Central Europe (IRR 1·36, 95%CI 1·03-1·78, p=0·02) and East Europe (IRR 1·75, 95%CI 1·18-2·60, p=0·05), compared to South Europe. No significant difference was observed between West Central Europe (p=0.83), Argentina (p=0.50) and South Europe after adjustment.

After including age, hepatitis B and C status, hypertension, diabetes, anaemia, smoking status, CD4 count, year of follow-up and on cART viral load in the model as time-updated covariates the increased rate was no longer significant in East Central Europe (IRR 1·22, 95% CI 0·94-1·58,p=0·12) or East Europe (IRR 1·.24, 95% CI 0·91-1·68, p=0·16) but there remained a significantly increased rate of mortality in North Europe (IRR 1·43, 95% CI 1·17-1·73, p<·0001).

Table 7.15 shows the other factors that were found to be associated with non-AIDS related mortality. Consistent with all-cause mortality, older age, hepatitis B and C status, diabetes, being a current smoker compared to having never smoked, prior AIDS diagnosis and calendar year of follow-up were all associated with an increased risk of non-AIDS related mortality. Additionally, a current CD4 count < 500 cells/mm^3 was associated with an increased risk of non-AIDS related mortality. Not being on cART was associated with a 2.3 times higher rate of non-AIDS related mortality compared to being on cART with a suppressed viral load. Interestingly, there was no significant difference observed in non-AIDS related mortality rate in individuals on cART with uncontrolled viral replication and those on cART with a suppressed viral load (p=0.43).

Table 7.15 Poisson regression analysis investigative factors associated with non-AIDS related mortality

		Univariable			Multivariable[a] (baseline)			Multivariable[b] (time -updated)		
		IRR	95% CI	Global p-value	IRR	95% CI	p-value	IRR	95% CI	p-value
Gender (male vs. female)		0.65	0.54-0.78	<.0001	0.89	0.73-1.09	0.27	0.94	0.77-1.16	0.56
Race (white Vs. other)		0.75	0.59-0.96	0.02						
Age	10 year older	1.57	1.48-1.68	<.0001	1.79	1.65-1.93	<.0001	1.76	1.63-1.89	<.0001
HIV exposure group	Homosexual	1.00		<.0001	1.00			1.00		
	IDU	2.07	1.75-2.44		2.24	1.73-2.90	<.0001	1.95	1.48-2.57	<.0001
	Heterosexual	0.71	0.58-0.58		0.95	0.75-1.19	0.64	0.90	0.71-1.13	0.35
	Other	1.30	0.98-1.72		1.24	0.93-1.66	0.14	1.13	0.85-1.51	0.38
CD4 count	≥500	1.00		<.0001	1.08	0.86-1.34	0.50	1.00		
	350-499	1.83	1.50-2.24		1.27	1.03-1.56	0.02	2.19	1.71-2.80	<.0001
	200-349	3.22	2.63-3.93		1.91	1.54-2.38	<.0001	5.06	3.97-6.45	<.0001
	<200	16.77	11.46-24.56		8.23	5.08-13.33	<.0001	3.63	2.75-4.80	<.0001
	missing	1.27	1.02-1.58		1.08	0.86-1.34	0.50	1.76	1.63-1.89	<.0001
Treatment	on cART VL ≤500	1.00		<.0001	1.00			1.00		
	Not on cART	1.31	1.11-1.56		1.51	1.25-1.82	<.0001	2.31	1.91-2.79	<.0001
	on cART VL >500	1.73	1.45-2.08		1.60	1.32-1.93	<.0001	1.10	0.86-1.42	0.43
	on cart missing VL	10.04	6.25-16.11		4.39	2.43-7.94	<.0001	1.20	0.92-1.57	0.18
Hepatitis B (negative vs. positive)		1.51	1.17-1.64	0.005	1.38	1.07-1.78	0.01	1.36	1.08-1.72	0.009
Hepatitis C (negative vs. positive)		2.29	1.95-2.68	<.0001	1.63	1.29-2.07	<.0001	2.29	1.90-2.76	<.0001
Hypertensive (no vs. yes)		3.82	2.12-6.91	<.0001	1.34	0.69-2.59	0.39	0.76	0.55-1.05	0.10
Diabetic (no vs. yes)		2.75	2.15-3.50	<.0001	2.08	1.61-2.69	<.0001	1.66	1.31-2.11	<.0001
Anaemic (no vs. yes)		2.19	1.87-2.55	<.0001	1.60	1.36-1.87	<.0001	3.19	2.58-3.95	<.0001
Smoking status	Never	1.00		<.0001	1.00			1.00		
	Current	1.90	1.53-2.36		1.50	1.19-1.90	0.0006	1.65	1.31-2.07	<.0001
	Previous	1.27	0.98-1.63		1.05	0.81-1.37	0.68	1.11	0.87-1.43	0.40
Prior AIDS diagnosis (no vs. yes)		1.70	1.47-1.97	<.0001	1.36	1.16-1.58	<.0001	1.32	1.14-1.54	0.0003
Calendar year		0.83	0.78-0.87	<.0001	0.81	0.76-0.87	<.0001	0.88	0.85-0.91	<.0001

a and b also adjusted for region of Europe

7.4.5 Additional investigatory analysis

In order to try and further understand the reasons behind these differences observed in mortality rate, the interaction between age or calendar year of follow-up and region was tested. Further, to identify whether the differences in non-AIDS related mortality were due to a higher rate of mortality from a specific cause of death in North Europe, where there were sufficient events, the rates of specific causes of non-AIDS related death across the regions were investigated.

7.4.5.1 Age and region

From the baseline demographics, individuals in North Europe were on average older, median age 43 (IQR 37-50) and those in East Europe younger, median age 30 (IQR 25-36). For all three endpoints older age was associated with an increased risk of mortality. The test for interaction with age and region was non-significant for all three endpoints (all-cause p=0.11, AIDS related p=0.59, non-AIDS related p=0.19), indicating that the effect of age on mortality was not significantly different across the regions.

7.4.5.2 Calendar year of follow-up and region

For all three endpoints, the test for interaction between region and calendar year of follow-up was significant (p=0·04, 0·02 and 0·04 respectively), indicating that there is evidence of a difference in effect of calendar time across the regions. The crude incidence rates for all-cause, AIDS related and non-AIDS related mortality and how they vary with calendar year of follow-up are shown in figure 7.14, 7.15 and 7.16 respectively. In univariable analysis, there was a significantly decreased rate of all-cause mortality with increasing calendar year of follow-up in South (IRR 0.89 per calendar year, 95%CI 0.85-0.93, p<.0001), West Central (IRR 0.83 per calendar year, 95%CI 0.78-0.87, p<.0001), North (IRR 0.85 per calendar year, 95%CI 0.82-0.89, p<.0001), East Central (IRR 0.93 per calendar year, 95%CI 0.87-0.99, p=0.02), East (IRR 0.85 per calendar year, 95%CI 0.79-0.92, p<.0001) and Argentina (IRR 0.76 per calendar year, 95% CI 0.66-0.87, p=0.0001). Similar trends were seen in both AIDS and non-AIDS related mortality across the regions.

Figure 7.14 Crude incidence rate of all-cause mortality by region and year of follow-up

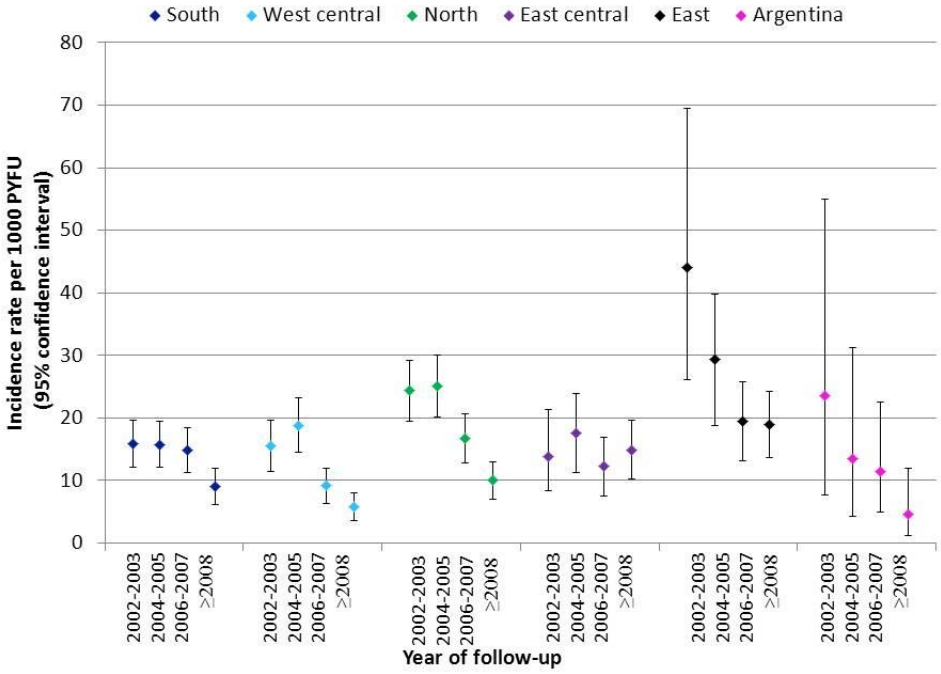


Figure 7.15 Crude incidence rate of AIDS related mortality by year of follow-up

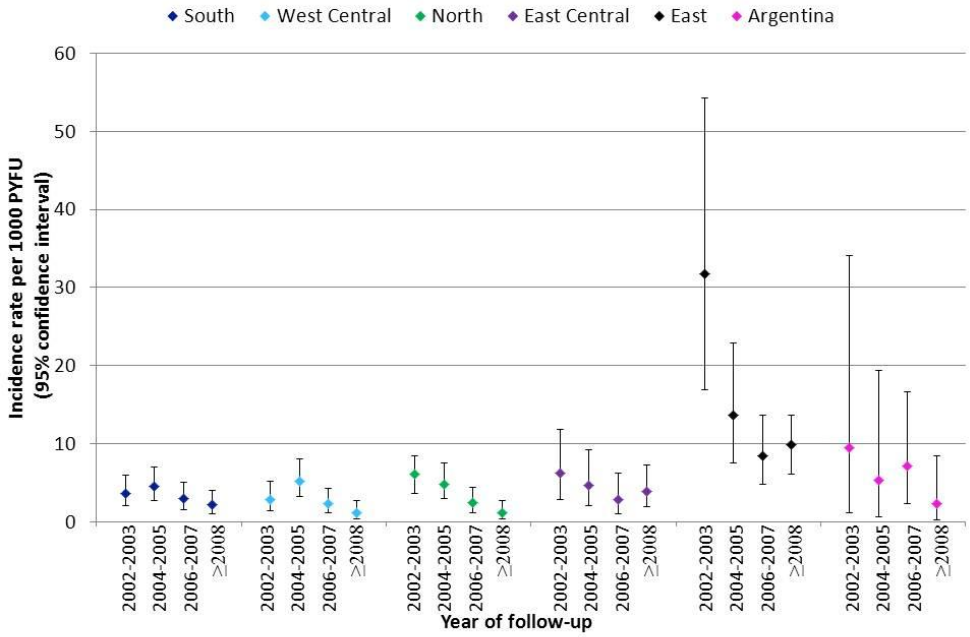


Figure 7.16 Crude incidence rate of non-AIDS related mortality by region and year of follow-up

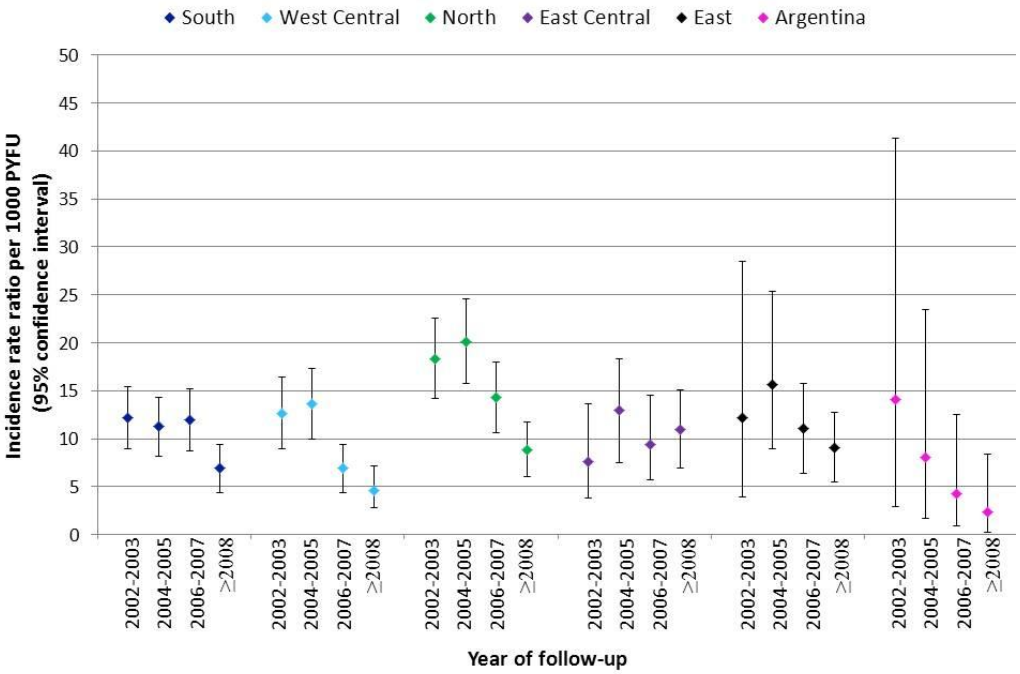
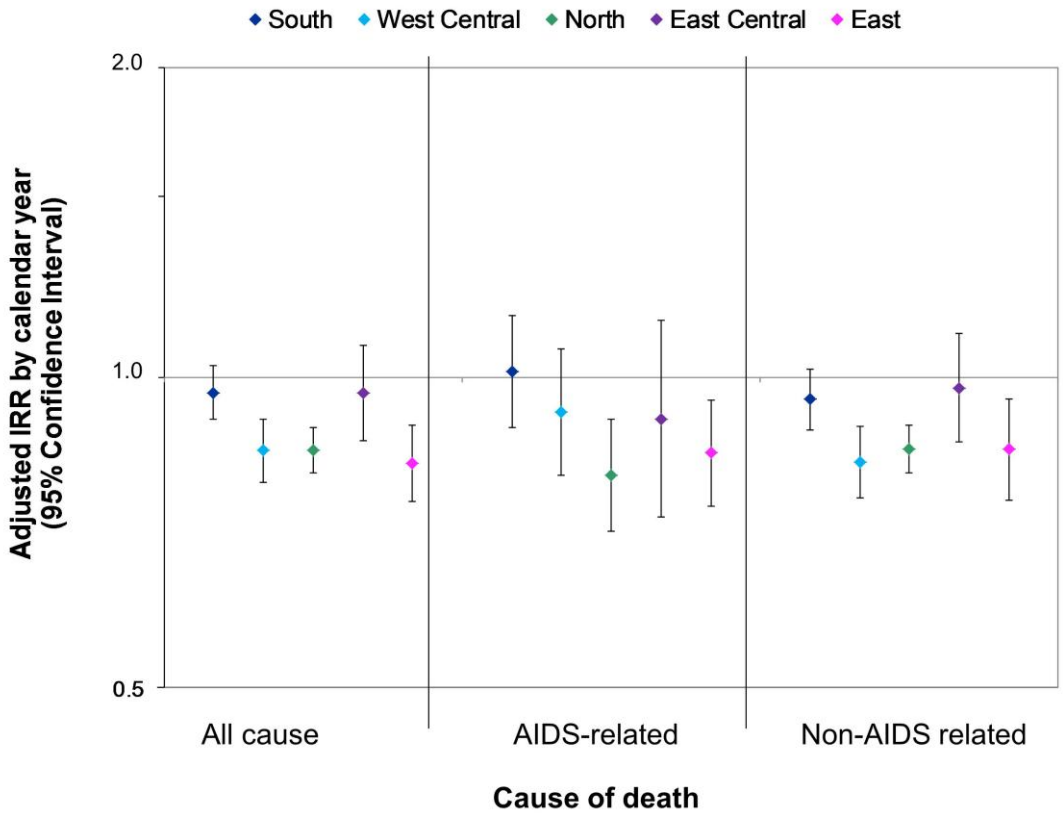


Figure 7.17 shows the association with mortality and calendar year of follow-up stratified by region, after adjustment. In multivariable adjusted analysis, significant decreases in all-cause mortality rates over time were observed in West Central (IRR 0.85 per calendar year later, 95%CI 0.79-0.91, p<.0001), North (IRR 0.85 per calendar year later, 95%CI 0.80-0.89, p<.0001), and East Europe (IRR 0 0.83 per calendar year later, 95%CI 0.76-0.90, p<.0001). No significant decreases were observed in South (IRR 0.96 per calendar year later, 95%CI 0.90-1.02, p=0.21) or East Central Europe (IRR 0.96 per calendar year later, 95%CI 0.87-1.07, p=0.46). Argentina was excluded from this analysis due to the limited number of patients. Despite the observed decrease in mortality in East Europe over time, in the most recent period, 2008-2010, there remained a significantly higher rate of all-cause (1·73, 95%CI 1·05-2·84, p=0·03) and AIDS-related mortality (IRR 4·68, 95%CI 2·20-10·97, p=0·0004) compared to South Europe in adjusted analysis. However, the higher rate observed in North Europe compared to South Europe was no longer significant in 2008-2010 for all-cause (IRR 1·34, 95%CI 0·87-2·11, p=0·18) or non-AIDS related mortality (IRR 1·43, 95%CI 0·86-2·38, p=0·16), suggesting that the regional differences between North Europe and South Europe may be decreasing over time.

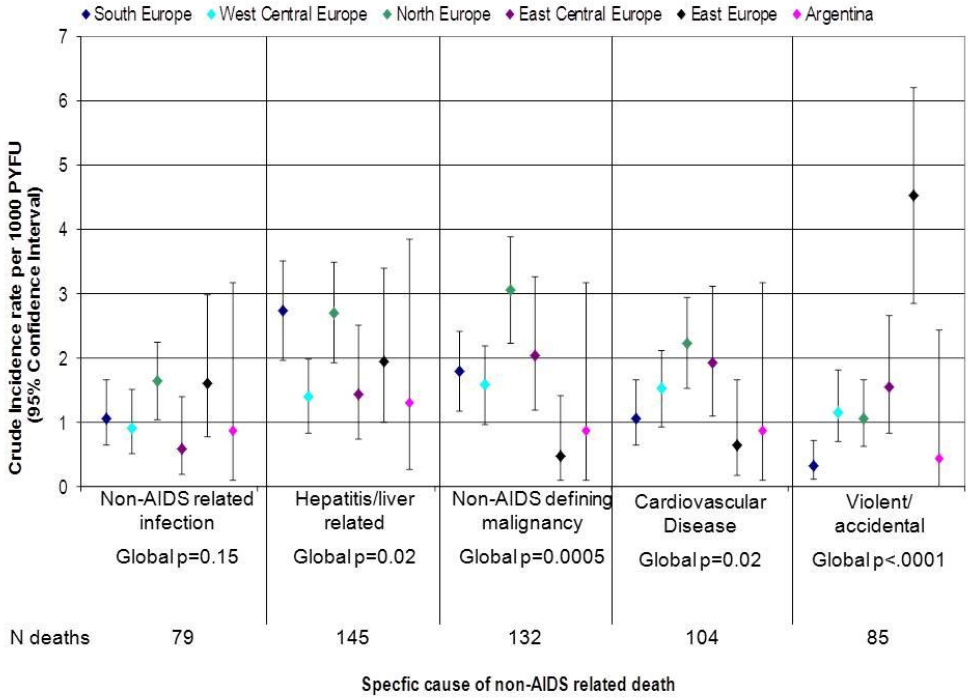
Figure 7.17 Adjusted incidence rate ratio by calendar year of follow-up stratified by region



7.4.5.3 Specific causes of non-AIDS related mortality

Figure 7.18 shows the crude death rates for specific non-AIDS related causes of death. The most common specific cause of non-AIDS related death was due to liver related causes (n=145, 19.2%), followed by non-AIDS defining malignancies (n=132, 17.5%), and cardiovascular disease (m=104, 13.8%). In univariable analysis, patients from North Europe had a significantly higher rate of death due to non-AIDS defining malignancies (IRR 1.71, 95% CI 1.10-2.65, p=0.01) and cardiovascular events (IRR 2.10 per 1000 PYFU, 95%CI 1.21-3.64, p=0.008) compared to South Europe. In East Europe, the main cause of non-AIDS related death was due to violence/accident, the rate of violence/accidental death was significantly higher compared to South Europe (IRR 13.49 per 1000 PFYU, 95%CI 5.58-32.57, p<.0001). The number of events for each specific cause of death was too small to investigate these differences further in adjusted analysis.

Figure 7.18 Crude incidence rate of specific non-AIDS related deaths by region of Europe



7.4.6 Sensitivity analysis

To investigate whether the overall differences in the incidence rates observed across the regions were observed in different subgroups of patients, sensitivity analysis excluding those patients infected via IDU was performed. 10,109 patients were included in this analysis and 655 deaths were observed (193 AIDS related and 462 non-AIDS related). Table 7.16 summarises the results of this analysis. After adjustment, patients in East Europe had a 2.37 times higher AIDS related mortality rate than South Europe (95%CI 1.38-4.08, p=0.001), lower than the 3.26 times higher rate in the main analysis. The incidence rate of non-AIDS related mortality remained significantly higher in North Europe (IRR 1.36, 95%CI 1.06-1.75, p=0.01) compared to South Europe,

Table 7.16 Multivariable Poisson regression analysis excluding those with injection drug use as HIV transmission route

Region			**Multivariable (time-updated)**		
All-cause	**N included**	**PYFU**	**IRR**	**95% CI**	**p-value**
South	2280	13336	1.00	-	-
West Central	2512	15099	0.95	0.74-1.20	0.64
North	2548	16294	1.27	1.02-1.57	0.02
East Central	1252	6460	1.65	1.25-2.17	0.0003
East	1083	3684	1.60	1.13-2.27	0.008
Argentina	434	2158	0.72	0.43-1.21	0.21
AIDS related	**N included**	**PYFU**	**IRR**	**95% CI**	**p-value**
South	2280	13336	1.00	-	-
West Central	2512	15099	0.96	0.62-1.48	0.84
North	2548	16294	1.07	0.70-1.64	0.75
East Central	1252	6460	2.18	1.35-3.51	0.001
East	1083	3684	2.37	1.38-4.08	0.001
Argentina	434	2158	0.94	0.44-1.97	0.85
Non-AIDS related	**N included**	**PYFU**	**IRR**	**95% CI**	**p-value**
South	2280	13336	1.00		
West Central	2512	15099	0.93	0.70-1.23	0.60
North	2548	16294	1.36	1.06-1.75	0.01
East Central	1252	6460	1.49	1.06-2.08	0.02
East	1083	3684	1.31	0.82-2.11	0.25
Argentina	434	2158	0.57	0.27-1.20	0.14

To investigate biases caused by under ascertainment of death, we varied the right censoring interval from 6 months after last follow-up visit to 3 months and 1 year. The results remained consistent with the main analysis (table 7.17).

Table 7.17 Mulitvariable Poisson regression analysis varying the right censoring interval

Region	Censored 3 months after last follow-up visit			Censored 1 year after last follow-up visit		
All-cause	**IRR**	**95% CI**	**p-value**	**IRR**	**95% CI**	**p-value**
South	1.00	-	-	1.00	-	-
West Central	0.93	0.76-1.14	0.51	0.92	0.75-1.12	0.40
North	1.41	1.18-1.68	0.0001	1.42	1.19-1.69	<.0001
East Central	1.34	1.08-1.68	0.009	1.40	1.13-1.75	0.002
East	1.75	1.38-2.21	<.0001	1.86	1.47-2.34	<.0001
Argentina	0.73	0.47-1.15	0.17	0.86	0.55-1.34	0.50
AIDS related	**IRR**	**95% CI**	**p-value**	**IRR**	**95% CI**	**p-value**
South	1.00	-	-	1.00	-	-
West Central	0.86	0.58-1.26	0.43	0.83	0.57-1.23	0.35
North	1.09	0.75-1.58	0.64	1.09	0.76-1.58	0.63
East Central	1.58	1.03-2.42	0.03	1.63	1.07-2.50	0.02
East	3.25	2.18-4.84	<.0001	3.44	2.31-5.12	<.0001
Argentina	1.01	0.52-1.97	0.96	1.20	0.62-2.31	0.58
Non-AIDS related	**IRR**	**95% CI**	**p-value**	**IRR**	**95% CI**	**p-value**
South	1.00	-	-	1.00	-	-
West Central	0.90	0.71-1.14	0.37	0.88	0.70-1.11	0.28
North	1.42	1.17-1.73	0.0004	1.44	1.18-1.75	0.0003
East Central	1.21	0.96-1.57	0.14	1.27	0.98-1.64	0.07
East	1.23	0.91-1.67	0.18	1.30	0.96-1.77	0.08
Argentina	0.54	0.29-1.01	0.05	0.63	0.33-1.18	0.14

As a number of patients had missing CD4 count data a sensitivity analysis was performed excluding all those with missing CD4 counts. Patients excluded from this analysis because of missing CD4 count data were more likely to come from North or East Europe, be IDU, current smokers, have hepatitis C, hypertension and diabetes. Individuals excluded were also less likely to have a suppressed viral load whilst on cART. Indicating that part of the association between missing CD4 count and all-cause mortality may be due to some sicker individuals who stop having their CD4 count measured routinely prior to their death and also those from high risk groups such as IDU being less likely to have a CD4 count available. A total of 13,111 patients with 784 events (579 AIDS related deaths and 205 non-AIDS related deaths) in 62,522 PYFU were included. The results were broadly consistent with the main analysis (table 7.18) although a higher rate of all-cause mortality was also seen in East Central Europe after adjustment.

Table 7.18 Multivariate Poisson regression analysis excluding those with missing CD4 count measurements

Region	**Multivariable (time-updated)**		
All-cause	**IRR**	**95% CI**	**p-value**
South	1.00		
West Central	0.91	0.73-1.13	0.38
North	1.34	1.10-1.64	0.003
East Central	1.53	1.20-1.96	0.007
East	1.54	1.14-2.08	0.004
Argentina	0.83	0.46-1.53	0.55
AIDS related	**IRR**	**95% CI**	**p-value**
South	1.00		
West Central	0.91	0.58-1.42	0.67
North	1.05	0.68-1.63	0.83
East Central	2.15	1.33-3.49	0.001
East	3.36	2.04-5.52	<.0001
Argentina	1.14	0.47-2.74	0.77
Non-AIDS related	**IRR**	**95% CI**	**p-value**
South	1.00		
West Central	0.88	0.68-1.14	0.32
North	1.44	1.15-1.81	0.001
East Central	1.37	1.03-1.84	0.03
East	0.90	0.59-1.39	0.65
Argentina	0.66	0.29-1.53	0.38

7.5 Discussion

EuroSIDA is unique amongst studies of HIV-positive individuals in terms of its coverage across the entire continent of Europe, including the East. The results of this study with over 67,000 PYFU found significant differences in mortality rates across regions of Europe and Argentina. In particular, individuals in East Europe were found to have a higher mortality rate from AIDS related causes and individuals in North Europe a higher rate of non-AIDS related mortality. Similar rates of both AIDS and non-AIDS related mortality was seen across the other regions of Europe and Argentina. The mortality rate decreased over time particularly in West Central, North and East Europe.

Differences in the incidence of AIDS related mortality could partly be explained by variations in patient demographics, including CD4 count and use of effective cART regimens. The epidemic in East Europe is more recent, the first outbreaks were reported among injection drug users in southern Ukraine in 1995[733]. Since then a number of countries in the region have expanded access to antiretroviral coverage, although treatment coverage remains low[8;733]. UNAIDS estimated that <25% of patients in need of antiretroviral therapy in East Europe and Asia were receiving it in 2008[8]. This is below the global average for low and middle income countries which was 42%[741]. In our study, after adjustment, the rate of AIDS related mortality appeared to be decreasing over time in East Europe, which may in part be due to improvements in antiretroviral coverage. Continued efforts to improve access to treatment in this region may help to reduce the incidence of AIDS related mortality in East Europe to a level similar to that in the rest of Europe. Improving access to optimal cART regimens to allow patients to achieve and maintain virological suppression could help reduce the rates of AIDS related mortality in East Europe to a level closer to the rest of Europe. However, after adjustment for time updated CD4 count, use of cART, viral load and other factors, regional differences in AIDS related mortality were still significantly different, indicating that the differences cannot be fully explained by those variables collected and included in our models.

A particular risk factor for mortality in HIV-positive patients is the mode through which HIV transmission occurred.

Before cART became available, mortality in persons who acquired HIV infection via intravenous drug use was similar to mortality in other HIV-positive individuals[540]. However, in the cART era mortality is higher for IDUs than non-IDUs[538;540;701;707]. EuroSIDA has demonstrated previously that IDUs who started cART had a similar CD4 count and viral load response to those in other exposure categories, but that IDU were 27% less likely to start cART[742]. Further, studies have shown that HIV-positive individuals infected via IDU are at an increased risk of mortality from causes not directly related to HIV infection, including overdose, suicide and homicide[354;743]. The greatest differences have been observed in deaths as a result of hepatitis and liver failure, and deaths as a result of substance abuse[707]. Several factors have been identified as contributing to the excess risk of mortality observed in IDUs in the cART era, including decreased access and adherence to cART[744;745] and more comorbidities such as co-infection with hepatitis C[361;707;746]. Additionally, HCV co-infection has been found to increasingly contribute to the overall number of deaths in the cART period, with one study reporting that 66% of all patients who died were co-infected[703].

A high proportion of patients in East Europe are infected via IDU[150]. This was accounted for in the adjusted analysis, but IDU populations may differ across the regions. Some may have been infected several years ago and have access to opiate substitution therapy and treatment[9], rather than actively injecting drug users who are less likely to access cART, have lower treatment adherence[744] and are at an increased risk of AIDS related mortality[747;748]. In West Central Europe coverage of HIV services in IDU populations has been reported to be high, particularly access to cART for HIV-positive[749]. However, in East Europe services are low, largely due to low levels of needle exchange programmes[750]. Opiate substitution programs are available in most countries, with the exception of Russia. However, the programs have been found to be very limited in East Europe. Further, very few IDUs in East Europe have been found to be receiving cART[749]. Detailed information on opiate substitution programmes, or whether individuals were actively injecting drug users, was not available for individuals in this analysis. However, a sensitivity analysis, excluding those where the reported transmission route was IDU, found a slightly lower but still increased incidence rate for East compared to South Europe, indicating that the higher prevalence of IDU does not explain all the differences.

State-of-the-art care of HIV patients requires the utilisation of multiple health care interventions. These include laboratory and clinical procedures for disease monitoring, but also involving health system interventions such as the procurement of antiretroviral drugs,

laboratory equipment, and health care infrastructure in general. Not all differences in patient care will have been captured by the use of effective cART, and overall lower levels of patient care in East Europe may play a significant role in the higher rate of AIDS related mortality in East Europe. The increase in differences in mortality rates in East Europe compared to South, when time-updated variables were included in the model, indicates that current measurements of variables such as CD4 count, on cART viral load, are not the only measures of health. Further, there were quite high levels of missing values so it was difficult to fully adjust for CD4 count or viral load. These missing values were found to be strongly associated with risk of all-cause mortality. Part of the reason for this may be that sicker individuals particularly those who are near to death are maybe less likely to attend the clinic for the scheduled appointments and routine laboratory testing. However, these missing values may also be an indicator of poorer healthcare and less frequent monitoring of patients in some clinics. The results of the sensitivity analysis excluding those with missing CD4 count were consistent with the main analysis. There might be some factors, such as socio-economic and infrastructure aspects, which play a role and for which we do not have data[730].

An increased rate of non-AIDS mortality was found in patients in North Europe compared to South Europe. These differences could not be explained by adjusting for differences in patient demographics, treatment or other measured risk factors, such as smoking status, co-infection and CVD risk factors (diabetes and hypertension), either at baseline or time-updated. After adjustment for baseline factors, an increased rate of non-AIDS related mortality was also observed in East Central and East Europe compared to South Europe. However, after adjusting for time-updated variables these differences were no longer observed.

Geographical differences in non-AIDS mortality may be driven by underlying differences in population morbidity and mortality. For example, lifestyle factors associated with drug use and homosexuality may account for some of the differences in regions. An elevated risk of suicide has been observed in men in same sex partnerships[751]. However, a recent study in the USA found that mortality risk from non-HIV related causes including suicide was not elevated among homosexual men[752]. Smoking, excessive alcohol and drug use are found at an increased level in the HIV-positive population compared to the general population[18;364;372], and may also differ between the regions. This could not be fully adjusted for in our models. However, we have recently started to collect data on alcohol intake.

A recent study using data from 12 Western European countries found that, in the general population, middle-aged men in North Europe had a 5% higher risk of all-cause mortality and woman 28% higher risk compared to the South Europe[753]. Additionally, in the general population, some North European countries are reported to be at a higher risk of CVD[754] and have poorer cancer survival rate[755] than those in other regions. Mediterranean diet has been found to be associated with a lower risk of all-cause mortality, and in particular, mortality due to CVD and cancer in the general population[756] although we have accounted for CVD factors such as diabetes, and hypertension these data were not available for all patients. Hypertension and diabetes have previously been identified in EuroSIDA[356], and in other studies[696], as potential modifiable risk factors associated with liver, CVD and NADM related deaths.

Unsurprisingly, older age was associated with an increased risk of all-cause, AIDS and non-AIDS related mortality. The effects of age on the risk of mortality were similar in each region. It has been proposed the HIV-positive patients may suffer from accelerated aging driven by residual immune activation[720;757]. Despite years of successful treatment and virological suppression, the immune system may have persistent defects[720]. These defects are similar to those seen in normal aging, but in HIV-positive patients occur at an earlier age than normal[758]. Most of these abnormalities have been seen in patients who start cART in the later stages of the disease, when the CD4 count has already fallen to low levels (<200cells/mm^3)[720]. Certain biomarkers that predict morbidity in the very old are higher than expected in younger HIV-positive patients, and this consistent observation provides indirect evidence that HIV infection might accelerate the ageing process[757]. The National Institute of Aging has sponsored a collaborative program aimed at identifying possible therapeutic agents that delay physiologic aging[759]. It may be that differences in lifestyle factors, discussed above, such as dietary intake or exercise levels of exercise, may be adding to this accelerated aging process in North Europe and having a greater impact on mortality.

It has been reported that on-going viral replication is associated with an excess risk of opportunistic infection and death after accounting for CD4 count[534]. In this analysis, due to a high proportion of missing viral load measurements in individuals off cART, only viral load levels in patients receiving cART were included.

For each endpoint, being off cART was associated with a significantly higher rate of mortality compared to those on cART with a suppressed viral load. Individuals on cART

with uncontrolled viral replication also had a higher risk of AIDS related mortality which is supported by other studies[696;699;711]. In our analysis, no significant difference in the rate of non-AIDS related mortality was observed in patients on cART, with uncontrolled viral replication compared to those on cART with a suppressed viral load. Some studies support this for all non-AIDS mortalitiy[711] and others only for specific non-AIDS related causes such as NADM[363]. An association with viral load and deaths due to infections and cardiovascular disease has been reported in a number of studies[363;699;699]. Due to the number of deaths we could not investigate the impact of viral replication on specific causes, so we are unable to fully investigate this. However, the previous chapter found no association with viral replication and the risk of developing an NADM.

It is unlikely that the higher rate of non-AIDS mortality in North Europe is driven by poor patient care. EuroSIDA has previously found, through different measures of healthcare in HIV-positive individuals, that North Europe has a high level of healthcare[735]. It has been speculated that an increase in the prevalence of certain diseases, such as cancer, may be expected with economic development and thus a higher mortality rate from certain causes might be observed despite a generally high quality of healthcare[760].

Long-term retention of individuals in care is important to ensure they receive the optimum level of care, and a better understanding of factors that prevent this is also needed. Factors that may contribute include overly centralised treatment programmes that limit geographical accessibility, shortages of healthcare workers, drug stock-outs and weak community treatment literacy[733]. In particular, it has been reported that Russia and the Ukraine, two countries in East Europe with the highest HIV prevalence, are spending relatively low levels on AIDS responses given their disease burden and ability to pay[733].

Another explanation, is the under ascertainment or incomplete report of deaths in other regions. Centres in some countries may be linked to national death registers allowing more complete reporting of mortality or have better methods of finding patients lost to follow-up (LTFU). As part of new survey, EuroSIDA is planning on collecting detailed information on this which may provide increased insight into the difference observed. The incidence of LTFU in EuroSIDA is low, and has previously been reported to be fairly consistent over time at < 5% per 100 PYFU, with individuals in North and West Central Europe having the lowest, and those in East Europe the highest rate of LTFU[427]. To investigate biases caused by under ascertainment of death, we varied the right censoring interval from 6

months after last follow-up visit to 3months and 1 year. The results remained consistent with the main analysis.

In the more recent time periods, it is more likely that the specific cause of death was more accurately diagnosed. Prior to CoDe being introduced in 2004, it is more likely that misclassification may have occurred. Countries with a high prevalence of AIDS events and a low prevalence of non-AIDS events screening, such as those in East Europe, may have overestimated the role of HIV infection in fatal outcome in this earlier period. In contrast, in North Europe it may be that in the early 2000s attention focused on the adverse events associated with cART and non-AIDS related events, which may have led to an underestimation of the causal relationship of HIV with mortality in this region.

It would be of interest to compare mortality rates to those of the general population for each of the regions. However, it is hard to find a suitable population as HIV-positive patients tend to have higher rates of many other risk factors associated with all-cause mortality[411;412]. Demographically, individuals enrolled in the EuroSIDA study are fairly representative of HIV-positive individuals in each region based on comparisons with data from UNAIDS[9]. However, centres included in EuroSIDA are often university-associated clinics in larger cities. Thus, individuals in these clinics may have better access to care and may not be representative of all clinics in Europe. This raised most concern in Eastern Europe, where the least favourable outcomes were seen, suggesting that improvements in this region are urgently needed. In future years, with expansion of the database to collect more detailed information on factors such as opiate substation therapy and alcohol use, and a better understanding of how deaths are ascertained at each of the centres, the regional differences observed can be further explored. This will be discussed more fully in the next chapter. As mentioned in the introduction to this chapter, previous EuroSIDA analyses identified four healthcare indicators (table 7.18) that measure adherence to current guidelines and outcome after cART initiation. These healthcare indicators may serve as a tool for benchmarking the clinical management of HIV infection in Europe and elsewhere[735]

Table 7.19 Healthcare indicators developed by EuroSIDA[735]

Healthcare Indicators
1. Compliance with current guidelines on when to start cART, based on CD4-cell count and presence of AIDS diagnosis 2. Compliance with current guidelines on prophylaxis of opportunistic infections, i.e. initiating of Pneumocystis jiroveci pneumonia (PCP) chemoprophylaxis at CD4-cell count <200 cells/µL and no prior diagnosis of PCP 3. Laboratory evaluation of HIV-disease status: median number of CD4-cell count and HIV-RNA measurements performed per patient per follow-up year, stratified by whether patients were off or on cART; 4. Virologic response to cART, assessed by the proportion of patients spending more than 90% of the follow-up time on cART with suppressed HIV-RNA

In conclusion, differences were observed in the rate of all-cause mortality among HIV-positive patients across different regions of Europe. Individuals in Eastern Europe had an increased risk of mortality from AIDS related causes in part due to differences in use of effective cART. Patients in North Europe had the highest rate on non-AIDS related mortality which could not easily be explained but did appear to decrease over time. Research investigating regional differences across Europe, particularly allowing a comparison with East Europe, is limited. EuroSIDA includes HIV-positive individuals from a wide range of countries across all the regions, including a significant proportion from Eastern Europe, typically underrepresented or absent in other studies of the HIV epidemic. There is an urgent need to better understand the poor outcomes in East Europe including a comprehensive measure of healthcare indicators that capture the wide aspect of HIV treatment and outcomes.

Chapter 8 Conclusions

8.1 Summary of main findings

The focus of HIV research has changed since the introduction of cART, which saw dramatic improvements in the quantity and quality of life for HIV-positive individuals. Once initiated, cART is a lifelong commitment; a high proportion of patients have now been on cART for over 10 years. It is therefore important that patients are on an effective, safe and durable cART regimen, that allows them to be monitored by clinicians closely enough to receive effective treatment, but not too closely that it impacts unnecessarily on the patient's quality of life. Effective durable cART has led to an increase in life expectancy and we now have an ageing HIV-positive population in Europe. This ageing population may be at an increased risk of developing a variety of different clinical events not traditionally seen as AIDS related, but that occur at a higher rate than in the HIV-negative population. It is important for cohort studies to identify new and emerging trends in clinical events, and to understand what factors are related to the development of these events. Cohort studies can also help investigate the impact HIV and non-HIV related risk factors are having on the development on these new clinical events and on mortality rates across the HIV positive population in Europe. The aim of this thesis was, therefore, to assess the long term durability of cART through assessing various clinical, virological and immunological outcomes including mortality in HIV-positive patients across Europe.

8.1.1 Investigation into whether frequency of monitoring can be reduced in a sub-group of HIV-positive patients across Europe

Treatment guidelines in developed countries such as Britain and the US have recommended that individuals on cART should be seen in clinics every 3-4 months for CD4 count and viral load monitoring, regardless of their response to therapy or how long they have been on their current cART regimen. In Chapter 3, a subgroup of otherwise healthy HIV-positive patients were identified who had responded well to therapy and were on a well-tolerated and fully suppressive cART regimen. This subgroup were found to have a low chance of immunological or virological failure or an AIDS, non-AIDS defining event or death, occurring in the next 3-6 months.

The most common event was viral rebound, which was defined as two consecutive viral load measurements above 500 copies/ml. Very few serious clinical events were observed, and the probability of developing an AIDS defining illness, non-AIDS defining illness, full suppressed cART regimen for a year was less than 1%. The probability of the CD4 count dropping substantially to either below 200/mm^3 or below the CD4 at starting cART was also less than 1%. Further, patients who had spent less than 70% of their follow-up whilst on cART (excluding the first four months after starting a new regimen) with uncontrolled viremia had an increased risk of experiencing disease progression.

These findings suggest that it may be reasonable to consider increasing visit intervals from 3 to 6 months in HIV-positive patients who are otherwise healthy, and have maintained a stable and fully suppressed cART regimen for at least one year, particularly if the patient has experienced sustained periods of virological suppression since cART initiation. Extending the time between clinic visits would benefit the patient, in that it would make treatment less intrusive on their lives, with fewer reminders to patients of their illness, and would benefit out-patient clinics through saving considerable resources, by freeing up clinicians to allocate more resources to those patients at greatest risk of treatment failure and clinical disease progression.

Since this analysis was performed, treatment guidelines[494;761] have been modified and currently recommend that, in patients with consistently suppressed viral loads whose CD4 cell count has increased well above the threshold for opportunistic infection risk, the CD4 count may be monitored every 6 to 12 months, unless there are changes in the patient's clinical status. The recommendations also state that adherent patients with suppressed viral load and stable clinical and immunologic status for >2–3 years, the interval between HIV RNA monitoring may be extended to every 6 months. Results from this analysis provide some direct evidence to support these recommendations[761].

A manuscript of this analysis was published in AIDS in May 2008, and can be found in Appendix IV.

8.1.2 History of viral suppression on cART as a predictor of virological failure after starting at least one new antiretroviral

The analysis performed in Chapter 3 looked at a subgroup of patients on a stable and fully suppressed cART regimen, and found that even in the subgroup of individuals who were doing well on therapy, patients who had spent < 70% of their follow-up time on cART with a suppressed viral load had a higher risk of experiencing an event, the most common of which was virological failure. In Chapter 4, the association between history of viral suppression and future virological failure was investigated in more detail. All patients who were on cART, and starting a new ARV(s), were included and the chapter investigated whether there was an association between different measures of describing a patient's history of viral suppression after cART initiation, and risk of future viral failure after starting a new ARV(s). The 6 measures of previous responses to cART investigated were, time in months to initial suppression (HIV-RNA ≤ 500 copies/ml) after starting cART, number of viral rebounds after initial suppression, size of the highest viral rebound, time since most recent viral rebound, viral rebound above viral load at first starting cART (yes versus no), and the proportion of time spent with a viral load ≤ 500copies/ml while receiving cART.

The most important factor for predicting virologic failure after starting a new ARV(s) was the percentage of time spent with a suppressed viral load whilst on cART prior to starting a new ARV(s), both in patients virologically suppressed and those virologically failing at the time of starting new ARV(s). In addition, in patients who were virologically suppressed at baseline, time since last viral rebound prior to starting new ARV(s) was also an important factor in predicting the risk for future virologically failure. Individuals who had spent <70% of their time on cART (excluding the first four months after starting a new regimen) with a suppressed viral load had a higher risk of future virological failure, consistent with the analysis in Chapter 3. In individuals virologically suppressed at the time of making a treatment switch, a higher rate of virological failure was also observed in those suppressed between 70-90% compared to >90% on the time on cART. Further, in those who were suppressed at the time of starting new ARV(s), the time since last virological rebound was also associated with risk of future virological failure after starting the new ARV(s). A decreasing risk of virological failure was found with increasing time since last virological rebound.

These findings suggest that when patients on cART are making a change in their treatment regimen, patients who have previously spent a low percentage of time on cART with a suppressed viral load and those who have recently rebounded may require more intensive monitoring and consideration should also be made to increasing adherence counselling.

These results have also been used to provide support to treatment recommendations and compliment the results from Chapter 3. Based on results from both these analyses, guidelines now recommend that, once viral replication is suppressed, monitoring intervals may be extended up to every 6 months, among patients who remain virologically suppressed and have CD4 cell counts greater than 350cells/mm^3. However, more frequent monitoring is required for patients who have changed therapy because of virologic failure[761].

A manuscript of this analysis was published in HIV Medicine in August 2010 and can be found in Appendix V.

8.1.3 A comparison of the long term durability of nevirapine, efavirenz and lopinavir in routine clinical practice across Europe

In Chapter 3, patients who had maintained a fully suppressed cART regimen for at least a year had a very low risk of disease progression in the next 6 months. Additionally, results for Chapters 3 and 4 found that an important predictor of virological rebound and disease progression in patients on cART was the proportion of time patients had spent on cART with a suppressed viral load. The virological efficacy of a number of ARVs has already been demonstrated in a number of clinical trials and prior cohort studies. However, it is not only important for a regimen to be virologically effective; it also needs to have long term durability. Durability can be assessed by the rate and reasons for treatment discontinuation and the risk of developing long term adverse events, such as such as cardiovascular disease or pancreatitis.

Nevirapine, efavirenz, or lopinavir, together with two NRTIs are three of the most commonly used regimens across Europe.

Previous studies have investigated the virological efficacy and incidence of AIDS related events in patients on these three regimens, but less is known about the long term durability of each regimen. Therefore, the analysis in Chapter 5 compared the long term durability of regimens including nevirapine, efavirenz and lopinavir. As the focus was long-term durability, patients were only included once virological suppression had been achieved, and after at least 3 months exposure to either nevirapine, efavirenz or lopinavir based cART, to exclude discontinuations due to early-onset potentially treatment limiting toxicities, such as central nervous system (CNS) disturbances or hypersensitivity reactions (rash, hepatotoxicity).

No significant difference was found in the rate of discontinuation for any reason between the three treatment regimens, although differences were found in the rate of discontinuation for specific reasons. Patients on nevirapine had a higher rate of discontinuation due to reported treatment failure, and a lower rate of discontinuation due to toxicity or patient/physician choice compared to those on efavirenz and lopinavir. There was no significant difference in the development of any non-AIDS clinical events, worsening in anaemia, severe weight loss, or increasing in ALT or AST levels. Patients on lopinavir had a higher rate of low HDL cholesterol compared to patients on nevirapine. However, there was no difference in low HDL cholesterol between patients on efavirenz and nevirapine. Furthermore, patients on lopinavir had a marginally higher mortality rate compared to nevirapine, but there was no significant difference between efavirenz and nevirapine.

This analysis, based on data from patients under routine follow-up across Europe, found that nevirapine based cART regimens have similar durability based on risk of all-cause discontinuation and development of serious clinical events compared to efavirenz and lopinavir. However, patients on nevirapine had a higher rate of discontinuation due to reported treatment failure, and those on efavirenz and lopinavir had a higher rate of discontinuation due to toxicity or patient physician choice. As a choice of first-line cART, all three regimens appear to have similar long-term durability. However, it is also worth noting that treatment for HIV is currently expected to be lifelong, and it is possible that there will be long-term differences in outcomes for those who discontinued treatment due to treatment failure, and those who discontinue due to toxicities or patient/physician choice due to the rate at which available alternative regimens are used and new drugs

become available. A real strength of this analysis was the long term follow-up available for each of the ARVs investigated, something rarely available in clinical trials where the results focus on short-term outcomes, particularly virological response.

A manuscript to this analysis was published in HIV medicine in May 2011 and can be found in Appendix VI.

8.1.4 The relationship between current level of immunodefiency and non-AIDS defining malignancies

In developed countries, the use of effective, durable cART has led to a decrease in the incidence of many AIDS-defining illnesses, including two AIDS defining malignancies (ADM); Kaposi's sarcoma (KS) and non-Hodgkin's lymphoma (NHL). In the current treatment era, a higher portion of malignancies in HIV-positive patients are non-AIDS defining malignancies (NADM) rather than ADM. Further, many of these NADM have been found to occur at higher rates in HIV-positive patients compared to the general population. Although some of this increased risk can be explained by the higher prevalence of associated risk factors, such as smoking, alcohol, drug use and co-infection with a number of oncogenic viruses, such as EBV, HPV, and hepatitis, it does not account for all of the increased risk. One theory is that impaired immune function may result in the general reduced immune surveillance for malignant cells, or it may impair the ability to suppress oncogenic viruses, and this may result in a higher risk of developing some cancers. To explore the link between immune suppression and the development of NADM, the incidence of NADM across Europe in HIV-positive patients and the risk factors associated with the development of NADM were investigated in Chapter 6.

The incidence of NADM in EuroSIDA from 1994-2007 was 4.3 per 1000 PYFU. After adjustment, a higher current CD4 count was independently associated with a decreased incidence of NADM. In addition, an increased rate of 'virus-related' cancers and 'non-virus related epithelial' cancers was found in immunodeficient patients. Hodgkin's lymphoma, anal, and lung cancers were all found at a higher rate in patients with lower current CD4 counts after adjustment for other demographic and traditional risk factors.

Further, individuals in East Europe had a significantly lower risk of developing an NADM which may be due in part to competing risks from other events such as a higher rate of mortality.

This analysis demonstrated a link with immunodeficiency and the development of certain NADM, particularly those that are virus-related. Starting cART earlier to reduce the proportion of patients with a low CD4 count may decrease the rate of developing many common non-AIDS related malignancies, although the potential harms for longer exposure to drugs and risk of toxicities would have to be balanced against the potential benefits. Current guidelines using evidence from this study, together with a number of other cohort studies, agree that the evidence suggests that initiating ART to suppress HIV replication and maintain CD4 counts at above 350–500 cells/mm3 may reduce the risk of both AIDS defining and non-AIDS-defining malignancies[494]. The European AIDS clinical society guidelines include details on the prevention and management of non-infectious co-morbidites in HIV. Within this section there are guidelines on the screening of six NADM (anal cancer, breast cancer, cervical cancer, colorectal cancer, hepatocellular carcinomas, and prostate cancer)[762]. The screening recommendations are derived from the general population, and reflect the increasing importance of co-morbidities for HIV-postitive persons as a consequence of increased life expectancy resulting from durable and effective cART, and concerns that several risk factors including immunodeficiency may result in an increased risk of these of events in HIV-positive persons.

A manuscript of this analysis was published in Cancer in November 2010 and can be found in Appendix VII.

8.1.5 Regional differences in the incidence of AIDS and non-AIDS related mortality in HIV-positive patients across Europe

Eastern Europe has one of the fastest growing HIV epidemics worldwide[8]. The uptake of cART in Eastern Europe remains low, and previous studies have reported substantial differences in access to care and treatment compared to the rest of Europe[424;735].

In Chapter 3, a subgroup of patients were selected for the analysis who had maintained a stable and fully suppressed cART regimen for at least 1 year, and compared to the other regions a higher proportion of patients in East Europe were excluded from this analysis. This was due to a higher proportion of patients in East Europe having missing CD4 count and viral load measurements and being unable to maintain a stable and fully suppressed cART regimen. In Chapter 5, long term durability of nevirapine, efavirenz and lopinavir based cART regimens, although no differences were observed across the different regimens in the incidence of serious non-AIDS clinical events, a higher rate of non-AIDS events was observed in North Europe compared to South Europe. Additionally in the last chapter, patients in East Europe had a lower risk of developing an NADM and it was speculated that this may, in part, be due to competing risks from AIDS related events and mortality. All these factors indicate that there may be differences in the mortality rates in the current treatment era, across different regions of Europe and Argentina and, in particular, differences in the specific causes of death. Chapter 8, the final chapter, aimed to investigate whether differences are seen on the rate of all-cause, AIDS-related, and non-AIDS related mortality across the regions.

Significant differences in all-cause, AIDS and non-AIDS related mortality rates were seen across the 6 different regions. In particular, individuals in East Europe were found to have a higher mortality rate from AIDS related causes compared to South Europe. Some, but not all, of this increased rate could be explained by variations in patient demographics, including CD4 count and use of effective cART regimens. Further, an increased rate of non-AIDS mortality was found in patients in North Europe compared to South Europe, and these differences could not be explained by adjusting for differences in patient demographics, treatment or other risk factors. However, the rate of non-AIDS related mortality appeared to be decreasing over time in North Europe, with no difference observed in the most recent time period. Similar rates of both AIDS and non-AIDS related mortality were seen across the other regions of Europe and Argentina.

Differences in the incidence of AIDS related mortality could potentially be explained by variations in patient demographics, including CD4 count and use of effective cART regimens.

The differences in the rate of non-AIDS mortality, particularly the higher rate observed in North Europe compared to South Europe, could not be explained by adjusting for differences in patient demographics, treatment or other risk factors that were measured such as smoking status, co-infection and CVD risk factors (diabetes and hypertension), either at baseline or time-updated.

Some of the differences in non-AIDS mortality may be driven by underlying differences in population morbidity and mortality, which could not be accounted for in this analysis. However, another, perhaps more likely, explanation is the under ascertainment or incomplete report of deaths in other regions. Centres in some countries may be linked to national death registers allowing more complete reporting of mortality or have better methods of finding patients lost to follow-up (LTFU).

Research investigating regional differences across Europe, particularly allowing a comparison with East Europe, is limited. EuroSIDA includes HIV-positive individuals from a wide range of countries across all the regions, including a significant proportion from Eastern Europe, typically underrepresented or absent in other studies of the HIV epidemic. There is an urgent need to better understand the poor outcomes in East Europe, including a comprehensive measure of healthcare indicators that capture the wide aspect of HIV treatment and outcomes. To fully understand these differences, a comparison with the HIV-negative population is probably needed.

8.2 Limitations

8.2.1 Observational studies

To answer the research questions addressed in this thesis, data were selected from a large prospective observational cohort study. Although statistical methods, such as adjusting for confounding variables, can account for some of the biases, there may remain unmeasured or unknown confounding that cannot be adjusted for, and therefore we cannot fully exclude the possibility of some bias[763].

For example, in Chapter 5, the long term durability of nevirapine, efavirenz and lopinavir based cART regimens were compared. Randomised controlled trials are the gold standard for comparisons of drug interventions, and this analysis would possibly benefit from trial data to address this question, as observational cohort studies are likely to be affected by confounding because of the non-randomized selection treatment regimen in clinical practice[764]. For example, factors such as the patients disease status or underlying risk factors may influence which treatment is selected for a given patient[764]. However, the focus of this analysis was comparing the long-term durability of the three treatment regimens. The median follow-up time for this analysis was 2.6 years, and a number of different clinical outcomes were assessed. To carry out the same analysis in a randomised controlled trial with enough power to detect differences in clinical outcomes would be very costly and time consuming.

The benefits of observational studies include longer follow-up time, the lack of strict inclusion/exclusion criteria, that patients are under routine clinical management, the opportunity to study long-term or rare effects of treatment and more power to detect clinical outcomes.

8.2.2 Data limitations

There were a number of limitations to these analyses, some of these are discussed as the end of each of the chapters but there are a few that require discussion in more detail. Firstly, a number of the endpoints that were analysed were rare, and in order for there to be sufficient power to perform the analysis some endpoints were grouped together to form a composite end point. A consequence of this may be that there are other risk factors associated with some rarer events that could not be identified, and the risk factors that were identified are most likely to be those associated with the most common events in the composite endpoint. For example, in Chapter 3, the most common event was virological failure, so the risk factors that were identified are most likely to be predictive of virological failure, and there may have been other factors that were associated with, for example, the risk of death that were not identified. Similarly, in the analysis looking at the association between NADM and immunosuppression, a lot of consideration was put into grouping the malignancies, with input from an expert oncologist.

However, in the virus-related group, Hodgkin's lymphoma, anal and liver cancer made up the majority of the events, and it may be the associations identified are different for some of the other rarer cancers.

Another limitation that was of concern when looking at the long term durability of nevirapine, efavirenz and lopinavir was the information collected on the reason for patients making a treatment switch. Table 8.11 lists the reasons that are collected on the EuroSIDA follow-up forms (see Appendix I) and were used in the analysis in Chapter 5. Reasons for discontinuation of antiretrovirals have been collected since 1999. This information is only collected for the ARV(s) that are being stopped, not those that are started. Additionally, only one reason is given per ARV.

Table 8.1 Possible reasons reported for stopping ARVs

Reason for stopping antiretroviral
1: Treatment failure (i.e. virological, immunological and/or clinical failure)
2: Abnormal fat redistribution
3: Concern of cardiovascular disease
3.1: Dyslipidemia
3.2: Cardiovascular disease
4: Hypersensitivity reaction
5: Toxicity, predominantly from abdomen/GI tract
5.1: Toxicity - GI tract
5.2: Toxicity - Liver
5.3: Toxicity - Pancreas
6: Toxicity, predominantly from nervous system
7: Toxicity, predominantly from kidneys
8: Toxicity, predominantly from the endocrine system
8.1: Diabetes
9: Hematological toxicity
10: Hyperlactataemia/lactic acidosis
90: Toxicity, not mentioned above
91: Patient's wish/decision, not specified above
92: Physician's decision, not specified above
94: Other causes, not specified above
94.1:Out of stock
93: STI - Structured Treatment Interruption
99: Unknown

Focusing on treatment failure first, only one category was available, and this covers a range of possible reasons for treatment failure, including virological, immunological or clinical reasons. These reasons likely have quite different consequences in terms of clinical outcome and patient management. In addition, it was not based on a predefined definition, but at the clinician's discretion as to whether they felt this was the reason for stopping. One possible solution is to look at whether the patient was failing virologically at the time of stopping treatment. This was done in Chapter 5, and the majority of those who stopped due to treatment failure were virologically failing at the time of stopping. Further, if the treatment change was due to patient or physician choice, this was grouped with stopping due to toxicities, as this had been found to be a good marker for generalized toxicities causing treatment interruption[400;589]. The exact reasons for the patient or physician choosing to stop treatment are not known.

In a number of the chapters, Chapter 4 in particular, the analysis was focused around viral suppression or viral rebound. Arbitrary decisions on how to measure variation in viral load, and what constitutes a failure, may have a serious effect on how the results of a study are interpreted[126]. Throughout the thesis when viral load was used as an outcome, a number of sensitivity analyses were performed looking at different cut offs for the outcome. In particular, sensitivity analyses were performed in a subgroup of patients who had their viral load consistently measured with a lower limit of detection of 50copies/ml. However, this substantially reduced our power to detect a difference, but the results remained consistent. European AIDS clinical society guidelines now define virological failure as a confirmed viral load > 50 copies/l (or > than the lower limit of detection of the viral load assay) 6 months after starting therapy (initiation or modification)[762].

A further general limitation of the EuroSIDA data set is that there were missing values for a number of the variables used in each analysis. There are a number of reasons why individuals may have had missing values. If the data are missing at random, then this should not impact on the results of our analysis. However, some of the missing values may have been down to differences in monitoring strategies and availability of tests and diagnostic facilities across the centres.

Patients in East Europe were found to have higher rates of missing CD4 counts and viral loads than the other regions. This is likely due to less frequent monitoring of patients in this region. In order to account for this in the analysis comparing mortality rates across Europe, both CD4 count and viral load were included as categorical variables with a category for missing values. Alongside this, in order to reduce the number of individuals lost to follow-up, at each 6-monthly update of the database, the percentage lost to follow-up in each centre is reported back to the co-ordinating centre. Individual centres with the highest rates of loss to follow-up are contacted, and often a site monitor will visit these sites to try to address any issues they may be having with completing the follow-up forms. As mentioned in the last chapter, loss to follow-up in EuroSIDA is low, it has been reported to be <5% per year. Older patients, those with higher CD4 count, and those who have started cART had lower incidence of loss to follow-up and has been found to be higher in those from East Europe[427].

8.2.3 Changes in data collection

As the focus of HIV research has changed, so have the variables collected on the EuroSIDA follow-up forms, and the attention placed on ascertaining and verifying certain clinical events. For example, data on NADM has been systematically collected and included as part of the quality assurance process from 2001, after it emerged that NADM were increasing in HIV-positive patients. Therefore, any association observed with an increase in the number of events over time may simply reflect changes in data collection and better ascertainment of events, rather than a true increase in the rate of NADM over time.

Further changes include the use of CoDe for assigning the cause of death. CoDe was developed by a multidisciplinary team of experts in 2004 to help with accurately coding the causes of death in HIV-positive patients. Since then, clinics are requested to complete a CoDe form for any patient that dies, together with completing a final follow-up form. Thus from 2004 onwards, more detailed and accurate information has been available on causes of death. Further work more recently has allowed the development of an algorithm to classify deaths as AIDS or non-AIDS. These changes may have had an impact on our results, as some countries may have focused on monitoring non-AIDS related causes.

It is therefore important to adjust for calendar year of follow up, as it may reflect underlying factors such as treatment management practices, concomitant care and available drugs[764].

Since the analysis in this thesis began, the EuroSIDA follow-up forms have again been modified. In particular, it has become apparent that it is important for observational studies to not only collect information on risk factors thought to be associated with HIV disease progression, but also on risk factors associated with the risk of developing other serious clinical events. The latest version of the EuroSIDA follow-up form is available online at www.cphiv.dk and in Appendix I of this thesis.

An additional limitation of the EuroSIDA data is that we cannot adjust for current IDU status, as this information was not collected. This lead to a number of sensitivity analyses being performed, excluding individuals where the transmission route was IDU. The updated follow up forms now also collect information on current intravenous drug use, and opiate maintenance therapy. Numerous studies have reported the difference in treatment adherence and outcome in IDUs compared to the rest of the HIV-positive population, and once there is sufficient data available it would be interesting to compare outcomes and the long term durability of cART specifically in this subgroup of patients, as they are probably at the highest risk of virological failure, disease progression, and mortality. Additionally, if there were sufficient active IDUs enrolled across the regions, it would be interesting to repeat the regional comparisons in this subgroup of patients.

Another addition to our data collection concerns alcohol abuse, and whether or not the patient is currently suffering from alcohol abuse or has done in the past. This will help provide information on a modifiable lifestyle factor that is likely to significantly contribute to an individual's risk of non-AIDS events and mortality. It may also have an impact on individual's adherence to treatment and drug tolerability, which are all important for long-term durable cART regimens. However, this information is collected in a similar way to smoking status and therefore has some limitations. For example, we do not collect data on the amount of alcohol being consumed, frequency of binge drinking or type of alcohol consumed.

As mentioned in Chapter 7, no information is currently available on which centres are routinely linked with death registries and would therefore have complete information on death. It is important for any analysis looking at death rates, particularly comparing regions, that data are as complete as possible. Future work could include a sensitivity analysis only including centres known to link to death registries. It is unlikely that this occurs equally in different countries or regions however, which would introduce a further bias.

8.3 Further Research

The analysis in Chapter 5 looked at the long term durability of three main cART regimens. It would be interesting to repeat this analysis with current state of the art regimens. Further, to look at whether the risk of certain clinical events, such as non-AIDS defining malignancies, is the same for different antiretrovirals at a given CD4 count and viral load.

As previously discussed, non-AIDS defining malignancies were split into three categories based on cancer type. These groups were still very heterogeneous; highlighted by the results of the limited analysis that was performed on specific NADM. With longer follow-up, and more events, it would be interesting to look at risk factors for specific type of malignancies. It may also have been interesting to split the CD4 count into smaller categories, but there was insufficient power for this type of analysis. As more events are reported in EuroSIDA allowing for analysis on specific types of malignancies it would also be interesting to use the plasma viral load samples that are stored for EuroSIDA patients to investigate whether certain bio-markers could be identified that predict a risk of developing a specific non-AIDS defining malignancy .

Similarly, when looking at mortality rates grouping all non-AIDS related deaths into one category resulted in a very heterogeneous grouping. With the improvement in the coding of death within EuroSIDA using the CoDe form, it will be important to investigate whether regional differences in mortality rates exist due to specific non-AIDS related events and also whether certain risk factors have different impacts on the risk of mortality from a specific cause. The additional information that is now being collected on alcohol abuse and current IDU status would also provide additional valuable information, which could be used to help develop a list of healthcare indicators.

These healthcare indicators could be for measuring and comparing the different regions of Europe, to identify areas where improvements could be made.

8.4 Concluding remarks

The aim of this thesis was to assess the long term durability of cART through assessing various clinical, virological and immunological outcomes, including mortality in HIV-positive patients across Europe. Results showed that HIV-positive patients on a well-tolerated and fully suppressive cART regimen have a small risk of treatment failure occurring over the next 6 months, and could therefore be monitored less frequently. In contrast, patients who have spent a low percentage of time with a suppressed viral load whilst on cART, or who have recently rebounded may require more intensive monitoring after making a treatment switch. In patients who have achieved an initial response and tolerated the first three months of treatment, nevirapine efavirenz and lopinavir based cART regimens all have similar durability based on risk of all-cause discontinuation and development of serious clinical events. Starting cART earlier to reduce the proportion of patients with a low CD4 count may decrease the rate of developing many common non-AIDS related malignancies. Individuals in Eastern Europe had an increased risk of mortality from AIDS related causes, in part due to differences in use of effective cART.

The continued expansion of EuroSIDA will allow for more powerful and detailed analysis on specific clinical events, and further stratifications of variables of interest. Several findings from my research have already been incorporated into treatment guidelines and used in the clinical management of patients. It is hoped that the results from this thesis will continue to provide evidence that will help improve the long term durability of cART and clinical outcomes for HIV-positive patients.

Appendix I. EuroSIDA sample follow-up form 2012

EuroSIDA D29

December 2008 / January 2009

Center/patient code

Completed by (investigator's initials) ____ Date of completion of this form (dd-mm-yyyy) - -

Section A. Demography

Date of Birth (dd-mm-yyyy): Gender: 1=male, 2=female

Section B1. Basic clinical information (if dead see also section H)

Height (999cm = unknown) ____ cm

Enrollment weight (999.0=unknown) ____ kg

Most recently measured weight ____ kg

Time of measurement (dd-mm-yyyy) - -

Not available (x) ☐

First seen at the department

Present visit (dd-mm-yyyy) - -
(if dead, present visit = time of death, see section H)

Last follow-up recorded in database

Time of AIDS diagnosis (dd-mm-yyyy) if applicable - -
(section F and/or G should be completed)

Most recent measurement since last follow-up:	Not done	Date of measurement (dd-mm-yyyy)	Value	Unit
Systolic and diastolic blood pressure:	☐	- -	/	

Smoking status:	Yes	No	Unknown
Is the patient currently a cigarette smoker?	☐	☐	☐
Was the patient a cigarette smoker at last follow-up?	☐	☐	☐
If NO - has he/she ever smoked cigarettes?	☐	☐	☐
Have any first degree relatives (genetic mother, father, brother, sister) experienced myocardial infarction or stroke before the age of 50 years? Please fill out if blank or new information is available:	☐	☐	☐
For women: Pregnancy in 2008?	☐	☐	☐

If Pregnancy, outcome:

☐ Spontaneous abortion ☐ Birth of HIV+ ☐ Birth of child with unknown HIV status

☐ Medical abortion ☐ Birth of HIV- child ☐ Still pregnant

Section B2. Clinical events

Center/patient code

Have any of the following serious events occured since last follow-up?: (Please see diagnosis definitions in the instructions)	Yes	No	Unknown	If yes, date of event: (dd-mm-yyyy)
Cardiovascular events*:				
Carotic endarterectomy*:	☐	☐	☐	- -
Coronary angioplasty/stenting*:	☐	☐	☐	- -
Coronary artery by-pass grafting*:	☐	☐	☐	- -
Myocardial infarction*:	☐	☐	☐	- -
Stroke*:	☐	☐	☐	- -
Metabolic events:				
Diabetes Mellitus*:	☐	☐	☐	- -
Lipodystrophy:				
Is the patient experiencing loss of fat from extremities, buttocks or face?	☐	☐	☐	
Is the patient experiencing accumulation of fat in abdomen, neck, breasts or other defined location?	☐	☐	☐	
Other organ events:				
Avascular necrosis in the femural head (by imaging):	☐	☐	☐	- -
Bone fracture (specify location:_____):	☐	☐	☐	- -
Chronic Liver Disease (failure) *:	☐	☐	☐	- -
Pancreatitis (symptoms + objective evidence):	☐	☐	☐	- -
End Stage Renal disease (dialysis/transplantation)*:	☐	☐	☐	- -

*** Please complete relevant DAD case report forms**

Section C. Laboratory values

Most recently measured:	Not done	Fasting	Date of measurement (dd-mm-yyyy)	Value	Unit
Serum total cholesterol:	☐	☐	- -		
Serum HDL cholesterol:	☐	☐	- -		
Serum triglycerides:	☐	☐	- -		
Peak value since last visit for:					
Plasma glucose:	☐	☐	- -		
Most recently reported s-creatinine value:			- -		

ALL measured s-creatinine values since last follow-up:

Date of measurement (dd-mm-yyyy)	Value	Unit	Date of measurement (dd-mm-yyyy)	Value	Unit
- -			- -		
- -			- -		
- -			- -		

Section C. Laboratory values

Center/patient code

Most recently measured since last follow-up:	Date of measurement (dd-mm-yyyy)	Value	Unit
Haemoglobin level:	- -		
Platelet count:	- -		
ALT value:	- -		
AST value:	- -		
INR value:	- -		
Bilirubin value:	- -		
Alkaline phosphatase:	- -		
Parathyroid hormone:	- -		
Prostate-specific antigen (PSA):	- -		
Peak value since last follow-up for:			
S-amylase value:	- -		

	Date of measurement (dd-mm-yyyy)	Value	Date of measurement (dd-mm-yyyy)	Value
Two most recently reported CD4 cell counts:	- -		- -	
ALL CD4 cell counts measured since last follow-up:	- -		- -	
	- -		- -	
	- -		- -	

Two most recently reported HIV-RNA values:	Date of measurement (dd-mm-yyyy)	Value	Below level of detection (X)	Detection limit	Assay (see list)
Please note that values reported below level of detection have been registered as detection limit minus 1 eg. Below 200 would be recorded as 199	- -				
	- -				
ALL HIV-RNA values measured since last follow-up:	- -				
	- -				
	- -				
	- -				
	- -				
	- -				

Assay:

1 - Roche
2 - Roche ultrasensitive
3 - NASBA
4 - Chiron/bDNA
5 - TaqMan
9 - Other, please specify:_____

Hepatitis virology/serology	Last reported		Most recent test (if any later than the last recorded)					
	Date	Result	Date	Positive	Negative	Unknown	Value	Unit
Hepatitis B surface antigen (HBsAg)	-		- -					
Hepatitis B surface antibody (HBsAb)	-		- -					
Hepatitis B IgG core antibody (HBcIgG)	-		- -					
Hepatitis B core antibody total (IgM + IgG anti-HBc)	-		- -					
HBV-DNA	-		- -					
Hepatitis C antibody (Anti-HCV IgG)	-		- -					
HCV-RNA	-		- -					
HCV-genotype			- -					

Section D. Antiretroviral treatment within the last 5 year

Center/patient code

1. Has the patient ever received antiretrovirals? **If no, index X [ ]**

If yes, please update this section which should include all data on antiretrovirals used within the last 5 years. (go to section E for other treatment)

2. Previous and/or present antiretroviral therapy from within the last 5 years	**First time of initiation**	**Most recent ended treatment**			**Ongoing treatment**			***If stopped drug since last visit,**	
		Date of start	**Date of stop**	**Reason**	**Latest date of start**	**On drug at last visit** indexed by X	**On drug at present visit** indexed by X	a. last date of stopping	b. reason for discontinuation

3. All initiated and/or restarted antiretroviral therapy since last follow-up
Please use the list of codes below or write full drug name for any drugs not listed

_____	- -			- -	
_____	- -			- -	
_____	- -			- -	
_____	- -			- -	

4. Has any comment(s) on adherence to ART been made in the patient records since last follow-up?	**Date of comment (dd-mm-yyyy)**	**"<70%", "poor", "inadequate", "not good", "intermittent"**	**anything inbetween**	**">95%", "perfect", "full", "excellent"**
☐ No ☐ Yes - please give date of comment(s) to the right:	- -			
	- -			
	- -			

Combination drugs:

COM:	Combivir (AZT/3TC)
KIV:	Kivexa (3TC/ABC)
LPV:	Kaletra (LPV/RTV)
TRP:	Atripla (TEN/EMT/EFV)
TRU:	Truvada (TEN/EMT)
TZV:	Trizivir (AZT/3TC/ABC)

Entry (fusion/CCR5) inhibitors:

ENF:	Enfurvirtide (Fuzeon/T20)
MAR:	Maraviroc

Integrase inhibitors:

RGV:	Raltegravir

NRTIs:

ABC:	Abacavir
AZT:	Zidovudine
3TC:	Lamivudine
D4T:	Stavudine
DDI:	Didanosine
EMT:	Emtricabine
TEN:	Tenofovir

NNRTIs:

EFV:	Efavirenz
ETV:	Etravirine (TMC-125)
NVP:	Nevirapine

PIs:

AMP:	(Fos-)Amprenavir
AZV:	Atazanavir
DAV:	Darunavir (TMC-114)
IDV:	Indinavir
LPV:	Lopinavir/r

PIs:

NFV:	Nelfinavir
RTV:	Ritonavir
SQH:	Saquinavir hard gel capsule
TPV:	Tipranavir

Other:

PBT:	Participant in blinded trial

***Reason for discontinuation:**

1: Treatment failure (i.e. virological, immunological and/or clinical failure)	5: Toxicity, predominantly from abdomen/GI tract	7: Toxicity, predominantly from kidneys	90: Toxicity, not mentioned above
2: Abnormal fat redistribution	5.1: Toxicity - GI tract	8: Toxicity, predominantly from the endocrine system	91: Patient's wish/decision, not specified above
3: Concern of cardiovascular disease	5.2: Toxicity - Liver	8.1: Diabetes	92: Physician's decision, not specified above
3.1: Dyslipidaemia	5.3: Toxicity - Pancreas	9: Haematological toxicity	93: STI - Structured Treatment Interruption
3.2: Cardiovascular disease	6: Toxicity, predominantly from nervous system	10: Hyperlactataemia/ lactic acidosis	94: Other causes, not specified above
4: Hypersensitivity reaction			94.1: Out of stock
			99: Unknown

Section E1. Treatment against infections

Center/patient code

1. Has the patient ever received drugs to prevent (both primary prophylaxis and maintenance therapy) or treat infections? **If no, index X [ ]**

If yes, complete this section (For drugs against opportunistic infections, only those used after enrollment in EuroSIDA).

2. Previous and/ or present therapy	First time of treatment	Most recent ended treatment: Date of start	Most recent ended treatment: Date of stop	Latest date of start (dd-mm-yyyy)	On drug at last clinical visit (indexed by X)	On drug at present visit (indexed by X)	If not on drug at pres. visit indicate date of stopping (dd-mm-yyyy)
Nothing previously reported							
3. All initiated and/or restarted therapy since last follow-up							
Please use the list of codes below or write full drug name for any drugs not listed							
_____				- -			- -
_____				- -			- -

CMV/HSV drugs

CIDO:	Cidofovir
CONA:	Continous Acyclovir
CONF:	Continous Famciclovir
CONV:	Continous Valaciclovir
GANC:	Ganciclovir
FOSC:	Foscarnet

Fungal drugs

AMPH:	Amphotericin B, i.v.
CASP:	Caspofungin
FLUC:	Fluconazole
ITRA:	Itraconazole
KETO:	Ketoconazole
VORI:	Voriconazole

HBV drugs

ADEF:	Adefovir dipivoxil
ENTE:	Entecavir
TELB:	Telbivudine

HCV drugs

PINT:	Peg-Interferon
RIBA:	Ribavirin

Immunomodulating therapy

IL2:	Interleukin 2
GCSF:	G-CSF
INTF:	Interferon
PINT:	Peg-Interferon

Mycobacterium drugs

CLAR:	Clarithromycin/azithromycin

Mycobacterium drugs

ETHA:	Ethambutole
ISON:	Isoniazide
PYRA:	Pyrazinamide
RIFA:	Rifabutine
RIFM:	Rifampicine
STRE:	Streptomycin

PCP/TOXO drugs

ATOV:	Atovaquone
BACT:	Bactrim (cotrimoxazole)
CLIN:	Clindamycin
DAPS:	Dapsone
PENT:	Pentamidine neb./inj.
PYRI:	Pyrimethamine
SULP:	Sulphadiazine

Section E2. Treatment related to risk of cardiovascular disease

1. Has the patient ever received medication related to risk of cadiovascular disease? **If no, index X [ ]**

If yes, complete this section .

2. Previous and/ or present treatment	First time of treatment	Most recent ended treatment: Date of start	Most recent ended treatment: Date of stop	Latest date of start (dd-mm-yyyy)	On drug at last clinical visit (indexed by X)	On drug at present visit (indexed by X)	If not on drug at pres. visit indicate date of stopping (dd-mm-yyyy)
Anabolic steroids/ appetite stimulants:	-	-	-	- -			- -
ACE inhibitors:	-	-	-	- -			- -
Antihypertensive agents, others:	-	-	-	- -			- -
Anti platelets:	-	-	-	- -			- -
Insulin or derivatives hereof:	-	-	-	- -			- -
Oral antidiabetic agents:	-	-	-	- -			- -
Lipid lowering agents:							
Statins:	-	-	-	- -			- -
Fibrates:	-	-	-	- -			- -
Other/unspecified:	-	-	-	- -			- -

Section F1. Severe opportunistic infections

Center/patient code

1. Any previous or new severe opportunistic infections (including AIDS defining)? **If no index X [ ]**
If yes, complete this section

2. Previously reported:	**Time of onset (dd-mm-yyyy)**	**Way of diagnosis (tick box)** Definitive	Presumptive	Autopsy
Nothing previously reported				
3. New severe opportunistic infections:				
_____	[- -]	[ ]	[ ]	[ ]
_____	[- -]	[ ]	[ ]	[ ]

DEM:	AIDS dementia complex	LEIS:	Leishmaniasis, visceral
BCNE:	Bacterial pneumonia, recurrent (>2 episodes within 1 year)	MCDI:	Microsporidosis diarrhoea (duration >1 month)
CANO:	Candidiasis, oesophageal	MC:	Mycobact. avium complex (MAC) or Kansasii, extrapulm.
CRCO:	Cryptococcosis, extrapulm.	MCP:	Mycobact. tuberculosis, pulm.
CRSP:	Cryptosporidosis (duration > 1 month)	MCX:	Mycobact. tuberculosis, extrapulm.
CMVR:	Cytomegalovirus (CMV) chorioretinitis	MCPO:	Mycobact. pulm., other type, specify
CMVO:	CMV - other location, specify	MCXO:	Mycobact. extrapulm., other type, specify
HERP:	Herpes simplex virus ulcers (duration >1 month) or pneumonitis/esophagitis	PCP:	Pneumocystis jiroveci pneumonia (PCP)
		LEU:	Progressive multifocal leucoencephalopathy
HIST:	Histoplasmosis, extrapulm.	SAM:	Salmonella bacteriaemia (non-typhoid) (>2 episodes)
WAST:	HIV wasting syndrome	TOX:	Toxoplasmosis, brain
ISDI:	Isosporiasis diarrhoea (duration >1 month)	FBLS:	Focal brain lesion

Section F2. Other severe infections - requiring hospitalisation

1. Any previous or new other severe infections? **If no index X [ ]**
If yes, complete this section

2. Previously reported:	**Time of onset (dd-mm-yyyy)**	**Way of diagnosis (tick box)** Definitive	Presumptive	Autopsy
Nothing previously reported				
3. New severe infections - requiring hospitalisation: **Please use codes below or write the full type for any severe infection not listed**				
_____	[- -]	[ ]	[ ]	[ ]
_____	[- -]	[ ]	[ ]	[ ]

BACT:	Bacteremia	MENI:	Meningitis	PERI:	Peritonitis	PYEL:	Pyelonephritis
ENDO:	Endocarditis	OSTI:	Ostitis	PNEU:	Pneumonia		Please specify full name for any other severe infections

Section G1. AIDS defining malignancies

Center/patient code

1. Any new AIDS defining malignancies? **If no index X [ ]**
If yes, complete this section

2. Previously reported:	**Time of onset (dd-mm-yyyy)**	**Way of diagnosis (tick box)** Definitive	Presumptive	Autopsy
Nothing previously reported				
3. New AIDS defining malignancies:				
_____	[- -]	[ ]	[ ]	[ ]
_____	[- -]	[ ]	[ ]	[ ]

KS: Kaposi´s sarcoma
CRVC: Cervical cancer

Non-Hodgkin lymphoma:
NHLB: Burkitt (Classical or Atypical)
NHLI: Diffuse large B-cell lymphoma (Immunoblastic or Centroblastic)
NHLU: Unknown/other histology
NHLP: Primary brain lymphoma (at diagnosis, involvement of the central nervous system without other organ affection - regardless of histology)

Section G2. Non-AIDS defining cancers

1. Any new non-AIDS defining cancers?* **If no index X [ ]**
If yes, complete this section

2. Previously reported*:	**Time of onset (dd-mm-yyyy)**	**Way of diagnosis (tick box)** Definitive	Presumptive	Autopsy
Nothing previously reported				
3. New non-AIDS defining cancers*: **Please use codes below or write the full type for any cancer not listed**				
_____	[- -]	[ ]	[ ]	[ ]
_____	[- -]	[ ]	[ ]	[ ]

*** Please complete relevant DAD case report forms**

ANUS: Anus cancer
BLAD: Bladder cancer
BRCA: Breast cancer
CERV: Cervical dysplasia/carcinoma in situ
COLO: Colon cancer
COTC: Connective tissue cancer
HDL: Hodgkin lymphoma
KIDN: Kidney cancer

Leukemia:
ALL: Acute lymphoid
AML: Acute myeloid
CLL: Chronic lymphoid
CML: Chronic myeloid
LIPC: Lip cancer
LIVR: Liver cancer
LUNG: Lung cancer
MALM: Malignant melanoma
MULM: Multiple myeloma

Metastasis:
MESC: of squamuos cell carcinoma
MEAC: of adenocarcinoma
MEOC: of other cancertype
PENC: Penile cancer
PROS: Prostate cancer
RECT: Rectum cancer
STOM: Stomach cancer
TESE: Testicular seminoma
UTER: Uterus cancer

Section H. For patients who died

1. Time of death [- -] **(dd-mm-yyyy)**

2. Presumed ilness causing terminal condition, index (X)

- [ ] (1) Myocardial Infarction
- [ ] (2) Stroke
- [ ] (3) Other cardiovascular disease
- [ ] (4) Symptoms caused by mitochondrial toxicity
- [ ] (4.1) Lactic Acidosis
- [ ] (5) Complications to diabetes mellitus
- [ ] (6) Pancreatitis
- [ ] (7) Liver failure
- [ ] (7.1) Hepatitis related
- [ ] (7.2) Liver failure not related to hepatitis or mitochondrial toxicity
- [ ] (8) HIV-related
- [ ] (8.1) AIDS defining event (which? _____)
- [ ] (8.2) Invasive bacterial infection
- [ ] (9) Renal Failure
- [ ] (10) Suicide
- [ ] (11) Drug overdose
- [ ] (90) Other, specify: _____
- [ ] (99) Unknown

Please complete the case report form for the CoDe project

Appendix II. The EuroSIDA study group

The multi-centre study group on EuroSIDA (national coordinators in parenthesis).

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Appendix III CoDe Case Report Form

Cause of Death Form (CRF)

CoDe

*Study:_____

*Patient ID code: _____

*Date of death : ___–_____– ___
(dd/mmm/yy eg 01-FEB-05)

Section 1 ♦ Background demographics

- * **A.** Year of birth (yyyy) _ _ _ _ **B**. Gender : male female
- **C.** Height (cm) : _ _ _ **D.** Weight (kg) : _ _ _ _ (most recent before death) **E.** Date :_ _ – _ _ _ – _ _ (dd-mmm-yy; weight measured)

Section 2 ♦ What data sources were available for the completion of this form? (please mark all that apply)

• **A.** Hospital files	Yes, complete	Yes, incomplete	No
• **B.** Outpatient clinic chart	Yes, complete	Yes, incomplete	No
• **C.** Autopsy report	Yes, complete	Yes, incomplete	No

If other, specify:

- **D.** Registry
- **E.** Obituary
- **F.** Patient's relatives or partner
- **G.** Patient's medical provider
- **H.** Nursing home
- **I.** Other:_____

Section 3 ♦ Risk factors:

A. Ongoing risk factors in the year prior to death:

1. Cigarette smoking	Yes	No	Unknown
2. Excessive alcohol consumption	Yes	No	Unknown
3. Active illicit injecting drug use	Yes	No	Unknown
4. Active illicit non-injecting drug use	Yes	No	Unknown
5. Opiate substitution (methadone)	Yes	No	Unknown

Section 4 ♦ Co-morbidities:

A. Ongoing chronic conditions:

1. Hypertension	Yes	No	Unknown
2. Diabetes mellitus	Yes	No	Unknown
3. Dyslipidemia	Yes	No	Unknown
B. Prior cardiovascular disease (*myocardial infarction, stroke or invasive cardiovascular procedure)*	Yes	No	Unknown
C. History of depression	Yes	No	Unknown
D. History of psychosis	Yes	No	Unknown

E. Liver disease:

1. Chronic elevation of liver transaminases	Yes	No	Unknown
2. Chronic HBV infection	Yes	No	Unknown
3. Chronic HCV infection	Yes	No	Unknown
4. HDV infection	Yes	No	Unknown
5. History of previous liver decompensation	Yes	No	Unknown
6. Clinical signs of liver failure in the 4 weeks before death	Yes	No	Unknown
7. Liver histology available (ever)	Yes	No	Unknown

If Yes, please indicate: the date of most recent biopsy _ _ _ – _ _ _ _ – _ _ _ _ (dd-mmm-yy eg 01-FEB-05) the stage of fibrosis (0-4): ☐

*** Please note that if any of the mandatory fields remain empty the CRF will not be registered**

Cause of Death Form

CoDe

*Study:___ _____

*Patient ID code: _____

Section 5 ♦ Cause of death

A. Was the death sudden? Yes No Unknown

B. Was the death unexpected? Yes No Unknown

C. Please complete the table below by recording all illnesses and conditions (acute and chronic) or injuries that the patient had at the time of death.

	Illness / Condition / Injury (text)	**Date of onset** dd/mmm/yy (eg 01-FEB-05)	**Certainty of diagnosis[a]**		
			Definite	Likely	Possible
1.		____ _____ ____			
2.		____ _____ ____			
3.		____ _____ ____			
4.		____ _____ ____			
5.		____ _____ ____			
6.		____ _____ ____			
7.		____ _____ ____			
8.		____ _____ ____			
9.		____ _____ ____			

[a]**Certainty of Diagnosis: Definite =** 95-100% certainty, **Likely =** 80-95% certainty, **Possible =** 50-80% certainty

* **D. Brief narrative of the sequence of events leading to death (please include means of diagnosis of illnesses):**

_____

_____

_____

_____

_____

_____

_____

_____

E. In summary, the causal relation between the conditions leading to death was (complete this section with the corresponding number from table C above):

1. Condition that directly caused death (immediate cause):_____ ____

2. Due to or as a consequence of:_____

3. Due to or as a consequence of:_____

4. Condition that initiated the train of morbid events (the underlying condition):_____

* **Please note that if any of the mandatory fields remain empty the CRF will not be registered**

Section 6 ♦ Post-mortem / Autopsy:

A. Has autopsy been performed: Yes No Unknown

B. Did the autopsy reveal any evidence of intoxication?

Yes, with the agent: _____ No Unknown

Please provide a brief summary of the findings from the autopsy report (please also include a copy of the full report):

Section 7 ♦ ART and laboratory values prior to death

A. Has the patient EVER received ART: Yes No Unknown

If YES, when was ART started (in months before death):

≤ 1 month before ≤ 3 months before ≤ 6 months before More than 6 months before

B. Did the patient receive ART at the time of death? Yes No Unknown

- ***If No***, Date of stopping __ __ – __ __ __ – __ __ **(dd/mmm/yy eg 01-FEB-05)**

C. Laboratory values (please complete all fields where data is available)

Laboratory values	Time	Value	Unit	Date dd/mmm/yy (eg 01-FEB-05)
CD4+ cell count	1. Most recent prior to last stopping ART		Cells/mm³	__ __ – __ __ __ – __ __
	2. Most recent prior to death		Cells/mm³	__ __ – __ __ __ – __ __
HIV RNA	1. Most recent at time of stopping ART		Copies/mL	__ __ – __ __ __ – __ __
	2. Most recent prior to death		Copies/mL	__ __ – __ __ __ – __ __
Haemoglobin	Most recent prior to death		/	__ __ – __ __ __ – __ __

*** Please note that if any of the mandatory fields remain empty the CRF will not be registered**

Cause of Death Form

CoDe

*Study:___ _____

*Patient ID code: _____

Section 8 ♦ Adverse effects to any type of medical treatment:

A. Was the death considered to be related to a medical treatment? Yes No Possibly

B. The suspected relation was to: Antiretroviral treatment Other medical treatment

Please provide a brief narrative of the suspected association including the name of the medication and the date of starting:

Please refer to the 'CoDe instructions' for definitions and guidelines for the completion of this form

Completed by: Name (in print):_____

Position : Physician Nurse Other (describe) : _____

Directly involved in the medical care of the patient around the time of death? Yes No

Date (dd/mmm/yy)**:** __ __ – __ __ __ – __ __

Signature: _____

*** Please note that if any of the mandatory fields remain empty the CRF will not be registered**

Appendix IV. Does less frequent routine monitoring of patients on a stable, fully suppressed cART regimen lead to an increased risk of treatment failure?

Appendix not available due to copyright restrictions

Appendix V. History of viral suppression on combination antiretroviral therapy as a predictor of virological failure after a treatment change

Appendix not available due to copyright restrictions

Appendix VI. A comparison of the long-term durability of nevirapine, efavirenz and lopinavir in routine clinical practice in Europe: a EuroSIDA study.

Appendix not available due to copyright restrictions

Appendix VII. Relationship between current level of immunodeficiency and non-AIDS defining malignancies.

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