Factors associated with different responses to combination antiretroviral therapy in an observational cohort study of HIV-1 infected patients

THESIS

presented for the

DEGREE

of

DOCTOR OF PHILOSOPHY

in the Faculty of Medicine

(Field of study: Epidemiology)

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May 2008

Declaration

I, Wendy Patricia Bannister, 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

The introduction of combination antiretroviral therapy (cART) into clinical practice for the treatment of HIV in 1995-1996 has led to dramatic reductions in mortality and morbidity. Factors linked to a positive response to therapy include a potent and tolerable regimen, good adherence and low levels of HIV drug resistance. The aims of this thesis were to investigate factors potentially associated with different responses to cART measured using virological and immunological predictive markers, and also to look at the development of toxicities to a specific regimen. The analyses were based on data from the EuroSIDA study, which is an observational cohort of 14,310 HIV-1 infected patients from Europe, Israel and Argentina. Data collected includes demographic history, CD4 cell counts, viral loads and details of all drugs taken. EuroSIDA also collects viral sequence data for its resistance database.

Investigation into virological response to first-line cART across geographical regions found evidence of variation, which was most apparent in early-cART years. Virological response improved over calendar time in all regions, especially in East Europe. Neither HIV-1 subtype nor transmitted drug resistance (TDR) were found to be associated with virological response to cART, however statistical power was limited. A significantly decreased risk of virological failure was found in patients starting efavirenz compared with nevirapine, which did not appear to be explained by baseline drug resistance. Finally, incidence of abacavir discontinuation due to a hypersensitivity reaction side effect of the drug appeared to be higher in patients starting abacavir as part of first-line therapy but decreased in recent years.

In conclusion, this thesis has compared a variety of different responses to antiretroviral therapy across subsets of a large heterogeneous population. It is hoped that these findings will contribute to research in monitoring trends in response to therapy and provide insight into association with the genetic variability of the virus.

Acknowledgements

This page gives me the chance to thank my supervisors Dr Amanda Mocroft and Dr Alessandro Cozzi-Lepri for all their support, guidance and encouragement throughout the preparation of this thesis. I really appreciate all the time and effort they put into reading the countless drafts, giving me helpful advice and constructive feedback, and in preparing tough question sessions to get me thinking!

This work would not have been possible without the extensive work of the entire study group who work on the EuroSIDA study. I would like to thank Professor Jens Lundgren for his clinical input into suggestions and ideas, all the EuroSIDA investigators, the team from the co-ordinating centre at the Copenhagen HIV Programme (especially Dr Ole Kirk, Jesper Kjær and Dr Nina Friis-Møller) and of course, the patients enrolled in EuroSIDA, for making it possible for me to carry out these analyses.

In addition, I would like to give my thanks to the HIV Epidemiology and Biostatistics Group at the Royal Free University College Medical School, in particular Professor Andrew Phillips for his input into the drug resistance aspects of this thesis, as well as Professor Caroline Sabin, Dr Fiona Lampe, Dr Colette Smith and Rebecca Lodwick for devoting time to reading and commenting on specific chapters. My other colleagues also gave me lots of encouragement: Dr Zoe Fox, Elaine Harris, Loveleen Bansi, Andy Gilroy Thomson, Dr Teresa Hill and Joanne Reekie. Last but not least, many thanks go to Jani Puradiredja for sharing our slightly chaotic office and for keeping me smiling throughout the writing up process.

Finally, I am very grateful to my parents for their continued support in my decisions, to my exceptional friends who lent me ears and shoulders when most needed, and to Antonye for his patience, emotional support and for making me cups of tea.

Contents

*Appendices removed due to copyright restrictions.

List of Figures

*Figures removed due to copyright restrictions.

List of Tables

Chapter 1. Introduction

1.1 Current worldwide statistics

Human immunodeficiency virus (HIV) is the virus responsible for a worldwide pandemic. It is the cause of the condition *acquired immune deficiency syndrome* (AIDS), which has killed more than 25 million people since the first diagnosis in 1981 [1]. According to the latest statistics from the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organisation (WHO) as of December 2007, 33.2 million (range: 30.6-36.1 million) individuals worldwide are estimated to be living with HIV, of which 15.4 million (46%) are women and 2.5 million (8%) are children under the age of 15 years [2]. This total is over 10 million more than 10 years ago and has been rising steadily each year (Figure 1.1) [2]. In 2007 alone, there were 2.5 million (range: 1.8-4.1 million) new infections of HIV and 2.1 million (range: 1.9-2.4 million) deaths from AIDS worldwide [2].

Figure 1.1: Estimated number of people living with HIV globally, 1990-2007.

Source: UNAIDS/WHO. AIDS epidemic update December 2007 [2].

A global view of the prevalence of HIV infection in 2007 is illustrated in the AIDS epidemic update December 2007 (UNAIDS/WHO) below (Figure 1.2) [2]. This highlights the concentrated epidemic in Africa, particularly in Southern parts, but also the relatively high prevalence in Eastern Europe, compared to Western and Central Europe.

Figure 1.2: Adults and children estimated to be living with HIV, 2007.

Source: UNAIDS/WHO. AIDS epidemic update December 2007 [2].

The data analysed in this thesis are from the large observational study, EuroSIDA, which collects data from HIV infected patients across Europe and from a minority in Argentina and Israel. UNAIDS/WHO report that in Western and Central Europe, in 2007, there were an estimated 760,000 individuals living with HIV. In Eastern Europe and Central Asia, where nearly 90% of HIV infections are in the Russian Federation and Ukraine, there were 1.6 million HIV infected individuals in 2007. During this year, a total of 31,000 new infections and 12,000 deaths occurred in Western and Central Europe, and 150,000 new infections and 55,000 deaths occurred in Eastern Europe and Central Asia. Eastern Europe has experienced one of the steepest increases in the number of people living with HIV over the past six years; this figure rose by over 150% from 630,000 in 2001 to 1.6 million in 2007 [2].

To understand how HIV has spread so rapidly worldwide, it is important to understand how the virus infects the human immune system and uses it to replicate.

1.2 Immunology and virology

1.2.1 The immune system

Infection with HIV leads to the progressive breakdown of the immune system in humans [3,4]. The immune system protects the body from potentially harmful substances by activating an immune response. Cells invading the body such as viruses and bacteria carry proteins called antigens that are recognised by white blood cells in the immune system. The white blood cells act by either directly attacking the foreign cells or by helping other cells in the immune response to destroy them [5]. This response is co-ordinated by a certain type of white blood cell called a *CD4+ "T-helper"*

lymphocyte [6]. HIV infected (HIV positive) individuals experience a significant depletion of these CD4 cells, which means that the body is left without defence and is vulnerable to infection [7,8]. CD4 cells die naturally as a consequence of immune activation but in healthy individuals the body produces more to take their place. How the vast depletion caused by HIV occurs is still unclear and could be caused directly or indirectly by the virus [7-9].

1.2.2 HIV replication

HIV infection is characterised by a very high turnover of virus, as many as one billion new virions produced per day (and over 10 million newly infected host cells a day) [10]. This occurs through the process of *reverse transcription*. HIV is a human retrovirus, which is defined by its capability to produce an enzyme called reverse transcriptase, and this enzyme catalyses the transformation of the viral genetic material, single-stranded RNA (ribonucleic acid), into double-stranded DNA (deoxyribonucleic acid) [11-13]. The virus contains two strands of RNA, which stores the genetic code for manufacturing viral proteins [14].

RNA is made up of a string of chemical units called *nucleotide bases*, of which there are four types: adenine (A), uracil (U), cytosine (C) and guanine (G). The specific position of a single base is referred to as a *site* [15]. There are approximately 9200 bases in HIV RNA [16] and the order in which they appear defines the genetic sequence. Much of the sequence has no function, however, certain triplets of bases (codons) encode for the production of one of 20 different amino acids, which are the building blocks of proteins [17]. An RNA *gene* comprises a section of RNA that encodes for one protein, for example the RT gene is responsible for the production of reverse transcriptase. The complete set of genes is called the *genome* [15].

In a similar way, DNA, which is contained in every cell of every organism, stores the genetic code for manufacturing proteins to allow the organism to grow and function [15]. When an individual is infected with HIV, the virus invades human cells by fusing with the cell surface. HIV RNA enters the human cell and the process of reverse transcription follows. The resulting HIV DNA is then able to enter the central nucleus of the host cell [4,13,18,19]. A further viral enzyme called integrase [20] allows integration of the viral DNA with the host cell's DNA [13,18] leading to the production of viral proteins including the enzyme protease, which is needed to convert other HIV proteins into functional forms. Together the proteins form new HIVs (virions) that leave the cell, free to invade others [4,13,18,19]. This is the way in which the virus replicates itself and multiplies in the body. Sometimes the host cell can remain in a resting state

during which time the cell is not producing any new virus or exhibiting signs of infection, i.e. it is latently infected. Thus HIV is able to remain undetected by the immune system that would normally destroy it [9,13].

1.2.3 Target host cells

CD4 cells are the major (but not exclusive) targets for HIV as the virus carries a glycoprotein (gp120) that can bind with the CD4 receptors present on the surface of these cells [21,22] in the presence of a co-receptor, usually either CCR5 or CXCR4 [23,24]. If the infected cells are active then viral replication is effective, however, the majority of CD4 cells in the body are in a resting state. Studies have shown that reverse transcription can still occur in these cells [25,26]. This provides a lifelong reservoir of latently infected CD4 cells, which makes the virus impossible to eradicate completely by the anti-HIV (antiretroviral) drugs that are currently available [27,28]. The HIV replication process is illustrated in Figure 1.3.

Figure 1.3: Replication cycle of HIV.

Figure not available due to copyright restrictions.

Source: Weiss (2001) [19].

1.2.4 Monitoring HIV progression

Quantitative measurements of the amount of circulating virus, i.e. how much of the virus is in the blood, as well as counts of CD4 cells, are the main predictive markers used to indicate the clinical progression of HIV infection [29-32]. The CD4 count is the

measurement of the number of CD4 cells detected in a cubic millimetre of blood (cells/mm^3). A healthy HIV negative individual can be expected to have a CD4 count of between 540 and 1120 cells/mm^3 (mean: 830 cells/mm^3) [33], although this can vary depending on factors such as age, gender and smoking status [34,35]. In untreated HIV positive individuals the CD4 count gradually declines over a number of years at a rate of around 50-80 cells/mm^3 per year [36-39] and if the CD4 count drops below 500 cells/mm^3 this indicates that the immune system is beginning to fail. The risk of AIDS related illness greatly increases if the CD4 count drops below 200 cells/mm^3 and individuals are advised to start antiretroviral therapy (ART) and disease specific prophylaxis (preventative treatment) [40-42].

Independent from this, another prognostic marker of HIV progression is the viral load, which refers to the amount of HIV genetic material (RNA) present in the blood plasma (the liquid part of the blood) and is measured in copies of RNA per millilitre (mL) [30,43,44]. The first assay used to measure this was the Amplicor HIV-1 Monitor Test approved by the United States Food and Drug Administration (US FDA) in 1996 [45,46]. This was used in clinical practice and could quantify HIV RNA to as low as 1000 copies/mL [47]. The most widely used ultra-sensitive viral load assays at present use one of three different methods to quantify HIV RNA in blood plasma, namely, reverse transcriptase-polymerase chain reaction (RT-PCR), nucleic acid sequence-based amplification (NASBA) and the branched DNA (bDNA) signal amplification technique. These have been found to give broadly consistent results and limits of detection are as low as 40 copies/mL [48,49].

1.3 HIV transmission

HIV can be transmitted via blood, semen, cervical secretions and breast milk, which puts individuals engaging in unprotected sexual intercourse, injecting drug users and babies born to HIV positive mothers at risk. Before 1985, when HIV testing became available, HIV was also transmitted via the donation of blood products, organs and tissue, which accounted for approximately 2% of AIDS cases in the USA [50,51]. This put individuals with haemophilia particularly at risk. For example, in the UK, between 1979 and 1985, 1227 of the 6278 haemophilic individuals (19.5%) were infected with HIV [52].

1.3.1 Sexual transmission

The most common mode of transmission worldwide is sexual intercourse [53,54], even though the probability of infection per contact is lower than that through other routes of exposure, as low as one in a thousand for female-to-male exposure [53,55].

Transmission susceptibility varies greatly according to a number of factors. It is increased when the infected partner's viral load is particularly high. This occurs when the individuals are in the primary stage or the late stage of infection in the absence of ART [55-57]. The presence of other sexually transmitted diseases (STDs), especially local infections and ulcerations in the genital region, has also been found to be a strong predictor of transmission [53,57-59]. A review of the scientific data investigating the relationship between the presence of STDs in HIV infected individuals to the risk of HIV transmission found that STDs increased the risk by two to five times, but ranging up to as much as 23.5 times [58]. This review included studies looking at ulcerative and non-ulcerative STDs, male-to-female, female-to-male and male-to-male transmission. Other factors associated with the risk of transmission (either positively or negatively) have been found to be genetic factors, ART, cervical ectopy (condition where cells extend beyond uterus into cervix), male circumcision, contraception and menstruation [54,55,59,60].

1.3.1.1 Heterosexual transmission

The per-contact risk of being infected with HIV through heterosexual intercourse with an HIV infected person has been estimated to be between 0.01% and 0.14% from studies of discordant couples (where one partner is infected with HIV and the other is not) in the USA and Europe [55,61-63]. For example, a ten-year study in northern California found that the per-contact risk of male-to-female transmission was 0.0009 (95% confidence interval (CI): 0.0005-0.001) and that male-to-female transmission was eight times more efficient than female-to-male [63].

1.3.1.2 Homosexual transmission

Estimates for the per-contact risk of transmission through male-to-male intercourse have been found to vary greatly. A study in the USA estimated the risk of anal transmission (with ejaculation) in men who have sex with men (MSM) during the asymptomatic phase of infection to be between 0.5% and 3.0% [64]. Another study found that during primary infection the risk was as high as 10-30% per contact [65]. Risk through orogenital contact (oral sex) was estimated in a cohort study of MSM predicting a 0.04% chance of infection through receptive oral sex with ejaculation [66]. This however included men whose HIV infection status was unknown. Factors likely to be associated with an increased risk of HIV transmission through oral sex are oral trauma, sores, inflammation, allergy, concomitant sexually transmitted infections, ejaculation in the mouth and systemic immune suppression [67].

1.3.2 Transmission via blood

The most efficient mode of transmission is through the transfer of blood. Intravenous drug users (IDUs) are particularly at risk if they regularly share or reuse needles and estimates of the risk of infection per injection with a contaminated syringe are between 0.7% and 0.8% [68,69]. Health care workers who come into contact with HIV infected blood and body fluids are also at risk. Transmission through needle stick injury has been estimated to be 0.3% per injury [70].

1.3.3 Mother-to-child transmission

HIV can also be passed on from mother to child (vertical transmission) either in the womb, at birth or through breastfeeding. The risk of transmission in the absence of any interventions has been estimated to be 15-25% in non-breastfeeding populations and 25-40% in breastfeeding populations, depending on a number of risk factors [71]. A systematic review of five placebo-controlled trials showed that the use of the drug zidovudine as prophylactic antiretroviral treatment reduced the risk of transmission by 43% [72]. In the same review, it was found that compared to a zidovudine regimen during labour and after delivery, nevirapine monotherapy given to mothers and babies as a single dose resulted in a 40% lower risk of transmission. Current guidelines by the WHO recommend the use of combination antiretroviral therapy (cART) to help prevent vertical transmission [73], which is a potent and effective combination of drugs, detailed later in section 1.6. However, a single dose of the drug nevirapine during and after delivery is advised as the absolute minimum in the absence of adequate resources to provide cART [74].

If the mother does not breastfeed, takes prophylactic ART during pregnancy and opts for a caesarean section delivery, the risk may be reduced to less than 1% [75-77]. Risk of vertical transmission has been shown to increase with maternal viral load [78,79]. The odds of vertical transmission, adjusted for significant covariates such as use of ART, were almost twice as high for mothers with a maternal viral load of 10,000-50,000 copies/mL compared to 1000-10,000 copies/mL, and almost four times as high for those with a viral load of more than 50,000 copies/mL [78].

A study investigating infection through breastfeeding estimated the risk to be 29% (95% CI: 16-42%) in mothers infected during or after delivery, and 14% (95% CI: 7-22%) in mothers with established infection [80].

1.3.4 Modes of transmission across Europe

Epidemics of HIV have arisen in populations throughout Europe with changing trends in the routes of HIV transmission. Figures from 2005 reported by the EuroHIV surveillance network showed that in the European Union, heterosexual contact accounted for 55% of all HIV transmissions, homo-/bisexual contact accounted for 34% and injecting drug use (IDU) for 10% [81]. These figures exclude Italy and Spain where surveillance data were not available. Together with Portugal, these were previously sites of epidemics amongst IDUs, however recent data from certain regions of Italy and Spain show a decline in HIV in this exposure group and an increase in heterosexual contact as the major mode of transmission [81,82]. The predominant mode of transmission varies between countries within Europe, for example in Germany, Greece, Iceland and the Netherlands, the prevailing transmission group is homo-/bisexual men [82]. In Eastern Europe, the major exposure group is IDUs [81]. A major increase in IDU followed the collapse of the Soviet Union during the 1990s when Afghanistan was the world's largest opium producer [83]. However this appears to have declined over the past few years, especially in the Russian Federation. In 2001, the number of new diagnoses amongst IDUs reached a peak of over 55,000, whereas in 2005 it had decreased to less than 20,000 [81].

1.4 Stages of infection

Although the course of HIV infection can vary widely between individuals, there are generally three main stages that occur in HIV positive individuals in the absence of antiretroviral treatment, characterised by virological and immunological events. [3,84-86]. These are described over the next few paragraphs. A typical plasma viral load and CD4 count over the duration of infection are shown in an illustration in Figure 1.4. However, recent studies have found evidence that examination of viral load and CD4 count from peripheral blood alone may be misleading to understanding the course of infection. The major sites of HIV infection are in the gut and mucosal tissues where CD4 cells are selectively infected and destroyed within days [87,88]. This could have major implications for treatment strategies, as to preserve these intestinal CD4 cells, therapy needs to be started immediately after infection. One study provided a historical comparison to compare viral load three years after HIV infection between individuals who did not start therapy and those who started cART in the first stage of HIV infection (primary HIV infection detailed below) [89]. Subjects in the CASCADE (Concerted Action on SeroConversion to AIDS and Death in Europe) study who had viral load measurements available before starting ART were compared to subjects from the Quest trial who received cART for an average of two and a half years. The results showed that cART taken during primary HIV infection might increase the probability of

a low viral load after treatment discontinuation, but the difference appeared modest and could represent a delay of natural history of the virus rather than a long-term benefit of treatment.

Figure 1.4: Typical course of HIV infection.

Source: Pantaleo (1993) [86].

1.4.1 Primary HIV infection

The first stage of HIV infection is known as *primary* or *acute* HIV infection (PHI), lasting approximately two to twelve weeks. During this time, the patient experiences a sharp rise in plasma viral load to as much as 10^7 copies/mL [90,91], before declining rapidly to a 'set point' [3,85]. This can differ greatly from person to person but in most people it is between 10^3 and 10^5 copies/mL [29,43,92]. At the same time, the CD4 count (in the blood) experiences an acute drop by as much as 50% [3,84-86]. This rises back towards normal levels (540-1120 cells/mm^3 [33]) within four to five months [3].

During this immune response, antibodies are produced that help to fight the virus and it is the presence of these antibodies that is detected in tests used to determine whether or not an individual has HIV. Antibody tests were traditionally carried out on blood samples but now saliva or urine samples may also be used [93]. The time when the body starts producing antibodies is called seroconversion, which follows the primary infection period. Antibodies can be detected in a sample one to six months following seroconversion depending on the sensitivity of the test used [94,95].

Individuals in the PHI stage are particularly infectious because of the initial high viral load [96]. Clinical symptoms have been estimated to emerge two to four weeks after exposure in 40-90% of individuals [97,98] and include fever, lethargy and malaise, anorexia, sore throat, myalgias (muscular pain), headaches, arthralgias (pain in the joints), weight loss, swollen glands, retro-orbital pain (behind the eye), dehydration, nausea, lymphadenopathy (abnormal enlargement of the lymph glands) and diarrhoea [98-100]. Findings indicate that those who develop illness in this early stage have a more rapid decline in CD4 count and may progress to AIDS more rapidly than those who do not show symptoms [101,102].

1.4.2 Asymptomatic HIV infection

The second stage is known as *asymptomatic* infection due to the general lack of clinical symptoms. This can last a number of years but in the continued absence of ART, the median time to development of AIDS from initial HIV infection is ten to eleven years [3,84-86]. During this stage the CD4 count generally remains within a healthy range but slowly decreases over time, eventually reaching borderline dangerously low levels around 200-250 cells/mm^3 [3,84-86,103]. It is reported that over this period, the plasma viral load stays at a fairly steady level around the individual's set point, mostly between 10^3 and 10^5 copies/mL [29,43,92,104]. However there is some evidence from observational studies that the plasma viral load may gradually increase over this period, as opposed to remaining stable [103,105,106]. Longitudinal studies have shown gradual increases of 0.08 $\log_{10}$copies/mL [105] and 0.11 $\log_{10}$copies/mL per year [103]. This supports an inverse relationship between the two variables.

1.4.3 Symptomatic HIV infection

If an HIV infected individual still does not receive ART, it is likely the individual will progress into the last stage of infection, the *symptomatic* phase. Over potentially a period of some years, the CD4 count diminishes towards zero leaving the body with little immune defence, at the same time as the viral load gradually rises back up above the set point [85]. Early symptoms to appear include skin rashes, fatigue, weight loss, night sweats and oral candidiasis (thrush in the mouth) [107]. As the immune system breaks down, more severe opportunistic infections that would not be normally seen in patients with a preserved immune function start to occur. In HIV positive individuals, the contraction of one of these diseases defines the condition known as AIDS, which ultimately results in the death of the individual [108].

1.4.4 AIDS defining illnesses

AIDS is signified by the contraction of one or more of a list of AIDS defining illnesses (ADIs). The Centers for Disease Control and Prevention (CDC) in the USA, with the support of the WHO, describe a staging system for HIV infection, which is based primarily on clinical criteria but also allows a subdivision of clinical stages to incorporate laboratory markers if available (total lymphocyte counts or CD4 counts) [109,110]. In Europe, this staging system has not been adopted but the list of AIDS defining illnesses, as defined by the CDC/WHO (Table 1.1), is used to diagnose patients with AIDS [111,112]. Factors associated with faster progression to AIDS include a high viral load set point at the end of the first stage [113,114] and older age at time of infection [115].

1.4.5 Prevention of opportunistic infections

In 1995, the United States Public Health Service (USPHS) and the Infectious Diseases Society of America (IDSA) developed guidelines for the use of prophylaxis against opportunistic infections, including AIDS defining illnesses, defining thresholds of CD4 counts below which people were recommended to start treatment [116]. Both primary prophylaxis (to prevent initial episodes) and secondary prophylaxis (to prevent subsequent episodes) for opportunistic infections such as *Pneumocystis jiroveci* pneumonia (PCP; formerly classified as *Pneumocystis carinii*), Mycobacterium avium complex (MAC) (affecting the lungs) and cytomegalovirus (a herpes virus) had already become standard of care at this time [117]. However the introduction in 1995-1996 of cART (detailed in section 1.6) reduced the incidence of opportunistic infections and lengthened survival time for patients considerably [118-121]. Updated guidelines therefore advised that it was safe to stop prophylaxis if cART led to an increase in CD4 count to above the defined thresholds, which were based on randomised controlled trial and observational data [116,122]. For example, it was concluded that it was safe to discontinue primary and secondary PCP prophylaxis after the CD4 count had remained above 200 cells/mm^3 for at least three months [123,124]. For *Mycobacterium avium* complex disease, a threshold of at least 100 CD4 cells/mm^3 was set [125,126].

Table 1.1: Definitive and presumptive diagnosis of AIDS defining illnesses.

AIDS defining illness	Definitive, autopsy or presumptive	Definitive diagnostic method or presumptive diagnostic criteria
AIDS dementia complex	Definitive	Disabling cognitive and/or motor dysfunction, or milestone loss in a child, and no other causes by CSF exam and brain imaging or by autopsy.
	Presumptive	Same as above but no CSF and brain imaging performed.
Bacterial pneumonia, recurrent (> 2 episodes within 1 year)	Definitive	New X-ray evidence not present earlier and culture of pathogen that typically causes pneumonia (other than *P. jiroveci* or M. tuberculosis).
	Presumptive	Acute radiological findings (new X-ray evidence not present earlier) and acute clinical findings.
Candidiasis (tracheal, bronchial, lung)	Definitive/autopsy	Gross inspection at endoscopy/autopsy or by microscopic evaluation of tissue, not only culture.
Candidiasis (oesophageal)	Definitive/autopsy	Gross inspection by endoscopy/autopsy or by microscopy (histology).
	Presumptive	Recent onset retrosternal pain on swallowing and confirmed oral or pharyngeal candidiasis.
Cervical cancer (only females)	Definitive/autopsy	Histology.
Coccidioidomycosis, disseminated or extrapulmonary	Definitive/autopsy	Microscopy, culture or detection of antigen in tissue/fluid from affected organ.
Cryptococcosis, extrapulm.	Definitive/autopsy	Microscopy, culture of, or antigen detection in affected tissue.
Cryptosporidiosis, > 1 month	Definitive/autopsy	Microscopy. Duration of diarrhoea for more then 1 month.
Cytomegalovirus retinitis	Presumptive	Loss of vision and characteristic appearance on serial ophtalmoscopy, progressing over serial months.
Cytomegalovirus (pneumonia, oesophagitis, colitis, adrenalitis, other organs)	Definitive/autopsy	Microscopy (histology or cytology).
Herpes simplex ulcers (duration > 1 month) or pneumonia/oesophagitis	Definitive/autopsy	Microscopy, culture of, or antigen detection in affected tissue.
Histoplasmosis (extrapulm.)	Definitive/autopsy	Microscopy, culture of, or antigen detection in affected tissue.
HIV wasting syndrome	Definitive	Weight loss (over 10% of baseline) with no other cause, and 30 days or more of either diarrhoea or weakness with fever
Isosporiasis, duration > 1 month	Definitive/autopsy	Microscopy (histology or cytology). Duration of diarrhoea for more than 1 month.
Kaposi's sarcoma	Definitive/autopsy	Histology.
	Presumptive	Characteristic erythematous/violaceous plaque-like lesion on skin or mucous membranes.

AIDS defining illness	Definitive, autopsy or presumptive	Definitive diagnostic method or presumptive diagnostic criteria
Malignant lymphoma	Definitive/autopsy Presumptive	Histology. (Only primary brain lymphoma). Recent onset of focal neurological symptoms and signs or reduced level of consciousness, CT/MR scan evidence of a lesion or lesions having mass effect, no response to toxo therapy, no evidence of lymphoma outside the brain.
Mycobacterium tuberculosis and MAC/Kansasii (pulmonary and/or extrapulm.) (Pulmonary MAC/Kansassii not AIDS defining)	Definitive	Culture.
Mycobacterium other type (extrapulm.)	Definitive Presumptive	Culture (indicate type). Acid fast bacteria (species not identified by culture) on microscopy of normally sterile body fluid/tissue.
Pneumocystis *jiroveci* pneumonia (PCP)	Definitive Presumptive	Microscopy (histology or cytology) Recent onset of dyspnoea on exertion or dry cough, and diffuse bilateral infiltrates on chest X-ray and pO2 <70 mmHg and no evidence of bacterial pneumonia.
Progressive multifocal leukoencephalopathy (PML)	Definitive/autopsy Presumptive	Microscopy (histology or cytology). Progressive deterioration in neurological function and CT/MR scan evidence.
Salmonella (non typhoid) bacteriaemia (> 2 episodes)	Definitive	Culture.
Toxoplasmosis, brain	Definitive Presumptive	Microscopy (histology/cytology). Recent onset focal neurological abnormalities or reduced level of consciousness, and mass effect lesion on scan, and specific therapy response.

Source: EuroHIV. 1993 Revision of the European and surveillance case definition [111,112].

1.4.6 Survival time

1.4.6.1 Pre-cART era

In the absence of any antiretroviral treatment, the average survival time following diagnosis of AIDS is approximately two years [85,86]. Factors associated with a longer period of survival following an AIDS diagnosis have been investigated in a number of studies. Younger age, more recent date of diagnosis and a higher CD4 count at time of diagnosis were consistently found to be associated with longer survival time [127-136]. However other findings were less consistent, most likely due to differences in analytical methods and the presence of confounding variables, for example, associations found between survival time and gender, transmission risk group, and ethnicity [127,129,130,132,135]. Survival time has also been shown to vary considerably depending upon which AIDS defining illness is diagnosed

[129,130,132,137,138]. Results from the AIDS in Europe study found that median survival time varied from 2-19 months with diseases such as progressive multifocal leukoencephalopathy (affecting central nervous system), malignant lymphoma (tumour of lymphoid tissue) and AIDS dementia complex having the shortest survival time and PCP, Kaposi's sarcoma and tuberculosis (extrapulmonary) having the longest survival time [137].

1.4.6.2 Post- introduction of cART

Since the introduction of cART the incidence of AIDS and death in HIV positive individuals has decreased significantly [119,120,139,140]. The risk of death for infected individuals in the era of cART has been estimated to be more than 85% lower than the risk before cART [139]. In patients receiving cART, the strongest predictor of clinical progression to AIDS or death has been found to be a lower CD4 count measured at the start of treatment [139,141-144]. Viral load [142,144], haemoglobin level (an iron-containing respiratory pigment of red blood cells) [144] and a diagnosis of severe AIDS before starting cART have also been independently linked to the risk of clinical progression [142]. In 2002, the EuroSIDA study incorporated these three factors, together with CD4 count, into a clinically prognostic scoring system for patients receiving cART, which aimed to assess the risk of clinical progression according to a patient's current clinical status. The system was validated against data from two other cohorts and was found to be highly predictive [141]. This risk score was updated in 2007 and included all predictive factors of short-term clinical disease progression. These factors were: lower current CD4 cell count, higher viral load, presence of anaemia, body mass index outside normal range, older age, steeper CD4 cell slope, ART experience prior to cART, not presently receiving ART and HIV infection via IDU [145]. It is planned that the score will be made publicly available via online calculation tools so that it can be implemented into clinical practice.

Age has been found to be another independent predictor of disease progression in patients starting cART with no previous treatment experience (ART-naïve) [142]. The association between survival and both gender and transmission risk group is less clear [142,146-149] with suggestion of interactions with calendar time [150].

1.5 History of AIDS and HIV

1.5.1 Origin of HIV

The origin of HIV is now accepted to be the simian immunodeficiency virus (SIV) that infects monkeys [18,151]. Evidence was found in 1999 that showed that a strain of SIV

in chimpanzees was almost identical to the most infectious and predominant strain of HIV, HIV-1 [152]. Co-infection of three different strains of SIV may have led to a *recombinant* form capable of infecting humans [18,153,154]. *Recombination* occurs when the genetic material from more than one viral strain is accidentally swapped during viral replication resulting in a strain that incorporates genetic code from both [18-20,155]. It is likely that cross-species transmission occurred through the hunting and butchering of monkeys [156]. The earliest recorded case of HIV-1 was in an African plasma sample from 1959 and it is estimated that the first human infection may have taken place not long before this [157]. A second genetically different strain of HIV, HIV-2, which is largely confined to West Africa and Asia, is thought to originate from the sooty mangabey monkey [18].

1.5.2 First cases of AIDS

The first documented cases of AIDS occurred in the USA in 1981. Deaths began to take place amongst homosexual men with reduced immune systems who had contracted the illnesses, Kaposi's sarcoma and/or PCP [158,159]. By the end of the year, it was recognised that this immune deficiency was caused by a new disease [160,161]. Cases of PCP also started occurring in IDUs and so it was apparent that the disease was not confined to just one population and could be transmitted via blood [162]. In July 1982, the CDC, gay-community leaders and federal bureaucrats met in Washington and agreed to name the disease *acquired immune deficiency syndrome* or AIDS to recognise the weakening of the immune system, characteristic of this condition [163]. Within the year, the first incidences of AIDS had occurred in haemophiliacs, which raised concerns with possible transmission via the transfusion of blood products [164,165]. Immune dysfunctions accompanied by opportunistic infections were also observed in infants and it was suggested that transmission of an 'AIDS agent' from mother to child, in utero or shortly after birth, could account for the early onset [166].

1.5.3 AIDS virus

It was not until 1983 that the virus believed to be the cause of AIDS was first discovered and at the time named LAV (lymphadenopathy-associated virus) [167]. However, in 1984, an independent laboratory similarly identified a virus designated HTLV-III (human t-cell leukaemia) and also provided convincing evidence that it was the virus behind AIDS [168]. By 1985 it was recognised that these viruses were actually the same [169]. In this same year, an antibody test using patients' blood serum to detect the presence of LAV/HTLV-III called ELISA (enzyme linked immunosorbent assay), was approved by the FDA [170], which led to the

recommendation that blood or serum from donors of organs, tissues or semen should be routinely screened for the virus [171].

In May 1986, a subcommittee commissioned by the International Committee on the Taxonomy of Viruses determined a definitive name for the AIDS virus. The names LAV and HTLV-III were dropped in favour of HIV (later to be called HIV-1 [172]) to settle the dispute over the two names [173-175]. Also this year, a genetically distinct strain of the virus was discovered in two AIDS patients in West Africa [176] (later to be designated HIV-2 [172]). The following year, the first antiretroviral drug was approved, which at the time was believed to be an important step towards bringing significant relief to those infected with the virus [177] (see section 1.7 on treatment strategies).

1.5.4 AIDS definition

The first formal definition of AIDS was in 1985 by the CDC/WHO as described in section 1.4.4. They defined it as an illness characterised by one of a list of opportunistic infections in the absence of all known underlying causes of immunodeficiency, other than the virus, HIV (at the time still called LAV/HTLV-III) [108]. This was first revised in 1987 to specify definitions of AIDS defining illnesses for those with laboratory evidence of HIV, those without and those with laboratory evidence against HIV [178]. In 1993 the definition was further extended to include extra diagnoses, i.e. pulmonary tuberculosis, recurrent pneumonia and invasive cervical carcinoma (malignant tumour of cellular tissue), and those who were severely immunosuppressed with a CD4 lymphocyte count less than 200 cells/mm^3 [109]. The 1993 European AIDS definition differs from this CDC definition (used in the USA) in that it does not include the CD4 count criteria [111].

1.6 Antiretroviral therapy

The use of combinations of antiretroviral drugs to treat HIV positive individuals has drastically reduced mortality and morbidity since its introduction into clinical practice in developed countries in 1995-1996 [118-121,139,140,179]. These drugs are designed to prevent the virus from successfully replicating, thereby preventing further damage to the immune system and allowing restoration of CD4 cells [40,180-186]. This protects the body from opportunistic infections, which could result in the development of AIDS or death [187]. However, the efficacy of antiretroviral drugs to achieve a successful response is restricted by a number of factors including drug absorption and distribution [188,189], the emergence of drug-resistant viral strains (which are discussed later) [172,190] and patient adherence to the regimen [191-196]. The HAART Observational Medical Evaluation and Research (HOMER) study run through the British Columbia

Centre for Excellence in HIV/AIDS Drug Treatment Program suggested that patients should be more than 95% adherent to conserve long-term CD4 count responses [192]. Factors affecting adherence include the severity of side effects experienced, convenience of the regimen, confidence in ability to take the medication correctly and amount of social support [197-201]. The side effects associated with the use of antiretroviral drugs can be severe and often result in treatment switches or discontinuation. Studies have shown that up to 50% of both ART-naïve and experienced patients who start a cART regimen discontinue one or more of the drugs within a year of starting, mostly due to toxicities or patient choice [202-205]. The risk of serious life threatening events such as liver failure [206] and myocardial infarction is also increased in patients receiving therapy, for example, the D:A:D study showed that cART was independently associated with a 16% relative increase in the rate of myocardial infarction per year of exposure to a class of antiretroviral drugs called protease inhibitors (detailed in section 1.6.2.3) during the first four to six years of use [207].

1.6.1 Studying the effects of antiretroviral drugs

The effects of antiretroviral drugs are studied in HIV research in a variety of ways. Randomised controlled trials (RCTs) are the gold standard for investigating the safety and efficacy of drug interventions due to the fact that randomisation ensures that comparisons between patients are unbiased. The allocation of patients to intervention arms is determined by chance and so any other factors that may affect the outcome should be balanced between the groups. However RCTs are not always possible for ethical or practical reasons and are often not powered for clinical endpoints and so there is a strong argument in favour of using the findings from large-scale cohort studies as a supplement to RCTs [208,209]. Cohort studies follow a group of individuals over time and the effects of exposure to factors are observed without any interventions.

When ART was first introduced, the rate of clinical events, i.e. AIDS defining illnesses and deaths, was high and so conclusions from RCTs could be reached within a few years [210-212], which is relatively short compared to the number of years that would be needed now. As more drugs were developed and treatment strategies changed, a problem of the long-term RCTs became that the data were irrelevant by the time the trial had finished and the results were published. Surrogate marker endpoints, e.g. proportion of patients with viral loads less than 400 copies/mL, were designed instead to draw conclusions about the efficacy of treatment more quickly, typically within 48 weeks [213,214]. However these short-term trials cannot assess the long-term effects

and toxicities of ART, especially those that rarely occur. Further problems with RCTs are that patients are almost always not representative of the general HIV infected population due to the strict inclusion/exclusion criteria set, for example limitations in CD4 count, viral load, previous drug experience. Patients are usually motivated and therefore more adherent than the general population and patients visit their clinics more frequently [208,209]. Since observational cohort studies can include a much more heterogeneous population and follow up patients over a long period of time, this allows all stages of treatment including salvage therapy to be observed.

Although observational studies have the disadvantage that comparisons of patients may be biased due to differences in baseline characteristics, statistical analyses can take into account the known, measured differences and produce adjusted results [208]. Thus observational cohorts are important for studying areas that are not feasible in an RCT, to support findings from RCTs in a more representative sample of the HIV infected population and to identify areas of research for which an RCT would be useful.

1.6.2 Available antiretroviral drugs

Before drugs become available for public use, they need to be given marketing authorisation by a governing public health agency, i.e. the FDA in the USA and the European Medicines Agency (EMEA) in Europe. There are three main classes of antiretroviral drug to treat HIV, classified according to the way in which they act to prevent replication: nucleoside (and nucleotide) reverse transcriptase inhibitors (NRTIs or nucs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs). There are also three more drugs from new classes that have been more recently licensed: a fusion inhibitor, an entry inhibitor (CCR5 co-receptor antagonist) and an integrase inhibitor [215,216].

1.6.2.1 Nucleoside (and nucleotide) reverse transcriptase inhibitors

One strategy to prevent HIV replication is to inhibit the viral enzyme reverse transcriptase. HIV RNA binds to the active site of reverse transcriptase, which allows the enzyme to catalyse the production of a copy of the viral RNA called HIV proviral DNA. The class of antiretroviral drugs called nucleoside reverse transcriptase inhibitors (NRTIs) work by competitively binding to this active site during reverse transcription so that the NRTI is incorporated into the HIV proviral DNA chain. This results in termination of the chain and thus the prevention of viral DNA production. The remaining viral RNA is then likely to be destroyed by cellular enzymes [217,218]. *Nucleotide* reverse transcriptase inhibitors (sometimes called NtRTIs) work in the same

way but because of their chemical structure, they bind more easily to the active site [219].

The first antiretroviral drug to be developed was an NRTI called zidovudine (ZDV), authorised in the USA in 1987 [177]. Since then, a further six NRTIs have been approved: didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), abacavir (ABC) and emtricitabine (FTC), and one NtRTI: tenofovir disoproxil fumarate (TDF) [215,216,220]. There are a number of toxicities associated with the use of NRTIs that can be severe [221,222]. Cohort studies have shown that asymptomatic hyperlactaemia (mild to moderate elevations of lactic acid in the circulating blood) has an estimated prevalence of 15-35% in HIV infected patients on NRTIs and symptomatic hyperlactaemia and lactic acidosis (a persistently high elevation of lactic acid in the circulating blood) have an incidence ranging from 1.7-25.2 cases per 1000 person-years of treatment [223]. Risk factors linked to these illnesses are long duration of NRTI therapy, female sex, pregnancy and high body mass index [223]. Hepatotoxicity (liver damage) with hepatic steatosis (fatty degeneration of the liver) is another complication of NRTI treatment [224]. Studies have shown incidence rates of severe hepatotoxicity (defined by elevation of liver enzymes) ranging from 8.5-17% [225-230] and patients co-infected with hepatitis B and C were found to be at considerably (approximately three times) higher risk [226,227,230]. Severe or life threatening hepatic events were observed in a cohort of 2947 patients receiving ART regimens at a rate of 2.6 per 100 person-years of follow-up over a five-year period [206]. Lipodystrophy and lipoatrophy (metabolic disorders involving the loss or gain of fat deposition in tissue) have also been linked to NRTI use [231,232], especially with use of stavudine, which is why this drug is no longer a preferred option for first-line therapy [233]. Of patients on a cART regimen, the prevalence of lipodystrophy has been estimated to be 25-50% with NRTI use as a risk factor [231,232]. Hypersensitivity (symptomised by rash with or without fever) is a further problem that occurs in about 4-8% of patients taking ABC [234-236]. This particular toxicity is investigated in Chapter 7. Table 1.2 lists all NRTIs and NtRTIs licensed in the USA and Europe with possible associated toxicities.

Table 1.2: Currently licensed nucleoside and nucleotide reverse transcriptase inhibitors.

Brand name	Generic name	Manufacturer name	FDA approval date [220]	EMEA approval date [216]	Possible side effects [215,216]
Atripla	Tenofovir/ emtricitabine /efavirenz (NNRTI)	Gilead Sciences and Bristol-Myers Squibb	12 Jul 2006	13 Dec 2007	See Emtriva, Viread and Stocrin.
Combivir	Zidovudine/ lamivudine	GlaxoSmithKline	27 Sep 1997	18 Mar 1998	See Retrovir and Epivir.
Emtriva	Emtricitabine	Gilead Sciences	2 Jul 2003	24 Oct 2003	Headache, dizziness, insomnia, nausea, diarrhoea, abdominal pain, rash, neutropenia (low white blood cell count), hyperglycaemia (excess of sugar in blood), elevated liver enzymes, hyperbilirubinaemia (excess of pigment bilirubin in blood).
Epivir	Lamivudine	GlaxoSmithKline	17 Nov 1995	8 Aug 1996	Headache, insomnia, nausea, vomiting, abdominal pain, diarrhoea, rash, fatigue, malaise, fever, arthralgia (pain in joints), muscle disorders. More rarely: hepatitis, pancreatitis, rhabdomyolysis (destruction of skeletal muscle tissue).
Epzicom	Abacavir/ lamivudine	GlaxoSmithKline	2 Aug 2004	*	See Epivir and Ziagen.
Hivid	Zalcitabine	Hoffmann-La Roche	19 Jun 1992	*	Peripheral neuropathy (pain, tingling, numbness, or burning in the hands and/or feet). More rarely: lactic acidosis (an increase of lactic acid in the blood), pancreatitis (inflammation of the pancreas).
Kivexa	Abacavir/ lamivudine	GlaxoSmithKline	*	17 Dec 2004	See Epivir and Ziagen.
Retrovir	Zidovudine	GlaxoSmithKline	19 Mar 1987	*	Headache, insomnia, nausea, stomach discomfort. More rarely: muscle wasting, anaemia (low red blood cell count), neutropenia.
Trizivir	Zidovudine/ lamivudine/ abacavir	GlaxoSmithKline	14 Nov 2000	28 Dec 2000	See Retrovir, Epivir and Ziagen.
Truvada	Tenofovir/ lamivudine	Gilead Sciences	2 Aug 2004	21 Feb 2005	See Viread and Emtriva.
Videx	Didanosine (buffered version)	Bristol-Myers Squibb	9 Oct 1991	*	Numbness, tingling, or pain in the hands or feet, nausea, diarrhoea, vomiting, headache, rash. More rarely: pancreatitis. Possible increased side effects if taken with Viread.

Notes: *Not licensed by agency.
FDA = Food and Drug Administration; European Medicines Agency.

Brand name	Generic name	Manufacturer name	FDA approval date [220]	EMEA approval date [216]	Possible side effects [215,216]
Videx EC	Didanosine (delayed release capsules)	Bristol-Myers Squibb	31 Oct 2000	*	See Videx.
Viread	Tenofovir	Gilead Sciences	26 Oct 2001	5 Feb 2002	Nausea, vomiting, diarrhoea, flatulence. More rarely: osteopenia (thinning bones).
Zerit	Stavudine	Bristol-Myers Squibb	24 Jun 1994	8 May 1996	Numbness, tingling, or pain in the hands or feet, nausea, diarrhoea, vomiting, headache, rash. More rarely: pancreatitis or lactic acidosis.
Ziagen	Abacavir	GlaxoSmithKline	17 Dec 1998	8 Jul 1999	Headache, nausea, vomiting, diarrhoea, loss of appetite, insomnia, fever, fatigue, rash, serious allergic reactions.

Notes: *Not licensed by agency.
FDA = Food and Drug Administration; European Medicines Agency.

1.6.2.2 Non-nucleoside reverse transcriptase inhibitors

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) work in a different way to NRTIs but with the same outcome. NNRTIs bind to another site on the reverse transcriptase enzyme, which results in changes to the active site that prevents HIV RNA from binding to it [218,237].

Four NNRTIs are currently approved by public health agencies: nevirapine (NVP), efavirenz (EFV), etravirine (TMC-125) and in the USA, delavirdine (DLV) [215,220]. Two further NNRTIs loviride and caprivirine reached development stages but were discontinued due to disappointing results in clinical trials [238,239]. The newest NNRTI, etravirine, was very recently approved by the FDA and is designed to work against HIV that is resistant to NVP and EFV, therefore this drug will be ideal for use in second-line therapy after initial failure on either of these NNRTIs [240].

The rate of toxicities occurring is relatively low in this class but symptoms include rash, headaches, fatigue, stomach upset, dizziness, vivid dreams and insomnia [215,241]. The most severe adverse effect of this drug class is hepatotoxicity. Studies have found incidences of severe hepatic events ranging from 7.8-15.6% in NNRTI-treated patients [242]. Hypersensitivity reactions may also occur after starting NNRTI treatment with research showing incidences of 10-17% in NNRTI-treated patients [241]. Table 1.3 lists all licensed NNRTIs and toxicities.

Table 1.3: Currently licensed non-nucleoside reverse transcriptase inhibitors.

Brand name	Generic name	Manufacturer name	FDA approval date [220]	EMEA approval date [216]	Possible side effects [215,216]
Atripla	Tenofovir/ emtricitabine/efavirenz	Gilead Sciences and Bristol-Myers Squibb	12 Jul 2006	13 Dec 2007	See Emtriva, Viread and Stocrin.
Intelence	Etravirine	Tibotec Therapeutics	18 Jan 2008	*	Rash, nausea, diarrhoea, vomiting, abdominal pain, tiredness, tingling or pain in hands and feet, numbness, headache, high blood pressure.
Rescriptor	Delavirdine	Pfizer	4 Apr 1997	*	Rash, headache, fatigue, stomach upset, elevated liver enzymes.
Stocrin	Efavirenz	Merck	*	28 May 1999	Rash, drowsiness, insomnia, confusion, inability to concentrate, dizziness, vivid dreams, nausea, stomach discomfort, fever, insomnia, elevated liver enzymes.
Sustiva	Efavirenz	Bristol Myers-Squibb	17 Sep 1998	28 May 1999	See Stocrin.
Viramune	Nevirapine	Boehringer Ingelheim	21 Jun 1996	5 Feb 1998	Rash, stomach upset, headaches, increased liver enzyme levels. More rarely: hepatitis.

Notes: *Not licensed by agency.
FDA = Food and Drug Administration; European Medicines Agency.

1.6.2.3 Protease inhibitors

Protease inhibitors (PIs) work by competitively binding to the active site of the HIV enzyme protease. This prevents the enzyme from processing and cleaving HIV proteins into functional forms and as a result, the new virions produced are immature and unable to function properly [243,244].

There are nine currently approved PIs: saquinavir (hard and soft gel formulations) (SQV), ritonavir (RTV), indinavir (IDV), nelfinavir (NFV), amprenavir (APV) (and newer version, fosamprenavir), lopinavir (LPV), atazanavir (ATV), tipranavir (TPV) and darunavir (DRV) [215,220]. RTV is now only used in low-dose to boost other PIs by inhibiting their metabolism and therefore boosting the levels in the blood [40-42].

Table 1.4: Currently licensed protease inhibitors.

Brand name	Generic name	Manufacturer name	FDA approval date [220]	EMEA approval date [216]	Possible side effects [215,216]
Agenerase	Amprenavir	GlaxoSmithKline	15 Apr 1999	20 Oct 2000	Rash, appetite loss, headaches, malaise, diarrhoea, nausea, vomiting, numbness/tingling around the mouth. More rarely: lipodystrophy, diabetes.
Aptivus	Tipranavir	Boehringer Ingelheim	22 Jun 2005	25 Oct 2005	Rash, nausea, vomiting, diarrhoea, stomach pain, tiredness, headache. More rarely: liver damage.
Crixivan	Indinavir	Merck	13 Mar 1996	4 Oct 1996	Kidney stones, nausea, vomiting, diarrhoea, stomach discomfort, headache, insomnia, rash, back pain.
Fortovase	Saquinavir (soft gel capsules)	Hoffmann-La Roche	7 Nov 1997	20 Aug 1998	Nausea, diarrhoea, stomach discomfort, insomnia, headache, increased liver enzyme levels.
Invirase	Saquinavir	Hoffmann-La Roche	6 Dec 1995	4 Oct 1996	See Fortovase.
Kaletra	Lopinavir/ Ritonavir	Abbott Laboratories	15 Sep 2000	20 Mar 2001	Diarrhoea, nausea, feeling weak/tired, headache. More rarely: pancreatitis.
Lexiva	Fos-amprenavir	GlaxoSmithKline	20 Oct 2003	*	Rash, diarrhoea, nausea, vomiting, abdominal pain, fatigue, dizziness, headache.
Norvir	Ritonavir	Abbott Laboratories	1 Mar 1996	26 Aug 1996	Nausea, vomiting, diarrhoea, loss of appetite, stomach discomfort, oral tingling and numbness, increased liver enzyme levels.
Prezista	Darunavir	Tibotec	23 Jun 2006	12 Feb 2007	
Reyataz	Atazanavir	Bristol-Myers Squibb	20 Jun 2003	2 Mar 2004	Increased levels of bilirubin (a pigment found in the liver), headaches, pain/tingling in the arms and legs, nausea, diarrhoea, rash. More rarely: changes in the way your heart beats.
Telzir	Fos-amprenavir	GlaxoSmithKline	*	12 Jul 2004	See Lexiva.
Viracept	Nelfinavir	Agouron Pharmaceuticals	14 Mar 1997	22 Jan 1998	Diarrhoea, nausea, stomach discomfort, gas, rash, increased liver enzyme levels.

Notes: *Not licensed by agency.
FDA = Food and Drug Administration; European Medicines Agency.

Common to all the PIs are the side effects, nausea, diarrhoea and vomiting [215]. Body fat and metabolic disorders have also been linked to PIs including lypodystrophy, high lipid levels, insulin resistance, diabetes mellitus, hyperglycaemia (increased blood levels of sugar), hypercholesterolaemia (increased cholesterol), hypertriglyceridaemia (increased levels of triglycerides) and increased bleeding in haemophiliacs [231,241,245,246]. For example, 7% of patients treated with LPV for 60 weeks had developed body fat changes, as had those on NFV [247]. In another study 35% of

patients experienced body fat changes after four years on LPV and RTV [248]. Table 1.4 displays all licensed PIs and associated toxicities.

1.6.2.4 Fusion, entry and integrase inhibitors

The most recent developments are three new drugs, which all work differently to the standard drug classes available. The fusion inhibitor enfuvirtide (T-20) works by interacting with sites on the virion that bind to the CD4 cells and so blocks entry into the potential host cells [249,250]. The entry inhibitor maraviroc is similar in that it prevents the virus from entering cells. This is only effective for certain strains of virus (R5) that bind with a CCR5 co-receptor on the surface of CD4 cells [251]. Finally, the most recent new drug is raltegravir, which is an inhibitor of the viral enzyme integrase and prevents integration of the viral DNA into the host DNA [252]. Table 1.5 shows the licensed drugs and associated side effects.

Table 1.5: Currently licensed fusion, entry and integrase inhibitors.

Brand name	Generic name	Manufacturer name	FDA approval date [220]	EMEA approval date [216]	Possible side effects [215,216]
Celsentri	Maraviroc	Pfizer	*	18 Sep 2007	Cough, fever, colds, rash, muscle and joint pain, stomach pain and dizziness. Less common side effects include cardiovascular problems and liver toxicity.
Fuzeon	Enfuvirtide	Hoffman-La Roche and Trimeris	13 Mar 2003	27 May 2003	Injection site reactions including itching, swelling, redness, pain or tenderness, hardened skin or bumps. More rarely: serious allergic reactions.
Isentress	Raltegravir	Merck & Co.	12 Oct 2007	*	Diarrhoea, nausea and headache. In some patients, blood tests showed abnormally elevated levels of a muscle enzyme, creatine kinase.
Selzentry	Maraviroc	Pfizer	6 Aug 2007	*	See Celsentri.

Notes: *Not licensed by agency.
FDA = Food and Drug Administration; European Medicines Agency.

1.7 Treatment strategies

1.7.1 Monotherapy (1987-1993)

When antiretroviral drugs first started to be developed, there were very limited options for patients and the long-term effects were unknown. For the first four years, there was only one drug available therefore if the patient found it to be ineffective or intolerable, there were no alternatives to switch to. This first antiretroviral drug was an NRTI called azidothymidine (AZT), now more commonly known as zidovudine (ZDV) [177]. The

FDA approved ZDV in 1987 after a clinical trial demonstrated its ability to increase CD4 count, to decrease incidence of mortality and to reduce the frequency of opportunistic infections amongst patients with AIDS or advanced AIDS related complex (ARC) [210,253] (now defined as CDC/WHO category B [109]). Further trials showed that the drug was also effective in asymptomatic patients with CD4 counts of less than 500 cells/mm^3 and could delay progression to symptomatic disease or AIDS in the short-term [211,254]. However, the largest study of ZDV monotherapy in asymptomatic HIV infection, called the Concorde study, published results that suggested that over the long-term, starting ZDV before symptoms developed did not increase the chances of AIDS-free survival [212,255].

The second NRTI, didanosine (dideoxyinosine or ddI), was authorised by the FDA in 1991 [256] based on data from clinical trials that showed its efficacy in increasing CD4 count [257,258]. This provided an alternative for patients who had not responded to ZDV treatment or had not been able to tolerate its side effects. However no survival benefits over continuing ZDV were found [259,260].

In 1992, a third drug, zalcitabine (ddC or dideoxycytidine), was given accelerated approval by the FDA (allowing marketing approval before the drug's clinical efficacy had been established) for use in the first combination therapy with ZDV for patients who had advanced HIV infection with signs of clinical or immunological deterioration [261]. Although there were no study results to indicate a higher rate of survival, a lower incidence of opportunistic infections or decreased progression to AIDS, there was evidence that the combination appeared to increase and sustain CD4 cell count more than ZDV alone [262]. This approval, however, was withdrawn a year later after it was concluded that there were insufficient data to support the efficacy of this treatment [263]. At the same meeting ddC monotherapy was approved based on preliminary results from clinical trials showing that ddC was as effective as ddI in delaying progression to death [264]. No difference was found in survival between ddC and continuing ZDV [265]. Table 1.6 displays some of these findings from the major clinical trials investigating the use of a single antiretroviral drug.

Table 1.6: Major clinical trials in antiretroviral monotherapy.

Study	Clinical trial	Authors, year	Main outcomes	Conclusions
ZDV vs placebo	ZDVCG studies	Fischl *et al.* 1987 [210] Fischl *et al.* 1989 [253]	Progression to death or opportunistic infection and increase in CD4 count in patients with AIDS and ARC.	ZDV decreases mortality and frequency of opportunistic infections in subjects with AIDS/ARC over 8-24 weeks of observation. Continued survival benefits with long-term ZDV therapy.
ZDV vs placebo	ACTG 019	Volberding *et al.* 1990 [211] Volberding *et al.* 1994 [254]	Progression to AIDS, ARC or death and increase in CD4 count in asymptomatic HIV infected patients.	ZDV delays disease progression in asymptomatic patients and CD4 <500 cells/mm^3, but there is no survival benefit. Earlier use was not associated with longer survival compared with delayed initiation of ZDV.
ZDV: Early vs deferred	Concorde	Concorde Coordinating committee 1994 [212] 1998 [255]	Progression to AIDS, ARC or death and increase in CD4 count in asymptomatic HIV infected patients.	Early use of ZDV in asymptomatic patients is not encouraged.
ddI vs continued ZDV	ACTG 116B	Kahn *et al.* 1992 [259]	Progression to disease/death and increase in CD4 count in ZDV-experienced patients.	Changing treatment from ZDV to ddI appears to slow progression of HIV disease but there was no survival benefit with change to ddI.
ddI vs continued ZDV	ISS 901	Vella *et al.* 1996 [260]	Kaplan-Meier estimates of survival and time to AIDS-defining event in ZDV-experienced patients with AIDS.	In patients with advanced disease, switching from ZDV to ddI does not produce apparent benefits.
ddC vs continued ZDV	ACTG 119	Fischl *et al.* 1993 [265]	Estimated 12-month event-free probabilities and survival, rate of CD4 count decline and progression to AIDS/death in ZDV-experienced patients.	No differences in survival and clinical endpoints were found. Slower rates of CD4 count decline were found in the ddC group.
ddI vs ddC	CPCRA	Abrams *et al.* 1994 [264]	Disease progression or death rates and CD4 count decline in ZDV-experienced patients.	ddC is at least as efficacious as ddI in delaying disease progression and death with similar CD4 count decline.

Notes: ZDV = zidovudine; ddI = didanosine; ddC = zalcitabine; ARC = AIDS related complex.

1.7.2 Dual therapy (1992-1996)

Over the following few years, the use of two NRTIs in dual combination therapy gradually replaced monotherapy as the standard of treatment. Three major trials, ACTG 175, DELTA and CPCRA compared treatments of ZDV/ddC, ZDV/ddI and ZDV monotherapy. The first two found that both dual therapies were superior to ZDV alone in preventing a significant reduction of CD4 counts, progression to AIDS or death [266,267]. The third, CPCRA, did not find the combinations to be superior than ZDV alone in patients with advanced HIV infection, however suggested that they might be more effective in patients with little or no previous ZDV experience [268].

Two more NRTIs were introduced during this period: stavudine (d4T) in 1994 [269] and lamivudine (3TC) in 1995 [270]. A study looking at patients on a 3TC/ZDV combination compared to either drug alone found more improvements in immunological and virological measures in patients on the dual therapy than those on monotherapy [271]. Table 1.7 displays the outcomes of some of the major clinical trials investigating dual therapy versus monotherapy.

Table 1.7: Major clinical trials in antiretroviral dual versus monotherapy.

Study	Clinical trial	Authors, year	Main outcomes	Conclusions
ZDV vs ZDV/ddI vs ZDV/ddC vs ddI	ACTG 175	Hammer *et al.* 1996 [266]	Progression to 50% CD4 drop, AIDS or death in ZDV-naïve (no previous experience of ZDV) or -experienced patients with CD4 200-500 cells/mm^3.	Treatment with ZDV/ddI, ZDV/ddC or ddI alone slows the progression of HIV disease more than ZDV alone.
ZDV vs ZDV /ddI vs ZDV/ddC	DELTA 1 and 2	Delta Coordinating Committee 1996 [267]	Progression to AIDS or death, relative reduction in mortality and change in CD4 median at week 8 in ZDV-naïve (DELTA 1) and ZDV-experienced (DELTA 2) patients.	Treatment with ZDV/ddI or ZDV/ddC prolongs life and delays disease progression compared with ZDV alone. The addition of ddI to ZDV-experienced patients also improves survival, although benefit appears less.
ZDV vs ZDV/ddI vs ZDV/ddC	CPCRA	Saravolatz *et al.* 1996 [268]	Progression to disease or death in ZDV-naïve or -experienced patients with CD4 <200 cells/mm^3.	In patients with advanced HIV infection, combination therapy with ZDV and either ddI or ddC is not superior to ZDV alone. They may be more effective in patients with little or no previous ZDV treatment.
ZDV vs 3TC vs ZDV/3TC	NUCA 3001	Eron *et al.* 1996 [271]	Change in CD4 count and viral load at 24 weeks in ZDV-naïve patients with CD4 200-500 cells/mm^3.	ZDV/3TC produce more improvement in immunological and virological response than either alone.

Notes: ZDV = zidovudine; ddI = didanosine; ddC = zalcitabine; 3TC = lamivudine.

1.7.3 Combination antiretroviral therapy (1995-current)

The major breakthrough in HIV therapy came with the advent of cART defined as a regimen consisting of at least three antiretroviral drugs from two different drug classes. This is also widely known as highly active antiretroviral therapy (HAART).

1.7.3.1 PI-containing regimens

The era of cART started with the development of the new drug class, PIs, which proved to be highly potent when combined with NRTIs. Compared with a dual NRTI regimen, combinations of two NRTIs and one PI were found to be significantly superior in reducing the number of patients progressing to AIDS or death [180,182], as well as in antiviral activity and CD4 cell increase in subjects with and without previous ART experience both in early and late stages of infection [184,213,272-275]. Table 1.8

summarises some of the major trials looking at PI-containing cART regimens compared to dual NRTI therapy.

Table 1.8: Major clinical trials in PI-containing cART versus dual NRTI therapy.

Study	Clinical trial	Authors, year	Main outcomes	Conclusions
IDV/ZDV/3TC vs ZDV/3TC	ACTG 320	Hammer *et al.* 1997 [180]	Progression to AIDS/death, CD4 increase and viral load decrease in ZDV-experienced subjects with CD4 ≤200 cells/mm^3.	Treatment with three-drug therapy containing IDV, ZDV and 3TC compared to ZDV and 3TC significantly slows the progression of HIV disease.
RTV/2 NRTIs vs 2 NRTIs	Advanced HIV disease Ritonavir Study Group	Cameron *et al.* 1998 [182]	Progression to AIDS/death in NRTI-experienced, PI-naïve subjects with CD4 ≤100 cells/mm^3.	RTV in combination therapy for patients with advanced disease and extensive previous antiretroviral use lowers risk of AIDS and prolongs survival more than two NRTIs alone.
SQV/ZDV/ddC vs SQV/ZDV vs ZDV/ddC	ACTG 229	Collier *et al.* 1996 [184]	CD4 increase and reduction in viral load in ZDV-experienced subjects with CD4 50-300 cells/mm^3.	SQV/ddC/ZDV reduced viral load and increased CD4 count more than ZDV with either SQV or ddC alone.
RTV/d4T/3TC vs d4T/ddI vs ZDV/ddI vs ZDV/ddC vs no treatment	Spanish Earth-1 Study	Garcia *et al.* 1999 [272]	Change in CD4 count and viral load in ART-naïve subjects with CD4 >500 cells/mm^3 and viral load >10000 copies/mL.	Three-drug therapy is the treatment of choice in very early stages of HIV infection.
IDV/ZDV/3TC vs ZDV/3TC vs IDV	MSD Protocol 039	Hirsch *et al.* 1999 [273]	CD4 increase and reduction in viral load in PI-naïve and extensively ZDV-experienced subjects with CD4 ≤50 cells/mm^3.	Patients with advanced HIV infection benefit from three-drug therapy with IDV/ZDV/3TC more than two NRTIs or IDV alone.
APV/ZDV/3TC vs ZDV/3TC	PROAB3001	Goodgame *et al.* 2000 [213]	Proportion reaching viral load <400 copies/mL at 48 weeks in ART-naïve subjects with CD4 ≥200 cells/mm^3 and viral load ≥10000 copies/mL.	APV/ZDV/3TC significantly better in terms of antiviral activity than ZDV/3TC in ART-naïve subjects.
IDV/ZDV/3TC vs ZDV/3TC	AVANTI 2	AVANTI study group 2000 [274]	CD4 increase and reduction in viral load in ART-naïve subjects with CD4 150-300 cells/mm^3.	IDV/ZDV/3TC results in considerable improvement compared with ZDV/3TC.
NFV/ZDV/3TC vs ZDV/3TC	AVANTI 3	Gartland on behalf of the AVANTI study group 2001 [275]	CD4 increase and reduction in viral load in ART-naïve subjects with CD4 150-300 cells/mm^3.	NFV/ZDV/3TC has significantly better benefits than ZDV/3TC.

Notes: IDV = indinavir; ZDV = zidovudine; 3TC = lamivudine; RTV = ritonavir; SQV = saquinavir; ddC = zalcitabine; d4T = stavudine; APV = amprenavir; NFV = nelfinavir.

The first PI was approved in late 1995 [276]. Saquinavir (SQV), a hard gel formulation, when combined with both ZDV and ddC was shown to increase CD4 counts and decrease viral loads more than SQV/ZDV or ZDV/ddC in a randomised double-blind trial consisting of patients with previous exposure to NRTIs, despite SQV hard gel's low bioavailability (rate of absorption) [184]. In 1996, two more PIs were licensed for use either alone or in combination with NRTIs: ritonavir (RTV) [277] and indinavir (IDV) [278]. A randomised placebo-controlled trial investigating the efficacy of RTV in combination with up to two NRTIs, in patients previously on NRTIs only and with advanced HIV disease, found that the combination including RTV lowered the risk of AIDS complications and prolonged survival [182]. In NRTI-experienced patients with less than 200 CD4 cells/mm^3, IDV with ZDV and 3TC was found to be significantly more effective at slowing progression to HIV disease than ZDV and 3TC alone [180].

Two new PIs were introduced over the next couple of years: nelfinavir (NFV) [279] and amprenavir (APV) [280], as was a new formulation of SQV as a soft gel to improve the degree and rate at which the drug was absorbed [281]. NFV, as part of a cART regimen, was found to have more virological and immunological benefit than dual NRTI therapy [275]. APV with ZDV/3TC was also found to have a significantly higher rate of viral suppression than the two NRTIs alone [213]. A new version of this drug was licensed in 2003: fosamprenavir (fAPV), which had a slower release and resulted in higher blood levels of the active drug [282].

Lopinavir (LPV) was licensed in 2000 for use in a combined drug called Kaletra that contained a low dose of RTV [283]. Low dose RTV inhibits the metabolism of LPV and therefore enhances the concentration of the PI in the blood to above the levels needed to inhibit HIV replication. Trials have since been carried out to compare the antiviral efficacy of different RTV-boosted PIs to each other and to non-boosted PIs. These are displayed in Table 1.9. In the M98-863 phase III clinical trial, regimens containing either the RTV-boosted LPV (LPV/r) or NFV, together with d4T and 3TC, were compared in patients with no more than 14 days previous ART experience at any time [284]. The LPV/r arm was found to have superior antiviral activity and hence is currently recommended for first-line therapy [40-42]. The KLEAN study found similar virological efficacy between a fAPV/r containing regimen and one containing LPV/r in ART-naïve patients [285], and results from the CASTLE study showed similar virological responses in ART-naïve patients starting ATV/r and LPV/r [286].

Table 1.9: Major clinical trials in ritonavir-boosted versus non-boosted PI-containing regimens and comparisons of ritonavir-boosted PI-containing regimens.

Study	Clinical trial	Authors, year	Main outcomes	Conclusions
LPV/r/ d4T/3TC vs d4T/3TC/NFV	M98-863	Walmsley *et al.* 2002 [284]	Proportion with viral load <400 copies/mL at week 24 and time to loss of virological response at week 48 in subjects with no previous ART experience of more than 14 days at a time.	A combination regimen including LPV/r has superior virological efficacy than a NFV-containing regimen.
fAPV/r (once daily)/ABC/ 3TC vs NFV (twice daily)/ABC/3TC	SOLO	Gathe *et al.* 2004 [287]	Proportion with viral load <400 copies/mL and <50 copies/mL, and change in CD4 count at week 48 in ART-naïve subjects.	fAPV/r (once daily) was well-tolerated and provided potent, durable virological suppression.
LPV/r plus >1 NRTI vs SQV/r plus >1 NRTI	MaxCMin2	Dragsted *et al.* 2005 [288]	Proportion with treatment failure, defined as composite of virological failure, withdrawal of consent to participate, loss to follow-up, and death.	Less patients on the LPV/r regimen experienced virological failure than on SQV/r, which could be due to patient adherence rather than potency of drugs.
fAPV/r/ABC/ 3TC vs LPV/r/ABC/ 3TC	KLEAN	Eron *et al.* 2006 [285]	Proportion with viral load <400 copies/mL at week 48 in ART-naïve subjects.	A regimen containing fAPV/r provides similar virological efficacy as LPV/r, when each is combined with ABC/3TC.
ATV/r/TDF/FTC vs LPV/r/TDF/FTC	CASTLE	Molina *et al.* 2008 [286]	Proportion with viral load <50 copies/mL and <400 copies/mL, and change in CD4 count and viral load at week 48 in ART-naïve subjects.	ATV/r has similar virological efficacy to LPV/r.

Notes: LPV/r = ritonavir-boosted lopinavir; d4T = stavudine; 3TC = lamivudine; NFV = nelfinavir; fAPV/r = ritonavir-boosted fosamprenavir; ABC = abacavir; SQV/r = ritonavir-boosted saquinavir; ATV/r = ritonavir-boosted atazanavir; TDF = tenofovir; FTC = emtricitabine.

1.7.3.2 NNRTI-containing regimens

Alternative treatment options became available with the introduction of a third drug class. In combination with NRTIs, NNRTIs were found to be at least as effective as PI-containing regimens at suppressing the virus [289-291], although it was uncertain at first as to whether this was the case in patients starting therapy with low CD4 counts and high viral loads [292]. NNRTI regimens were particularly beneficial for those who could not tolerate the toxicities associated with PIs. Table 1.10 displays some of the major clinical trials comparing the efficacy of NNRTI-containing regimens to dual NRTI and PI-containing cART regimens.

Table 1.10: Major clinical trials in NNRTI-containing cART versus dual therapy and PI-containing cART.

Study	Clinical trial	Authors, year	Main outcomes	Conclusions
NVP/ZDV/ddI vs ZDV/ddI	ACTG 241	D'Aquila *et al.* 1996 [293]	Change in CD4 count and viral load at 48 weeks in NRTI-experienced subjects with CD4 ≤350 cells/mm^3.	Adding NVP to ZDV/ddI improved long-term immunological and virological effects of therapy.
NVP/ZDV/ddI vs ZDV/ddI	ISS 047	Floridia *et al.* 1999 [214]	Change in CD4 count and viral load and proportion with viral load <400 copies/mL at 24 and 48 weeks in ART-naïve subjects with AIDS or CD4 <200 cells/mm^3.	Three-drug combination more effective at sustaining immunological and virological response.
NVP/ZDV/ddI vs NVP/ZDV vs ZDV/ddI	INCAS	Montaner *et al.* 1998 [294]	Change in CD4 count and viral load (average of measures from weeks 40, 44, 48 and 52) and proportion with viral load <20 copies/mL at week 52 in ART-naïve subjects free from AIDS and with CD4 200-600 cells/mm^3. Rate of disease progression or death.	Three-drug combination led to significantly greater and sustained decrease in plasma viral load than the two-drug regimens.
NVP/ZDV/3TC vs NFV/ZDV/3TC	COMBINE	Podzamczer *et al.* 2002 [289]	Change in CD4 count and viral load and proportion with viral load <200 and <20 copies/mL at 12 months in ART-naïve subjects.	NVP/ZDV/3TC is at least as effective as NFV/ZDV/3TC as first-line therapy for HIV disease.
DLV/ZDV/ddI vs DLV/ZDV vs DLV/ddI vs ZDV/ddI	ACTG 261	Friedland *et al.* 1999 [295]	Change in CD4 count and viral load at 4-12 and 40-48 weeks in ZDV- or ddI-experienced subjects with CD4 200-500 cells/mm^3.	Therapy with DLV/ZDV/ddI showed modest, but not always significant, antiviral activity and CD4 count benefit compare to two-drug regimens.
EFV/ZDV/3TC vs EFV/IDV vs IDV/ZDV/3TC	DMP 266-006	Staszewski *et al.* 1999 [290]	Change in CD4 count and proportion with viral load <400 and <50 copies/mL at 48,72 and 144 weeks in PI-, NNRTI- and 3TC-naïve subjects with CD4 ≥50 cells/mm^3 and viral load > 10000 copies/mL.	EFV/ZDV/3TC has superior efficacy with durability of response than IDV/ZDV/3TC.
Initial regimens: EFV/ddI/d4T vs NFV/ddI/d4T vs EFV/ZDV/3TC vs NFV/ZDV/3TC vs EFV/NFV/ddI/ d4T vs EFV/NFV/ZDV/ 3TC	ACTG 384	Shafer *et al.* 2003 [291]	Comparison of time to regimen failure between four-drug regimens and three-drug regimens followed sequentially by further three-drug regimen with same NRTIs and opposite third drug in ART-naïve subjects.	There was no significant difference in the duration of successful treatment between a single four-drug regimen and two consecutive three-drug regimens. Initiating therapy with EFV/ZDV/3TC was the optimal choice.

Notes: NVP = nevirapine; ZDV = zidovudine; ddI = didanosine; 3TC = lamivudine; DLV = delavirdine; EFV = efavirenz; IDV = indinavir; d4T = stavudine; NFV = nelfinavir.

The first NNRTI was licensed in 1996: nevirapine (NVP) [296]. NVP in combination with ZDV and ddI was found to improve the long-term virological and immunological effects of the treatment compared to the NRTIs alone [214,293]. It was also found to be at least as effective as NFV in a three-drug regimen as first-line therapy [289].

The FDA licensed another NNRTI, delavirdine (DLV), in 1997 [220]. However the following year, an application for licensing this drug in Europe was rejected by the European pharmaceutical licensing board, as it was not considered to offer any unique benefits when compared to other NNRTIs [297]. A randomised trial found that a three-drug combination of DLV, ZDV and ddI resulted in moderately improved antiviral activity and CD4 count increase compared to each of the two-drug combinations of the same drugs, however these differences were not all statistically significant [295].

In 1998, efavirenz (EFV) was given approval [298]. In one trial, NNRTI-, PI- and 3TC-naïve patients were randomised to either EFV or IDV in combination with ZDV and 3TC. It was found that patients in the EFV arm were significantly more likely to suppress their viral loads to less than 400 copies/mL (undetectable by standard RNA assays at that time) than those in the IDV arm and that this combination was better tolerated [290]. Another trial compared time to regimen failure between EFV-and NFV-containing regimens [291]. Four-drug regimens containing two NRTIs and both EFV and NFV were compared to initial three-drug regimens containing two NRTIs plus either EFV or NFV sequentially followed by the same NRTIs plus the opposite third drug. The optimal choice was found to be EFV/ZDV/3TC.

EFV and NVP were compared in the 2NN study (2004) in a randomised trial of first-line ART. No difference was found in virological efficacy between regimens containing d4T/3TC and either EFV or NVP [299]. However a number of cohort studies found an increased risk of virological failure in those starting NVP [300-304]. This is investigated further in Chapter 6.

1.7.3.3 Triple NRTI regimens

With the increasing development of NRTIs, clinical trials were carried out to investigate the potential advantages of triple NRTI regimens. These would not only reduce and simplify pill burden leading to possible improvements in adherence, but also would avoid the risks of the severe toxicities associated with PIs. Furthermore, starting a triple NRTI regimen as first-line treatment (with no previous use of ART) would keep

the efficacy of other drug classes optimal for use in later therapy options if the NRTI regimen started to fail [305].

However, the results from clinical trials looking at ART-naïve patients showed no benefits in terms of antiviral efficacy in comparison to both PI- and NNRTI-containing cART regimens [185,306-308]. Staszewski *et al.* (2001) found that similar proportions of patients starting regimens containing a combination pill of ZDV and 3TC (Combivir) and either abacavir (ABC) (an NRTI that was approved in 1998 [309]) or IDV, achieved virological suppression at 48 weeks [307]. Gulick *et al.* in 2004 found that the triple NRTI regimen ZDV/3TC/ABC was virologically inferior to ZDV/3TC/EFV [185]. Due to the suboptimal virological response observed in trials, current guidelines do not recommend triple NRTI regimens for ART-naïve patients starting first-line therapy [40-42].

In ART-experienced patients, the use of triple NRTI regimens was in the past suggested as a simplification strategy. In a number of trials, in patients with a high level of viral suppression, switching to a triple NRTI combination from PI- and NNRTI-containing regimen was found to maintain suppression at least as well as continuing on a PI regimen and improved lipid profiles in some patients (reducing cardiovascular risks) [310-312]. This could be attributed to the increased number of adverse side effects associated with the PI, withdrawals of consent and a desire to simplify treatment. They are now only recommended in highly selected circumstances due to their low potency and the ease of resistant strains emerging. There is evidence that in patients with undetectable viral load (i.e. suppressed to a level undetectable by RNA assays) before switching, the emergence of NRTI-resistant strains may result in subsequent virological failure, therefore using this as a simplification strategy may need careful monitoring [313]. Indeed, evidence from the I.Co.N.A. (Italian Cohort of Antiretroviral Naïve Patients) study showed that patients who achieved virological suppression whilst receiving ABC-containing cART were more likely to experience viral rebound (i.e. if the viral load increases and remains above the level of detection) than those receiving EFV instead [314]. Table 1.11 provides the results from major clinical trials looking at triple NRTI regimens compared to PI- and NNRTI-containing cART regimens.

A number of combination drugs were approved over this period with the aim of reducing pill burden. Following Combivir in 1997 were Trizivir in 2000 (ZDV/3TC/ABC), Epzicom (USA) and Kivexa (Europe) (both 3TC/ABC) in 2004, Truvada in 2004 (FTC/TDF) and most recently Atripla (FTC/TDF/EFV) in 2006 [215,216,220].

Table 1.11: Major clinical trials in triple NRTI therapy versus PI-/NNRTI-containing cART.

Study	Clinical trial	Authors, year	Main outcomes	Conclusions
ZDV/3TC/ABC vs ZDV/3TC/EFV vs ZDV/3TC/ABC/EFV	ACTG 5095	Gulick *et al.* 2004 [185]	Virological failure (two consecutive viral loads >200 copies/mL) at 32 weeks and time to virological failure in ART-naïve subjects.	The triple NRTI combination was virologically inferior to both EFV-containing regimens.
ddI/d4T/3TC vs ddI/d4T/IDV vs ddI/d4T/NVP	Atlantic study	Van Leeuwen *et al.* 2003 [306]	% with viral load <500 copies/mL and <50 copies/mL at 48 weeks and 96 weeks in ART-naïve subjects with CD4 >200 cell/mm^3, viral load >500 copies/mL.	A smaller percentage of subjects randomised to receive 3TC as third drug reached a viral load <50 copies at 48 and 98 weeks in an on-treatment analysis.
ZDV/3TC/ABC vs ZDV/3TC/IDV	CNAAB3005	Staszewski *et al.* 2001 [307]	% with viral load <400 copies/mL at 48 weeks in ART-naïve subjects with CD4 >100 cell/mm^3, viral load >10000 copies/mL.	Triple NRTI regimen was equivalent to IDV-containing one in achieving virological suppression.
Continuation of two NRTIs/PI vs switching PI to ABC	CNA30017	Clumeck *et al.* 1999 [310]	Time to virological failure (two consecutive viral loads >400 copies/mL or premature discontinuation of treatment) subjects with viral load <50 copies/mL.	The replacement of a PI with ABC in a triple combination regimen following prolonged suppression of viral load provides continued virological suppression.
Continuation of three NRTIs vs continuation of two NRTIs/PI vs continuation of two NRTIs/NNRTI vs switching to ZDV/3TC/ABC (Trizivir)	AZL30002	Katlama *et al.* 2002 [311]	Time to virological failure (two consecutive viral loads >400 copies/mL) in subjects with CD4 ≥100 cells/mm^3 and viral load <400 copies/mL throughout therapy.	Switching to Trizivir had equivalent efficacy to triple therapy.
Continuation of PI-based regimen vs switching to ABC or EFV from PI		Maggiolo *et al.* 2003 [312]	% with viral load <50 copies/mL at 52 weeks in ABC- and NNRTI-naïve subjects with viral load <50 copies/mL.	Switching from a PI-based regimen to ABC or EFV maintains optimal levels of virological suppression.

Notes: ZDV = zidovudine; 3TC = lamivudine; ABC = abacavir; EFV = efavirenz; ddI = didanosine; d4T = stavudine; IDV = indinavir; NVP = nevirapine.

1.7.4 Current aims of cART

The primary aim of cART for initial treatment of HIV infection is to suppress viral replication for as long as possible, as measured by the viral load [40-42]. Virological suppression enables recovery of the immune system, which is indicated by the CD4 lymphocyte count and hence reduces the risk of opportunistic infections and death [186,187].

Treatment guidelines offer advice to physicians providing HIV care with regards to when to start therapy, what drugs to start with, when to change regimens and what regimens to switch to after therapy fails [40-42]. Current guidelines recommend that patients should start ART if they are symptomatic or severely immunocompromised, i.e. with a CD4 count less than 350 cells/mm^3. The recommended initial antiretroviral treatment is currently an EFV-containing regimen with a dual NRTI backbone when good adherence is expected, due to the convenience, superior virological suppression, lower rates of toxic effects and fewer drug interactions [40-42]. Alternatively, if a PI-containing regimen is prescribed, it should be boosted with RTV to increase blood levels of the other PI. Triple NRTI regimens are no longer recommended as initial therapy due to the inferior potency compared to an EFV-containing regimen.

Patients may change regimens or switch components of a regimen due to toxic effects, intolerance, inconvenience or failure of treatment to suppress the viral load. Causes of treatment failure include inadequate adherence [191-196], emergence or pre-existence of drug resistance [172,190] or low drug absorption [188,189]. If a patient experiences failure, the patient's immunological condition and remaining treatment options, determined by how much resistance the virus has built up to specific drugs or drug classes, are both considered when determining the recommended course of action.

In early treatment failure, when the patient still has many treatment options available, the aim is to achieve re-suppression of viral replication by substituting one or more drugs in the regimen or by completely switching regimen, depending upon the cause of failure [41,315]. In late treatment failure, in patients who still have high CD4 counts but with limited treatment options, it is reasonable to continue with the same regimen but with close monitoring of CD4 count. CD4 count can remain high for prolonged periods, even when the viral load is detectable, therefore keeping the risk of opportunistic infections low [316]. In late treatment failure, in patients with low CD4 counts, it is recommended that the patient continue treatment with the hope that new drug classes will become available [41,315].

The introduction of ART has drastically improved the outlook for HIV patients both in the short-term and long-term. However, the development of drug-resistant strains remains a major barrier to successful treatment. This is discussed next.

1.8 Antiretroviral drug resistance

Drug-resistant strains of HIV develop due to the high frequency of genetic errors during viral replication. These errors can result in survival benefits in the viral copies over the original virus, e.g. resistance to antiretroviral drugs. Treatment with antiretroviral drugs may then fail to suppress this new strain of drug-resistant virus and consequently the viral load may increase [16,317-323]. To understand how this happens, the process by which these changes occur is detailed.

1.8.1 Genetic mutations

As detailed earlier, HIV infection is characterised by a very high turnover of virus production. Replication of the virus is highly error-prone and results in a high frequency of genetic mutations in the replicated copies of the virus [4,10,13,324]. A genetic *mutation* is defined as an alteration or mistake in the genetic code [15]. If a mutation occurs in the specific code needed to manufacture the viral proteins, reverse transcriptase or protease, this may affect whether or not the antiretroviral drugs designed to target these proteins are able to inhibit replication of this virus.

1.8.2 Selection

The concept of natural *selection* (introduced by Charles Darwin [325]) highlights that species with characteristics advantageous to survival are more likely to reproduce than those without, meaning that the genes responsible for these characteristics are more likely to be passed on. In a viral population, a strain with mutations conferring resistance to antiretroviral drugs has a survival benefit in the presence of ART compared to drug-sensitive HIV (*wild-type*), i.e. it has a higher level of *fitness*, which is the ability to replicate in a well-defined environment. However in drug-resistant strains the efficiency of the target enzymes (e.g. reverse transcriptase, protease) is lowered, which results in inefficient replication (a reduced *replicative capacity*) in comparison to wild-type [38,326-330]. In the absence of ART, wild-type virus has a higher level of fitness and thus produces more successful replicated copies than drug-resistant HIV and remains the dominant viral strain. If therapy is stopped after drug resistance has developed, the wild-type may again become the dominant strain [331-334], for example, if 3TC is stopped, the HIV variants with the M184I/V mutation rapidly

disappear [335]. Figure 1.5 shows in a diagram how drug-resistant virus populations are selected.

Figure 1.5: Resistance and viral load.

Source: www.aidsmap.com.

1.8.3 Reducing the risk of drug resistance

Drug-resistant strains of HIV may emerge when treatment fails to suppress viral replication effectively either through suboptimal treatment, e.g. monotherapy, or moderately high but incomplete adherence [16,317-323]. In suboptimal concentrations of antiretroviral drugs, the virus has more chance to replicate and therefore it is more likely that resistance-associated mutations will occur. The most effective strategy to prevent the development of drug resistance is to completely suppress viral replication and sustain an undetectable viral load with the initial cART regimen [336,337].

In addition, the use of more than one drug class in cART means that viruses that develop resistance to one drug class may still be sensitive to the others and so with a rapid switch of drugs virological suppression may be sustained. Current guidelines recommend that single drug substitutions should generally be avoided to ensure that minor resistant strains do not emerge [338,339]. Different drugs or drug classes have different *genetic barriers* to resistance, that is thresholds of evolving a sufficient number of resistance mutations in the viral population to overcome the efficacy of the drugs in keeping the viral load suppressed. For example, NNRTIs have a low genetic barrier and only one critical resistance mutation needs to occur for NNRTI-resistant strains to emerge, therefore in an NNRTI-containing cART regimen it is vital to keep the viral load

undetectable. RTV-boosted PI regimens have high genetic barriers and drug resistance appears to be limited if virological failure occurs [340].

1.8.4 Resistance testing

HIV infected individuals may have resistance tests carried out in routine clinical practice to see if their dominant HIV strain has drug resistance. There are two types of resistance testing: *phenotypic* and *genotypic* [338,339,341,342]. Phenotypic tests measure the concentration of a drug required to reduce viral replication by a set amount compared to wild-type virus. Genotypic tests extract and sequence HIV RNA to look for specific mutations thought to be associated with drug resistance. Currently only RT and PR genes can be sequenced by commonly available assays. Both types of tests have strengths and weaknesses. Phenotypic tests detect actual drug resistance rather than simply listing mutations and are therefore easier to interpret. However they take longer to do and are twice as expensive as genotypic tests. Genotypic tests report the specific mutations rather than just low, medium or high-level resistance, which can be an advantage, but the presence of mutations might not necessarily mean the virus has full resistance to a drug [343]. This is why interpretation systems have been developed to improve the understanding of mutational interactions. These are discussed further below.

1.8.4.1 Genetic sequencing

To establish the presence of drug resistance mutations, the genetic sequence from the test HIV RNA is compared to a reference or *consensus* sequence, obtained from the RNA of wild-type HIV [344]. A consensus sequence is a sequence of the most common nucleotide bases. The reference sequence generally used in HIV research is from the HIV-1 strain HXB2 [344,345]. Comparison of the test RNA sequence against this reference sequence highlights the positions where there are differences, that is, where mutations have occurred and certain mutations or combinations of mutations have been found to be associated with phenotypic resistance to certain antiretroviral drugs [346]. Drug resistance can be interpreted using one of the established interpretation systems. These have either been devised by experts in the field based on literature looking at correlations between genotypic and phenotypic data or between treatment history and clinical response (rule-based system), or have been based on statistical modelling on databases of genotypes and phenotypes [347,348].

1.8.4.2 Genotypic resistance and interpretation systems

The International AIDS Society (IAS-USA) publishes updated lists of point mutations, i.e. specific positions on the RT and PR RNA genes thought to be related to resistance

[346]. Each position relates to a codon that encodes an amino acid. The amino acids expected in the wild-type virus are specified, together with the amino acid substitutions resulting from mutations that would indicate antiretroviral resistance. Using this, researchers can specify how many NRTI-, NNRTI-, major PI- and minor PI-associated mutations a virus has from the RNA sequence extracted from a plasma sample of the infected individual's blood. See Appendix I for the current IAS-USA list of drug resistance mutations from Spring 2008 [346].

However, the aim of conducting a genotypic resistance test is to predict how a patient will respond to therapy. Interpreting how drug resistance will impact on this is more complicated than merely reporting the number of resistance-associated mutations. Certain combinations of mutations can increase or decrease viral susceptibility to drugs and so a number of algorithms to interpret resistance have been developed to try and take these into account. In particular they define sets of drug-specific rules that identify combinations of mutations that confer resistance to each drug. Each algorithm then outputs a genotypic sensitivity score (GSS), which tries to predict how sensitive or how resistant the virus is to the drug regimen the patient is starting [347]. Three of the main algorithms presently used are the Rega algorithm [349] (Appendix II), the ANRS (Agence Nationale de Recherche sur le SIDA) algorithm [350] and the Stanford University algorithm (HIVdb) [351] (both found at http://hivdb.stanford.edu/pages/asi/).

1.8.4.3 Guidelines

The most recent guidelines for the clinical use of drug resistance testing from the IAS-USA and from the British HIV Association (BHIVA) recommend resistance testing at the time of HIV diagnosis where possible, as well as in all cases of virological failure [42,338]. It is now accepted that identifying the presence of resistance is an important addition in the management of patients in order to provide optimal treatment. Genotypic tests are not favoured over phenotypic but are more commonly used due to their lower cost and faster turnaround time.

1.8.5 Transmitted drug resistance

Drug-resistant strains can be transmitted to other individuals therefore it is possible for HIV infected individuals who have never received ART to have viral drug resistance [337,352,353]. As drug-resistant HIV variants tend to have a reduced replicative capacity compared to wild-type, in ART-naïve patients they remain in the minority and so transmitted drug resistance (TDR) may be difficult to pick up by routine resistance tests [339]. Prevalence of TDR has been reported in numerous studies and is discussed in detail in Chapter 5. However a problem with comparing estimates of

prevalence is that different definitions of resistance are used. Shafer *et al.* (2007) suggested a standardised list of mutations to define TDR [345].

1.9 HIV genetic diversity

Within both types of HIV, HIV-1 and HIV-2, there are further subtypes based on clustering of genetically similar strains. The HIV-1 subtypes are discussed in Chapter 4. Phylogenetic analysis, which is the method used to recognise different HIV strains, is discussed below.

1.9.1 Phylogenetic analysis

Phylogenetic analysis identifies clusters of genetically similar viral strains and uses a hierarchical model to construct the most likely evolutionary history [354]. A phylogenetic *tree* resembles a family tree diagram in appearance, mapping a sample of RNA sequences back in time to the nearest point in history at which they all share a common ancestor. This point is where all the branches in the tree merge and is called the *most recent common ancestor*. Each branch represents an event (mutation or recombination) that has led to a change in the nucleotide base. The genetic *distance* between two sequences is measured by the expected number of nucleotide substitutions that have occurred over time between them and their most recent common ancestor and can be represented by branch lengths in the tree. A statistical method can then be applied to determine the most likely tree topology and branch lengths.

Phylogenetic analysis can be used to determine the HIV-1 subtype an individual is infected with. Viral RNA strands extracted from a patient's blood plasma are sequenced and aligned to make a site-by-site comparison. If the person is only infected with one strain of HIV, all of the HIV RNA will have an ancestral sequence in common. These ancestral sequences can be compared against consensus sequences that have been identified for each subtype [344].

1.9.2 The HIV genome

Maps of the HIV-1 and HIV-2 genomes have been constructed to display the different regions that code for different functions. These are displayed in Figure 1.6 [18]. Three of the major regions are known as *env*, *pol* and *gag* [344,355,356]. The *env* (envelope) region encodes the glycoproteins, which interact with host cell surface receptors and are needed to allow the virus to enter the cell. The *pol* (polymerase) region contains the genetic code for producing the enzymes essential for viral replication, i.e. HIV

reverse transcriptase for converting viral RNA into DNA, integrase for incorporating the viral DNA into the cell DNA and protease for processing other HIV proteins into functional forms. The *gag* region encodes the structural proteins of the virus [18].

Figure 1.6: Genomic organisation of HIV-1 and HIV-2.

Source: Weiss (2000) [18].

1.9.3 Implications for vaccine development

The vast genetic variability of HIV, together with the complex viral biology and a limited understanding of the immune responses that protect against HIV infection, pose major challenges in the development of potential HIV vaccines. So far, these obstacles have not been overcome but finding a vaccine is essential for prevention of the continuing worldwide spread. In 1987, President Bill Clinton set a ten-year goal for developing an HIV/AIDS vaccine, however this has not been accomplished. Nevertheless, clinical trials continue to evaluate new vaccination strategies in the hope of developing a safe, effective and affordable vaccine. Phase I and II trials of candidate vaccines are being conducted [357-359].

1.10 Aims and objectives of this thesis

The broad aim of this thesis was to investigate the factors potentially associated with different responses to cART measured using virological and immunological endpoints, as well as the development of toxicities to a specific regimen. The analyses were based on data from the EuroSIDA study, which is a large, prospective, observational cohort study that includes patients from all across Europe and a minority from Israel and Argentina. Details of the study and of the EuroSIDA patients are described in

Chapter 2, which then goes on to detail the statistical methods employed in the analysis of endpoints throughout this thesis.

Chapter 3 investigates both regional differences and changes over calendar time in the rate of virological response to first-line cART. The motivation for this was to see if the ongoing clinical success of cART due to an increasing availability of new antiretroviral drugs and improvements in clinical support and the management of associated toxicities, was also reflected in an improving rate of virological success over time, and if so, in which regions. Three time periods were compared: early-cART years (1996-1997), mid-cART years (1998-1999) and late-cART years (2000-2004). The analysis was stratified by geographical region within EuroSIDA, which was split into South, Central West, North and East. Response to cART in these four regions was also investigated within each time period to see if regional variability had decreased over the years since ART became more widely available.

Chapter 4 compares virological and immunological response to cART across patients infected with different HIV-1 subtypes in a population where subtype B is prevalent. The aim of this was to investigate whether or not the genetic differences that define different subtypes appear to affect the efficacy of antiretroviral drugs. Emerging differences in genetic mutations and selection of resistance pathways have been observed between subtypes in other studies that could affect drug susceptibility and response.

Chapter 5 investigates trends in transmitted drug resistance (TDR) across ART-naïve patients in different geographical regions, patients infected with different HIV-1 subtypes and over calendar time. It also looks at the factors associated with detection of TDR and goes on to investigate whether or not the presence of baseline TDR has a negative impact on virological and immunological response rates following initiation of cART. The findings from analyses such as these are important for assessing the value of resistance testing in ART-naïve patients.

Chapter 6 specifically compares two NNRTIs in terms of virological response following initiation of treatment. A previous EuroSIDA analysis compared NVP-containing regimens with EFV-containing regimens and found that patients starting NVP were twice as likely to experience virological failure than patients starting EFV [300]. The analyses in this chapter aimed to re-investigate this difference and see if ART resistance present at the start of treatment could explain this finding. Resistance profiles at the time of virological failure were also compared between the two groups.

Chapter 7 looks at the toxic effects of cART. It investigates reasons for discontinuation of the NRTI, ABC, in particular the potentially fatal toxicity, hypersensitivity reaction (HSR). The incidence of ABC related HSR was investigated according to the line of therapy within which ABC was received, geographical region, calendar time, and co-formulation of ABC (as part of Kivexa or as part of either Trizivir or as a single tablet). The rate of death associated with ABC HSR was also determined. This is important to identify patients most at risk and to monitor trends.

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

Chapter 2. Patients and methodology

2.1 Patients

2.1.1 EuroSIDA and AIDS in Europe

The data analysed in this thesis are from the EuroSIDA study, which is a prospective observational cohort of 14,310 patients with HIV-1 infection. The project is one of the largest international cohort studies, so far spanning 95 centres across 31 European countries, Israel and Argentina. EuroSIDA began in May 1994 to continue the work generated by its predecessor, the AIDS in Europe study [360]. AIDS in Europe gathered retrospective data on every patient with AIDS diagnosed between 1979 and 1989 in 52 centres across 17 European countries, giving a total of 6572 patients enrolled into the study. It collected data from patient case-notes on demographics, HIV antibody status, CD4 lymphocyte counts, use of zidovudine, PCP (now known as *Pneumocystis jiroveci* pneumonia) prophylaxis, details of AIDS defining illnesses and opportunistic infections. Like EuroSIDA, the study was coordinated in Copenhagen by the project leader, Professor Jens Lundgren, director of the Copenhagen HIV Programme (CHIP) at Panum Institute in Copenhagen, Denmark. Details of CHIP can be found at http://www.cphiv.dk. EuroSIDA evolved from this initial study with the aim of collecting more detailed prospective data.

Primary support for EuroSIDA is provided by the European Commission BIOMED 1 (CT94-1637), BIOMED 2 (CT97-2713), the fifth Framework (QLK2-2000-00773) and the sixth Framework (LSHP-CT-2006-018632) programmes. Current support also includes unrestricted grants by Bristol-Myers Squibb, GlaxoSmithKline, Roche, Gilead, Pfizer, Merck and Co., Tibotec and Boehringer-Ingelheim, and the participation of centres from Switzerland was supported by a grant from the Swiss Federal Office for Education and Science. The study is guided by the steering committee, which consists of regional representatives from across Europe and is led by the chair, Bruno Ledergerber from Zürich in Switzerland. Regular elections are held for positions on the steering committee. Appendix III provides a full list of the members of the EuroSIDA study group. The study receives statistical contribution and support from the statistical centre in the Royal Free and University College Medical School, London and also data input relating to genotypic resistance and subtype diversity from the virology laboratory group, based in the central virology centres in London, UK (up to 2004) and in Badalona, Spain. Both the statistical centre and the virology coordinators are also involved in the proposal and development of new EuroSIDA projects.

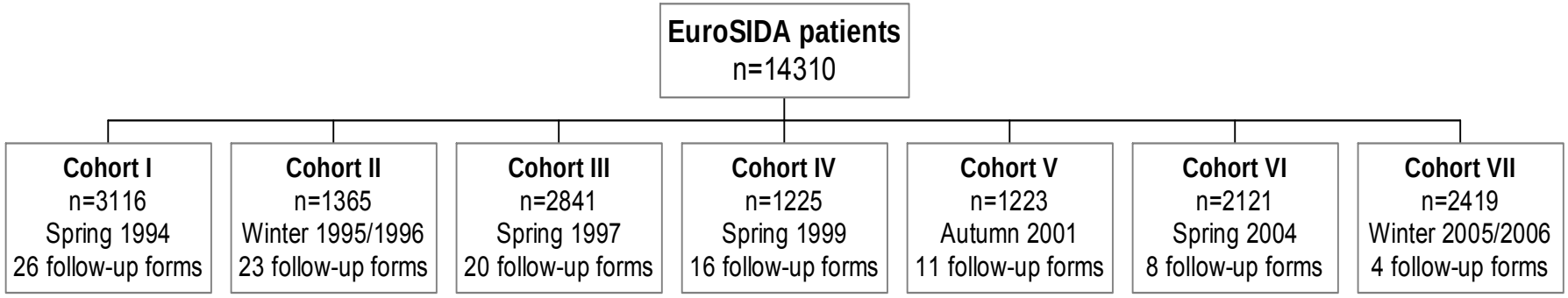
EuroSIDA is also one of the major contributors of data to the D:A:D study (Data collection on Adverse events of anti-HIV Drugs), which has the primary purpose of assessing the risk of myocardial infarction in HIV infected patients and is looking at cardiovascular risk factors associated with ART [361,362].

2.1.2 Data collection

The EuroSIDA study has so far enrolled seven cohorts of consecutive HIV-1 infected patients with a pre-booked clinic appointment, aged 16 or over, for which data were collected at their clinical centres. This ensures that the selection of patients under current follow-up is not based on any characteristics or background and so is unbiased to gain a representative sample from each clinic. Patients were enrolled until a predefined number was attained from each centre, starting with Cohort I in May 1994 (Figure 2.1). For Cohort I-III, eligible patients were those with a CD4 lymphocyte count of below 500 cells/mm^3 in the previous four months. This restriction was removed for Cohorts IV-VII. A further specification for Cohorts VI and VII were that half of the patients enrolled should be from East European countries. The reason for this was to boost the numbers in East Europe in order to gain a more comprehensive view of the epidemic in this region. To date, EuroSIDA has collected 74,581 person years of patient follow-up.

Information is provided on a standardised data collection form at baseline and every six months thereafter with follow-up to (median date of last visit) January 2007 at present. Examples of the current forms can be found in Appendix IV. At each follow-up visit, details on all CD4 counts and viral loads measured since the last follow-up, including methods used and the lower limits of detection, are collected. A total of 310,503 CD4 counts from 14,118 patients and 257,749 viral load measurements from 12,094 patients have been collected so far (including retrospective and prospective data), with a median average of 18 (interquartile range (IQR): 8-34) CD4 counts per patient (median 3.0 months (IQR: 2.0-4.1 months) between each measurement) and 19 (IQR: 9-32) viral load measurements per patient (median 3.0 months (IQR: 2.0-4.0 months) between each measurement). The dates of starting and stopping each antiretroviral drug, reasons for discontinuation of drugs, and the use of drugs for prophylaxis against opportunistic infections are also recorded, as are dates of diagnosis of all AIDS defining illnesses, including those diagnoses made subsequent to the initial diagnosis, using the 1993 clinical definition of AIDS from the CDC [109]. Information on dosing levels of drugs is not collected in EuroSIDA, however it is assumed that if patients start ritonavir (RTV) plus another PI at the same time, then this is likely to be a boosted PI regimen with low-dose RTV.

Figure 2.1: Patients, enrolment dates and number of follow-up forms available according to cohort in EuroSIDA.



Members of the coordinating centre visit all centres participating in EuroSIDA to ensure correct patient selection and to verify that accurate data are supplied by checking the information provided against case-notes for all patients with clinical events and 10% randomly selected patients per year. Centres have ethical approval according to their own local and national requirements and as part of the new contract with the European Commission, the coordinating centre is required to have copies on file of the approvals. The data collected in EuroSIDA is summarised in Table 2.1, which also indicates when new items were added to the follow-up forms.

Table 2.1: Summary of EuroSIDA data collected.

Demographics	Last HIV-subtyping ***ADDED Jun 1999***
Date of birth	Last HCV-subtyping ***ADDED Jun 2005***
Gender	Last resistance test ***ADDED Jun 2001***
Mode of infection	Hepatitis virology/serology results and dates ***ADDED Jan 1997***
Country of origin	HBV antibody test
Race	HBVsAg
Basic clinical information	HBV-DNA
Height	HCV antibody test
Weight	HCV-RNA
Blood pressure ***ADDED Jun 2000***	**Antiretroviral treatment within the last 5 years**
Smoking ***ADDED Jun 2000***	History of antiretrovirals taken:
Family history of MI ***ADDED Jun 2000***	Starting and stopping dates
Pregnancy in women ***ADDED Jan 1997***	If discontinued, reason for discontinuation ***ADDED Jan 1999***
Clinical events *ADDED Jun 1998*	Adherence rating ***ADDED Jan 2005***
Diagnosed since last follow-up of (with date of diagnosis):	**Treatment against infections**
Cardiovascular events	Drugs to prevent or treat opportunistic infection:
Metabolic events	Starting and stopping dates
Other organ events	**Treatment related to risk of cardiovascular disease**
Laboratory values (and dates of measurement)	Medication related to risk of cardiovascular disease:
Serum total and HDL cholesterol ***ADDED Jun 1999***	Starting and stopping dates ***ADDED Jun 2000***
Serum triglycerides ***ADDED Jun 1999***	**Severe opportunistic infections**
Plasma glucose ***ADDED Jun 2004***	Dates and diagnosis (definitive, presumptive, autopsy)
S-creatinine ***ADDED Jun 2004***	**Other severe infections**
Haemoglobin	Dates and diagnosis (definitive, presumptive, autopsy) ***ADDED Jan 2006***
Platelet count ***ADDED Jun 2005***	**AIDS defining malignancies**
ALT ***ADDED Jun 2000***	Dates and diagnosis (definitive, presumptive, autopsy)
AST ***ADDED Jun 2000***	**Non-AIDS defining cancers**
INR ***ADDED Jun 2000***	Dates and diagnosis (definitive, presumptive, autopsy)
Bilirubin ***ADDED Jun 2005***	**For patients who died**
S-lactate (not LDH) ***ADDED Jun 2000***	Date of death
S-amylase ***ADDED Jun 2002***	Autopsy performed
CD4 counts	Presumed cause
HIV RNA ***ADDED Jan 1997***	CoDe case report form ***ADDED Jan 2005***

All deaths and causes of death are collected in EuroSIDA. Due to the lack of a uniform classification of deaths in HIV patients, especially in the increasing number of non-AIDS deaths, in July 2004 the CoDe (Coding of Death in HIV) project was initiated to provide a standardised approach to collecting and reviewing data on causes of death in HIV. A pilot study was carried out to test the CoDe case report forms and guidelines at clinics in the D:A:D study and a revised version was released in February 2005.

2.1.3 Resistance database

EuroSIDA also requests that plasma samples from patients are collected every six months and shipped intermittently for storage in the central repository at the coordinating centre. This repository currently holds 59,590 plasma samples from 8349 patients. Samples are then selected based on patient inclusion criteria for a number of ongoing projects and viral RNA is extracted for sequence analysis. Genotypic resistance mutations can then be identified. This process is conducted retrospectively, even though the collection of plasma samples is done prospectively, and so resistance test results are not communicated to clinicians at the time of storing the sample. At present, the genotypic resistance database contains 4427 partial or full sequences of the HIV-1 RT (reverse transcriptase) and PR (protease) genes from 2535 patients. Sequence analysis of RT and PR reading frames is performed using the Trugene HIV-1 genotyping Kit and OpenGene DNA Sequencing System according to the manufacturer's recommendations (Bayer, Barcelona, Spain) and mutations are identified by comparison against a reference sequence of the subtype B isolate, HXB2. The database also holds information on mutations identified in resistance tests performed at the clinical sites, as requested on the follow-up forms. A total of 2143 paper copies of resistance test results from 1417 patients have been collected.

HIV-1 subtypes in EuroSIDA are primarily ascertained using the plasma samples analysed in the central virology laboratories. Phylogenetic analysis of the RT and PR genes is used to determine subtype. So far, 634 subtypes have been identified in this way. When sequences have been incomplete, serology methods have previously been used to determine subtype, which do not give as reliable results as phylogenetic analysis. The database holds 564 subtypes determined using a serology method. In the absence of plasma samples, clinics can provide information on subtypes based on the results of genetic sequencing conducted in the local laboratories. A total of 183 have been provided so far. If these are also not available, any subtype assignment in the patients' case-notes reported on the follow-up forms is taken. At present there are 2348 patients with HIV-1 subtype determined on the forms. This gives an overall total of 3729 subtypes identified in EuroSIDA.

2.1.4 Publications

As of April 2008, 92 publications have appeared in peer-reviewed journals, which have described analyses investigating a wide range of predictive markers for survival and response rates following initiation of ART. Analyses have used virological, immunological and clinical endpoints [147] and include looking at trends over time [140], response according to specific regimens [300], triple class failure [363], influence of hepatitis B [364] and C [365] on disease progression, drug resistance [366] and incidence of AIDS defining illnesses [367].

2.1.5 Patient characteristics in EuroSIDA at January 2007

To give a recent overview of the patients in EuroSIDA, the characteristics of 7932 patients who were under prospective follow-up at 1st January 2007 are summarised in Table 2.2. Three quarters of the study population were male and the majority were of white ethnicity. A total of 40.6% of individuals were exposed to HIV via homo/bisexual contact (men who have sex with men (MSM)), 21.0% were injecting drug users (IDUs) and 31.3% were heterosexual. Almost a third had been previously diagnosed with an AIDS defining illness, 6.0% were co-infected with hepatitis B and 24.5% were co-infected with hepatitis C. The median date of enrolment into EuroSIDA was June 2000 (interquartile range (IQR): March 1997-March 2004) and the median age was 44 (IQR: 38-51) years. Median CD4 count and viral load at the date 1st January 2007 were 480 (IQR: 330-661) cells/mm^3 and 1.7 (IQR: 1.6-2.1) log_{10}copies/mL respectively.

EuroSIDA is divided into broad geographical regions based on the location of the clinical centres, the borders of which were chosen arbitrarily early on in the study in order to make comparisons between areas of Europe. These are defined as South (Argentina, Greece, Israel, Italy, Portugal, Spain), Central West (Austria, Belgium, France, south Germany, Luxembourg and Switzerland), North (Denmark, Ireland, north Germany, Netherlands, Norway, Sweden, UK, Finland), and the newly established East region (Belarus, Russia, Ukraine, Serbia, Croatia, Poland, Hungary, Romania, Slovakia, Czech Republic, Bulgaria, Estonia, Latvia and Lithuania). Although not in Europe, Argentina and Israel were included after links were established with centres that offered to provide data and were added into the South region due to similarities in characteristics. The East region has further been split into Central East (Serbia, Croatia, Poland, Hungary, Romania, Slovakia, Czech Republic, Bulgaria) and East (Belarus, Russia, Ukraine, Estonia, Latvia and Lithuania), however numbers are still limited at present in both groups and therefore the aggregated East region is used in many analyses. At January 2007, 31.4% of patients were from the South region,

24.8% were from the Central West, 23.8% were from the North and 20.0% from the East.

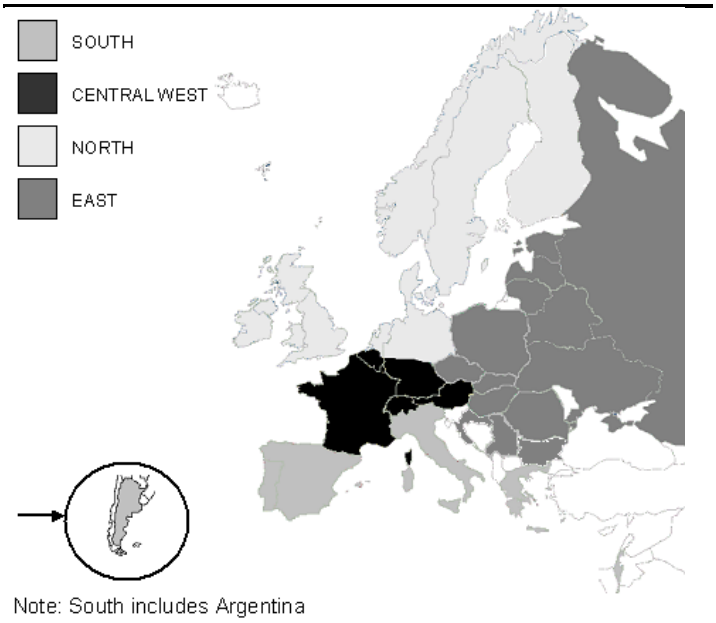
Table 2.2: Demographics and laboratory data of 7932 EuroSIDA patients under follow-up at 1st January 2007.

	n	%
All	7932	100
Male	5843	73.7
HIV exposure group		
MSM	3183	40.1
IDU	1665	21.0
Heterosexual	2481	31.3
Other	603	7.6
Ethnicity		
White	7007	88.3
Asian	153	1.9
Black	420	5.3
Unknown	352	4.4
EuroSIDA region*		
South	2493	31.4
Central West	1968	24.8
North	1886	23.8
East	1585	20.0
Origin		
Same country as centre	5758	72.6
Other European	409	5.2
Africa	435	5.5
America	341	4.3
Asia	114	1.4
Unknown	875	11.0
Prior AIDS	2422	30.5
Hepatitis B status		
Negative	6729	84.8
Positive	479	6.0
Unknown	724	9.1
Hepatitis C status		
Negative	5347	67.4
Positive	1943	24.5
Unknown	642	8.1

	Median	Interquartile range
Date of enrolment	Jun 00	Mar 97-Mar 04
Age (years)	44	38-51
CD4 count (cells/mm^3)		
At Jan 2007[(1)]	480	330-661
Nadir[(2)]	260	135-409
Time from nadir (years)	9	5-11
Viral load (log_{10}copies/mL)		
At Jan 2007 [(3)]	1.7	1.6-2.1
Maximum ever[(4)]	4.9	4.1-5.4
Time from HIV+ve diagnosis (years)	12	7-16

Note: Median CD4 counts and viral loads based on measurements from [(1)]6849, [(2)]7668, [(3)]6260 and [(4)]7566 patients.
MSM = men who have sex with men ; IDU = injecting drug use.

***EuroSIDA geographical regions**



Note: South includes Argentina

Table 2.3 summarises ART history and current regimens. At January 2007, 1117 (14.1%) of the 7932 patients were not receiving any ART, of which 639 (57.2% or 8.1% of all patients under follow-up) were ART-naïve. The remaining 478 (42.8% or 6.0% of all under follow-up) had discontinued therapy sometime before this date. The vast majority (90.4%) had previously received at least three different drugs and nearly two thirds had received at least six (not necessarily in the same regimen). Just over half had previously received all three drug classes (NRTIs, PIs and NNRTIs) and 84.1%

had received a cART regimen defined as at least three drugs including a PI, an NNRTI or abacavir (ABC). Current regimens mostly consisted of three or four drugs and approximately a third of patients were taking a ritonavir (RTV)-boosted regimen and another third, a single NNRTI regimen.

Table 2.3: History of ART and current regimens of 7932 EuroSIDA patients under follow-up at 1st January 2007.

	n	%		n	%
All	7932	100	**Current ART at Jan 07**		
Previous ART at Jan 07			Number of drugs		
ART-naïve	639	8.1	0	1117	14.1
Number of different drugs			1	42	0.5
0-2	760	9.6	2	184	2.3
3-5	2033	25.6	3	3456	43.6
6-8	2244	28.3	4	2443	30.8
9-11	1791	22.6	5+	690	8.7
12+	1104	13.9	Number of NRTIs		
Number of NRTIs			0	1382	17.4
0-1	726	9.2	1	451	5.7
2-3	2500	31.5	2	5187	65.4
4-5	2773	35.0	3+	912	11.5
6+	1933	24.4	Number of PIs		
Number of PIs			0	4059	51.2
0-1	3008	37.9	1	696	8.8
2-3	2861	36.1	2	2887	36.4
4-5	1596	20.1	3+	290	3.7
6+	467	5.9	Number of NNRTIs		
Number of NNRTIs			0	4959	62.5
0	2513	31.7	1-2	2973	37.5
1	4233	53.4	On cART regimen	6528	82.3
2+	1186	15.0	Single PI	573	8.8
Received NRTIs, PIs and NNRTIs	4388	55.3	RTV-boosted PI	2206	33.8
			Single NNRTI	2390	36.6
Received cART	6671	84.1	Triple NRTI	299	4.6
			Other	1060	16.2

Note: RTV = ritonavir.

2.2 Statistical methods

To analyse the data in this thesis a number of statistical tests and modelling techniques were used, which are described below. SAS software version 9.1 (SAS Institute, Cary, NC, USA, 2002-2003) was used to carry out all analyses.

2.2.1 Initial analyses

For initial exploratory purposes, descriptive methods and tests of significance were used to compare subsets or groups of patients for a number of variables. Pearson's chi-squared and Fisher's exact tests were used for categorical data and non-parametric

Wilcoxon rank sum and Kruskal-Wallis tests for continuous data, which all compare independent groups of data. The Fisher's exact test was used when more than 5% of the events had expected frequencies of less than five, which causes problems when using the chi-squared test as the approximation to the chi-squared distribution breaks down. The Wilcoxon rank sum test is used when there are just two groups (with unpaired data) and the Kruskal-Wallis is an extension of this for more than two groups. Non-parametric tests do not make any assumptions about the underlying probability distribution of the data, which is useful when it is impossible to assess this or when the data are clearly not normally distributed [368]. All tests were two-sided and a p value of less than 0.05 was taken to be statistically significant, i.e. to indicate that there is enough evidence to reject the null hypothesis of no difference between subsets of the data.

To obtain 95% confidence intervals (CIs) of proportions, p, with sample size, n, for np and $n(1-p)$ greater than five, normal approximations with continuity corrections (using the simple asymptotic method) were used [369]. Otherwise, exact binomial CIs were calculated.

2.2.2 Statistical models

Statistical models aim to provide an understanding of the relationships between variables and can be applied for both descriptive and predictive purposes. 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 HIV epidemiology research, models are used to look at a number of virological, immunological and clinical outcomes. Univariable models contain only one explanatory variable and are used to investigate what effect this variable alone has on the response. However, there may be additional factors that have associations with both the response variable and the explanatory variable that *confound* their relationship. For example, a univariable analysis may find that IDUs have a higher risk of death, but IDUs also typically have a lower CD4 cell count due to lifestyle factors, which is also associated with a higher risk of death. Therefore it is possible that the relationship between IDU and death could be due to CD4 cell count instead. To prevent bias of potentially confounding factors, multivariable analyses are used that contain more than one variable and adjust the effects of each variable to take account of the others in the model. Unfortunately in observational studies, there may still be unknown or unmeasured confounding variables, which is a limitation of analyses such as these.

Statistical *interactions* may also be adjusted for in multivariable analyses. These occur when the relationship between the explanatory and the response variable is stronger in some groups than in others, e.g. the cumulative effect of treatment exposure on cholesterol is worse in men than in women meaning that there is a statistical interaction between gender and length of time on treatment. As a general rule, statistical interactions between all variables are not routinely tested, because of the possibility of a false positive result due to repeated statistical testing. Interactions of interest are normally decided *a priori* based upon clinical suspicion.

Three main methods of statistical modelling were used in this thesis: logistic regression, survival analysis and Poisson regression. Linear regression is also used in some sensitivity analyses. Formal descriptions can be found in Appendix V. Overviews of what the models are used for and the reasons for choosing these particular methods are briefly outlined below.

2.2.2.1 Logistic regression

Logistic regression models are used when the response variable under investigation is of a binary form, i.e. with only two different outcomes [370,371]. These are often labelled 'success' and 'failure' usually corresponding to a positive and negative outcome, for example, a successful reduction of viral load to an undetectable level following ART as opposed to failure to reach an undetectable level. For simple analyses, the proportion of successes can be compared between groups. However, this probability of a success, p, is a number between 0 and 1 and in order to use regression methods for analysis, this has to be mathematically transformed into something that takes a number between minus infinity ($-\infty$) and infinity (∞). Hence, logistic regression models predict the log *odds* of observing a 'success', which can take values in $(-\infty,\infty)$, based on observed explanatory variables. The *odds* of observing a success are defined as the ratio of the probability of success to the probability of failure, i.e. $p/(1-p)$. This number is similar to the probability of a success when the outcome is rare.

This type of model is called a linear logistic model and assumes a linear relationship between the explanatory variables and the log odds of a 'success'. It can be used to estimate odds ratios (and 95% CIs) that compare the outcomes from two groups of patients. An *odds ratio* is defined as the ratio of the odds in one group to the odds in a second group. When the value is greater than one, this indicates that the first group

has greater odds of a success than the second. When it is less than one, the second has greater odds.

2.2.2.2 Survival analysis

Survival analysis methods are used to investigate time to an event from a well-defined time origin [372]. If the event is death, the data are literally survival times. Survival data are often highly skewed and have a non-normal distribution. However, their main feature is that they can be *censored* when the event has not been observed for an individual, for example, if for the duration of patient follow-up, the event has not been observed the patient can be censored at their last visit, which indicates that from that point in time onwards there was no information available. The assumption of this is that the actual survival time is independent of any mechanism that causes the censoring. Censoring that occurs after the last known survival time is called *right censoring* (as it is to the right when plotted on a graph) and gives a right censored survival time that is less than the unknown actual survival time. Data can also be *left censored* when the actual survival time is less than that observed.

A mathematical function that summarises a distribution of survival times is called the *survivor function*. This can be estimated using a Kaplan-Meier estimate, which can be displayed visually as a plot showing the cumulative rates of those experiencing an event. The median survival time (and other percentiles) can be read from this plot. The log-rank test is a non-parametric test that can be used to compare survival times in independent groups.

To assess the effects of explanatory variables on survival times, a proportional hazards regression model can be used. This models a *hazard function* that predicts the instantaneous risk or hazard of the event occurring at a given time point after the time origin and is conditional on the individual having survived up to that point. The model can estimate *hazard ratios* or *relative hazards* (and 95% CIs) to compare the risk of an event between groups of patients, which are comparable to odds ratios in a logistic regression model.

A proportional hazards model assumes that for different values of an explanatory variable, the relative hazards are proportional over time. This can be checked by including in the model an interaction term between the log survival time and the variable of interest. If the model fits significantly better after including this term, there is evidence of non-proportionality. It also assumes a log-linear relationship between the hazard and the explanatory variables. In this thesis, the Cox proportional hazards

regression model is used that makes no assumption about the shape of the distribution of the hazard function.

2.2.2.3 Poisson regression

Poisson regression models are used for count data to predict *rates* of an event and hence can be used to calculate *rate ratios* (and 95% CIs) [373]. As with a logistic regression model, a function is needed to transform the data into a form that can take values in $(-\infty,\infty)$ and the appropriate function for this is log-linear.

2.2.2.4 Linear regression

Linear regression is the most straightforward of the linear models, used when the data are continuous with a normal distribution and assuming a linear relationship with the explanatory variables [374]. Differences in the response can be compared between values of an explanatory variable.

2.2.3 Choice of statistical methods

All of the above methods are used in this thesis. Survival analysis is the most appropriate method to use for time-to-event data as it takes into account the proportion of patients at risk at each time point. However, the results may be biased when there are varying time intervals between measurements as patients who have a higher frequency of measurements have a higher chance of the analysis registering an event. Most of the main analyses investigate virological and immunological response to therapy and due to differences in the frequency of viral load measurements and CD4 counts between analysis subgroups, logistic regression is used in most of the main analyses. Unlike survival analysis, it is relatively robust to the number of measurements available.

In one particular analysis in Chapter 5, *generalised estimating equations* are used to fit the parameters of the logistic regression model due to the inclusion of repeated measurements on patients. This method takes into account the within-subject correlation between measurements from the same patient by using weighted combinations of observations and is detailed by Hanley *et al.* (2003) [375].

2.2.3.1 Choice of endpoint

Treatment guidelines indicate that successful therapy will result in an undetectable viral load within 16-24 weeks and suggest that viral suppression leads to a CD4 cell increase of 100-150 cells/mm^3 per year with an accelerated response in the first three months [40]. Throughout this thesis, the logistic regression models estimate the odds

that the first viral load measurement within the period six to twelve months after the defined baseline is at an undetectable level. As EuroSIDA data have been collected for a number of years and some early viral load assays only measured as low as 500 copies/mL [376], this is defined as the undetectable level for the main analyses. The study population was limited to patients with an unsuppressed baseline viral load of at least 500 copies/mL, measured within the six-month period prior to the defined baseline. To analyse immunological response, the first CD4 count within six to twelve months after the defined baseline was examined to estimate the odds that this was at least 100 cells/mm^3 more than at baseline so patients were required to have a baseline CD4 count. These endpoints were chosen to be consistent with previous EuroSIDA analyses [147,377], however different endpoints were investigated in sensitivity analyses.

2.2.3.2 Missing measurements

Two conventional approaches were investigated for patients with missing measurements in this six-month period: patients were either defined to be virological/immunological failures or they were excluded [378]. The first approach, 'missing = failure', was used in the main analysis in all chapters to maximise the number of patients included. This gives a conservative estimate of the failure rate, as the assumption is that measurements are missing due to patients being too ill to visit the clinic (and hence having a worse response to treatment). The second approach, 'missing = excluded', was used in a sensitivity analysis. These were intent-to-treat analyses in the sense that no adjustments were made for stopping or changing any component of the regimen.

2.2.3.3 Alternative methods used

Cox proportional hazards models were used in this thesis to conduct sensitivity analyses to check how robust the findings are to the statistical method used. They were also used in Chapter 6 as the main analysis. This was due to the frequency of viral load measurements being similar between the two groups being studied and also due to the fact that this chapter was following up a previous EuroSIDA analysis that used this method. For comparison with these previous results, it was most suitable to use the same method, however both logistic regression and linear regression were used to check the robustness of findings. The censoring used is defined in each chapter where appropriate.

Poisson regression was used in the final chapter as this was investigating a different aspect of the effects of ART. Incidence of discontinuation of the drug abacavir was

calculated and was compared between subsets of patients in a multivariable Poisson regression model.

2.2.4 Model building strategies

Multivariable models were generally developed throughout this thesis including all variables of interest in addition to any potentially confounding variables. Potentially confounding variables were chosen *a priori* to be those which had been found in previous studies or analyses to be associated with the outcome or where there was a valid reason to believe that there could be an association. Those found to be significantly associated with the outcome with a *p* value of less than 0.1 (to be conservative) were included in the multivariable model. A stepwise selection method was then used to confirm whether or not after adjustment for these variables, any further variables might also be explanatory. Any found to be significant significantly associated with the outcome (with a *p* value of less than 0.1) were added in. This meant that all variables that were associated with the outcome and therefore could potentially confound the findings related to the variables of interest, were adjusted for in the models.

2.2.5 Summary

The statistical methods described in this chapter have been employed throughout this thesis. Further details of the patients selected, the assumptions made and the specific details of endpoints analysed are described in each chapter. Sensitivity analyses have been used to check the robustness of the results to different assumptions.

Chapter 3. Virological response to first-line combination antiretroviral therapy across geographical regions and over calendar time

3.1 Introduction

The introduction of cART into clinical practice in 1995-1996 in developed countries such as North America and Western Europe resulted in dramatic reductions in mortality and morbidity rates in HIV infected individuals [118-121,139,140,179]. These have been sustained into recent years despite the long-term adverse effects potentially associated with treatment, such as liver damage, lactic acidosis, increased risk of cardiovascular disease and selection of drug resistance [172,223,224,361]. The increasing availability of new, more potent antiretroviral drugs, improvements in clinical support, management of associated toxicities, as well as a greater understanding of issues relating to non-adherence, all appear to be increasing the clinical success of cART over time [139,140]. This is also reflected in improvements in virological response rate [379-382].

Regional differences across Europe in the use of antiretroviral drugs, death rates and virological failure following the initiation of cART have been highlighted in EuroSIDA reports [383-385]. With the increasing widespread use of antiretroviral drugs across Europe, it is speculated that virological response rates may be more similar between different regions in later years as compared to periods closer to the time of the first introduction of cART. There may also be different temporal trends in response to cART across regions, according to when cART was introduced. This chapter investigates both of these hypotheses in patients starting cART for the first time with no previous ART experience.

3.1.1 Regional differences in use of ART and virological failure

Kirk *et al.* (1998) found a decrease over time in regional variation in the use of ART over the period 1994-1997 [383]. The odds of being on triple ART including a protease inhibitor in March 1996 were approximately double in patients visiting a clinic in Central and North Europe, compared to patients in South Europe, but by September 1997 these differences had diminished. These results indicated a quicker introduction of new treatments in Central and North Europe compared to South, which could be due to varying availability of drugs after marketing approval between countries and also socio-economic factors such as income and education. Another EuroSIDA study compared virological failure across South, Central and North regions and found significantly

higher failure rates in the South region at week 16 after starting cART [384]. This could be due to differences in treatment strategies and availability of new drugs.

3.1.2 Changes over time in use of ART and virological failure

Virological response rates have increased over calendar time since the introduction of cART [379-382]. Risk of virological failure following initiation of first-line cART was investigated in a large multi-cohort analysis over a seven-year period in Europe and Canada including 3825 patients from five HIV clinic cohorts [379]. The results of this analysis indicated a significant decrease in virological failure over calendar time, which appeared to be partly due to improvements in cART regimens. It could also be explained by factors such as increased clinical experience, improved toxicity profile of drugs and better patient adherence due to more effective clinical management and knowledge of treatment.

3.1.2.1 Guidelines for first-line cART regimens

Improvements in cART regimens have been achieved through increased knowledge of the combinations of drugs that are most effective from clinical trial and observational data. This is reflected in the changing guidelines for what drugs to start as first-line therapy. Guidelines published in 1996 suggested NRTI combinations as an initial regimen, reserving PIs for patients at higher progression risk [386]. In 1998, a PI and two NRTIs was the preferred initial option [387]. By 2000, with the wider variety of antiretroviral drugs available, guidelines recognised the need to individualise regimens based on factors such as tolerability, convenience and adherence likelihood, and possibly baseline resistance test results, but recommended regimens containing two NRTIs and either a PI (or two PIs) or an NNRTI [292]. As discussed in Chapter 1 (section 1.7.4), current treatment guidelines recommend that patients should start a combination of two NRTIs and either a single NNRTI (efavirenz (EFV)) or a ritonavir (RTV)-boosted PI (atazanavir (ATV), fosamprenavir (fAPV) or lopinavir (LPV)) [40,41]. The EFV-containing regimen is the preferred option for first-line cART recommended by the British HIV Association (BHIVA) guidelines [42].

3.1.2.2 Transmitted drug resistance

Another factor that may influence the rate of virological response in ART-naïve patients over calendar time is the prevalence of transmitted (or primary) drug resistance (TDR). ART-naïve patients do not have drug pressure encouraging the selection of drug-resistant viral strains but may acquire a drug-resistant HIV strain through transmission [337,352,353]. As detailed in Chapter 1 (section 1.8), viral drug resistance reduces the efficacy of antiretroviral drugs and limits future treatment options [172,338].

It is unclear from research as to whether TDR is increasing or decreasing over calendar time. An increase could be due to more people in the HIV infected population developing drug resistance as a result of exposure to ART, hence increasing the probability that it is transmitted [388-393]. The UK Group on Transmitted HIV Drug Resistance found an increase in prevalence of TDR over the period 1996-2003 [389]. However, the latest data from this group between 1996 and 2004 showed a decrease in TDR in later years [394]. These results could be explained by the fact that there is more accessibility to regimens that suppress the viral load, which reduces transmission rates.

3.1.3 Motivation and aims for chapter

Virological response to first-line cART has not previously been compared across the current four geographical regions defined in EuroSIDA (South, North, Central West and East). As EuroSIDA has longitudinal data spanning a period of over 13 years it is also the ideal dataset in which to monitor trends over calendar time. Few cohort studies have the capacity to look at both aspects in the same analysis. Therefore the aims of this analysis were to examine both temporal changes within the four regions, and also regional differences within calendar time periods (corresponding to treatment initiation in early-, mid- and late-cART years, i.e. 1996-1997, 1998-1999 and 2000-2004) in rates of virological suppression following initiation of cART in previously ART-naïve patients.

3.2 Methods

3.2.1 Inclusion criteria

The EuroSIDA dataset used for the analyses in this chapter was the update completed in December 2005. It included data on 11,928 patients with follow-up to (median date of last visit) May 2005. Patients were included who started cART, defined as at least three antiretroviral drugs including a PI, an NNRTI, or abacavir (ABC), between January 1996 and May 2004. This allowed patients to have the potential for at least one year's follow-up providing a suitable length of time to analyse response to cART. They were also required to have no previous ART experience and to have a viral load of at least 500 copies/mL measured in the six months before starting cART.

3.2.2 Statistical methods

3.2.2.1 Definitions

EuroSIDA geographical regions were defined similarly to Chapter 2 (section 2.1.5), except that there were less clinical centres involved in EuroSIDA at the time of this

earlier dataset. Data was included from 83 centres across 26 European countries, Israel and Argentina: South (26 centres in Spain, Portugal, Italy, Greece, Serbia-Montenegro, Israel, and Argentina), Central West (20 centres in France, Belgium, south Germany, Luxembourg, Switzerland, and Austria), North (18 centres in the UK, Ireland, Netherlands, north Germany, Denmark, Sweden, and Norway) and East (19 centres in Poland, Czech Republic, Slovakia, Hungary, Romania, Estonia, Latvia, Lithuania, Belarus, Ukraine, and Russia). Calendar time periods were defined according to the starting date of cART: early-cART (1996-1997), mid-cART (1998-1999) and late-cART (2000-2004). These were consistent with previous EuroSIDA analyses [140,179]. Baseline was defined as the date of starting cART and treatment discontinuation was defined as the date of the first drug in the initial regimen to be discontinued.

For patients with blood plasma samples taken within a year before the date of cART initiation that were subsequently tested for genotypic HIV drug resistance, resistance was defined as at least one NRTI, NNRTI or major PI resistance mutation according to the IAS-USA 2005 figures of HIV-1 drug resistance mutations [395].

3.2.2.2 Virological response to cART

Logistic regression was used to investigate the odds of a successful virological response to cART (defined as a viral load less than 500 copies/mL) at the first viral load measurement six to twelve months after initiation of the cART regimen, as discussed in Chapter 2 (section 2.2.3). Patients were required to have a baseline viral load of at least 500 copies/mL and baseline measurements were defined as those taken within the six-month period prior to starting cART. A 'missing = failure' approach was used in the main analysis.

Stratified multivariable models were developed to investigate the effects of calendar time periods within regions and regions within time periods on virological response. Time period was fitted in different ways: as a categorical variable (with two dummy variables) and as an ordered categorical variable. A further model was developed including time as a continuous variable instead of as a categorical variable.

Explanatory variables were identified as those significantly associated with the odds of a virological response in univariable analysis (p<0.1) or in a stepwise selection procedure. These were included in the multivariable model to ensure that any that could potentially confound the relationship between calendar time or region and the outcome were adjusted for. Factors investigated were: gender, age, HIV exposure

group (defined as ‘men who have sex with men (MSM)’, ‘injecting drug use (IDU)’, ‘heterosexual’ or ‘other’), country of origin (defined as ‘same as clinical centre’, ‘another European country’, ‘Africa’, ‘America’, ‘Asia’ or ‘other’), ethnicity (defined as ‘white’ or ‘other’), viral load measurements (baseline and maximum ever at baseline), CD4 counts (baseline and nadir), time from CD4 nadir, hepatitis B/C co-infection status, prior AIDS diagnosis, time from HIV diagnosis, type of regimen (defined as containing ‘a single PI: not saquinavir (SQV) hard gel’, ‘a single PI: SQV hard gel’, ‘a RTV-boosted PI’, ‘a single NNRTI’, ‘triple NRTI’ or ‘other’) and number of drugs in regimen. For pairwise comparisons, the pre-chosen reference categories were mid-cART years and the North region due to having the largest number of patients.

3.2.2.3 Sensitivity analyses

A sensitivity analysis was carried out using a ‘missing = excluded’ approach. Further sensitivity analyses were conducted, using both approaches to missing data, on the subset of patients who started cART after enrolment into EuroSIDA, excluding patients whose treatment data were collected retrospectively. These excluded patients who had already survived for a period up to enrolment implying that their treatment was successful enough to keep them alive, therefore the observed proportion of virological suppression in this group could have been greater than that of those starting cART after enrolment.

Virological response, measured as a viral load less than 50 copies/mL rather than 500 copies/mL, was also investigated. This analysis was conducted on a subset of patients whose first viral load measurements in the six to twelve month period after starting cART were measured using an assay with a level of detection as low as at most 50 copies/mL. This required all those with missing values to be excluded.

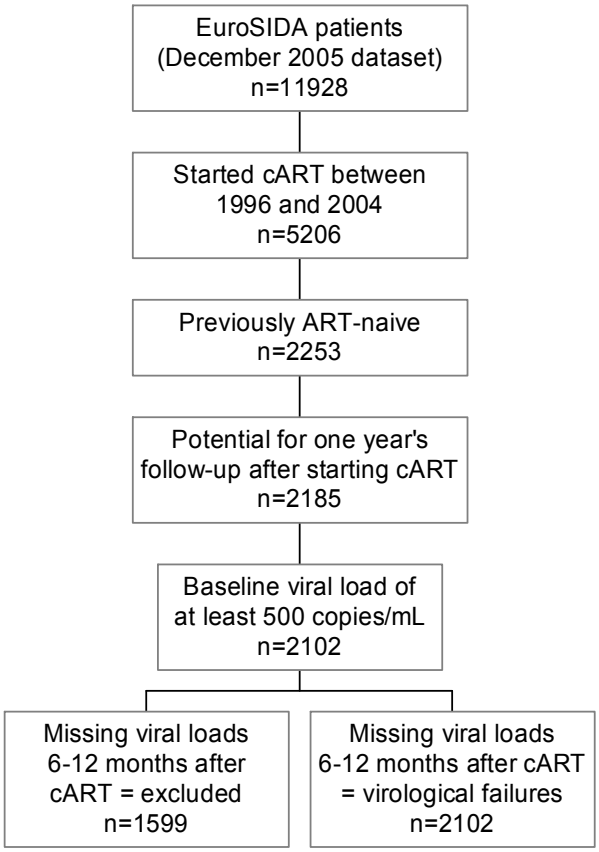
Survival analysis using Kaplan-Meier estimates and Cox proportional hazards models was used to check that the findings were consistent using a different statistical method. Time to virological suppression (a viral load less than 500 copies/mL), with right censoring at the date of patients’ last viral load measurement was investigated. Left censoring at date of enrolment into EuroSIDA was also investigated, again to exclude retrospective treatment data from patients.

3.3 Results

3.3.1 Patient numbers

A total of 2102 (17.6%) patients met the inclusion criteria, shown in Figure 3.1. Of these, 560 (26.6%) were from the South region (48 (8.6%) of which were from Israel and 88 (15.7%) of which were from Argentina), 466 (22.2%) were from the Central West, 606 (28.8%) were from the North and 470 (22.4%) were from the East. A total of 645 (30.7%) patients started cART in the early-cART years, 601 (28.6%) in the mid-cART years and 856 (40.7%) in the late-cART years.

Figure 3.1: Patient numbers in analyses according to inclusion criteria.



3.3.2 Patient characteristics at date of starting cART

3.3.2.1 Comparison of regions

Table 3.1 demonstrates the considerable heterogeneity in characteristics between regions. A significant difference was found in the dates of starting cART: April 1999 (median) in the South compared to January 1998 in the Central West, May 1998 in the North and March 2001 in the East (p<0.001). Median baseline CD4 counts ranged

between 200 and 244 cells/mm^3 (p=0.005) and viral loads between 4.8 and 5.1 log_{10}copies/mL (p<0.001). HIV transmission exposure groups differed significantly between regions (p<0.001). MSM were the main group in Central West and North regions (45.9% and 59.7% respectively), followed by heterosexuals (29.4% and 23.9% respectively). These groups were more evenly distributed in the South region (38.8% MSM and 36.6% heterosexual). In the East, there were similar proportions of patients exposed via IDU (31.9%) as MSM (28.7%) and heterosexual (31.3%) groups.

A total of 249 (11.9%) patients had plasma samples available within a year before starting cART that had been successfully tested for drug-resistant HIV: 23 (4.1%) in the South region, 34 (7.3%) in the Central West, 142 (23.4%) in the North and 50 (10.6%) in the East (p<0.001). Only 1 (4.4%) patient in the South had HIV with drug resistance (to NRTIs), 3 (8.8%) patients in the Central West (all with NRTI resistance), 9 (6.3%) in the North (6 of which had NRTI resistance, 2 of which had NNRTI resistance and 3 of which had PI resistance) and 4 (8.0%) in the East (3 with NRTI resistance, 1 with NNRTI resistance).

Time to treatment discontinuation or switches were compared across regions. Kaplan-Meier estimates of the median times to first treatment discontinuation were 25 months (95% CI: 23-30 months) in the South region, 19 months (95% CI: 16-21 months) in Central West and North, and 41 months in East (95% CI: 36-44 months) (p<0.001).

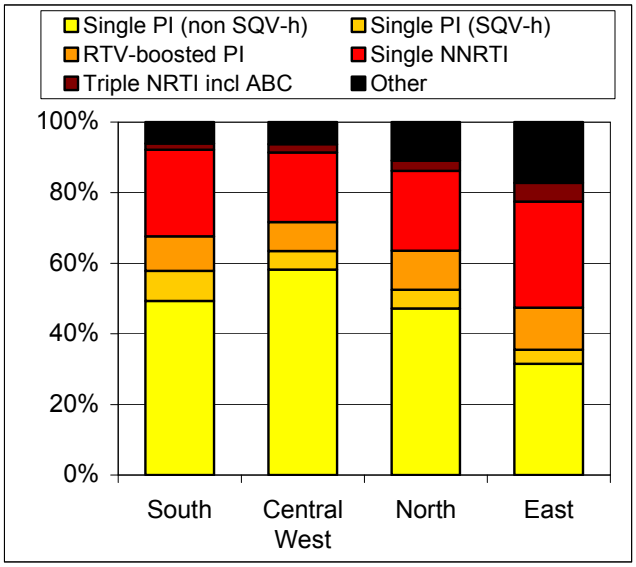
Table 3.1: Patient characteristics at start of cART according to geographical region within EuroSIDA.

	Total		South		Central West		North		East		*p*
n %											
All	2102	100	560	26.6	466	22.2	606	28.8	470	22.4	-
Male	1590	75.9	407	72.7	350	75.1	496	81.8	337	71.7	<0.001
HIV exposure group											<0.001
MSM	928	44.1	217	38.8	214	45.9	362	59.7	135	28.7	-
IDU	387	18.4	112	20.0	57	12.2	68	11.2	150	31.9	-
Heterosexual	634	30.2	205	36.6	137	29.4	145	23.9	147	31.3	-
Other	153	7.3	26	4.6	58	12.4	31	5.1	38	8.1	-
White ethnicity	1819	86.5	489	87.3	372	79.8	504	83.2	454	96.6	<0.001
Previous AIDS	413	19.6	145	25.9	80	17.2	118	19.5	70	14.9	<0.001
Hepatitis B status											<0.001
Negative	1299	61.8	293	52.3	289	62.0	435	71.8	282	60.0	-
Positive	103	4.9	26	4.6	15	3.2	41	6.8	21	4.5	-
Unknown	700	33.3	241	43.0	162	34.8	130	21.5	167	35.5	-
Hepatitis C status											<0.001
Negative	949	45.1	247	44.1	230	49.4	301	49.7	171	36.4	-
Positive	313	14.9	76	13.6	46	9.9	52	8.6	139	29.6	-
Unknown	840	40.0	237	42.3	190	40.8	253	41.7	160	34.0	-
Genotypic resistance test results available	249	11.8	23	4.1	34	7.3	142	23.4	50	10.6	<0.001
Median (IQR)											
Date started cART	Mar 99	(Sep 97-May 01)	Apr 99	(Jan 98-May 01)	Jan 98	(Mar 97-Mar 00)	May 98	(May 97-Apr 00)	Mar 01	(Dec 99-Dec	<0.001
Age (years)	36	(30-43)	36	(30-42)	37	(31-44)	38	(33-46)	33	(27-40)	<0.001
CD4 count (cells/mm^3)											
Baseline[(1)]	214	(98-345)	233	(99-374)	244	(103-368)	200	(101-300)	206	(84-346)	0.005
Nadir	193	(90-310)	205	(93-358)	220	(100-330)	177	(88-260)	183	(81-303)	<0.001
Viral load (log_{10}copies/mL)											
Baseline[(2)]	5.0	(4.5-5.5)	4.8	(4.3-5.3)	5.0	(4.5-5.5)	5.1	(4.6-5.5)	5.0	(4.5-5.6)	<0.001
Max ever	5.1	(4.6-5.5)	4.9	(4.4-5.4)	5.1	(4.6-5.6)	5.2	(4.8-5.5)	5.1	(4.6-5.7)	<0.001

Notes: *P* values obtained from Chi-squared, Fisher's Exact and Kruskal-Wallis tests.
Median CD4 counts and viral loads based on measurements from [(1)]2022 patients and [(2)]2046 patients.
MSM = men who have sex with men; IDU = injecting drug use; IQR = interquartile range.

There was also significant variation in types of cART regimen started (p<0.001), illustrated in Figure 3.2. Single PI-containing regimens were started by 57.9% of South patients, 63.5% of Central West, 52.5% of North and 35.5% of East patients. The next most widely used regimen contained an NNRTI and was started by 24.5% of South patients, 19.7% of Central West, 22.6% of North and 30.0% of East patients.

Figure 3.2: Type of cART regimen started according to geographical region within EuroSIDA.



Notes: SQV-h = saquinavir hard gel; RTV = ritonavir; ABC = abacavir.

3.3.2.2 Comparison of cART initiation periods

Table 3.2 summarises the characteristics of patients starting cART in the three defined time periods, early-, mid- and late-cART years. There was no evidence of a significant difference in median baseline CD4 counts across the three periods (p=0.170). Median baseline viral loads were between 4.9 and 5.0 $\log_{10}$copies/mL (p=0.005). Over time, the proportion of patients in the MSM exposure group decreased and IDUs and heterosexuals increased (p<0.001), which could be confounded by the increased number of East Europeans enrolled into EuroSIDA in later years. The percentage of patients with a previous AIDS diagnosis was reduced from 21.1% in early-cART to 17.9% in late-cART years (p<0.001).

Table 3.2: Patient characteristics at date of starting cART according to cART initiation period.

	Total		Early-cART 1996-1997		Mid-cART 1997-1998		Late-cART 2000-2004		*p*
n %									
All	2102	100	645	30.7	601	28.6	856	40.7	-
Male	1590	75.9	529	82.0	469	78.0	592	69.2	<0.001
HIV exposure group									<0.001
MSM	928	44.1	345	53.5	277	46.1	306	35.7	-
IDU	387	18.4	99	15.3	122	20.3	166	19.4	-
Heterosexual	634	30.2	147	22.8	172	28.6	315	36.8	-
Other	153	7.3	54	8.4	30	5.0	69	8.1	-
Ethnicity									0.107
White	1819	86.5	543	84.2	528	87.9	748	87.4	-
Other	283	13.5	102	15.8	73	12.1	108	12.6	-
Previous AIDS	413	19.6	136	21.1	124	20.6	153	17.9	<0.001
Hepatitis B status									0.232
Negative	1299	61.8	409	63.4	358	59.6	532	62.1	-
Positive	103	4.9	36	5.6	24	4.0	43	5.0	-
Unknown	700	33.3	200	31.0	219	36.4	281	32.8	-
Hepatitis C status									0.261
Negative	949	45.1	282	43.7	245	40.8	422	49.3	-
Positive	313	14.9	67	10.4	91	15.1	155	18.1	-
Unknown	840	40.0	296	45.9	265	44.1	279	32.6	-
Genotypic resistance test results available	249	11.8	99	15.3	76	12.6	74	8.6	<0.001
Median (IQR)									
Age (years)	36	(30-43)	36	(31-43)	35	(30-43)	37	(30-44)	0.236
CD4 count (cells/mm^3)									
Baseline[(1)]	214	(98-345)	220	(98-339)	230	(100-370)	205	(93-336)	0.170
Nadir	193	(90-310)	208	(90-315)	190	(91-332)	187	(86-296)	0.239
Viral load (log_{10}copies/mL)									
Baseline[(2)]	5.0	(4.5-5.5)	5.0	(4.5-5.5)	4.9	(4.4-5.4)	5.0	(4.5-5.5)	0.005
Max ever	5.1	(4.6-5.5)	5.1	(4.6-5.5)	5.0	(4.5-5.5)	5.1	(4.7-5.6)	<0.001

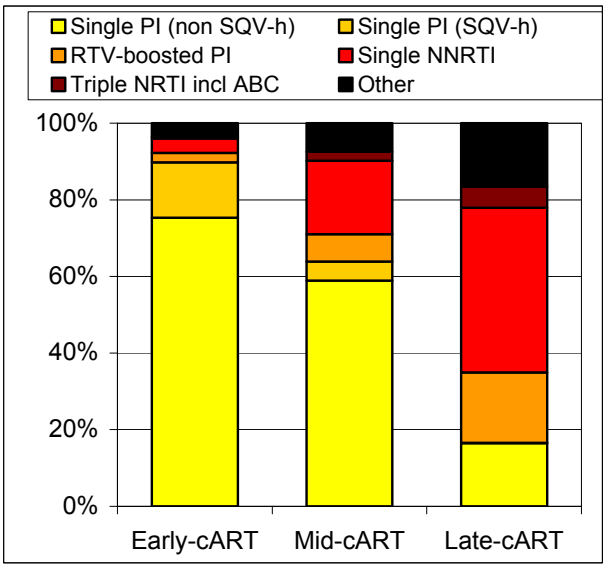
Notes: *P* values obtained from Chi-squared, Fisher's Exact and Kruskal-Wallis tests.
Median CD4 counts and viral loads based on measurements from [(1)]2022 patients and [(2)]2046 patients.
MSM = men who have sex with men; IDU = injecting drug use; IQR = interquartile range.

The proportion of patients with plasma samples available within a year before starting cART, which had been tested for HIV drug resistance, decreased over time: 99 (15.3%) patients in early-cART years, 76 (12.7%) in mid-cART years and 74 (8.6%) in late-cART years (p<0.001). A total of 7 (7.1%) patients in the early-cART period had HIV with drug resistance (6 of which had NRTI resistance, 2 of which had NNRTI resistance and 1 of which had PI resistance), 5 (6.6%) patients in mid-cART years (3 of which had NRTI resistance, 1 of which had NNRTI resistance and 1 of which had PI resistance) and 5 (6.8%) in late-cART years (4 of which had NRTI resistance and 1 of which had PI resistance).

Kaplan-Meier estimates of the median times to first treatment discontinuation were 18 months (95% CI: 16-20 months) in early-cART, 22 months (95% CI: 19-26 months) in mid-cART and 37 months (95% CI: 33-40 months) in late-cART years (p<0.001).

The types of cART regimen started also changed significantly over time (p<0.001), shown in Figure 3.3. Single PI regimens decreased from 89.8% in the early-cART period to 16.6% in late-cART, in favour of those containing a RTV-boosted PI and more commonly, an NNRTI. Single NNRTI regimens were started by 43.0% of patients in the late-cART period. The use of SQV hard gel, without low-dose RTV, as part of a single PI regimen was phased out over time. Triple NRTI regimens containing ABC were started by comparatively few patients in all three periods.

Figure 3.3: Type of cART regimen started according to cART initiation period.

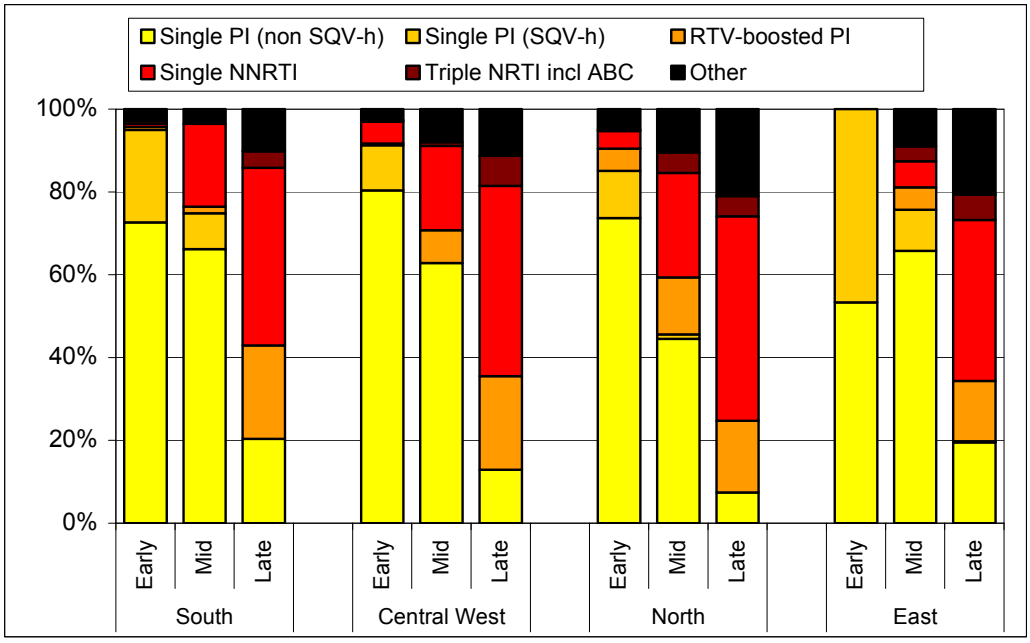


Notes: SQV-h = saquinavir hard gel; RTV = ritonavir; ABC = abacavir.

3.3.2.3 cART regimens over time within regions

Figure 3.4 illustrates how cART regimens have changed over time within each region. Similar patterns emerged in each region with single PI regimens decreasing in favour of RTV-boosted PI and single NNRTI regimens. However significant regional differences in type of cART regimen were found in early-, mid- and late-cART years (p<0.001 in each period).

Figure 3.4: Type of cART regimen started in each cART initiation period according to geographical region within EuroSIDA.



Notes: SQV-h = saquinavir hard gel; RTV = ritonavir; ABC = abacavir.

3.3.3 Virological response to cART: viral load less than 500 copies/mL at months six to twelve

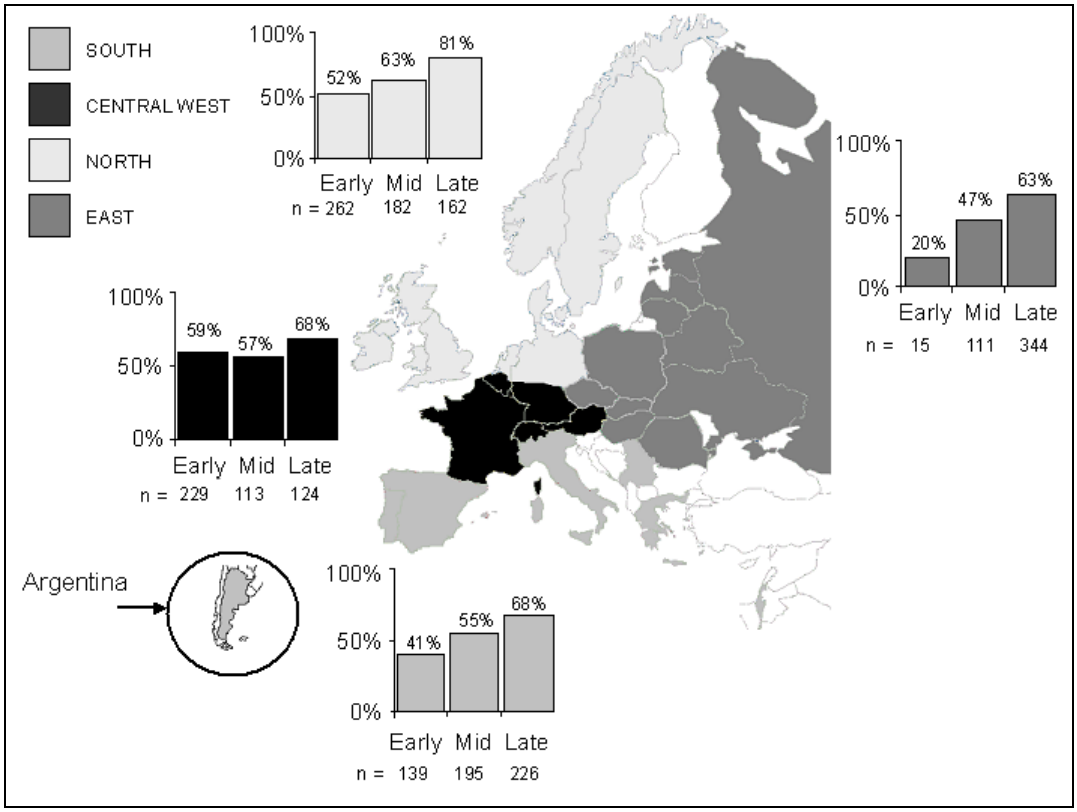
3.3.3.1 Main analysis: missing viral load = failure

A total of 1256 (59.8%) out of the 2102 patients included achieved virological suppression (less than 500 copies/mL) at the first measurement during the six to twelve month period after starting cART. In this same time window, 503 (23.9%) of the 2102 patients included had no viral load measurement available and so were counted as virological failures, although 432 (85.9%) of these had a subsequent measurement recorded a median of 19 months (IQR: 15-28 months) after starting cART. Median times to first viral load measurement in the six to twelve month period in each region were 33 weeks (IQR: 28-39 weeks), 31 weeks (IQR: 27-35 weeks), 32 weeks (IQR: 27-37 weeks) and 35 weeks (IQR: 26-40 weeks), in the South, Central West, North and East, respectively. In each time period, median times were 31 weeks (IQR: 28-37

weeks), 32 weeks (IQR: 26-39 weeks) and 33 weeks (IQR: 28-39 weeks), in patients starting in early-cART years, mid-cART years and late-cART years, respectively.

Response rates were found to be similar across regions: 317 (56.6%; 95% CI: 52.7-80.6%) of 560 South patients, 283 (60.7%; 95% CI: 56.5-65.4%) of 466 Central West patients, 384 (63.4%; 95% CI: 59.7-67.4%) of 606 North patients and 272 (57.9%; 95% CI: 53.6-62.5%) of 470 East patients (p=0.091). Over time, an increase in response rate was observed: 332 (51.5%; 95% CI: 47.8-55.5%) of 645 patients starting in early-cART years, 338 (56.2%; 95% CI: 52.4-60.4%) of 601 mid-cART patients and 586 (68.5%; 95% CI: 65.5-71.7%) of 856 late-cART patients (p<0.001). This increase was also reflected within each region (Figure 3.5), most noticeably in the East, rising from 20.0% in early-cART to 63.1% in late-cART years.

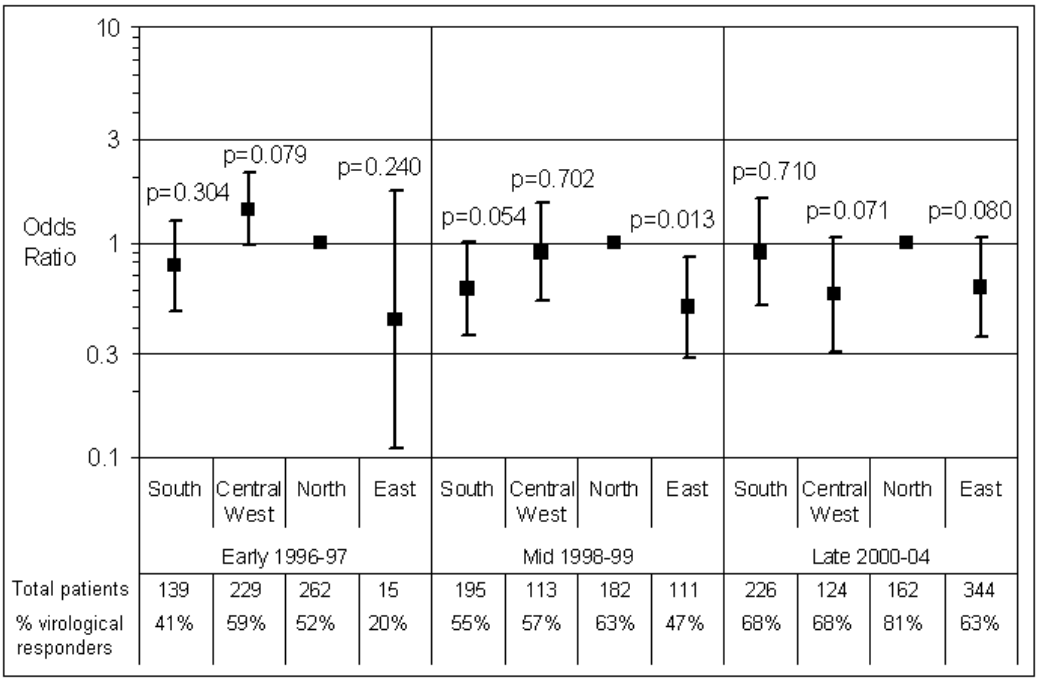
Figure 3.5: Virological response rates (less than 500 copies/mL) after starting cART over time within regions (missing = failure).



Variables significantly associated with the odds of a virological response were identified as: gender, age, HIV exposure group, origin, baseline CD4 count and viral load, hepatitis B/C co-infection, previous AIDS diagnosis, type of cART regimen and number of drugs in regimen. A multivariable logistic regression model adjusting for these variables was developed to look at the effects of region and time period (of cART initiation) on virological response. After adjustment for time period, region was found to have a significant effect (p=0.025 when time period was fitted as categorical, p=0.026 when it was fitted as ordered categorical, and p=0.037 when time was fitted as a continuous variable). Time period was also significant after adjustment for region (p<0.001). Although the interaction between time period and region was not found to be highly significant, possibly due to the low power for this test (p=0.088) stratified analyses were carried out as decided *a priori*.

To compare virological response in different regions within time periods, models stratified by time period and containing region were developed. Before adjustment for potential confounders, there were significant regional differences in the odds of achieving virological suppression in early-, mid- and late-cART periods with global p values p=0.001, p=0.054 and p<0.001 respectively. However, after adjustment for the variables identified above, these differences between odds of virological response by region were not observed in mid- or late-cART years (p=0.291 and p=0.163 respectively) and in early-cART the difference was borderline significant (p=0.068). Pairwise comparisons of regions with the North region within time periods showed one significant difference in the odds of a virological response in the East in mid-cART years (multivariable odds ratio (OR): 0.50; 95% CI: 0.29-0.86; p=0.013). However there was a tendency for a poorer response rate in the East within all time periods. These comparisons should be treated with caution due to multiple testing over time. Figure 3.6 displays these multivariable ORs with 95% CIs.

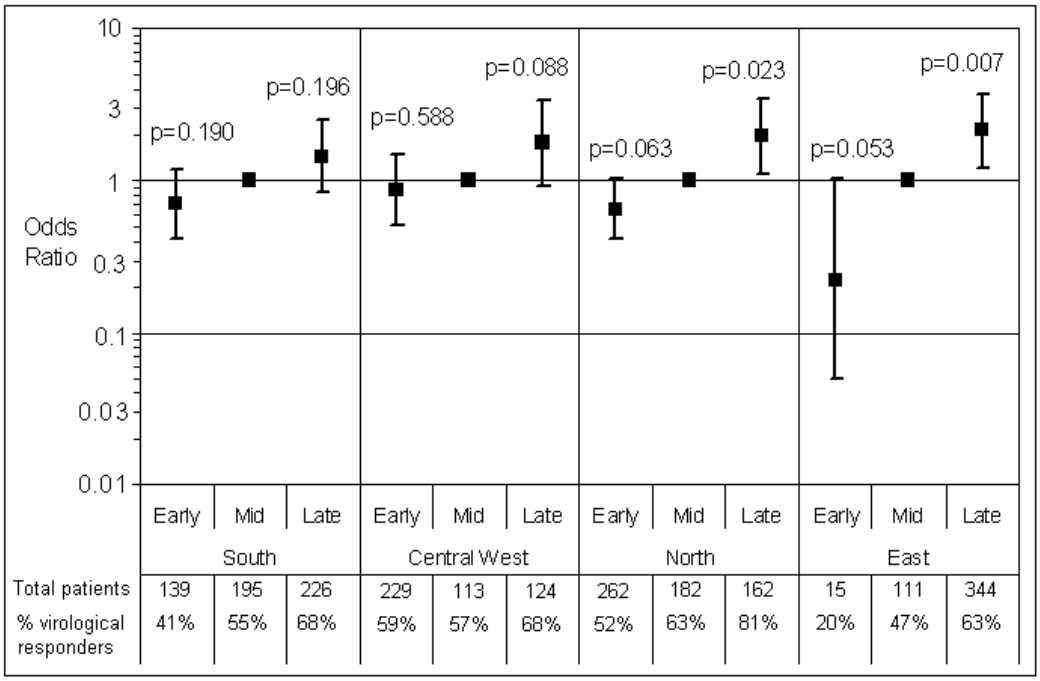
Figure 3.6: Multivariable odds ratios and 95% confidence intervals of virological response (less than 500 copies/mL) after starting cART in each cART initiation period according to region (missing = failure).



Note: Analysis adjusted for gender, HIV exposure group, previous AIDS diagnosis, origin, hepatitis B/C co-infection, baseline CD4 count and viral load, age, type of cART regimen and number of drugs in regimen.

To compare virological response in time periods within each region, models stratified by region and containing time period (ordered categorical) were developed. Before adjustment, there were significant differences over time between the odds of virological suppression in South, North and East regions, with global *p* values $p<0.001$ in each case (no significant difference in Central West, $p=0.150$). After adjustment, these differences were borderline significant in South and North regions ($p=0.061$ and $p=0.070$ respectively) and significant in Central West and East ($p<0.001$ and $p=0.001$ respectively). Pairwise comparisons between mid-cART and early-/late-cART showed that North and East regions had significantly higher odds of virological suppression in the late-cART period (OR: 1.96; 95% CI: 1.10-3.49; $p=0.023$; and OR: 2.14; 95% CI: 1.23-3.73; $p=0.007$ respectively). These are displayed in Figure 3.7.

Figure 3.7: Multivariable odds ratios and 95% confidence intervals of virological response (less than 500 copies/mL) after starting cART in each region according to treatment initiation period (missing = failure).



Note: Analysis adjusted for gender, HIV exposure group, previous AIDS diagnosis, origin, hepatitis B/C co-infection, baseline CD4 count and viral load, age, type of cART regimen and number of drugs in regimen.

3.3.3.2 Sensitivity analysis: missing viral load = excluded

Excluding patients with missing viral load measurements at six to twelve months provided mostly similar findings to those of the main analysis (Table 3.3). The number of eligible patients in this analysis was reduced from 2102 to 1599 patients and response rates were higher as expected. Virological suppression was achieved by 78.5% overall: 74.4% (95% CI: 47.8-55.5%) of 426 South, 80.6% (95% CI: 76.8-85.0%) of 351 Central West, 77.4% (95% CI: 73.9-81.3%) of 496 North and 83.4% (95% CI: 79.7-87.8%) of 326 East patients (p=0.017). Response rates increased over time with 67.8% (95% CI: 63.8-72.1%) of 490 early-cART patients, 78.6% (95% CI: 75.0-82.7%) of 430 mid-cART patients and 86.3% (95% CI: 83.9-89.0%) of 679 late-cART patients achieving viral load suppression (p<0.001). These rates remained almost identical when including patients with missing viral loads between months six to twelve who had a subsequent measurement after this time; overall 1578 (78.1%) of 2031 patients had a positive virological response.

Table 3.3: Multivariable odds ratios and 95% confidence intervals of virological response (less than 500 copies/mL) after starting cART (missing = excluded).

		Total patients	No. VL responders	% VL responders	Multivariable OR	95% CI	*p*
Early 1996-97 (n=490)	South	102	57	56%	0.83	(0.46-1.47)	0.514
	Central West	173	135	78%	2.14	(1.28-3.59)	0.004
	North	206	137	67%	1.00	-	-
	East	9	3	33%	0.51	(0.10-2.60)	0.416
Mid 1998-99 (n=430)	South	139	107	77%	0.97	(0.48-1.95)	0.934
	Central West	79	64	81%	1.38	(0.63-3.01)	0.419
	North	147	115	78%	1.00	-	-
	East	65	52	80%	1.90	(0.78-4.62)	0.158
Late 2000-04 (n=679)	South	185	153	83%	0.73	(0.30-1.76)	0.481
	Central West	99	84	85%	0.93	(0.35-2.46)	0.876
	North	143	132	92%	1.00	-	-
	East	252	217	86%	0.88	(0.37-2.10)	0.773
South (n=426)	Early	102	57	56%	0.46	(0.23-0.92)	0.028
	Mid	139	107	77%	1.00	-	-
	Late	185	153	83%	0.93	(0.42-2.08)	0.864
Central West (n=351)	Early	173	135	78%	1.01	(0.47-2.18)	0.983
	Mid	79	64	81%	1.00	-	-
	Late	99	84	85%	1.38	(0.48-4.00)	0.556
North (n=496)	Early	206	137	67%	0.62	(0.34-1.12)	0.111
	Mid	147	115	78%	1.00	-	-
	Late	143	132	92%	2.24	(0.94-5.32)	0.069
East (n=326)	Early	9	3	33%	0.06	(0.01-0.40)	0.004
	Mid	65	52	80%	1.00	-	-
	Late	252	217	86%	2.69	(0.99-7.30)	0.053
Total		1599	1256	79%			

Notes: Multivariable analysis adjusted for gender, HIV exposure group, previous AIDS diagnosis, origin, hepatitis B/C co-infection, baseline CD4 cell count and viral load, age, type of cART regimen and number of drugs in regimen.
VL = viral load; OR = odds ratio; CI = confidence interval.

After adjustment for the variables previously identified as significantly associated with the response, in addition to time period (ordered categorical), region was found to have a significant effect on virological response (p=0.028) as was time period after adjustment for region (p<0.001). This corresponds to the findings from the main analysis. Testing for an interaction between time period and region gave a significant result (p=0.039).

Within mid- and late-cART periods after adjustment, there were no significant regional differences in the odds of a virological response (p=0.447 and p=0.644 respectively) but within the early-cART period, there was a significant difference (p=0.016). This was borderline significant in the main analysis. Pairwise comparisons with the odds of virological suppression in the North region showed one significant difference with the odds in the Central West in the early-cART period (OR: 2.14; 95% CI: 1.28-3.59; p=0.004).

Significant differences were found over time in North and East regions after adjustment (p=0.008 and p=0.006 respectively). No significant differences were found in the South (p=0.134) or Central West (p=0.751). This differed from the main analysis that found a significant temporal trend in the Central West. Compared to the odds of a virological response in mid-cART years, the odds in early-cART years were significantly lower in the South region (OR: 0.46; 95% CI: 0.23-0.92; p=0.028) and particularly in the East (OR: 0.06; 95% CI: 0.01-0.40; p=0.004). Borderline significant differences were observed between the odds in mid- and late-cART years in North and East regions (p=0.069 and p=0.053 respectively).

3.3.3.3 Sensitivity analysis: subset of patients with prospective treatment data

Analyses with the same endpoint as the main analyses, but excluding patients who started cART before enrolment into EuroSIDA, were carried out. Using a 'missing = failure' approach, a total of 607 patients were included, of which 64.1% experienced a virological response. The proportion of virological responders across regions differed significantly in this subset: 47.2% (95% CI: 39.7-56.1%) of 144 South patients, 72.4% (95% CI: 64.6-82.3%) of 98 Central West, 71.2% (95% CI: 66.2-77.0%) of 271 North and 60.6% (95% CI: 51.8-71.6%) of 94 East patients (p<0.001). However the proportion of virological responders over time did not change significantly: 60.6% (95% CI: 54.6-67.6%) of 216 early-cART patients, 62.1% (95% CI: 55.4-69.8%) of 174 mid-cART and 69.1% (95% CI: 63.4-75.7%) of 217 late-cART patients (p=0.149). Adjusting for the previously identified potentially confounding variables, as well as time period (ordered categorical) and region, there was again an overall significant regional difference (p=0.023) but unlike in the main analysis there was no significant temporal difference (p=0.635). As there were no patients in the East region who started cART before enrolment into EuroSIDA in early-cART years, stratified analyses were carried out in the remaining regions and time periods. There was no significant regional difference found in mid-cART years (p=0.580) but a borderline difference in late-cART years (p=0.073). Stratified by region, there was one borderline significant temporal difference in the North (p=0.083).

Using a 'missing = excluded' approach left a total of 506 patients to be included, with a higher response rate of 76.9%. The global p values after adjustment for potential confounders were p=0.044 for the regional difference and p=0.407 for the temporal difference.

3.3.4 Virological response to cART: viral load less than 50 copies/mL at months six to twelve

Taking the subset of patients whose first viral loads in the period six to twelve months after initiation of the cART regimen were measured using an assay with a lower limit of detection of 50 copies/mL or less, gave a total of 1408 patients. The overall virological response rate was found to be 48.7%. Across regions, a significant difference was found: 44.7% (95% CI: 40.0-50.0%) of 380 patients in the South, 42.9% (95% CI: 37.9-48.4%) of 336 Central West patients, 48.6% (95% CI: 44.3-53.2%) of 484 North patients and 65.9% (95% CI: 59.9-72.8%) of 208 East patients (p<0.001). There was also a significant difference between time periods: 15.9% (95% CI: 12.8-19.4%) of 471 patients starting in early-cART, 52.3% (95% CI: 47.6-57.6%) of 384 patients starting in mid-cART and 74.1% (95% CI: 70.7-78.0%) of 553 patients in late-cART years (p<0.001). After adjustment for potential confounders, there appeared to be an overall borderline significant regional difference (p=0.062) and a significant temporal difference (p<0.001) in virological response to cART. Stratified analyses showed no significant regional differences in virological response in mid- and late-cART years (p=0.655 and p=0.205 respectively). No patients in the East in early-cART years achieved virological suppression, most likely due to the small number and the lack of more sensitive viral load assays. However there were significant temporal differences in South, Central West and North regions (p<0.001 for all).

3.3.5 Virological response to cART: time to viral load less than 500 copies/mL

Binary endpoints were investigated in the main analyses due to potential differences in the frequency of viral load measurements between regions or time periods. Survival analysis techniques were used as an alternative method to see how robust the findings were. The same inclusion criteria applied and so 2102 patients were included in these analyses.

3.3.5.1 Frequency of measurements

A significant difference was found between regions in the median frequency of viral load measurements in the first year following the start of cART. Median frequencies of viral load measurements were 2 (IQR: 1-4) in the South region, 4 (2-5) in the Central West, 4 (2-5) in the North and 2 (1-3) in the East region (p<0.001).

There was also a statistically significant difference in the median numbers of viral load measurements in the first year of cART comparing people who started in different

calendar time periods. Median numbers of viral load measurements were 3 (IQR: 1-5) in early-cART years, 3 (1-4) in mid-cART and 3 (2-5) in late-cART years (p<0.001).

3.3.5.2 Kaplan-Meier estimates

Figure 3.8 displays Kaplan-Meier estimates of the percentage of patients achieving virological suppression from date of starting a cART regimen, according to region and stratified by time period. The log rank tests showed significant regional differences in each of the time periods (p<0.001 in early- and late-cART years, p=0.002 in mid-cART years). Kaplan-Meier estimates according to time period and stratified by region showed significant temporal differences in all regions (all p<0.001).

3.3.5.3 Cox proportional hazards models

Cox proportional hazards models, adjusted for the same potential confounders as in the main logistic regression analysis, revealed overall that both region adjusted for time period, and time period adjusted for region had significant effects on the risk of virological response (p<0.001 for both).

Stratified by time periods, and again after adjustment, there was a significant regional difference in the risk of virological response in early-cART years (p=0.013). In mid- and late-cART years, there were no regional differences (p=0.104 and p=0.154 respectively). Pairwise regional comparisons of the relative hazards (RHs) of virological response (with 95% CIs) are displayed in Figure 3.9.

Figure 3.8: Kaplan-Meier estimates of the percentage of patients achieving a virological response (less than 500 copies/mL) by date from start of cART regimen, according to region in (a) early-cART years, (b) mid-cART years and (c) late-cART years.

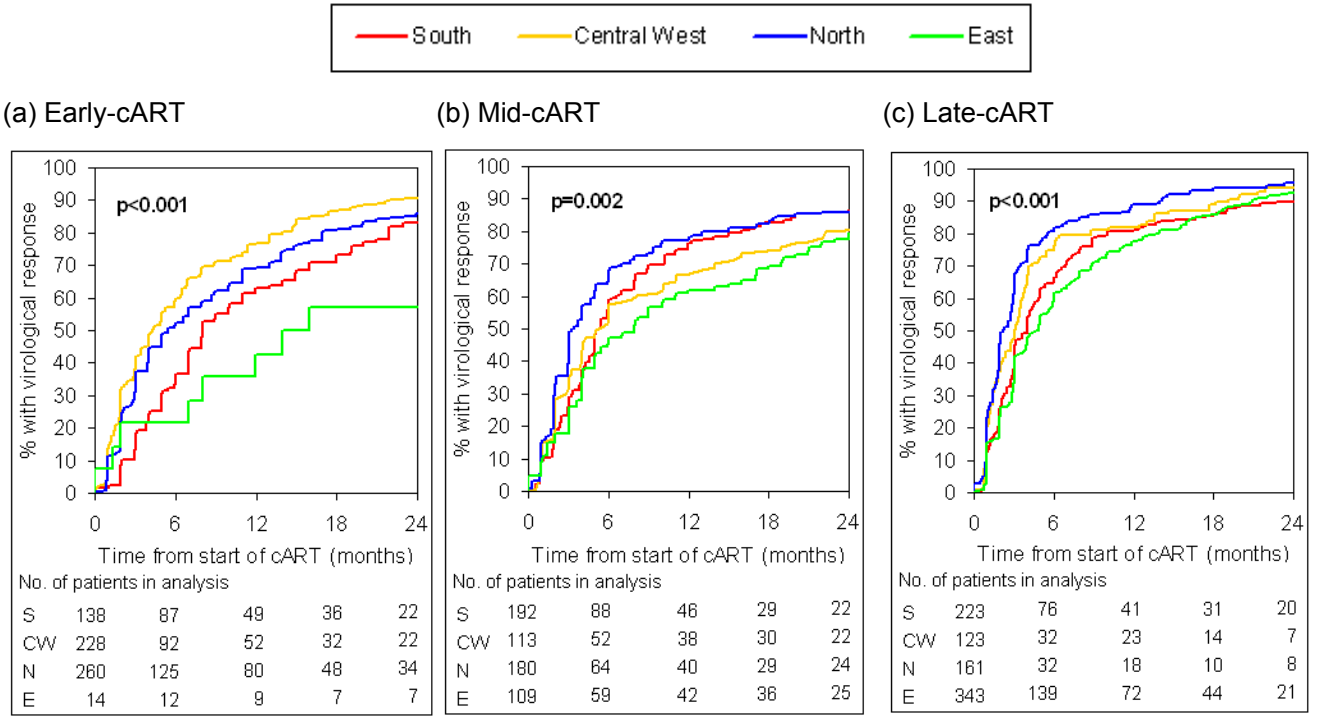
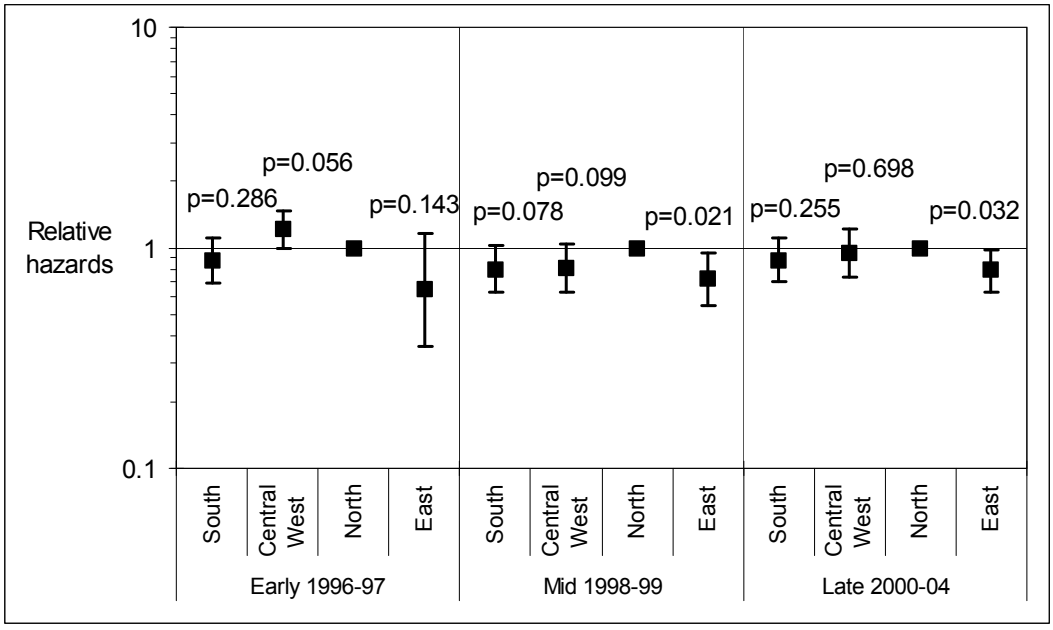


Figure 3.9: Multivariable relative hazards and 95% confidence intervals of virological response (less than 500 copies/mL) after starting cART in each cART initiation period according to region.

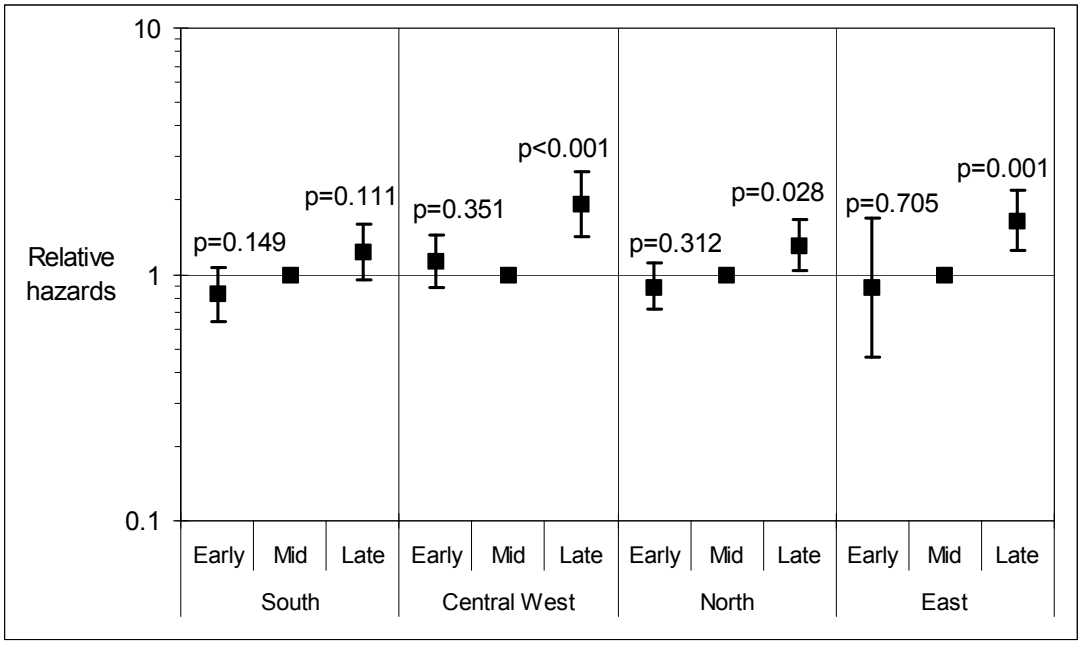


Note: Analysis adjusted for gender, HIV exposure group, previous AIDS diagnosis, origin, hepatitis B/C co-infection, baseline CD4 count and viral load, age, type of cART regimen and number of drugs in regimen.

Stratified by regions, and after adjustment, there were significant temporal differences in all regions in the risk of virological response (South, p=0.040, Central West, p<0.001, North, p=0.019 and East, p=0.001). Pairwise comparisons of the RHs of virological response over time are displayed in Figure 3.10.

Both stratifying by clinical centre and left censoring at date of enrolment resulted in similar findings (results not shown).

Figure 3.10: Multivariable relative hazards and 95% confidence intervals of virological response (less than 500 copies/mL) after starting cART in each region according to cART initiation period.



Note: Analysis adjusted for gender, HIV exposure group, previous AIDS diagnosis, origin, hepatitis B/C co-infection, baseline CD4 count and viral load, age, type of cART regimen and number of drugs in regimen.

3.4 Discussion

The focus of this chapter was to assess two potential predictors of virological response to first-line cART: geographical region and calendar time period of initiation of cART. The findings showed that overall there was some indication of regional differences in initial virological response to cART that cannot be explained by differences in cART regimen started. These were most evident in early-cART years, 1996-1997. Improvements in virological response rates over time were also clearly observed, most noticeably in the East region.

3.4.1 Regional differences in virological response

3.4.1.1 Interpretation of findings

An overall significant regional difference in initial virological response to cART was found, after adjustment for potentially confounding variables including the type of cART regimen, suggesting that the difference could be linked to unmeasured factors such as differing levels of patient care and management of associated toxicities that may affect patient adherence. In the main analysis, there was a borderline significant regional difference in early-cART years and no regional differences in mid- or late-cART years, which could indicate that these factors have become more uniform throughout Europe

in more recent years. This finding was more pronounced in the 'missing = excluded' analysis implying that there was more variability between regions in the proportion with missing viral load measurements in early-cART years. The East region was found to have the lowest odds of achieving virological suppression in all three calendar time periods, however this did increase over time to become more consistent with other regions. The Central West region appeared to have the highest odds of virological response in early-cART years compared to the North but the lowest in late-cART years. This may be an indication of comparatively higher improvements in treatment strategies and adherence and increased access to new antiretroviral drugs in the other regions. It could also indicate increasing drug resistance in Central West. However, these trends were not statistically significant and so may be explained by a chance result.

3.4.1.2 Previous research

As expected, these findings are broadly consistent with previous regional comparisons in EuroSIDA, however the chosen endpoint of a successful virological response to cART has not been compared between EuroSIDA regions before. In an analysis comparing South, Central and North Europe (before the addition of East European countries into EuroSIDA), Chiesi *et al.* (1999) found that rate of mortality following a low CD4 cell count was significantly lower in Central Europe than in North and South Europe over the period 1994-1999 [179]. Mocroft *et al.* (2000) showed that in patients mostly starting cART before 1996-1997, Central and North Europe had significantly lower odds of virological failure at 16 and 48 weeks following initiation of cART than South Europe [384]. These studies both found evidence of a better response to cART in Central Europe in early years, which, as mentioned above was found in the analyses in this chapter albeit that the effects observed were not statistically significant.

Another EuroSIDA analysis by Kirk *et al.* (1998) examined the use of ART over the period 1994-1997 and found a decrease in regional variation over time [383]. The findings in this chapter indicated a significant regional variation in the type of cART regimen started in all three time periods, however the magnitude of differences did decrease over time, which supports these previous results. The decreasing regional differences could be due to treatment policies becoming more consistent throughout Europe, resulting from the increasing widespread availability of antiretroviral drugs.

3.4.2 Changes over time in virological response

3.4.2.1 Interpretation of findings

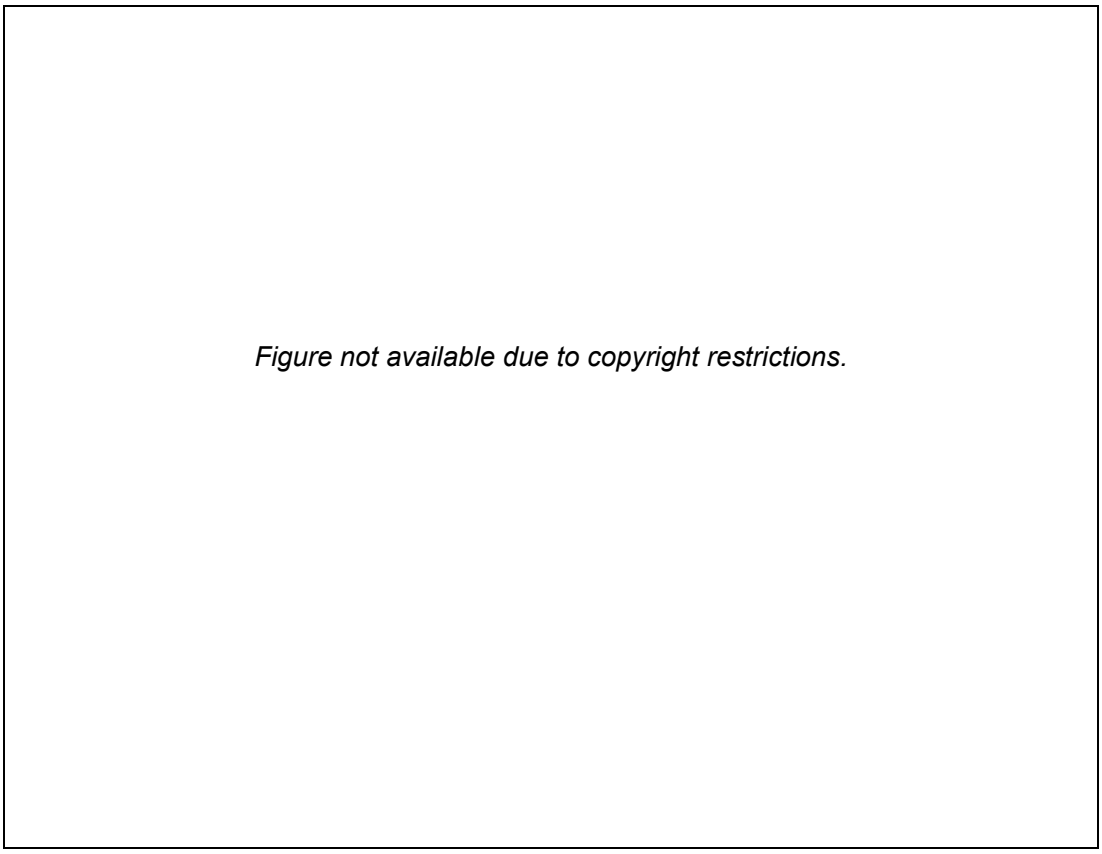
The improvements in initial virological response to cART over time observed in this analysis could be attributed to changes in clinical support, patients' attitude towards therapy, management of toxicities linked to improved adherence, a better understanding of drug resistance and an increased availability of new antiretroviral drugs [40-42]. The analyses were adjusted for type of cART regimen, suggesting that changes in first-line regimens are not solely responsible for the improvements in response. Increases in virological response rates were observed in all four regions. These were only statistically significant in the Central West and East regions in the main analysis, although they were borderline significant in the South and North. The analysis excluding patients with missing viral loads gave somewhat different results with no significant changes over time in virological response in South or Central West regions. This indicates that the proportion with missing values declined over time in these regions, possibly due to improved clinical management. This finding was also true in the analyses excluding patients with retrospective treatment data, possibly due to those with retrospective data being more likely to have missing measurements. The number of patients included in this analysis was reduced to just over a quarter and so had reduced power to detect true differences. As East European countries were enrolled into EuroSIDA relatively recently, the proportion with retrospective data was highest in this region and all patients who started cART in early-cART years were excluded.

3.4.2.2 Previous research

Few studies have quantified trends over calendar time in virological response to cART. The ART Cohort Collaboration 2006 found a steady improvement in the odds of a virological response (viral load less than 500 copies/mL) six months after starting cART in ART-naïve patients over the years 1995 to 2003 [382]. Moore *et al.* (2005) found significant improvements in virological suppression (viral load less than 400 copies/mL) to cART: 43.8% in 1996 compared to 72.4% in 2001-2002 by six months and 60.1% in 1996 compared to 79.9% in 2001-2002 by twelve months [380]. Ledergerber *et al.* (1999) looked at virological suppression and progression to AIDS or death in the Swiss HIV Cohort Study and found that patients starting PI-containing cART regimens in 1997-1998 were 30% more likely to reach virological suppression than those starting in 1995-1996 [381]. Lampe *et al.* (2006) investigated risk of initial virological failure after starting cART whilst ART-naïve in a multi-cohort analysis over a seven-year period in Europe and Canada and found a significant decrease over time (results shown in

Figure 3.11) [379]. The results in this chapter support the conclusions of improvements over time in treatment success of initial cART regimens.

Figure 3.11: Unadjusted and adjusted risk ratios (with 95% confidence intervals) of virological failure by year of starting triple-combination therapy (missing = failure).



Source: Lampe *et al.* (2006) [379].

Since an increase in odds of virological response to cART over time was observed and the majority of patients' regimens were not chosen according to their resistance profiles (only 12% of patients had plasma samples available within a year before starting cART that had been successfully tested for HIV drug resistance), this indicates that the transmission of drug-resistant HIV may not have had a great impact or may be decreasing over time, which supports recent data from the UK [394]. However, it is possible that if TDR was increasing, it may have reduced the rate of improvement in virological success rates. This is investigated further in Chapter 5 that specifically investigates the prevalence of TDR in EuroSIDA.

3.4.3 Limitations of analysis

The limitations of this analysis should be recognised when interpreting these results. The clinics involved in EuroSIDA were not selected at random and could differ from others in terms of clinician experience and availability of ART. However due to the large number of clinics in the study, the sample should be broadly representative of centres across Europe. Regions were defined according to the grouping of countries historically used by EuroSIDA [179] as there was not enough power to compare countries or clinical centres. Countries were allocated into regions at the initiation of EuroSIDA in 1994 according to geographical location and the same groupings have been maintained in all analyses. There may be variation in virological response between countries within regions however this analysis aims to compare broad regions of Europe to gain an overview of any existing differences.

Pairwise comparisons of regions within time intervals should be treated with caution as multiple testing over time carries an increased risk that significant results could be due to chance. Adjustments can be made to the *p* values (such as the Bonferroni correction) to compensate for this. The pairwise comparisons made in this analysis were conducted to illustrate regional differences in more detail and conclusions were not drawn from these *p* values.

The sensitivity analysis using the subset of patients including only those who started cART after enrolment into EuroSIDA showed different results to the main analysis as no significant trend over time was found in virological response. This is likely to be due to the fact that no patients from the East region started cART before enrolment into EuroSIDA in the early-cART years. As this was the subset of patients with the lowest virological response rate in the main analysis, this could account for the difference. The alternative is that the validity of the retrospective data could be questioned as patients who started cART before enrolment have already proved that treatment was successful enough to allow them to survive a period up to enrolment and therefore they may be more likely to have a successful virological response. Retrospective data are also more likely to contain missing information, such as viral load measurements. In a 'missing = failure' analysis, this would bias the findings in favour of virological failure. In order to increase numbers in this sensitivity analysis, patients could only be excluded who started cART more than six months before enrolment so that they had the potential for a prospective viral load measurement in the six to twelve month period after starting cART.

The frequency of viral load measurements in the year after starting cART was significantly different across regions and time periods, which could account for missing values during months six to twelve, although it cannot be ruled out that missing values may be related to the outcome of treatment, e.g. patients being too ill to visit a clinic. Most patients with missing values during this time had a subsequent one after. The difference in frequency of measurements was the reason for choosing a binary endpoint rather than a time-to-event endpoint for the main analyses. However to compare the results found from using an alternative statistical method, survival analysis was also applied and even though there was potential for bias the conclusions were the same.

3.4.4 Implications of findings

The findings of this analysis show that the differences in virological response rate following initiation of first-line therapy between regions that existed when cART was first introduced have now diminished, which suggests that the standard of patient care, toxicity management, the level of information available to patients and availability of new antiretroviral drugs have become more uniform across Europe. Increasing virological response rates were observed in all regions, which indicate that improvements in clinical management have made an important difference over the years. The analysis was carried out on ART-naïve patients as they do not have a history of ART use that would confound the results due to the accumulation of HIV drug resistance. Nowadays, patients generally start cART for the first time from ART-naïve and so these results are applicable to the population of newly diagnosed HIV infected individuals across Europe.

3.4.5 Further research

The continued expansion of EuroSIDA will increase the number of patients eligible for inclusion in analyses such as this. As more clinical centres in East European countries join EuroSIDA, this will provide a better picture of the impact of cART in this region. Ideally it would be interesting to compare response to cART across individual countries rather than regions, as well as using a year-by-year stratification.

Immunological response could also be investigated across regions and over time using logistic regression to analyse endpoints such as a 100 cells/mm^3 or 50% increase in CD4 count from initiation of cART at the first measurement six to twelve months after starting cART in those with CD4 counts available. To study the long-term effects of cART, the risk of clinical outcomes (new AIDS or death) should be compared using survival analysis methods. A previous EuroSIDA analysis compared pre-cART (1994-

1995), early-cART (1996-1997) and late-cART years (1998-2002) in terms of mortality and morbidity, finding that there was a significantly higher risk of AIDS and death in the pre-cART years compared to the early-cART years and a significantly lower risk in the late-cART years [140]. This showed that the initial drop following the introduction of cART was sustained in later years. A EuroSIDA analysis comparing death rates between regions in EuroSIDA was mentioned earlier, which found Central Europe to have a significantly lower death rate than South and North [179]. EuroSIDA has already grown extensively since these analyses were carried out and in the future the dataset may be large enough to compare clinical outcomes across regions within time periods and over time within regions.

3.4.6 Conclusions

Some regional variation in initial virological response to cART was observed in this analysis, which was most apparent in early-cART years. Virological response appears to have improved over calendar time in all regions, especially in East Europe. These findings could be a consequence of better adherence to regimens due to improved management of toxicities, more clinical support and increased access to new antiretroviral drugs that are virologically more potent and have better tolerability profiles. There was no evidence to support a clinically significant increasing emergence of TDR and so this may be yet to have a significant impact. This will be investigated further in Chapter 5.

A manuscript of this analysis was published by the Journal of Acquired Immune Deficiency Syndrome in June 2006 and can be found in Appendix VI.

Chapter 4. HIV-1 subtypes and virological and immunological response to combination antiretroviral therapy

4.1 Introduction

HIV infection is characterised by a very high turnover of virus [10]. Viral replication is highly error-prone and results in a high frequency of genetic mutations, consequently HIV has evolved into numerous different strains and has become a genetically diverse group of retroviruses [18,344,396,397].

4.1.1 Classification of HIV-1

HIV-1 is the predominant form of the virus worldwide, classified into three groups according to its genetic structure: 'M' the main or major group, 'O' the outliers and 'N' the new or non-M/O group [344,355,398,399]. Group 'O' is currently restricted to countries in West Central Africa and 'N' has only been identified in a few individuals from Cameroon [396,400]. The 'M' group represents the vast majority of all infections and currently nine distinct subtypes within this group have been identified: A-D, F-H, J and K, with further sub-subtypes A1, A2, A3, F1 and F2 [344,355,399,401]. Infection with two or more different HIV strains has led to the evolution of circulating recombinant forms (CRFs) [402-404], which incorporate genetic material from more than one subtype through the process of recombination during replication (recombination is described in Chapter 1, section 1.5.1). There are currently 43 known CRFs (CRF01-CRF43) [405], of which CRF01_AE and CRF02_AG are the most common, predominant in South East Asia and Western/Central Africa respectively [344,396,406]. Subtype E was originally identified as one of the two major strains causing epidemics in Thailand [407,408] but in more recent years it has been found that all representatives of this subtype are actually the recombinant or mosaic form, CRF01_AE, made up of subtypes E and A [344,403,404]. The non-recombinant form, E, is no longer thought to exist. Likewise subtype I has now been recognised as a complex mosaic form. It was first reclassified as a CRF comprising A, G and I [409], however it is now recognised that CRF04_cpx, as it is now known, additionally comprises subtypes H and K [410].

4.1.2 Genetic sequencing

As discussed in Chapter 1 (section 1.9.1), HIV-1 subtypes can be determined via the method of phylogenetic analysis. Each subtype has a consensus sequence, which is the sequence of the most common nucleotide bases. To determine the HIV strain an individual is infected with, viral RNA is extracted from a plasma sample, sequences are

aligned and by constructing a phylogenetic tree, the most recent common ancestor can be compared against consensus sequences to find out to which one it is most closely matched [344,354]. For most accurate results, the entire HIV genome should be sequenced and compared [344,355,396]. However, the major regions of the genome that are often focused upon are known as *env*, *pol* and *gag* [344,355,356]. As it is essential for the functions of these proteins to be preserved, the variation that can occur between subtypes is limited in these genetic regions, but is sufficient to allow phylogenetic identification of subtypes. HIV-1 subtypes have been found to differ by approximately 20-30% in the *env* region, 10-12% in the *pol* region and 15-22% in the *gag* region [399,411].

4.1.3 Antiretroviral resistance

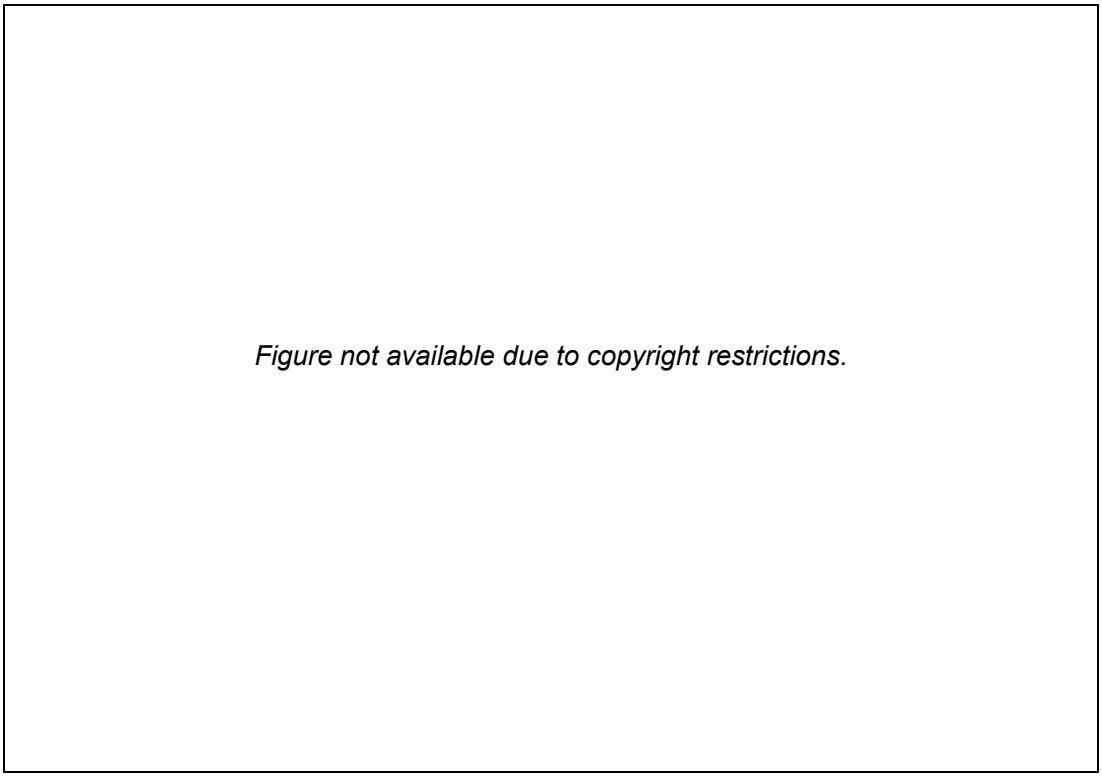
Mutations in the reverse transcriptase (RT) and protease (PR) genes in the *pol* region have been shown to be associated with resistance to inhibitors of these enzymes [412,413]. These resistance mutations are defined according to subtype B consensus sequences but are also selected by ART in non-B subtypes. Although drug resistance evolution appears to be fairly comparable between B and non-B subtypes according to current research, emerging differences in drug susceptibilities have been observed that may result in difficulties interpreting genotypic resistance in non-B subtypes [399,406,411,413-417]. These differences could be driven by different patterns of nucleotide changes existing before therapy initiation or by selection of resistance along different pathways, which could affect drug susceptibility and therapy response. For example, the selection of the K65R mutation by tenofovir (TDF) appears to be more rapid in subtype C than subtype B [418]. Another example is that greater genetic variability at NNRTI resistance sites, e.g. mutations V106M and A98S, has been observed in subtype C strains than in subtype B [419,420]. This indicates that differences in resistance to different regimens may exist between subtypes, i.e. there could be an interaction between HIV-1 subtype and type of cART regimen in terms of response to therapy.

4.1.4 Global distribution of HIV-1 subtypes and response to ART

Historically, antiretroviral drugs have mostly been developed in North America and Western Europe where subtype B is the most prevalent strain [416]. In 2004, Hemelaar *et al.* estimated the global distribution and regional spread of HIV-1 subtypes by combining molecular epidemiological data on subtype distribution in individual countries with country-specific estimates of the number of individuals living with HIV from UNAIDS and the WHO [401]. This is displayed in Figure 4.1. It was estimated that 98% of individuals living with HIV in North America and 88% in Western Europe

were infected with subtype B [401]. Consequently drugs have been developed based on the biological and clinical findings of their effects on patients largely infected with this strain [13,25], even though in 2004, subtype B was estimated to contribute to just 10% of the worldwide epidemic [401]. As non-B subtypes are genetically different and as already mentioned, may develop different patterns of resistance, there is reason to hypothesise that patients infected with different subtypes will have different response rates to therapy.

Figure 4.1: Global distribution of HIV-1 subtypes and recombinants in 2004.



Source: Hemelaar *et al.* (2004) [401].

Non-B subtypes are widespread in Africa (subtypes A, C, D, G, CRF02_AG and other CRFs and recombinants), Asia (subtypes A, CRF01_AE and other CRFs and recombinants) and much of East Europe (subtype A) [401,415]. However, as travel and migration are on the increase, non-B subtypes are spreading worldwide [421]. Previous studies researching response to therapy across HIV-1 subtypes have generally so far been small-scale and have not found any striking differences, although most have only been able to compare subtype B with an aggregated group of non-B subtypes [399,422-429].

4.1.5 Motivation and aims for chapter

EuroSIDA represents an ideal setting for comparing response to therapy across HIV-1 subtypes in an area where B is most prevalent. The study prospectively collects plasma samples from patients all across Europe from which viral RNA can be sequenced to determine subtype. Whether or not cART is as effective at reducing viral load and promoting immune regeneration in patients infected with non-B subtypes as it is in those with subtype B remains an important, yet unresolved question in HIV research. Therefore the aim of this analysis was to compare both virological and immunological response to cART between patients infected with different HIV-1 subtypes.

4.2 Methods

4.2.1 Inclusion criteria

The EuroSIDA dataset used for the analyses in this chapter, as in Chapter 3, was the update completed in December 2005 including data on 11,928 patients with follow-up to (median date of last visit) May 2005. Patients were eligible for analyses if they started cART, defined as at least three antiretroviral drugs including a PI, an NNRTI, or abacavir (ABC), with no previous PI/NNRTI/ABC experience and had the potential for at least one year's follow-up after starting cART (i.e. started cART at least one year before May 2005). They were also required to have pre-cART blood plasma samples stored, upon which genotypic sequencing and phylogenetic analysis were retrospectively performed to determine the HIV-1 subtype they were infected with. Alternatively, if this was not available, pre-cART subtype test results provided by the clinical centres were used. Plasma samples or genotypic sequence data may have been obtained when a patient was tested for drug resistance therefore patients with subtype test results after starting cART may be more likely to have been failing their regimen. This was the reason for selecting only those with samples obtained *before* starting cART, to prevent a biased selection of patients for the analysis who may be more likely to fail.

4.2.1.1 Identification of HIV-1 subtype

As detailed in Chapter 2 (section 2.1.3), HIV-1 subtypes in EuroSIDA are primarily ascertained using phylogenetic analysis on viral RNA extracted from plasma samples analysed in central virology laboratories. In some cases when sequences were incomplete, serology methods were used instead to determine subtype, which are not as accurate as phylogenetic analysis. In the absence of plasma samples, the results of genetic sequencing conducted in the local laboratories were used if available and as

long as they were dated before the patient started cART. If these were also not available, any subtype assignment in the patients' case-notes reported on the follow-up forms was taken, again, as long as this was before the patient started cART.

4.2.2 Statistical methods

4.2.2.1 Definitions

Patients infected with HIV-1 subtypes A, B, C and 'other' were compared, where 'other' included all other HIV-1 subtypes and CRFs. For the main analysis, A, C and 'other' subtypes were aggregated into a non-B subtype group due to lack of statistical power to compare the four groups. Baseline was defined as the date of starting cART.

For patients with blood plasma samples taken within a year before the date of cART initiation that were subsequently tested for genotypic HIV drug resistance, resistance was defined as at least one NRTI, NNRTI or major PI resistance mutation according to the IAS-USA 2005 figures of HIV-1 drug resistance mutations [395].

4.2.2.2 Infection with an HIV-1 B or non-B subtype

Multivariable logistic regression models were developed to investigate the factors affecting whether or not patients were infected with an HIV-1 non-B subtype, as opposed to subtype B. Explanatory variables were identified as those significantly associated with the odds of being infected with subtype B in univariable analysis (p<0.1) and those investigated were: gender, age, HIV exposure group (defined as 'men who have sex with men (MSM)', 'injecting drug use (IDU)', 'heterosexual' or 'other'), country of origin (defined as 'Africa' or 'non-Africa'), geographical region (defined in Chapter 2, section 2.1.5), ethnicity (defined as 'white' or 'other'), hepatitis B/C co-infection status and how subtype was determined ('phylogenetic analysis' or 'other').

4.2.2.3 Virological and immunological response to cART

Logistic regression was also used to compare the odds of a virological and immunological response to cART at the first viral load measurement and CD4 count respectively, six to twelve months after initiation of the cART regimen, between patients infected with B and non-B subtypes. A virological response was defined as a viral load less than 500 copies/mL. An immunological response was defined as a CD4 count increase of at least 100 cells/mm^3 from baseline. For analyses of virological response, patients were required to have a baseline viral load of at least 500 copies/mL and for analyses of immunological response, patients needed to have a baseline CD4 count available. Baseline measurements were defined as those taken within the six-month

period prior to starting cART. A 'missing = failure' approach was taken as the main analysis.

Multivariable models were developed to investigate the effects of HIV-1 subtype on virological and immunological response. Variables were identified that were significantly associated with the response (p<0.1 in univariable analyses or using a stepwise selection procedure) and therefore potentially could confound the relationship between subtype and the outcome. Variables investigated were as in section 4.2.2.2 with additions of: viral load measurements (baseline and maximum ever at baseline), CD4 counts (baseline and nadir), time from CD4 nadir, prior AIDS diagnosis, time from HIV diagnosis, calendar year of starting cART regimen, type of regimen (defined as containing 'a single PI: not saquinavir (SQV) hard gel', 'a single PI: SQV hard gel', 'a ritonavir (RTV)-boosted PI', 'a single NNRTI' or 'other'), number of new drugs in regimen (according to the patient inclusion criteria any previous drugs would have been taken as monotherapy or dual therapy, not cART), whether or not ART-naïve at baseline and whether or not the patient had genotypic resistance test results available within a year before starting cART.

4.2.2.4 Sensitivity analyses

A sensitivity analysis was carried out using a 'missing = excluded' approach. Further sensitivity analyses using a 'missing = failure' approach were conducted on subsets of (i) ART-naïve patients, to minimise any differences in drug resistance caused by previous NRTI experience; (ii) patients with subtypes determined by phylogenetic analysis as opposed to other less reliable methods; (iii) patients who had their subtypes identified post-1999, in order to exclude from the analysis potentially less reliable measures of viral load due to subtype-specific problems of viral load assays commonly used before then [430-432] (for virological response only); and (iv) patients who started cART after enrolment into EuroSIDA to exclude retrospective data. Another sensitivity analysis looking at immunological response was carried out on a subset of only patients who had achieved virological suppression.

A further endpoint for the virological response analysis was also investigated: a viral load less than 50 copies/mL. This analysis was conducted on a subset of patients whose first viral load measurements in the six to twelve month period after starting cART were measured using an assay with a level of detection as low as at most 50 copies/mL. This required all those with missing values to be excluded. In addition, an alternative immunological response was investigated: a CD4 count increase of 25% from baseline. A 'missing = failure' approach was used for this.

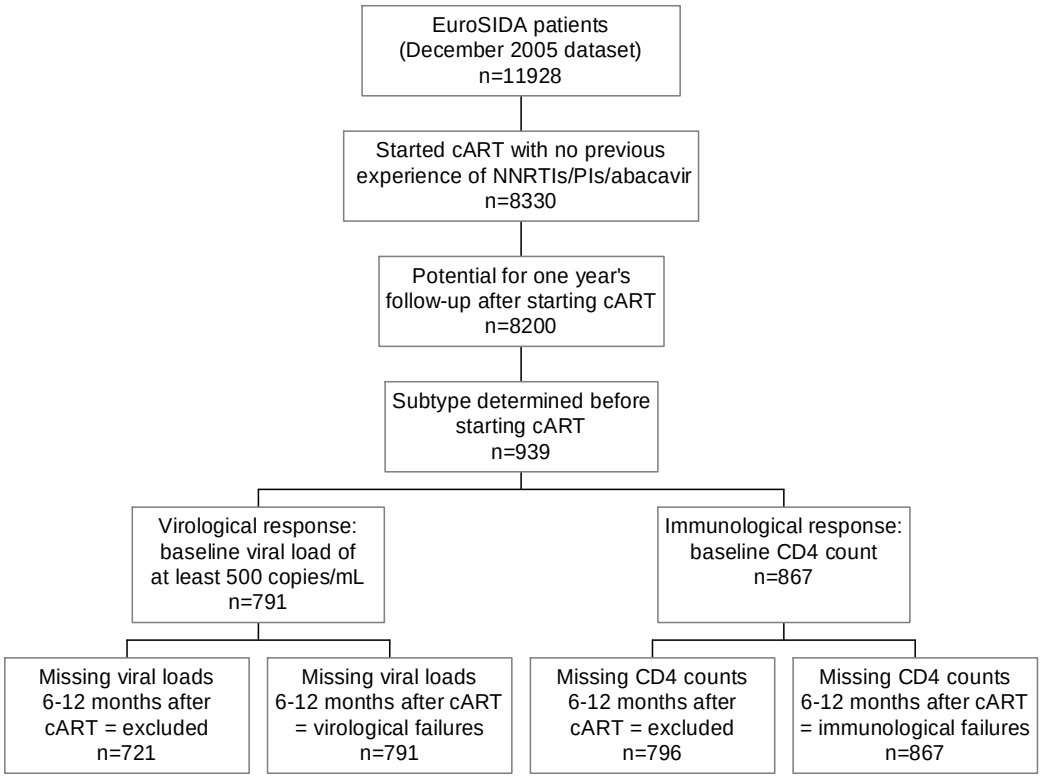
Survival analysis using Kaplan-Meier estimates and Cox proportional hazards models was used to check the consistency of results. Time to virological suppression (a viral load less than 500 copies/mL) and time to a CD4 cell increase of 100 cells/mm^3, with right censoring at the date of patients' last viral load measurement or CD4 cell count respectively were investigated. Stratifying by clinical centre was investigated to adjust for potential differences between centres. Left censoring at date of enrolment into EuroSIDA was also investigated.

4.3 Results

4.3.1 Patient numbers

A total of 8200 (68.7%) patients met the inclusion criteria, displayed in Figure 4.2. Pre-cART HIV-1 subtype results were available for 939 (11.4%) patients, therefore 7261 (88.5%) patients were excluded for having unknown subtypes.

Figure 4.2: Patient numbers in analyses according to inclusion criteria.



4.3.2 Patient characteristics at date of starting cART

4.3.2.1 Patients with HIV-1 subtypes known versus unknown

Table 4.1 compares the characteristics of patients with HIV-1 subtype test results available to those without. Due to the large sample size, there were a number of statistically significant differences. Those with known subtypes on average started cART later than those with unknown subtypes (median: January 1998 versus July 1997; $p<0.001$). They had higher median baseline CD4 counts (228 versus 185 cells/mm^3; $p<0.001$) and slightly higher median viral loads (4.7 versus 4.5 $\log_{10}$copies/mL; $p<0.001$). There was also a lower proportion with a previous AIDS diagnoses (21.9% versus 29.5%; $p<0.001$) and a higher proportion of patients who were ART-naïve at baseline (44.6% versus 36.5%; $p<0.001$).

4.3.2.2 Regional distribution of HIV-1 subtypes

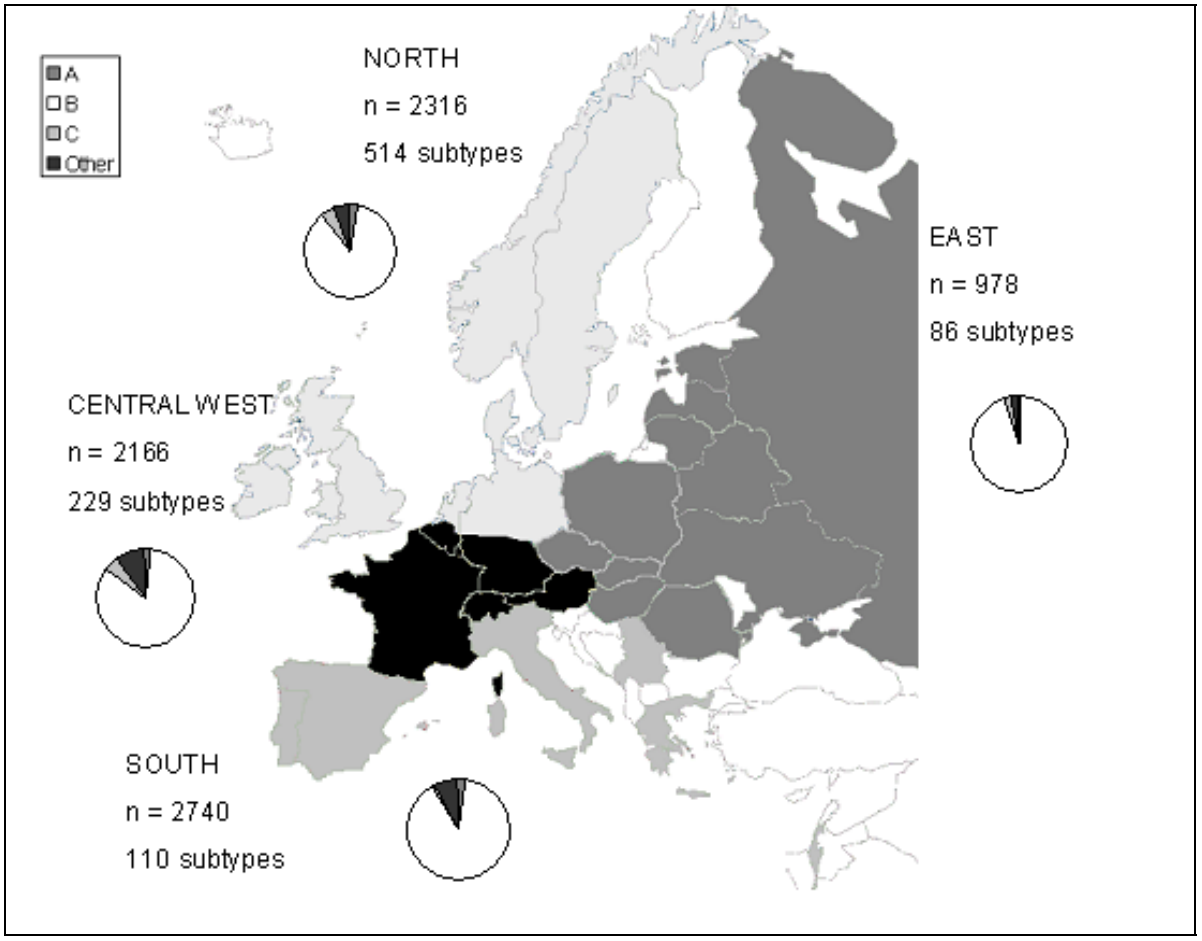
Figure 4.3 illustrates the distribution of the subtypes identified in the 939 patients included in the analyses across geographical regions within EuroSIDA. These patients were widespread across Europe with the addition of 16 (1.7%) patients from Israel (no patients from Argentina). The large majority of patients who met the inclusion criteria in all the regions (South, Central West, North and East) were infected with HIV-1 subtype B (89.1%, 82.5%, 86.4% and 94.2% respectively), however there were also non-B subtypes found in all regions. Overall the analyses included 812 (86.5%) patients infected with subtype B, 23 (2.4%) with A, 42 (4.5%) with C and 127 (6.6%) with any other subtype, including 16 (1.7%) with CRF01_AE and 14 (1.5%) with CRF02_AG. Of all patients who started a cART regimen (n=8200), those visiting clinical centres in the North region were over twice as likely to have pre-cART subtype test results available than those in the South, Central West or East (22.2% compared to 4.0%, 10.6% and 8.8% respectively).

Table 4.1: Patient characteristics at date of starting cART according to whether or not pre-cART HIV-1 subtype test results were available.

	Total		Pre-cART subtype test results		No subtype test results		*p*
n %							
All	8200	100.0	939	11.5	7261	88.5	-
Male	6318	77.0	725	77.2	5593	77.0	0.901
HIV exposure group							0.001
MSM	3601	43.9	467	49.7	3134	43.2	-
IDU	1820	22.2	179	19.1	1641	22.6	-
Heterosexual	2180	26.6	223	23.7	1957	27.0	-
Other	599	7.3	70	7.5	529	7.3	-
Ethnicity							0.007
White	7073	86.3	783	83.4	6290	86.6	-
Other	1127	13.7	156	16.6	971	13.4	-
Region							<0.001
South	2740	33.4	110	11.7	2630	36.2	-
Central West	2166	26.4	229	24.4	1937	26.7	-
North	2316	28.2	514	54.7	1802	24.8	-
East	978	11.9	86	9.2	892	12.3	-
Previous AIDS	2348	28.6	206	21.9	2142	29.5	<0.001
Hepatitis B status							<0.001
Negative	4524	55.2	662	70.5	3862	53.2	-
Positive	409	5.0	68	7.2	341	4.7	-
Unknown	3267	39.8	209	22.3	3058	42.1	-
Hepatitis C status							<0.001
Negative	2525	30.8	418	44.5	2107	29.0	-
Positive	1168	14.2	153	16.3	1015	14.0	-
Unknown	4507	55.0	368	39.2	4139	57.0	-
Type of cART regimen							<0.001
Single PI (non-SQV hard gel)	4461	54.4	465	49.5	3996	55.0	-
Single PI (SQV hard gel)	1118	13.6	67	7.1	1051	14.5	-
RTV-boosted PI	447	5.5	78	8.3	369	5.1	-
Single NNRTI	1188	14.5	185	19.7	1003	13.8	-
Triple NRTI incl ABC	155	1.9	27	2.9	128	1.8	-
Other	155	1.9	27	2.9	128	1.8	-
ART-naïve	3069	37.4	419	44.6	2650	36.5	<0.001
Median (IQR)							
Date started cART regimen	Jul 97	(Nov 96-Mar 99)	Jan-98	(Apr 97-Dec 99)	Jul 97	(Nov 96-Feb 99)	<0.001
Age (years)	37	(32-44)	37	(32-45)	37	(32-44)	0.208
CD4 count (cells/mm^3)							
Baseline[(1)]	191	(81-323)	228	(113-333)	185	(78-321)	<0.001
Nadir[(2)]	150	(60-254)	172	(87-260)	148	(59-253)	<0.001
Viral load (log_{10}copies/mL)							
Baseline[(3)]	4.6	(3.8-5.2)	4.7	(4.0-5.2)	4.5	(3.7-5.2)	<0.001
Max ever[(4)]	4.8	(4.2-5.3)	5.0	(4.5-5.4)	4.8	(4.1-5.3)	<0.001

Notes: *P* values obtained from Chi-squared, Fisher's Exact and Kruskal-Wallis tests.
Median CD4 counts and viral loads based on measurements from [(1)]7974 patients, [(2)]8122 patients, [(3)]5391 patients and [(4)]5711 patients.
MSM = men who have sex with men; IDU = injecting drug use; SQV = saquinavir; RTV = ritonavir; ABC = abacavir; IQR = interquartile range.

Figure 4.3: HIV-1 subtypes identified in patients with pre-cART HIV-1 subtype test results available according to geographical region within EuroSIDA.



4.3.2.3 Patients with HIV-1 B versus non-B subtypes

Table 4.2 compares the characteristics between the 812 (86.5%) patients infected with B and the 127 (13.5%) infected with a non-B virus at the date of starting cART. Dates of starting cART were similar for patients infected with B and non-B subtypes (medians: February 1998 and November 1997 respectively), as were dates of subtype tests (medians: May 1997 and April 1997 respectively). A total of 656 (80.8%) of the patients infected with B had their subtype determined by phylogenetic analysis compared to 73 (57.5%) of the non-B infected patients (p<0.001). There was a significant difference in country of origin between B and non-B infected patients (p<0.001), for example 52.8% of patients with a non-B subtype originated from Africa (15 patients with subtype A, 28 with C, 24 with another subtype) compared to just 1.7% of patients with subtype B and these were mostly infected through heterosexual contact (78.5%). The majority of the remaining patients originated from the same country as the clinical centre they visited. Significant differences were also found between groups in gender, HIV exposure group and ethnicity (all p<0.001). For those with baseline CD4 cell counts (n=923), medians were found to be similar between B and non-B infected patients (230 versus 210 cells/mm^3; p=0.881). Baseline viral loads (for the 637 patients with measurements) were slightly higher for B infected patients (medians: 5.0 versus 4.9 $\log_{10}$copies/mL; p=0.017).

A total of 533 (56.8%) patients had genotypic resistance test results available from tests carried out on plasma samples collected within one year before starting cART. Amongst these patients, 466 (87.4%) were infected with subtype B. Overall, 218 patients (40.9% of those with test results) had at least one IAS-USA NRTI resistance mutation (41.2% of B infected versus 38.8% of non-B infected patients; p=0.709), of whom 193 (88.5%) patients had previous NRTI experience. Major PI resistance mutations were found in 35 (3.7%) patients and NNRTI resistance mutations in 24 (2.6%) patients.

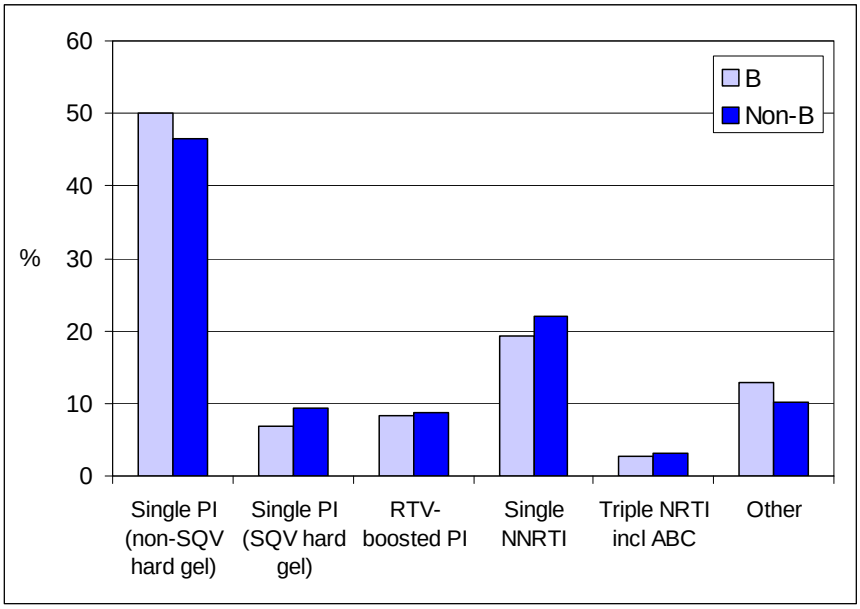
Table 4.2: Patient characteristics at date of starting cART according to HIV-1 subtype B versus non-B.

	Total		B		Non-B		*p*
n %							
All	939	100.0	812	86.5	127	13.5	-
Source of subtype identification							-
Phylogenetic analysis	729	77.6	656	80.8	73	57.5	<0.001
Other:	210	22.4	156	19.2	54	42.5	-
Serology methods	6	0.6	6	0.7	0	0.0	-
Local lab results	30	3.2	21	2.6	9	7.1	-
EuroSIDA forms	174	18.5	129	15.9	45	35.4	-
Male	725	77.2	666	82.0	59	46.5	<0.001
HIV exposure group							<0.001
MSM	467	49.7	457	56.3	10	7.9	-
IDU	179	19.1	170	20.9	9	7.1	-
Heterosexual	223	23.7	128	15.8	95	74.8	-
Other	70	7.5	57	7.0	13	10.2	-
Ethnicity							<0.001
White	783	83.4	725	89.3	58	45.7	-
Other	156	16.6	87	10.7	69	54.3	-
Region							0.045
South	110	11.7	98	12.1	12	9.4	-
Central West	229	24.4	189	23.3	40	31.5	-
North	514	54.7	444	54.7	70	55.1	-
East	86	9.2	81	10.0	5	3.9	-
Previous AIDS	206	21.9	184	22.7	22	17.3	0.177
Hepatitis B status							0.582
Negative	662	70.5	574	70.7	88	69.3	-
Positive	68	7.2	56	6.9	12	9.4	-
Unknown	209	22.3	182	22.4	27	21.3	-
Hepatitis C status							0.013
Negative	418	44.5	351	43.2	67	52.8	-
Positive	153	16.3	143	17.6	10	7.9	-
Unknown	368	39.2	318	39.2	50	39.4	-
ART-naïve	419	44.6	366	45.1	53	41.7	0.481
Genotypic resistance test results available	533	56.8	466	57.4	67	52.8	0.327
NRTI resistance[1]	218	40.9	192	41.2	26	38.8	0.709
PI resistance[1]	35	3.7	26	5.6	8	11.9	0.022
NNRTI resistance[1]	24	2.6	17	3.6	7	10.4	0.059
Median (IQR)							
Date started cART regimen	Jan 98	(Apr 97-Dec-99)	Feb 98	(Apr 97-Dec 99)	Nov 97	(Mar 97-Mar 00)	0.607
Date pre-cART subtype test	May 97	(Jul 96-Dec-98)	May 97	(Jul 96-Dec 98)	Apr 97	(Jul 96-Sep 98)	0.752
Age (years)	37	(32-45)	37	(32-45)	35	(32-43)	0.124
CD4 count (cells/mm^3)							
Baseline[2]	228	(113-333)	230	(113-333)	210	(116-330)	0.881
Nadir[3]	172	(87-260)	171	(87-260)	176	(84-257)	0.843
Viral load (log_{10}copies/mL)							
Baseline[4]	4.7	(4.0-5.2)	4.7	(4.0-5.2)	4.5	(3.8-5.0)	0.045
Max ever[5]	5.0	(4.5-5.4)	5.0	(4.5-5.4)	4.9	(4.4-5.2)	0.017

Notes: *P* values obtained from Chi-squared, Fisher's Exact and Kruskal-Wallis tests.
[1]In subset of patients with a successful genotypic resistance test within one year pre-cART.
Median CD4 counts and viral loads based on measurements from [2]923 patients, [3]936 patients, [4]637 patients and [5]659 patients.
MSM = men who have sex with men; IDU = injecting drug use; IQR = interquartile range.

A total of 366 (45.1%) B infected patients and 53 (41.7%) non-B infected patients were ART-naïve at initiation of cART (p=0.481). The types of regimen started by the two groups were also similar, displayed in Figure 4.4 (p=0.786). Amongst patients infected with subtype B, single PI-containing regimens were started by 56.8%, RTV-boosted PI regimens were started by 8.3% and single NNRTI regimens were started by 19.3%. Amongst patients infected with a non-B subtype, single PI-containing regimens were started by 55.9%, RTV-boosted PI regimens were started by 8.7% and single NNRTI regimens were started by 22.0%.

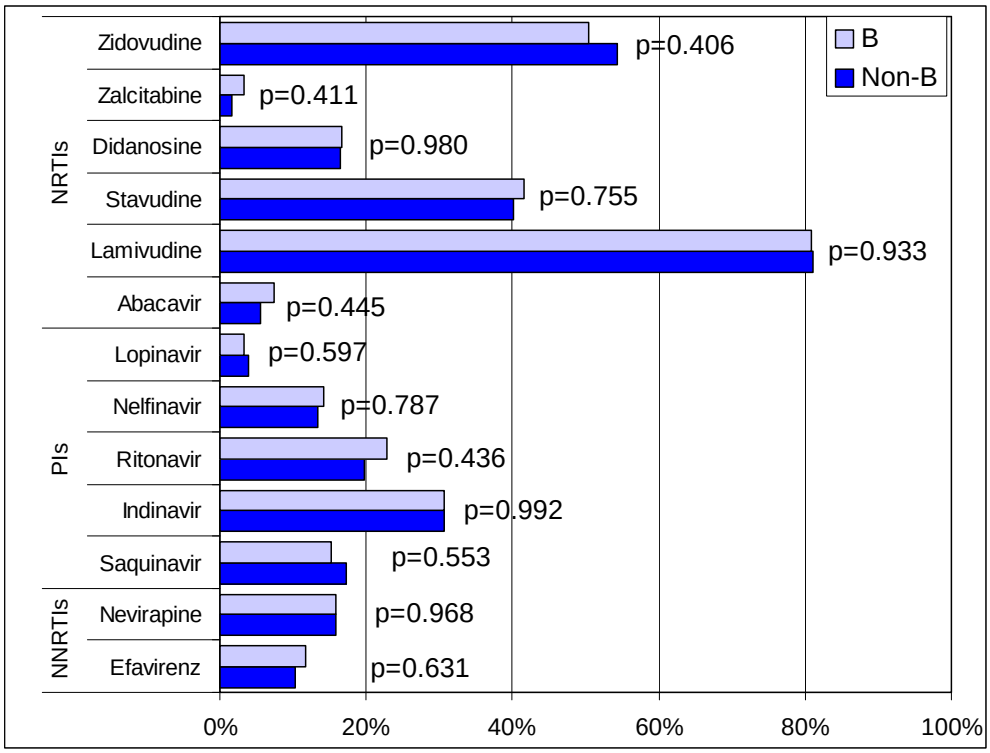
Figure 4.4: Type of cART regimen started according to HIV-1 subtype B versus non-B.



Notes: SQV = saquinavir; RTV = ritonavir; ABC = abacavir.

Figure 4.5 displays the specific antiretroviral drugs started as part of the cART regimen. The proportions of patients starting each drug were similar between B and non-B infected patients for all drugs (p>0.1). The most widely used NRTIs were zidovudine (ZDV), stavudine (d4T) and lamivudine (3TC) with 50.9%, 41.4% and 80.8% of patients starting each respectively. Indinavir (IDV) and RTV were the most common PIs started: 30.7% and 22.4% respectively. Slightly more patients started nevirapine (NVP) than efavirenz (EFV) (15.9% compared to 11.5%).

Figure 4.5: Antiretroviral drugs in cART regimen according to HIV-1 subtype B versus non-B.



4.3.2.4 Factors associated with infection with an HIV-1 non-B subtype

Factors associated with being infected with a non-B subtype, as opposed to subtype B virus, were investigated further and those found to be significant (p<0.1) in univariable analyses are displayed in Table 4.3. Before adjustment for other variables, determination of subtype by a non-phylogenetic analysis method, female gender, HIV exposure group other than MSM, non-white ethnicity and African origin were all associated with higher odds of infection with non-B virus. The East region and positive hepatitis C status were associated with lower odds of infection with a non-B subtype. In a multivariable model containing these variables, significant differences in odds of infection with a non-B subtype remained between different methods of subtype determination, HIV transmission exposure groups, country of origin and hepatitis C status. Subtype determination by non-phylogenetic analysis methods, non-MSM transmission exposure groups and African origin were independently associated with higher odds of infection with a non-B subtype and positive hepatitis C status was associated with lower odds (borderline significant).

Table 4.3: Odds ratios and 95% confidence intervals of infection with an HIV-1 non-B subtype.

	Univariable OR (95% CI)	*p*	Multivariable OR (95% CI)	*p*
Source of subtype id				
Phylogenetic analysis	1.00		1.00	
Other	3.11 (2.10-4.61)	<0.001	2.22 (1.26-3.91)	0.006
Gender				
Male	1.00		1.00	
Female	5.26 (3.55-7.78)	<0.001	1.12 (0.62-2.01)	0.710
HIV exposure group				
MSM	1.00		1.00	
IDU	2.42 (0.97-6.06)	0.059	4.69 (1.58-13.97)	0.006
Heterosexual	33.92 (17.18-66.98)	<0.001	16.70 (7.39-37.70)	<0.001
Other	10.42 (4.37-24.86)	<0.001	6.90 (2.46-19.36)	<0.001
Ethnicity				
White	1.00		1.00	
Other	9.91 (6.55-15.00)	<0.001	1.33 (0.59-2.96)	0.491
Region				
North	1.00		1.00	
South	0.78 (0.41-1.49)	0.446	1.37 (0.62-3.02)	0.441
Central West	1.34 (0.88-2.05)	0.174	1.50 (0.81-2.77)	0.198
East	0.39 (0.15-1.00)	0.050	0.85 (0.30-2.43)	0.762
Origin				
Non-Africa	1.00		1.00	
Africa	63.65 (33.80-19.85)	<0.001	23.02 (9.12-58.10)	<0.001
Hepatitis C status				
Negative	1.00		1.00	
Positive	0.37 (0.18-0.73)	0.005	0.38 (0.14-1.05)	0.061
Unknown	0.82 (0.55-1.23)	0.338	0.84 (0.48-1.47)	0.537

Notes: MSM = men who have sex with men; IDU = injecting drug use; OR = odds ratio; CI = confidence interval.

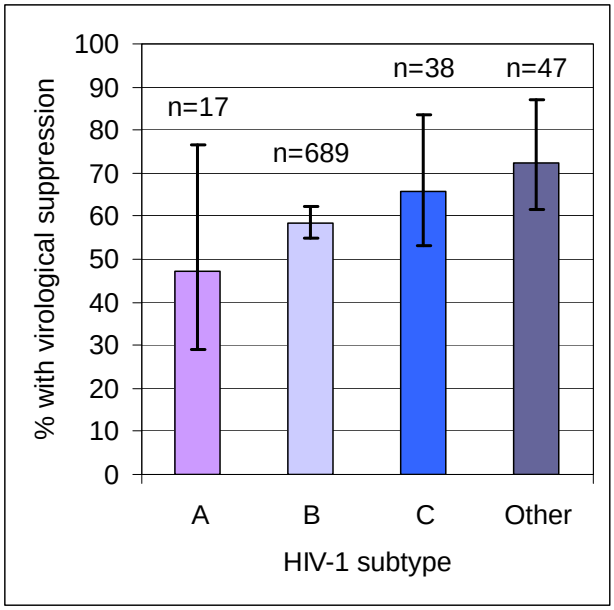
4.3.3 Virological response to cART: viral load less than 500 copies/mL at months six to twelve

4.3.3.1 Main analysis: missing viral load = failure

A total of 791 (84.2%) of the 939 patients with subtype test results available had baseline viral loads of at least 500 copies/mL. Of these 469 (59.3%) achieved virological suppression (viral load less than 500 copies/mL) at the first measurement during the six to twelve month period after starting cART: 402 (58.3%; 95% CI: 54.8-62.2%) of 689 B infected compared to 67 (65.7%; 95% CI: 57.4-75.9%) of 102 non-B infected patients (p=0.159). Median time to first measurement was 7 months (IQR: 6-8 months) for both B infected and non-B infected patients (p=0.779). During months six to twelve, 70 (8.8%) of the 791 patients included had no viral load measurement available and so were counted as virological failures, although 47 (67.1%) of these had a subsequent measurement recorded a median of 15 months (IQR: 13-19 months) after starting cART, 28 (59.6%) of whom had a viral load less than 500 copies/mL. Within the non-B subtypes, 8 (47.1%; 95% CI: 29.1-76.6%) of 17 patients infected with subtype A, 25 (65.8%; 95% CI: 53.3-83.5%) of 38 patients with C and 34 (72.3%; 95%

CI: 61.6-87.2%) of 47 patients with 'other' achieved virological suppression (p=0.152). Response rates are displayed in Figure 4.6.

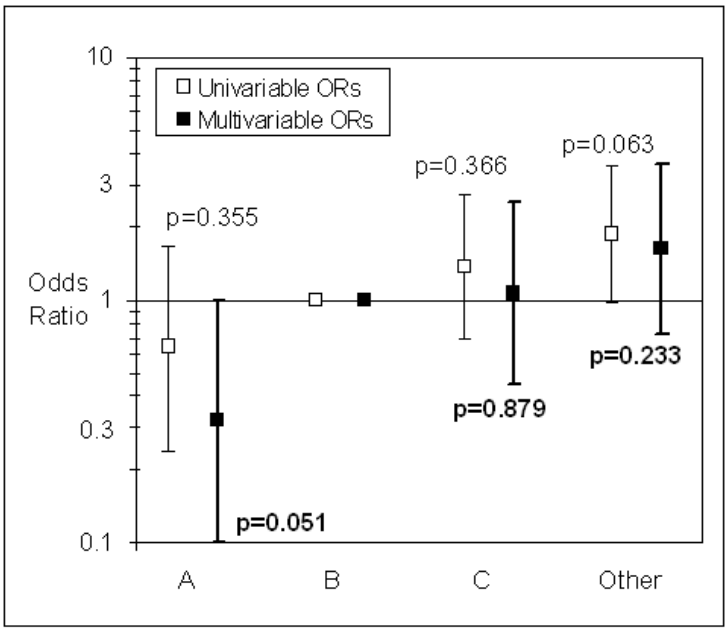
Figure 4.6: Virological response rates (less than 500 copies/mL) after starting cART and 95% confidence intervals according to HIV-1 subtype (missing = failure).



The odds of achieving virological suppression were investigated in patients infected with a non-B subtype compared to B subtype. Before adjustment for potentially confounding variables, the data showed no evidence of a significant difference between them (odds ratio (OR): 1.37; 95% CI: 0.88-2.11; p=0.160). Further analysis of the non-B subtypes, A, C, and 'other' compared to B resulted in ORs of 0.64 (95% CI: 0.24-1.67; p=0.355), 1.37 (95% CI: 0.69-2.73; p=0.366) and 1.87 (95% CI: 0.97-3.60; p=0.063) respectively.

A multivariable model was developed adjusting for baseline maximum ever viral load, CD4 nadir, time from CD4 nadir, hepatitis B and C co-infection status, calendar year of starting cART, type of regimen, number of new drugs in regimen, whether or not ART-naïve at baseline, age, HIV exposure group, geographical region and ethnicity. After adjustment, there remained no significant difference in the odds of achieving virological suppression (OR: 1.05; 95% CI: 0.58-1.93; p=0.866). Further investigation into A, C and 'other' subtypes showed a borderline significantly lower odds in patients infected with subtype A compared to B (multivariable OR: 0.32; 95% CI: 0.10-1.00; p=0.051). There was no significant difference in C or 'other' compared to B (multivariable ORs: 1.07; 95% CI: 0.45-2.54; p=0.879; and 1.64; 95% CI: 0.73-3.67; p=0.233, respectively). Univariable and multivariable ORs are displayed in Figure 4.7.

Figure 4.7: Odds ratios and 95% confidence intervals of virological response (less than 500 copies/mL) after starting cART according to HIV-1 subtype (missing = failure).



Notes: Multivariable analysis adjusted for baseline maximum ever viral load, baseline CD4 nadir, time from CD4 nadir, hepatitis B and C co-infection status, calendar year of starting cART regimen, type of regimen, number of new drugs in regimen, whether or not ART-naïve at baseline, age, HIV exposure group, EuroSIDA region and ethnicity.

Interactions between type of cART regimen and HIV-1 subtype were also considered. The power for this was limited, however interactions between PI-containing versus other regimens and subtype (A, B, C or 'other') were not found to be significant (*p*=0.841).

4.3.3.2 Sensitivity analysis: missing viral load = excluded

Excluding patients with missing viral load measurements at six to twelve months provided similar findings to those of the main analysis. The number of eligible patients in this analysis was reduced from 791 (84.2%) to 721 (76.8%) patients and response rates were higher as expected. Virological suppression was achieved by 469 (65.0%) patients: 63.9% (95% CI: 60.3-67.8%) of 629 infected with B and 72.8% (95% CI: 64.8-83.0%) of 92 infected with non-B (*p*=0.094). Within the non-B subtypes, 57.1% (95% CI: 38.2-90.1%) of 14 patients infected with subtype A, 69.4% (95% CI: 57.1-87.2%) of 36 patients with C and 81.0% (95% CI: 71.4-95.2%) of 42 patients with 'other' experienced a virological response.

No significant difference was found in the odds of achieving virological suppression in patients infected with a non-B subtype compared to B, after adjustment for the

potentially confounding variables identified in the main analysis (OR: 1.37; 95% CI: 0.69-2.70; *p*=0.367). These results are included in Table 4.4, which compares the results of all sensitivity analyses (non-B versus B), to those of the main analysis.

Looking at non-B subtypes A, C and 'other' compared to B gave multivariable ORs of 0.42 (95% CI: 0.11-1.57; *p*=0.198), 1.00 (95% CI: 0.39-2.57; *p*=0.997) and 2.38 (95% CI: 0.90-6.31; *p*=0.081) respectively.

Table 4.4: Odds ratios and 95% confidence intervals of virological response (less than 500 copies/mL) after starting cART for HIV-1 subtype B versus non-B.

	B		Non-B		Non-B versus B			
	n	% VL responders	n	% VL responders	Univariable OR (95% CI)	*p*	Multivariable OR (95% CI)	*p*
Main analysis, missing = failure	689	58%	102	66%	1.37 (0.88-2.11)	0.160	1.05 (0.58-1.93)	0.866
Missing = excluded	629	64%	92	73%	1.51 (0.93-2.46)	0.096	1.28 (0.65-2.54)	0.476
Sensitivity analyses, missing = failure:								
ART-naïve	326	71%	46	72%	1.04 (0.53-2.07)	0.903	1.09 (0.44-2.70)	0.849
Subtypes by phylogenetic analysis	573	59%	58	62%	1.15 (0.66-2.01)	0.613	1.22 (0.58-2.56)	0.597
Subtypes post-1999	139	63%	35	83%	2.80 (1.09-7.20)	0.033	3.51 (0.80-15.47)	0.098
Excluding retrospective data	565	62%	72	67%	1.23 (0.73-2.06)	0.437	1.13 (0.56-2.29)	0.730
Endpoint viral load <50 copies/mL	689	29%	102	42%	1.76 (1.15-2.69)	0.010	1.39 (0.72-2.67)	0.330

Notes: Multivariable analysis adjusted for baseline maximum ever viral load, baseline CD4 nadir, time from CD4 nadir, hepatitis B and C co-infection status, calendar year of starting cART regimen, type of regimen, number of new drugs in regimen, whether or not ART-naïve at baseline, age, HIV exposure group, EuroSIDA region and ethnicity.
VL = viral load; OR = odds ratio; CI = confidence interval.

4.3.3.3 Further sensitivity analyses

Repeating the main analysis in a subset of ART-naïve patients, of patients with subtypes determined by phylogenetic analysis only and excluding patients with retrospective data gave consistent results as those from the main analyses. There were no significant differences in the odds of a virological response in patients infected with non-B compared to B after adjustment for potential confounders, ORs: 1.09 (95% CI: 0.44-2.70; *p*=0.849), 1.22 (95% CI: 0.58-2.56; *p*=0.597), and 1.13 (95% CI: 0.56-2.29; *p*=0.730) respectively. Within the subset of patients with subtypes determined post-1999, there was a much greater odds of virological suppression (although not significant at the 5% level) in patients infected with non-B compared to B (multivariable OR: 3.51; 95% CI: 0.80-15.47; *p*=0.098). However the number of non-B patients in this subset was small (n=35), as reflected by the large confidence interval.

The alternative endpoint of a viral load less than 50 copies/mL instead of 500 copies/mL at six to twelve months also gave consistent results in the multivariable analysis of all patients with a viral load measurement during this time (OR: 1.39; 95% CI: 0.72-2.67; *p*=0.330).

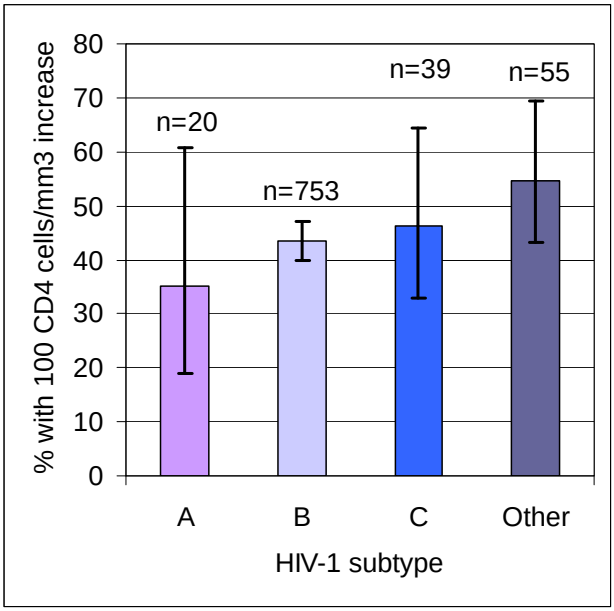
4.3.4 Immunological response to cART: 100 CD4 cells/mm^3 increase at months six to twelve

4.3.4.1 Main analysis: missing CD4 count = failure

A total of 867 (92.3%) of the 939 patients with pre-cART subtype test results had baseline CD4 counts available. Of these 382 (44.1%) experienced a successful immunological response (CD4 count increase of at least 100 cells/mm^3) at the first measurement during the six to twelve month period after starting cART: 327 (43.4%; 95% CI: 38.4-49.1%) of 753 B infected compared to 55 (48.2%; 95% CI: 36.8-63.2%) of 114 non-B infected patients (*p*=0.334). Median time to first measurement was 7 months (IQR: 6-8 months) for both B infected and non-B infected patients (*p*=0.973). During months six to twelve, 71 (8.2%) of the 867 patients included had no CD4 count available and so were counted as immunological failures, although 47 (66.2%) of these had a subsequent measurement recorded a median of 14 months (IQR: 13-17 months) after starting cART, 27 (57.5%) of whom had a CD4 count increase of at least 100 cells/mm^3. Within the non-B subtypes, 8 (47.1%; 95% CI: 29.1-76.6%) of 17 patients infected with subtype A, 25 (65.8%; 95% CI: 53.3-83.5%) of 38 patients with C and 34 (72.3%; 95% CI: 61.6-87.2%) of 47 patients with 'other' experienced an immunological response (*p*=0.476,) displayed in Figure 4.8.

Comparing the odds of experiencing an immunological response in non-B to B infected patients, before adjustment for potentially confounding variables, showed there was no evidence of a significant difference (OR: 1.21; 95% CI: 0.82-1.80; *p*=0.335). Analysis of A, C, and 'other' subtypes also showed no significant differences with ORs of 0.70 (95% CI: 0.28-1.78; *p*=0.455), 1.12 (95% CI: 0.59-2.13; *p*=0.738) and 1.56 (95% CI: 0.90-2.71; *p*=0.111) respectively.

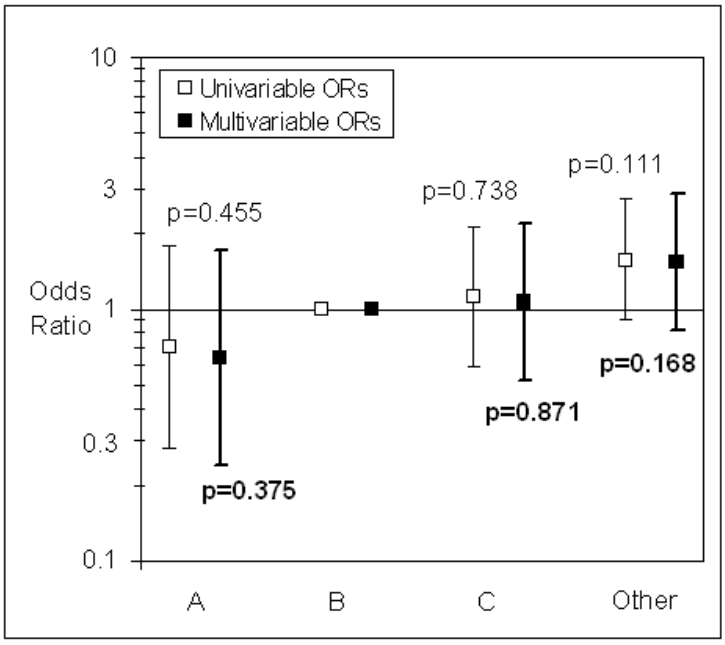
Figure 4.8: Immunological response rates (at least 100 CD4 cells/mm^3 increase) after starting cART and 95% confidence intervals according to HIV-1 subtype (missing = failure).



A multivariable model was developed adjusting for baseline viral load (less than 500 copies/mL, 500-10000 copies/mL, more than 10000 copies/mL or missing), baseline CD4 nadir, time from CD4 nadir, calendar year of starting cART, type of regimen, number of new drugs in regimen, whether or not ART-naïve at baseline and transmission exposure group. After adjustment, there was no significant difference in the odds of an immunological response (non-B compared to B) (OR: 1.17; 95% CI: 0.73-1.87; *p*=0.524). Multivariable ORs for A, C and 'other' subtypes compared to B were 0.64 (95% CI: 0.24-1.72; *p*=0.375), 1.06 (95% CI: 0.52-2.18; *p*=0.871) and 1.54 (95% CI: 0.83-2.86; *p*=0.168) respectively, which are displayed in Figure 4.9.

Interactions between type of cART regimen (PI-containing versus other) and subtype (A, B, C or 'other') were again considered, however these were not found to be significant (*p*=0.506).

Figure 4.9: Odds ratios and 95% confidence intervals of immunological response (at least 100 CD4 cells/mm^3 increase) after starting cART according to HIV-1 (missing = failure).



Notes: Multivariable analysis adjusted for baseline viral load, baseline CD4 nadir, time from CD4 nadir, calendar year of starting cART regimen, type of regimen, number of new drugs in regimen, whether or not ART-naïve and HIV exposure group.

4.3.4.2 Sensitivity analysis: missing CD4 count = excluded

Excluding patients with missing CD4 counts at six to twelve months resulted in consistent findings to those in the main analysis. The number of eligible patients was reduced from 867 (92.3%) to 796 (84.8%) patients. A successful immunological response was achieved by 382 (48.0%) patients: 47.3% (95% CI: 43.7-51.2%) of 691 B patients and 52.4% (95% CI: 43.8-62.9%) of 105 non-B patients (p=0.334). Within the non-B subtypes, 38.9% (95% CI: 21.8-66.9%) of 18 with subtype A, 51.4% (95% CI: 37.7-70.8%) of 35 with subtype C and 57.7% (95% CI: 46.1-73.0%) of 52 with 'other' experienced an immunological response.

Before adjustment, there were no significant differences in the odds of experiencing an increase of 100 CD4 cells/mm^3 between non-B subtypes compared to B (OR: 1.23; 95% CI: 0.81-1.85; p=0.334). In the multivariable analysis, the results were very similar (OR: 1.17; 95% CI: 0.72-1.90; p=0.537). Comparing the odds of an immunological response in non-B subtypes A, C and 'other' compared to B subtype resulted in ORs of 0.68 (95% CI: 0.25-1.88; p=0.457), 1.14 (95% CI: 0.53-2.43; p=0.738) and 1.44 (95% CI: 0.76-2.72; p=0.265) respectively. Table 4.5 displays the

results of this analysis and those from other sensitivity analyses, compared to the main results.

Table 4.5: Odds ratios and 95% confidence intervals of immunological response (at least 100 CD4 cells/mm^3 increase) for HIV-1 subtype B versus non-B.

	B		Non-B		Non-B versus B			
	n	% CD4 responders	n	% CD4 responders	Univariable OR (95% CI)	*p*	Multivariable OR (95% CI)	*p*
Main analysis, missing = failure	753	43%	114	48%	1.21 (0.82-1.80)	0.335	1.17 (0.73-1.87)	0.524
Missing = excluded	691	47%	105	52%	1.23 (0.81-1.85)	0.334	1.17 (0.72-1.90)	0.537
Sensitivity analyses, missing = failure:								
ART-naïve	353	48%	49	55%	1.31 (0.72-2.38)	0.383	1.35 (0.67-2.75)	0.401
Subtypes by phylogenetic analysis	623	43%	65	48%	1.21 (0.72-2.02)	0.470	1.27 (0.70-2.31)	0.425
Excluding retrospective data	614	44%	85	49%	1.26 (0.80-1.99)	0.317	1.34 (0.78-2.31)	0.285
Excluding non VL responders	434	54%	72	58%	1.20 (0.72-1.98)	0.486	1.02 (0.56-1.86)	0.953
Endpoint 25% CD4 cell increase	753	57%	114	61%	1.20 (0.80-1.79)	0.387	1.28 (0.78-2.09)	0.333

Notes: Multivariable analysis adjusted for baseline viral load, baseline CD4 nadir, time from CD4 nadir, calendar year of starting cART regimen, type of regimen, number of new drugs in regimen, whether or not ART-naïve and HIV exposure group.
VL = viral load; OR = odds ratio; CI = confidence interval.

4.3.4.3 Further sensitivity analyses

Further sensitivity analyses using subsets of patients also gave results consistent with those from the main analyses (results shown in Table 4.5). The alternative endpoint of a 25% CD4 cell increase from baseline also showed no significant difference in the odds of an immunological response between patients infected with non-B compared to B (multivariable OR: 1.28; 95% CI: 0.78-2.09; *p*=0.333).

4.3.5 Response to cART: time to viral load less than 500 copies/mL or 100 CD4 cells/mm^3 increase

Survival analysis techniques investigating time to virological suppression and time to immunological response, as defined in the main analysis, were used to check the robustness of these findings. The frequencies of viral load measurements and CD4 counts were compared between patients infected with B and non-B subtypes before adopting this alternative approach as a difference between the groups could bias the results.

4.3.5.1 Frequency of measurements

Median numbers of viral load measurements in the first year following the start of cART were 4 (IQR: 3-5) for both patients infected with subtype B and with non-B subtypes (p=0.095). Median numbers of CD4 counts were 4 (IQR: 3-5) for B infected patients and 4 (IQR: 3-4) for non-B infected patients (p=0.365). The median times between measurements in this first year also did not differ significantly. Median times between viral loads were 3.0 months (IQR: 1.9-4.0 months) for both B and non-B infected patients (p=0.729) and between CD4 counts were 3.0 months (IQR: 2.0-5.0 months) for B and non-B infected patients (p=0.195). As there were similar frequencies of measurements in each group, survival analysis methods could make an unbiased comparison between groups.

4.3.5.2 Kaplan-Meier estimates

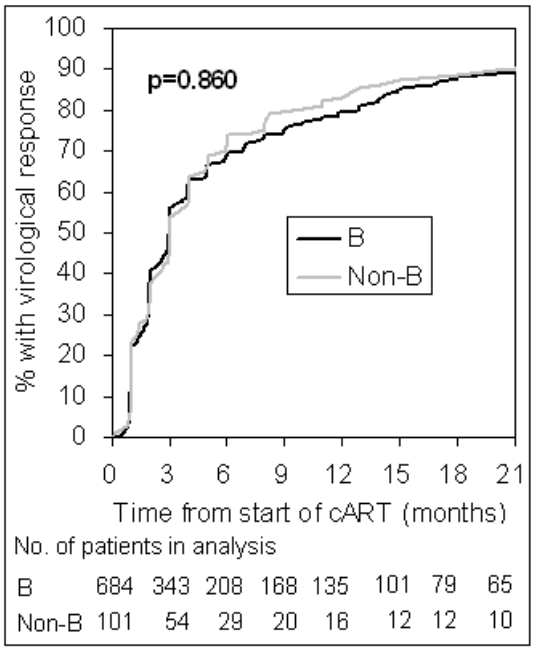
Figure 4.10 (a) displays a Kaplan-Meier estimate of the percentage of patients achieving virological suppression from date of starting a cART regimen. The log rank test showed no significant difference between B and non-B infected patients (p=0.860). Estimates of median time to virological suppression were 3.0 months (95% CI: 3.0-3.0 months) in those infected with B compared to 3.0 months (95% CI: 2.6-4.0 months) in those infected with non-B. Figure 4.10 (b) displays a Kaplan-Meier estimate of the percentage of patients achieving an immunological response (CD4 cell increase of 100 cells/mm^3) from date of starting cART. The log rank test showed no significant difference between B and non-B infected patients (p=0.384) and estimates of median time to immunological response were 8.0 months (95% CI: 7.0-8.9 months) in B infected patients compared to 6.0 months (95% CI: 5.0-7.6 months) in non-B infected patients.

4.3.5.3 Cox proportional hazards models

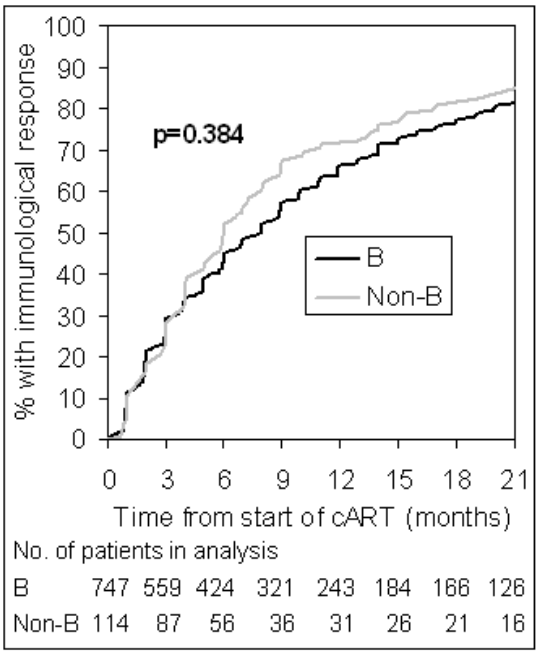
Both univariable and multivariable (adjusted for same potential confounders as in main analysis) Cox proportional hazards models revealed no significant effect of B versus non-B subtype on time to virological response. Univariable relative hazards (RH) were 1.02 (95% CI: 0.82-1.26; p=0.862) and multivariable RH were 0.96 (95% CI: 0.72-1.29; p=0.799) shown in Figure 4.11. Both stratifying by clinical centre and left censoring at date of enrolment into EuroSIDA resulted in very similar findings (multivariable RH: 0.89; 95% CI: 0.63-1.24; p=0.485 and RH: 0.96; 95% CI: 0.70-1.31; p=0.795 respectively).

Figure 4.10: Kaplan-Meier estimates of the percentage of patients achieving (a) a virological response (less than 500 copies/mL) and (b) an immunological response (at least 100 CD4 cells/mm^3 increase) by time from cART initiation for HIV-1 subtype B versus non-B.

(a) Virological response

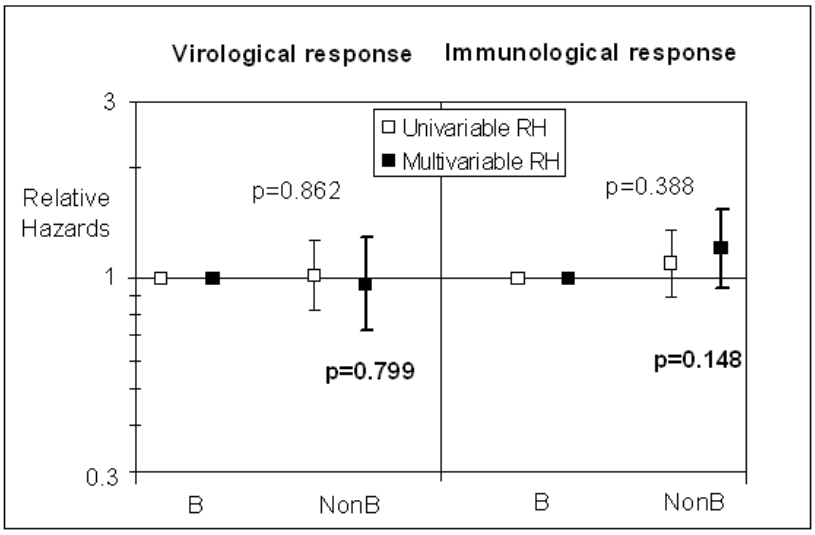


(b) Immunological response



For immunological response, univariable RH were 1.10 (95% CI: 0.89-1.35; *p*=0.388) and multivariable RH were 1.20 (95% CI: 0.94-1.53; *p*=0.148) indicating no significant difference between B and non-B infected patients. These are also illustrated in Figure 4.11. Stratifying by clinical centre gave consistent results (multivariable RH: 1.12; 95% CI: 0.85-1.49; *p*=0.412) however the multivariable analysis with left censoring at date of enrolment showed a significantly higher chance of achieving an immunological response in the non-B infected patients (multivariable RH: 1.37; 95% CI: 1.06-1.78; *p*=0.015). The median time between CD4 counts in the first year from baseline in the analysis (if patient started cART before enrolment, time of enrolment was taken as baseline) was investigated to see if this could explain the difference in response in this sensitivity analysis. A difference was found between B and non-B infected patients (3.0 months (IQR: 2.0-4.0 months) versus 2.9 months (IQR: 1.9-3.9 months); *p*=0.003), which could account for the significant difference observed.

Figure 4.11: Relative hazards and 95% confidence intervals of virological response (less than 500 copies/mL) and immunological response (at least 100 CD4 cells/mm^3 increase) after starting cART for HIV-1 subtype B versus non-B.



Notes: For virological response, multivariable analysis adjusted for baseline maximum ever viral load, baseline CD4 nadir, time from CD4 nadir, hepatitis B and C co-infection status, calendar year of starting cART regimen, type of regimen, number of new drugs in regimen, whether or not ART-naïve at baseline, age, HIV exposure group, EuroSIDA region and ethnicity.
For immunological response, multivariable analysis adjusted for baseline viral load, baseline CD4 nadir, time from CD4 nadir, calendar year of starting cART regimen, type of regimen, number of new drugs in regimen, whether or not ART-naïve and HIV exposure group.

4.4 Discussion

This chapter investigated potential differences in virological and immunological response to cART between patients infected with HIV-1 B and non-B subtypes. No significant differences between the two groups were found in this dataset and no interaction between HIV-1 subtype and type of cART regimen (PI-containing versus other) were found either. There were relatively few non-B subtypes but further investigation suggested that there were no significant differences in virological or immunological response between patients infected with C or 'other' subtypes, compared to those with B, although response rates were slightly higher than in patients infected with B subtype. There were borderline significantly lower odds of a virological response in patients infected with subtype A. These varied responses in patients infected with non-B subtypes suggests that they should not be aggregated together and shows that the overall response rate in non-B infected patients was averaged out, which could be responsible for the similar response rate to B infected patients. Due to the small numbers, the analysis lacked the statistical power to be able to draw firm conclusions.

4.4.1 Interpretation of findings

4.4.1.1 Factors associated with infection with an HIV-1 non-B subtype

The method by which HIV subtype was determined, HIV transmission exposure group and African origin were found to be associated with the odds of infection with a non-B subtype in this analysis. Patients who were enrolled into EuroSIDA more recently were less likely to have plasma samples stored in the EuroSIDA central laboratories and so were less likely to have had their HIV subtypes determined by phylogenetic analysis. Cohorts VI and VII included many patients from East Europe where subtype A is known to be prevalent [401] and the most common route of transmission is IDU [81]. This could explain the association of non-B subtypes with these variables. Non-B subtypes are also widespread across Africa so it was expected that patients with African origin would also mostly be infected with non-B virus [401,415].

4.4.1.2 Virological response to cART

No significant differences in virological response to cART were found between patients infected with B or non-B subtypes, except for a borderline significant difference in patients whose subtypes were determined after 1999 due to less reliable viral load assays potentially measuring low in patients infected with non-B subtypes before then [430-432]. Even though the potential bias was expected to be over-estimating the virological response rate in these patients, patients infected with non-B subtypes appeared to have higher odds of virological suppression in this analysis. However the sample size was small and the finding could be due to chance. Investigation into differences between A, B, C and 'other' subtypes showed a borderline significantly lower virological response rate in patients infected with A. The analysis would need more statistical power to determine if this was a true difference. 'Other' subtypes including CRFs appeared to have a slightly higher response rate but this was not statistically significant. This could account for the similar virological response rates between B infected patients and the aggregated group infected with non-B subtypes.

4.4.1.3 Immunological response to cART

There were also no significant differences in immunological response to cART between B and non-B subtypes. There was a similar trend for patients infected with subtype A to have a lower response rate than those with subtype B and for those with 'other' subtypes to have a higher response rate, but neither of the differences were significant. A significant difference was found in a sensitivity analysis using a Cox proportional hazards model with left censoring at the date of enrolment into EuroSIDA. This was conducted to exclude retrospective data from patients. This may be explained by a

difference that was found in the median time between CD4 count measurements when CD4 count data before enrolment was excluded between B and non-B subtypes.

No interaction between HIV-1 subtype and type of cART regimen started was found. However again, the number of patients infected with non-B subtypes that started each type of cART regimen was small.

Although the statistical power for these analyses was limited, these results suggest that the HIV genetic diversity and potential differences in drug resistance between HIV-1 subtypes do not appear to have affected patients' response to therapy so far.

4.4.2 Previous research

The findings are consistent with previous research, summarised in Table 4.6 [422-429]. Comparisons between patients infected with B and non-B subtypes generally found no significant differences in virological response, both short- and long-term. Bocket *et al*. (2005) [423] compared time to virological suppression between 317 B and 99 non-B infected ART-naïve patients starting ART in a French cohort and found no significant difference. A retrospective, case-control study of 50 B infected and 50 non-B infected UK patients starting cART looked at both initial and long-term virological response, finding no significant differences [428]. However, De Wit *et al.* (2004) [422] did find a significantly lower median CD4 cell increase 24 months after starting a PI-containing regimen in non-B infected patients, which they suggested could be due to viral or host characteristics and should be explored further. A comparison of patients seen at a UK clinic whose country of origin was either 'European' or 'African' was carried out by Frater *et al.* (2002) [429], with 'European' representing B subtype and 'African' representing non-B subtypes. The longer-term virological response was found to be poorer in the African cohort, which they suggested could be related to cultural and language barriers leading to non-adherence. In studies looking at specific non-B subtypes, no associations were found between subtype and virological response or incidence of disease progression [399,424-426].

Table 4.6: Previous research investigating the effect of HIV-1 subtypes on response to ART.

Authors, year	Patient numbers with each subtype	Main outcomes	Conclusions
Lawrence *et al.* 2006[399]	45 B, 22 CRF02_AG	Changes in viral load and CD4 cell count at 1, 3, 6, 12 and 24 months after starting ART. % with therapeutic failure defined as a rebound in viral load of at least 5000 copies/mL over 24 month period.	No significant differences in short or longer term response. No difference in therapeutic failure over study period.
Atlas *et al*. 2005 [424]	32 A, 44 B, 34 C, 18 D, 5 G, 19 CRF01_AE	% with undetectable viral loads six months after starting cART.	In univariable analyses, no evidence of association between subtype and short-term virological response.
Bocket *et al*. 2005 [423]	317 B, 99 non-B	Time to viral load <400 copies/mL after starting ART for first time. Incidence of clinical progression (AIDS or death), changes in CD4 cell count from baseline and % achieving viral load <400 copies/mL at 3, 6 and 12 months.	No significant differences in virological response or clinical progression.
De Wit *et al*. 2004 [422]	56 B, 119 non-B	% with viral loads <400 copies/mL and median CD4 cell increase 24 months after starting PI-containing cART for first time.	No significant difference in virological response but significant difference in immunological response.
Pillay *et al*. 2002 [425]	Children: 16 A, 44 B, 17 C, 10 D, 8 F, G, and H, 13 CRF01_AE and CRF02_AG	Change in viral load from starting dual ART to 24 and 48 weeks.	No evidence of association between subtype and virological response.
Alexander *et al*. 2002 [427]	459 B, 19 non-B	Virological response after up to 18 months after starting ART for first time.	No significant difference in virological response.
Frater *et al*. 2002 [429]	265 'European', 97 'African' (random % sequenced to check 'European' = B, 'African' = non-B)	Kaplan-Meier survival analysis on time to viral load <500 copies/mL after starting cART and changes in CD4 cell count and viral load from baseline.	Initial virological and immunological responses similar between European and African cohorts. Longer-term virological response was poorer in African cohort.
Loveday *et al*. 2001 [428]	50 B, 50 non-B	Retrospective case-controlled comparison of time to a viral load <400 copies/mL and <50 copies/mL, and mean minimum duration of response <400 copies/mL after starting cART.	No significant differences in initial or long-term virological response.
Alaeus *et al*. 1999 [426]	28 A, 59 B, 21 C, 18 D	Rate of CD4 cell decline from date of diagnosis, incidence of clinical disease progression, viral load (latest in follow-up period) over a mean follow-up period of 44 months.	No evidence of association between subtype and disease progression.

4.4.3 Limitations of analysis

The main limitation of the analyses in this chapter was the limited statistical power to detect true differences in response to therapy between HIV-1 subtypes due to the relatively small number of patients infected with a non-B subtype. For example, with the 689 subtype B infected patients and 102 non-B infected patients included in the

virological response analysis, there was 80% power (with alpha = 0.05) to detect a true difference in virological response rate of 15%. For the group of 38 patients infected with subtype C, there would only be 41% power to detect a difference with subtype B patients and for the 17 patients infected with subtype A, there would be only 19% power. This means that it is not yet possible to draw any firm conclusions of no real difference in response to therapy between B subtypes and specific non-B subtypes.

4.4.4 Implications of findings

Analyses such as this address whether or not ART results in better rates of virological and immunological response in patients infected with certain subtypes, which has implications for increasing the availability of drugs in developing countries (where non-B subtypes dominate) and for future drug development [356,396,406,411]. Current treatment guidelines do not make any specific recommendations according to HIV-1 subtype [40-42] and to date, clinical trials investigating antiretroviral drugs also do not report on subtype, therefore it is unknown whether or not newer therapies are equally effective for all subtypes. Although this analysis did not find any differences in response to cART between subtypes nor any interaction with type of cART regimen, it is important for research to continue to investigate potential differences as evidence of variation would support the use of subtype testing as a standard procedure before starting therapy to optimise a patient's subsequent treatment strategy.

4.4.5 Further research

As the EuroSIDA resistance database continues to expand, all available patient plasma samples will be used to obtain HIV RT and PR gene sequences. Consequently, these sequences will allow determination of HIV-1 subtypes by phylogenetic analysis and the use of other sources of subtype identification can eventually be eliminated. This will ensure the highest standard of subtype determination for future analyses. It will also increase the number of patients who will be eligible for inclusion in these analyses. With more patients included, it may be possible to make specific comparisons between subtypes instead of grouping them into B versus non-B. As non-B subtypes are genetically very different from each other, the effect of grouping them together may have concealed true differences in response. It may also be possible to focus on exclusively ART-naïve patients for the main analyses, which minimises the effects of drug resistance mutations that may have been acquired from previous NRTI experience. In addition, an increased number of patients could provide the power to stratify analyses by type of cART regimen.

Investigating virological and immunological response provides insight into the short-term outcome of ART. However, ultimately it is the long-term clinical outcome that is important. Due to lack of power it was not possible to analyse clinical endpoints (AIDS or death), however other studies have so far not found any significant difference between patients infected with different HIV-1 subtypes [422-429]. More data could provide enough power to conduct multivariable analyses looking at clinical endpoints. Collaborations of studies are an ideal way to pool data in order to maximise power to answer specific research questions. This analysis is a project that could be addressed in such a way.

4.4.6 Conclusions

The findings from these analyses showed no evidence of significant differences in virological or immunological response to cART between patients infected with HIV-1 B and non-B subtypes. Patients infected with non-B subtypes did not show a detrimental outcome compared to those with B as suggested by differences both in HIV diversity (in untreated patients), and in resistance development on treatment, found in other research. The continued expansion of the EuroSIDA resistance database and the exclusive use of phylogenetic analysis to determine subtypes will allow more sensitive analyses in the future with increased power to detect any true differences. Increasingly it will be possible to focus on individual non-B subtypes and on responses to particular regimens. A collaborative study may be needed in order to fully address this important research question.

A manuscript of this analysis was published by Antiviral Therapy in September 2006 and can be found in Appendix VII.

Chapter 5. Transmitted drug-resistant HIV-1 and association with virological and immunological response to first-line combination antiretroviral therapy

5.1 Introduction

Resistance of HIV-1 to antiretroviral drugs is a major challenge for the continued efficacy of ART and emerges when treatment fails to suppress viral replication effectively, either through suboptimal treatment or incomplete adherence. It is associated with virological failure in patients undergoing treatment and limits subsequent therapy options [16,317-320].

5.1.1 Transmission of drug-resistant HIV

As discussed in Chapter 1 (section 1.8.5), individuals may acquire a drug-resistant HIV strain through infection [337,352,353]. In the absence of therapy, wild-type HIV will rapidly become dominant due to its increased replicative capacity (efficiency in replication) compared to resistant HIV, and the resistant virus will become negligible, although still "archived". Under selective drug pressure, the resistant strain could once again emerge rapidly [336,339]. However, if only the resistant strain is transmitted, reversion to wild-type virus can only occur through *back mutations*, i.e. mutations that occur at the site of the resistance-associated mutations, which result in a viral copy closer to wild-type. This takes a much longer period of time to happen [433].

It has been suggested that drug-resistant HIV is less transmissible than wild-type HIV [434,435]. A number of reasons have been proposed for this. Patients who have developed resistance whilst on therapy may have lower viral loads than untreated individuals, lowering the transmission risk. In addition, patients regularly attending a clinic to receive ART may engage in less "risky" behaviour than those who do not attend a clinic. Resistant HIV also has a reduced level of fitness in patients not on therapy (the ability to replicate in a well-defined environment), which could lower the probability of transmission [434]. Despite this, transmission of many different resistant strains has been observed over the past few years [435,436].

5.1.2 Prevalence of transmitted drug resistance in Europe

The prevalence of transmitted drug-resistant HIV (TDR) in ART-naïve patients has been estimated to range from as little as 2.1% to 51.5% across Europe [437,438]. Recent data from the CATCH (The Combined Analysis of Resistance Transmission over Time of Chronically and Acute Infected HIV Patients) study, a substudy of the

European scientific surveillance programme, SPREAD (Strategy to Control Spread of HIV Drug Resistance) [436], showed a mean prevalence of TDR of 10.4% across recently and chronically infected ART-naïve patients in 19 European countries and gives an estimate close to the majority of smaller studies performed across Europe [348,435,439-444]. There are many reasons to explain this wide range of estimates of TDR prevalence including varying time periods of study, populations, designs, methods and definitions of resistance. The rates may also be affected by the distribution of HIV-1 subtypes in the population. Non-B subtypes are likely to have originated from countries where ART is not readily available resulting in a population that is likely to have a lower prevalence of TDR. Therefore, HIV-1 subtype may be a marker for exposure to ART [337].

As mentioned in Chapter 3 (section 3.1.2.2), temporal trends in prevalence of TDR appear to vary across cohorts. More HIV infected individuals may be developing drug resistance as a result of the wider access to ART, use of treatment interruptions or increasing high-risk behaviour, which could lead to an increase in transmission rates [388-393,445-448]. The UK Group on Transmitted HIV Drug Resistance found an increase in prevalence of TDR in a sample of 2357 UK HIV infected patients (high or medium level according to the Stanford genotypic resistance algorithm (Version 2004.04)) over the period 1996-2003 from 11.0% to 19.2%, most noticeably in NNRTI resistance [389]. However, the latest data from this group between 1996 and 2004 showed a decrease in TDR in later years. TDR was defined as at least one major mutation from the 2005 IAS-USA guidelines plus selected additional mutations, and reached a peak of approximately 14% in 2001-2002, falling to 8% by the end of 2004, mostly driven by NRTI resistance [394]. Improved virological control in patients receiving ART, management of treatment failure and the fact that most infectious people are not receiving ART could explain this latest trend. Blower *et al.* (2003) used a mathematical model to predict that the percentage of new drug-resistant HIV infections would increase over the years 1996 to 2001, with only very slight increases over 2001 to 2005 [449]. Estimates matched well with data from Grant *et al.* (2002) who found increasing TDR over 1996 to 2001 in California [392].

5.1.3 Resistance testing and response to first-line ART

If an ART-naïve patient who has acquired a drug-resistant strain through transmission is prescribed an initial regimen without knowledge of the resistance-associated mutations present, the selection of drugs may not be optimal, which could potentially lead to an increased risk of virological failure. Little *et al.* (2002) found that those infected with a drug-resistant virus in a sample of 202 ART-naïve North American

patients, experienced a longer time to viral suppression after initiation of therapy than in those with no TDR (p=0.05) [393]. Grant *et al.* (2002) also found a similar result in 225 patients in California [392]. Chaix *et al.* (2007) found that the presence of TDR led to a significantly inferior virological outcome in patients from the French ANRS PRIMO Cohort or ANRS Resistance Group who started cART during primary HIV infection [448]. This reasoning supports the implementation of resistance testing in patients starting therapy whilst ART-naïve [338,436,450,451]. Current guidelines recommend the clinical use of drug resistance testing in all newly diagnosed ART-naïve patients due to suboptimal virological responses found to be associated with TDR [42,338].

5.1.4 Motivation and aims for chapter

The question of whether or not TDR has a significant effect on the ability of cART to suppress viral load and to boost immune regeneration is yet unresolved, although some evidence has suggested that its presence leads to a suboptimal virological response to cART [348,392,393,448,452]. As EuroSIDA captures a geographically diverse population, it is in a position to monitor trends in TDR and to study its impact on the short-term outcomes of ART. Therefore, the aims of these analyses were to investigate the prevalence of TDR in the EuroSIDA cohort, the factors associated with its detection and to compare virological and immunological response to cART between patients infected with HIV with resistance to at least one drug prescribed in their regimens and patients infected with fully susceptible HIV.

5.2 Methods

5.2.1 Inclusion criteria

The EuroSIDA dataset used for the analyses in this chapter was the update completed in February 2008. It included data on 14,310 patients with follow-up to (median date of last visit) January 2007. The patients included in this analysis were those who had blood plasma samples taken whilst ART-naïve from which viral RNA was subsequently extracted and analysed for genotypic resistance. Samples selected for analysis were not based on suspicion of resistance and the results of these tests were not used to guide future treatment nor communicated to clinicians at the time of storing the sample. As mentioned in Chapter 2 (section 2.1.3), EuroSIDA also collects information on mutations identified in resistance tests performed by the clinical centres. It is possible that patients who received a resistance test in the clinic may have had suspected resistance, therefore to eliminate any potential selection bias, these resistance test results were excluded from the analyses.

To investigate virological and immunological response to cART, a subset of the ART-naïve patients who later started a cART regimen (defined as at least three antiretroviral drugs including a PI, an NNRTI, or abacavir (ABC)), without starting mono-/dual ART first, were selected. Patients were also required to have the potential for at least one year's follow-up time after starting cART (i.e. started cART at least one year before January 2007) to provide a suitable length of time to analyse response.

5.2.2 Statistical methods

5.2.2.1 Definitions

The results from genotypic resistance tests performed on HIV RNA from plasma samples taken whilst the patient was ART-naïve were used to estimate prevalence of TDR in the EuroSIDA population. If the patient had more than one sample available before starting ART, results were cumulated. TDR was defined as the detection of at least one HIV-1 mutation from a list proposed for genotypic TDR surveillance by Shafer *et al*. (2007) [345], which included 31 PI resistance mutations at 14 PR positions, 31 NRTI resistance mutations at 15 RT positions and 18 NNRTI resistance mutations at 10 RT positions. For comparison purposes, the IAS-USA 2008 figures of HIV-1 drug resistance mutations [346] (which include naturally occurring polymorphisms that may not have been present at transmission), the Stanford University algorithm (HIVdb) Version 4.3.4 [351], the Rega algorithm Version 7.1 [349] and the ANRS algorithm July 2006 [350] were also used to interpret resistance and to obtain estimates of TDR prevalence.

Date of HIV infection was estimated in patients who had dates for HIV negative and HIV positive serostatus as the midpoint of the two. Chronic HIV infection was defined as HIV infection longer than a year (similarly to the CATCH Study [436]).

As in previous chapters, a cART regimen was defined as at least three drugs including a PI, an NNRTI or abacavir (ABC). Baseline was defined as the date of starting cART.

5.2.2.2 Availability of resistance test results whilst ART-naïve

Multivariable logistic regression models were developed to investigate the factors affecting whether or not patients had plasma samples available whilst ART-naïve that were subsequently tested retrospectively for genotypic resistance. Variables investigated (at date of enrolment) were gender, age, HIV exposure group (defined as 'men who have sex with men (MSM)', 'injecting drug use (IDU)', 'heterosexual' or 'other'), geographical region (defined in Chapter 2, section 2.1.5), country of origin (defined as 'same as clinical centre', 'another European country', 'Africa', 'America',

'Asia' or 'other'), ethnicity (defined as 'white' or 'other'), CD4 count and viral load, hepatitis B/C coinfection status, prior AIDS diagnosis and date of enrolment.

5.2.2.3 Detection of transmitted drug resistance

Multivariable logistic regression was also used to assess the effect of calendar time (divided into 1994-1995, 1996-1997, 1998-1999, 2000-2001 and 2002-2004), HIV-1 subtype (A, B and 'other') and geographical region (South, Central West, North and East), on the detection of TDR after adjustment for potentially confounding factors. All resistance test results were included until the first detection of TDR for each patient and the analysis was adjusted for repeated tests per patient using generalised estimating equations. Potential confounders investigated were as above but at the date of the plasma sample analysed, instead of at date of enrolment. Those that were significantly associated with the detection of TDR in univariable analyses (p<0.1) were included in the multivariable model.

5.2.2.4 Virological and immunological response to cART

Response to cART was analysed via multivariable logistic regression models taking the first measurements six to twelve months after initiation of cART. Patients infected with HIV with full/intermediate resistance to at least one drug started according to genotypic sensitivity scores calculated using the Stanford algorithm (the 'resistant group') were compared to those with HIV susceptible to all drugs started (the 'susceptible group'), after adjustment for potentially confounding factors. These were identified as those significantly associated with the odds of a response in univariable analysis (p<0.1) and any additional variables identified using a stepwise selection procedure. Variables investigated were similar to above but at date started cART.

A successful virological response was defined as a viral load less than 500 copies/mL and immunological response as a 100 CD4 cells/mm^3 or more increase from the start of cART. For analyses of virological response, patients were required to have a baseline viral load of at least 500 copies/mL and for immunological response patients needed a baseline CD4 count available measured within six months prior to starting cART. A 'missing = failure' approach was taken as the main analysis.

5.2.2.5 Sensitivity analyses

A sensitivity analysis was carried out using a 'missing = excluded' approach. Further sensitivity analyses using the 'missing = failure' approach were conducted on the subset of patients who started cART after enrolment into EuroSIDA (excluding patients whose treatment data were collected retrospectively), using the Rega algorithm to

calculate genotypic sensitivity scores instead of Stanford, and using the definition of TDR by Shafer *et al*. (2007) to define groups based on whether or not patients had TDR mutations regardless of the drugs they were starting.

For the analysis of immunological response, a sensitivity analysis was carried out adjusting for the change in viral load from baseline to the first measurement six to twelve months after starting cART. This excluded any patients with missing viral load measurements during this time.

Alternative endpoints were also investigated. A virological endpoint of a viral load less than 50 copies/mL was analysed on a subset of patients whose first viral load measurements in the six to twelve month period after starting cART were measured using an assay with a level of detection as low as 50 copies/mL. This required all those with missing values to be excluded. A further immunological endpoint of a 50% CD4 cells/mm^3 increase or more from baseline was also analysed.

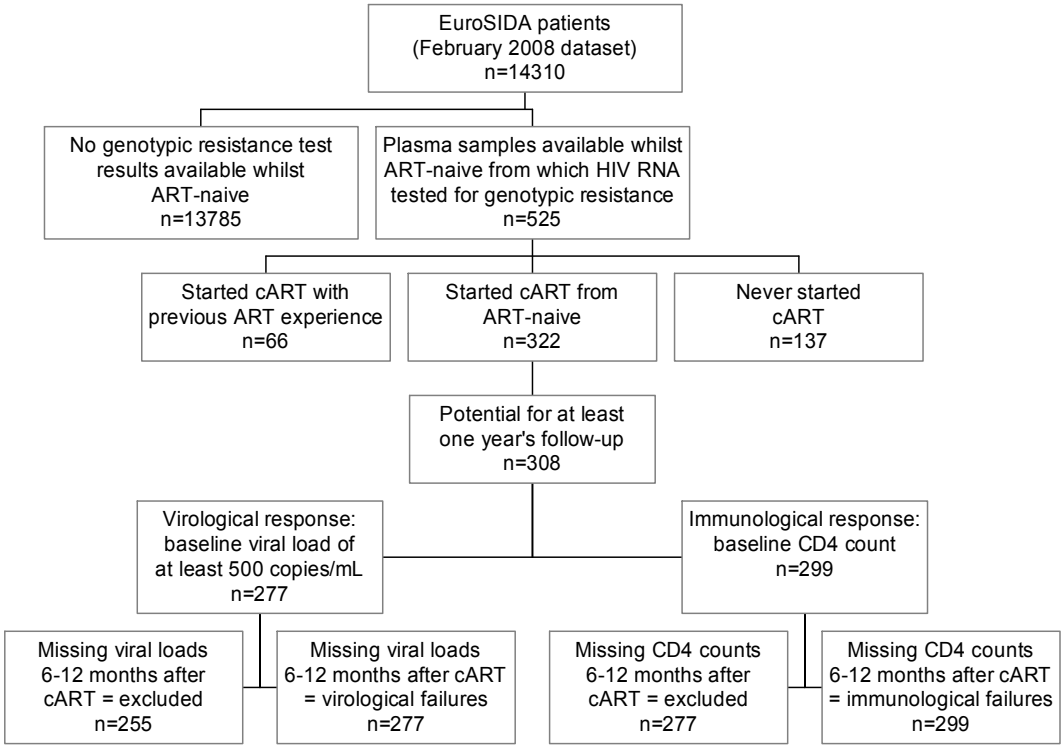
5.3 Results

5.3.1 Patient numbers

The patient numbers according to the inclusion criteria are displayed in Figure 5.1. Amongst 14,310 patients, 525 (3.7%) were included who had plasma samples available whilst ART-naïve, which were subsequently tested retrospectively for genotypic HIV drug resistance.

Date of HIV infection could be estimated for 118 (22.5%) of the 525 ART-naïve patients tested for resistance. The median time between estimated date of infection and earliest resistance test was 51.0 months (IQR: 24.6-83.5 months) for these patients and was more than one year for 105 (89.0%) patients. In 395 (75.2%) patients whose date of infection could not be estimated but the date of HIV positive diagnosis was known, the median time between HIV positive diagnosis and earliest resistance test was 48.4 months (IQR: 17.5-97.1 months) and was more than one year for 325 (82.3%) patients. Therefore the majority of this study population (at least n=430, 81.9%) were chronically infected patients.

Figure 5.1: Patient numbers in analyses according to inclusion criteria.



5.3.2 Patient characteristics at date of enrolment

5.3.2.1 Resistance test results available versus not

Table 5.1 shows the differences between these 525 patients who met the inclusion criteria compared to those excluded at the date of enrolment into EuroSIDA. Over half of the patients included with resistance test results were from the North region, whereas amongst those excluded, patients were more evenly distributed between all regions (p<0.001). Patients with resistance test results available were less likely to have a previous AIDS diagnosis (10.7% versus 29.1%; p<0.001), more likely to be co-infected with hepatitis B (68.2% versus 60.0%; p<0.001) and were diagnosed with HIV more recently (33 versus 52 months; p<0.001). They also had a higher median CD4 count (370 versus 284 cells/mm^3; p<0.001) and a higher median viral load (4.4 versus 2.7 $\log_{10}$copies/mL; p<0.001).

Table 5.1: Patient characteristics at date of enrolment according to whether or not patients had plasma samples available whilst ART-naïve that were subsequently tested retrospectively for genotypic HIV drug resistance.

		Total		Tested for resistance		No resistance test		*p*
All	**n %**	14310	100.0	525	3.7	13785	96.3	-
Male		10840	75.8	395	75.2	10445	75.8	0.780
HIV exposure group								0.021
	MSM	5809	40.6	239	45.5	5570	40.4	-
	IDU	3641	25.4	140	26.7	3501	25.4	-
	Heterosexual	3848	26.9	117	22.3	3731	27.1	-
	Other	1012	7.1	29	5.5	983	7.1	-
Ethnicity								0.588
	White	12678	88.6	469	89.3	12209	88.6	-
	Other	1632	11.4	56	10.7	1576	11.4	-
Region								<0.001
	South	4562	31.9	52	9.9	4510	32.7	
	Central West	3481	24.3	84	16.0	3397	24.6	-
	North	3707	25.9	282	53.7	3425	24.9	-
	East	2560	17.9	107	20.4	2453	17.8	-
Origin								0.003
	Same as EuroSIDA centre	10893	76.1	429	81.7	10464	75.9	-
	Other European country	793	5.5	26	5.0	767	5.6	-
	Africa	771	5.4	29	5.5	742	5.4	-
	Other/unknown	1853	13.0	41	7.8	1812	13.1	-
Previous AIDS		4068	28.4	56	10.7	4012	29.1	<0.001
Hepatitis B status								<0.001
	Negative	8628	60.3	358	68.2	8270	60.0	-
	Positive	706	4.9	33	6.3	673	4.9	-
	Unknown	4976	34.8	134	25.5	4842	35.1	-
Hepatitis C status								0.241
	Negative	5762	40.3	224	42.7	5538	40.2	-
	Positive	2780	19.4	108	20.6	2672	19.4	-
	Unknown	5768	40.3	193	36.8	5575	40.4	-
Median (IQR)								
Date of enrolment		Aug 97	(Dec 95-Nov 03)	Apr 97	(Jan 96-May 99)	Sep 97	(Dec 95-Nov 03)	<0.001
Age (years)		36	(31-44)	35	(29-41)	36	(31-44)	<0.001
CD4 count (cells/mm^3)								
	At enrolment[(1)]	289	(144-440)	370	(256-475)	284	(140-439)	<0.001
	Nadir[(2)]	176	(67-300)	320	(220-415)	170	(63-294)	<0.001
Time from nadir (months)		5	(1-16)	3	(0-8)	5	(1-17)	<0.001
Viral load (log_{10}copies/mL)								
	At enrolment[(3)]	2.7	(1.8-4.2)	4.4	(3.8-5.0)	2.7	(1.7-4.1)	<0.001
	Maximum ever[(4)]	4.6	(3.6-5.2)	4.6	(4.1-5.1)	4.6	(3.6-5.2)	0.016
Time from HIV positive diagnosis (months)		51	(20-96)	33	(11-78)	52	(21-96)	<0.001

Notes: *P* values obtained from Chi-squared, Fisher's Exact and Kruskal-Wallis tests.
Median CD4 counts and viral loads based on measurements from [(1)]13552 patients, [(2)]13918 patients, [(3)]8129 patients, [(4)]8725 patients.
MSM = men who have sex with men; IDU = injecting drug use; IQR = interquartile range.

5.3.2.2 Factors affecting availability of resistance test results

A multivariable logistic regression model was developed to investigate further factors that may have affected whether or not patients had plasma samples available whilst ART-naïve that were subsequently tested retrospectively for genotypic HIV drug resistance. Odds ratios (ORs) of all factors investigated are displayed in Table 5.2. The multivariable odds of having resistance test results available were significantly higher in: IDUs versus MSM (OR: 1.39; 95% CI: 1.01-1.91; p=0.044), patients from East versus North Europe (OR: 1.47; 95% CI: 1.02-2.12; p=0.039), patients with higher CD4 counts at date of enrolment compared to less than or equal to 200 cells/mm^3 (OR for 201-350 cells/mm^3: 3.08; 95% CI: 2.30-4.13; p<0.001; OR for higher than 350 cells/mm^3: 6.39; 95% CI: 4.81-8.50; p<0.001; and OR for missing CD4 count: 2.85; 95% CI: 1.46-5.57; p=0.002) and patients with a viral load at time of enrolment of more than 10,000 copies/mL versus less than or equal to this amount (OR: 5.70; 95% CI: 1.02-2.12; p=0.039).

The multivariable odds of having resistance test results available were significantly lower in: patients from South and Central West versus North Europe (OR for South: 0.13; 95% CI: 0.09-0.18; p<0.001 and OR for Central West: 0.31; 95% CI: 0.24-0.41; p<0.001), patients with a prior AIDS diagnosis (OR: 0.43; 95% CI: 0.32-0.57; p<0.001) and patients with positive or unknown hepatitis C coinfection status versus a negative status (OR for hepatitis C positive: 0.69; 95% CI: 0.49-0.97; p=0.032 and OR for unknown status: 0.71; 95% CI: 0.54-0.92; p=0.011).

Table 5.2: Odds ratios and 95% confidence intervals of patients having plasma samples available whilst ART-naïve that were subsequently tested retrospectively for genotypic HIV drug resistance.

	Univariable			Multivariable		
	OR	95% CI	*p*	OR	95% CI	*p*
Gender						
Male	1.00					
Female	1.03	(0.84, 1.26)	0.778	1.17	(0.90, 1.52)	0.229
HIV exposure group						
MSM	1.00			1.00		
IDU	0.93	(0.75, 1.15)	0.516	1.39	(1.01, 1.91)	0.044
Heterosexual	0.73	(0.58, 0.92)	0.006	0.95	(0.70, 1.28)	0.722
Other	0.69	(0.47, 1.02)	0.061	0.93	(0.60, 1.45)	0.759
Ethnicity						
White	1.00			1.00		
Other	0.93	(0.70, 1.23)	0.588	0.85	(0.56, 1.30)	0.449
Region						
North	1.00			1.00		
South	0.14	(0.10, 0.19)	<0.001	0.13	(0.09, 0.18)	<0.001
Central West	0.30	(0.23, 0.39)	<0.001	0.31	(0.24, 0.41)	<0.001
East	0.53	(0.42, 0.67)	<0.001	1.47	(1.02, 2.12)	0.039
Origin						
Same as EuroSIDA centre	1.00			1.00		
Other European country	0.83	(0.55, 1.24)	0.355	0.67	(0.44, 1.04)	0.072
Africa	0.95	(0.65, 1.40)	0.807	1.02	(0.58, 1.79)	0.941
Other/unknown	0.55	(0.40, 0.76)	<0.001	1.01	(0.68, 1.49)	0.961
Prior AIDS	0.29	(0.22, 0.39)	<0.001	0.43	(0.32, 0.57)	<0.001
Hepatitis B status						
Negative	1.00			1.00		
Positive	1.13	(0.79, 1.63)	0.504	1.22	(0.83, 1.80)	0.318
Unknown	0.64	(0.52, 0.78)	<0.001	0.97	(0.74, 1.27)	0.804
Hepatitis C status						
Negative	1.00			1.00		
Positive	1.00	(0.79, 1.26)	0.995	0.69	(0.49, 0.97)	0.032
Unknown	0.86	(0.70, 1.04)	0.120	0.71	(0.54, 0.92)	0.011
CD4 count at time of enrolment (cells/mm3)						
≤200	1.00			1.00		
201-350	2.69	(2.04, 3.54)	<0.001	3.08	(2.30, 4.13)	<0.001
>350	3.34	(2.59, 4.31)	<0.001	6.39	(4.81, 8.50)	<0.001
Missing	0.88	(0.46, 1.66)	0.685	2.85	(1.46, 5.57)	0.002
Viral load at time of enrolment (copies/mL)						
≤10000	1.00			1.00		
>10000	5.91	(4.65, 7.52)	<0.001	5.70	(4.41, 7.36)	<0.001
Missing	1.87	(1.46, 2.38)	<0.001	0.92	(0.69, 1.25)	0.601
Date of enrolment (per year increase)	0.91	(0.88, 0.93)	<0.001	0.73	(0.70, 0.77)	<0.001
Age (per 10 year increase)	0.75	(0.68, 0.82)	<0.001	0.83	(0.74, 0.93)	0.001

Notes: MSM = men who have sex with men; IDU = injecting drug use; OR = odds ratio; CI = confidence interval.

5.3.3 Prevalence of transmitted drug resistance

5.3.3.1 Resistance profiles in ART-naïve patients

TDR defined according to Shafer *et al*. (2007) was detected in 60 of the 525 patients (11.4%; 95% CI: 8.9-14.3%). A total of 49 (9.3%; 95% CI: 7.0-12.0%) had HIV with one or more mutations associated with NRTI resistance, 5 (1.0%; 95% CI: 0.3-2.2%) had NNRTI resistance mutations and 16 (3.0%; 95% CI: 1.8-4.7%) had PI resistance

mutations. A total of 9 patients (1.7%; 95% CI: 0.8-3.0%) were infected with multi-class drug-resistant HIV. The specific TDR mutations that were detected are displayed in Figure 5.2.

Figure 5.2: HIV-1 drug resistance mutations identified for surveillance of transmitted drug resistance in 525 ART-naïve patients.

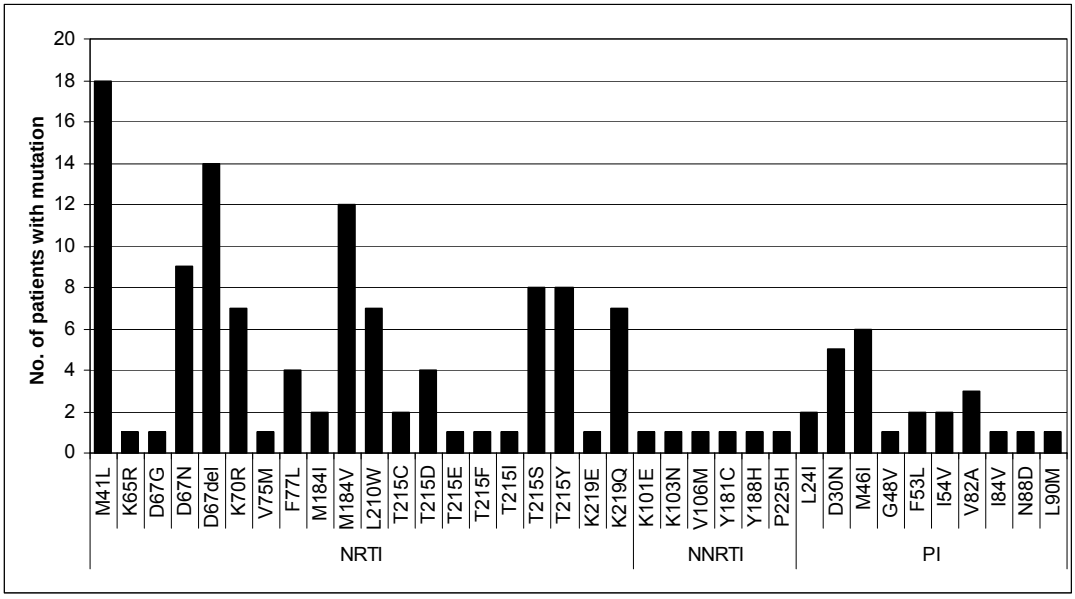


Table 5.3 compares the prevalence of TDR estimated by Shafer *et al.*'s list of mutations to other interpretation systems. Using the IAS-USA list of HIV-1 mutations to interpret resistance, at least one NRTI, NNRTI or major PI mutation linked to drug resistance was detected in HIV from 71 patients (13.5%; 95% CI: 12.7-18.9%). A total of 53 (10.1%; 95% CI: 7.7-12.9%) had NRTI resistance mutations, 21 (4.0%; 95% CI: 2.5-5.9%) had NNRTI resistance mutations and 17 (3.2%; 95% CI: 1.9-4.9%) had major PI resistance mutations. Using the Stanford algorithm to interpret genotypic resistance gave the highest estimate: 84 patients (16.0%; 95% CI: 13.1-19.3%) were infected with HIV with full or intermediate drug resistance. The Rega algorithm resulted in a similar estimate to that found using Shafer *et al.*'s list (9.9%; 95% CI: 7.5-12.6%) and the ANRS algorithm gave the lowest estimate of TDR prevalence (6.3%; 95% CI: 4.4-8.5%).

Table 5.3: Prevalence of transmitted drug resistance and 95% confidence intervals in 525 ART-naïve patients according to various interpretation systems.

Resistance interpretation system	Prevalence of TDR (95% CI)			
	Any	NRTI	NNRTI	PI
Shafer *et al.* 2007 [345]	11.4% (8.9-14.3%)	9.3% (7.0-12.0%)	1.0% (0.3-2.2%)	3.0% (1.8-4.7%)
IAS-USA 2008 [346] (1)	13.5% (12.7-18.9%)	10.1% (7.7-12.9%)	4.0% (2.5-5.9%)	3.2% (1.9-4.9%)(1)
Stanford algorithm Version 4.3.4[351](2)	16.0% (13.1-19.3%)	9.5% (7.2-12.2%)	4.8% (3.1-6.8%)	4.0% (2.5-5.9%)
Rega algorithm Version 7.1 [349](2)	9.9% (7.5-12.6%)	6.7% (4.7-9.0%)	1.0% (0.3-2.2%)	3.8% (2.4-5.6%)
ANRS algorithm July 2006 [350](2)	6.3% (4.4-8.5%)	3.4% (2.1-5.2%)	1.0% (0.3-2.2%)	3.6% (2.2-5.4%)

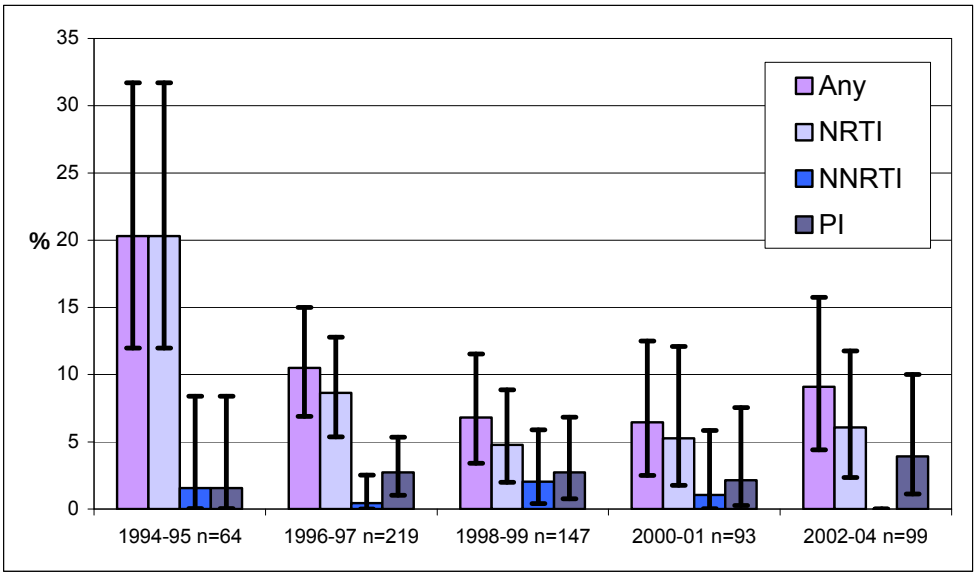
Notes: (1)Major PI mutations only.
(2)Full or intermediate resistance.
TDR = transmitted drug resistance; CI = confidence interval.

As an alternative to cumulating all resistance test results up to the date of starting therapy, only the results from the resistance test performed on each patient's earliest plasma sample were taken to obtain a further estimate of TDR prevalence. This gave a very similar estimate (using Shafer *et al.*'s definition) of 10.9% (95% CI: 8.4-13.7%). NRTI resistance was detected in 9.1% (95% CI: 6.9-11.8%), NNRTI resistance was detected in 0.6% (95% CI: 0.1-1.7%) and PI resistance was detected in 2.7% (95% CI: 1.5-4.2%).

5.3.3.2 Resistance profiles over calendar time

Figure 5.3 displays TDR according to calendar year of resistance test. Over calendar time, the difference in overall prevalence of detected TDR between two-year periods between 1994 and 2004 was borderline significant (p=0.050). After an initial relatively high TDR prevalence of 20.3% (95% CI: 12.0-31.7%) in 64 patients with plasma samples available for genotypic resistance testing in 1994-1995, the prevalence remained fairly stable. TDR prevalence was 10.5% (95% CI: 6.9-15.0%) in 219 patients in 1996-1997, 6.8% (95% CI: 3.4-11.5%) in 147 patients in 1998-1999, 6.5% (95% CI: 2.5-12.5%) in 93 patients in 2000-2001 and 9.1% (95% CI: 4.4-15.7%) in 99 patients in 2002-2004. No resistance test results were available in 2005 onwards. NRTI resistance ranged from 4.8% to 20.3%, NNRTI resistance from 0.0% to 2.0% and PI resistance from 1.6% to 3.9%.

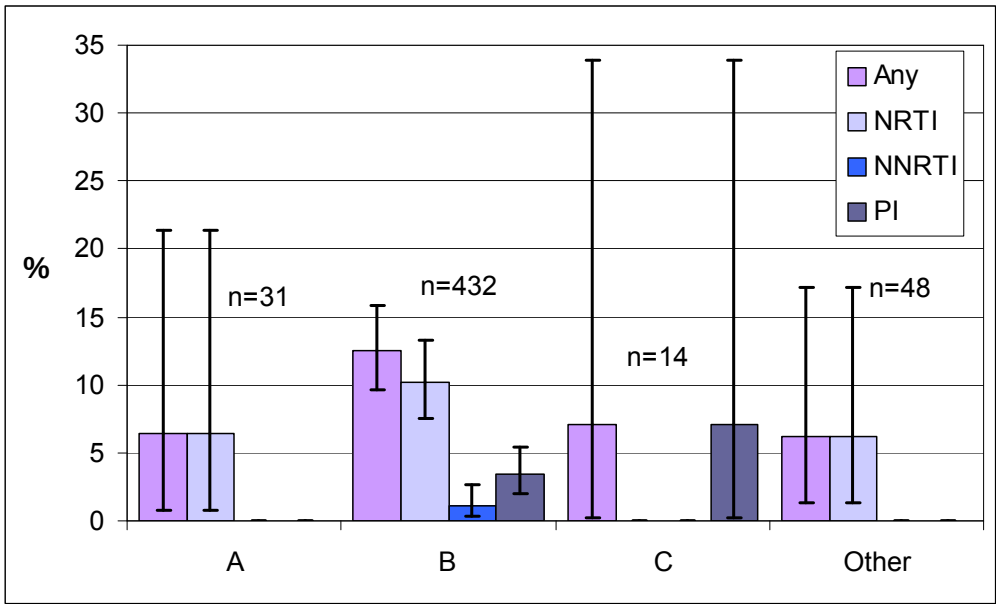
Figure 5.3: Prevalence of transmitted drug resistance and 95% confidence intervals according to calendar year.



5.3.3.3 Resistance profiles according to HIV-1 subtype

A total of 31 (5.9%) patients were infected with HIV-1 subtype A, 432 (82.3%) with subtype B, 14 (2.7%) with subtype C and 48 (9.1%) were infected with another subtype or circulating recombinant form. TDR was highest in those infected with subtype B, 12.5% (95% CI: 9.6-15.8%), followed by 7.1% (95% CI: 0.1-33.9%) of those with subtype C, 6.5% (95% CI: 0.8-21.4%) of the subtype A group and 6.3% (95% CI: 1.3-17.2%) of patients in the 'other' subtype group, displayed in Figure 5.4. The overall prevalence of TDR in non-B subtypes was 6.5% (95% CI: 2.5-12.5%) (6 out of 93 patients), which did not differ significantly compared to patients infected with subtype B (p=0.096). Resistance was predominantly NRTI-associated in patients infected across all subtypes, except for those with HIV-1 subtype C where the one patient with resistance had PI resistance mutations only. NNRTI resistance was only observed in patients infected with subtype B.

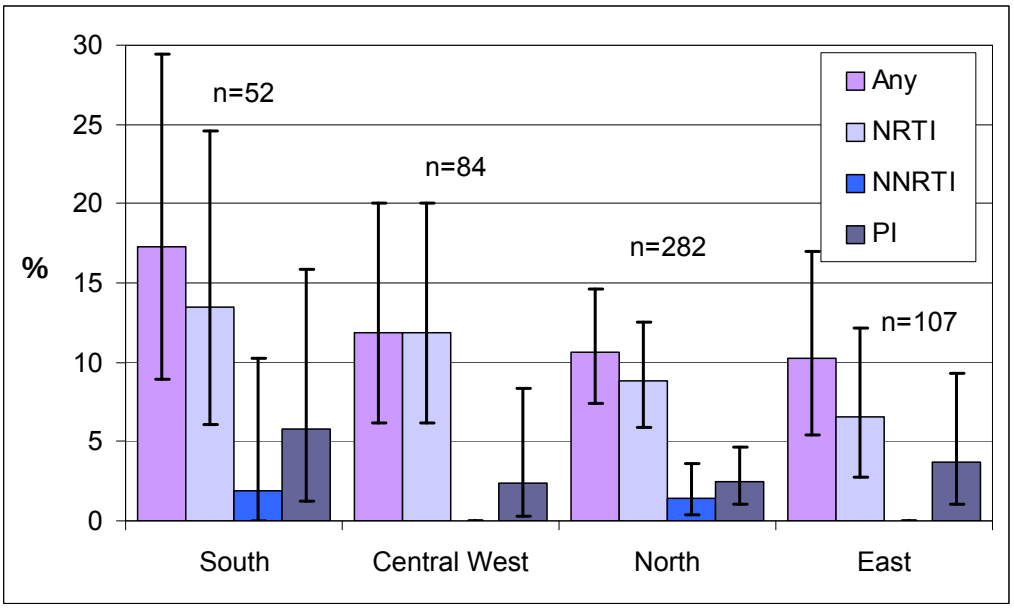
Figure 5.4: Prevalence of transmitted drug resistance and 95% confidence intervals according to HIV-1 subtype.



5.3.3.4 Resistance profiles according to geographical region within EuroSIDA

Amongst the 525 patients, 52 (9.9%) visited clinics in the South region, 84 (16.0%) in the Central West, 282 (53.7%) in the North and 107 (20.4%) in the East. There was no significant overall difference in TDR prevalence between regions (p=0.550). The South region had the largest prevalence of TDR, 17.3% (95% CI: 8.9-29.5%), followed by the Central West with 11.9% (95% CI: 6.1-20.0%), the North with 10.6% (95% CI: 7.4-14.6%) and the East region with 10.3% (95% CI: 5.4-17.0%). These are displayed in Figure 5.5. NRTI resistance was lowest in the East (6.5%) and highest in the South (13.5%). NNRTI resistance was similarly low across regions. PI resistance ranged from 2.4% to 5.8%.

Figure 5.5: Prevalence of transmitted drug resistance and 95% confidence intervals according to geographical region within EuroSIDA.

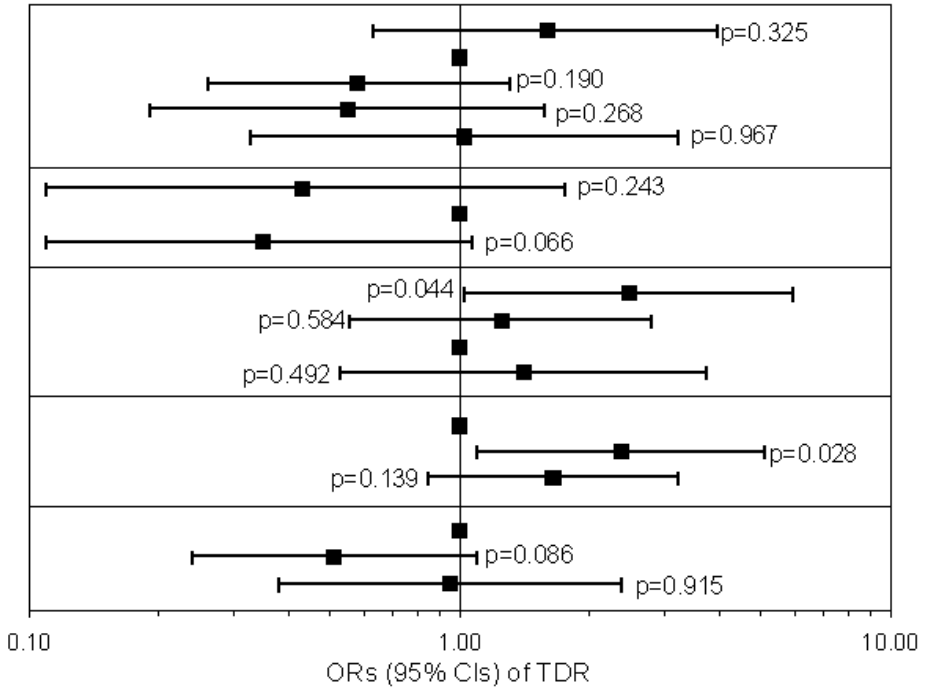


5.3.3.5 Multivariable model of detection of transmitted drug resistance

A total of 622 genotypic resistance test results for the 525 ART-naïve patients were included in a multivariable logistic regression analysis (using generalised estimating equations to adjust for repeated tests per patient), to investigate the effect of calendar time, HIV-1 subtype and geographical region on the detection of TDR. TDR was detected in 61 (9.8%) resistance tests and multivariable odds ratios are displayed in Figure 5.6. After adjustment for viral load at the date of the plasma sample analysed and hepatitis C status that were found to be significantly associated with detection of TDR in univariable analyses (p<0.1), no statistically significant differences could be demonstrated in the detection of TDR between any two-year time periods compared to 1996-1997. There was also no significant difference in the odds of detection of TDR in patients infected with subtype A compared to subtype B (p=0.243) and a borderline significantly lower odds in patients infected with 'other' subtypes compared to B (OR: 0.35; 95% CI: 0.11-1.07; p=0.066). In the South region, there were significantly higher odds of detection of TDR than in the North region (OR: 2.47; 95% CI: 1.02-5.95; p=0.044). The wide confidence intervals indicate the limited power of these analyses due to small patient numbers.

Figure 5.6: Multivariable odds ratios and 95% confidence intervals of detection of transmitted drug-resistant HIV in 622 genotypic resistance tests performed on plasma samples from 525 ART-naïve patients.

Factor in multivariable model		No. of tests	No. (%) detecting TDR
Total		622	61 (9.8)
Calendar year	1994-1995	64	13 (20.3)
	1996-1997	219	23 (10.5)
	1998-1999	147	10 (6.8)
	2000-2002	93	6 (6.5)
	2002-2004	99	9 (9.1)
HIV-1 subtype	A	34	2 (5.9)
	B	516	55 (10.7)
	Other	72	4 (5.7)
Region	South	57	9 (15.8)
	Central West	101	10 (9.9)
	North	352	31 (8.8)
	East	112	11 (9.8)
Hepatitis C status	Negative	258	18 (7.0)
	Positive	108	14 (13.0)
	Unknown	256	29 (11.3)
Viral load at date of plasma sample analysed (copies/mL)	500-10000	117	14 (12.0)
	>10000	344	22 (6.4)
	Missing	161	25 (15.5)



Notes: TDR = transmitted drug resistance; OR = odds ratio; CI = confidence interval.

5.3.4 Transmitted drug resistance at initiation of cART

5.3.4.1 Genotypic sensitivity scores

Out of the 525 patients with resistance test results, 308 (58.7%) then initiated a cART regimen whilst they were still ART-naïve with the potential for one year's follow-up. TDR mutations (defined according to Shafer *et al*. (2007)) were detected in 29 (9.4%; 95% CI: 6.5-13.0%) patients in this subset. Genotypic sensitivity scores calculated using the Stanford algorithm showed that 13 (4.2%; 95% CI: 2.3-6.8%) patients had HIV with full resistance to at least one drug prescribed in their regimens at the time of starting cART (baseline) or intermediate resistance to at least two drugs started and 24 (7.8%; 95% CI: 5.1-11.1%) had HIV with intermediate resistance to just one drug started. A total of 23 (7.5%; 95% CI: 4.8-10.7%) had NRTI resistance mutations to an NRTI started including M41L, D67G/N/deletion, K70R, F77L, M184I/V, L210W, T215Y /D/E/S/I and K219Q, 5 (1.6%; 95% CI: 0.5-3.7%) had NNRTI resistance including V106M and 11 (3.6%; 95% CI: 1.8-6.0%) had PI resistance including L24I, M46I and V82A.

Alternatively, using the Rega algorithm, 14 (4.6%; 95% CI: 2.5-7.2%) patients had HIV with full or intermediate resistance to at least one drug started. Using the ANRS algorithm, 5 (1.6%; 95% CI: 0.5-3.7%) patients had HIV with full or intermediate resistance to at least one drug started.

5.3.4.2 Patient characteristics at date of starting cART

Table 5.4 shows that the patient characteristics were mostly similar between these 37 patients with resistant HIV interpreted using the Stanford algorithm (the 'resistant' group) and the 271 patients with HIV susceptible to all drugs started (the 'susceptible' group). Both groups had a similar median time between their most recent resistance test whilst ART-naïve and starting cART: 8 months (IQR: 4-23 months) for the resistant group and 7 months (IQR: 3-19 months) for the susceptible group (p=0.482).

Table 5.4: Patient characteristics at date of starting cART according to whether patients had HIV with full or intermediate resistance to at least one drug started ('resistant') or HIV susceptible to all drugs started ('susceptible').

		Total		Resistant		Susceptible		*p*
All	**n, %**	308	100.0	37	12.0	271	88.0	-
Male		230	74.7	30	81.1	200	73.8	0.340
HIV exposure group								0.071
	MSM	154	50.0	26	70.3	128	47.2	-
	IDU	63	20.5	5	13.5	58	21.4	-
	Heterosexual	71	23.1	5	13.5	66	24.4	-
	Other	20	6.5	1	2.7	19	7.0	-
Ethnicity								0.149
	White	278	90.3	36	97.3	242	89.3	-
	Other	30	9.7	1	2.7	29	10.7	-
Region								0.135
	South/East	92	29.9	15	40.5	77	28.4	-
	Central West	36	11.7	6	16.2	30	11.1	-
	North	180	58.4	16	43.2	164	60.5	-
Previous AIDS		35	11.4	3	8.1	32	11.8	0.782
Hepatitis B status								0.959
	Negative	246	79.9	30	81.1	216	79.7	-
	Positive	20	6.5	2	5.4	18	6.6	-
	Unknown	42	13.6	5	13.5	37	13.7	-
Hepatitis C status								0.977
	Negative	176	57.1	21	56.8	155	57.2	-
	Positive	61	19.8	7	18.9	54	19.9	-
	Unknown	71	23.1	9	24.3	62	22.9	-
cART regimen (including ≥ 2 NRTIs)								0.134
	Single PI	128	41.6	13	35.1	115	42.4	-
	RTV-boosted PI	38	12.3	9	24.3	29	10.7	-
	Single NNRTI	81	26.3	7	18.9	74	27.3	-
	Triple NRTI including ABC	15	4.9	1	2.7	14	5.2	-
	Other	46	14.9	7	18.9	39	14.4	-
Median (IQR)								
Date of starting cART		Jul 99	(Oct 97-Feb 02)	Oct 00	(Jan 98-Jul 02)	Jun 99	(Oct 97-Dec 01)	0.148
Date of enrolment		Apr 97	(Feb 97-May 99)	Mar 99	(Mar 97-Nov 00)	Apr 97	(Nov 96-Mar 99)	0.052
Age (years)		37	(31-44)	36	(30-43)	37	(31-44)	0.735
CD4 count (cells/mm^3)								
	Baseline[(1)]	251	(147-330)	261	(164-314)	250	(140-331)	0.785
	Nadir[(2)]	207	(122-283)	222	(144-273)	205	(110-288)	0.470
Time from nadir (months)		2	(1-7)	1	(1-3)	2	(1-8)	0.004
Viral load (log_{10}copies/mL)								
	Baseline[(3)]	4.9	(4.4-5.3)	4.8	(4.5-5.3)	4.9	(4.3-5.3)	0.978
	Maximum ever[(4)]	5.1	(4.7-5.5)	5.1	(4.6-5.5)	5.1	(4.7-5.5)	0.719
Time from HIV positive diagnosis (months)		61	(34-113)	64	(32-113)	60	(35-113)	0.877
Time from most recent resistance test (months)		7	(3-19)	8	(4-23)	7	(3-19)	0.482

Notes: *P* values obtained from Chi-squared, Fisher's Exact and Kruskal-Wallis tests.
Resistance interpreted by the Stanford algorithm Version 4.3.4.
Median CD4 counts and viral loads based on measurements from [(1)]299 patients, [(2)]308 patients, [(3)]289 patients, [(4)]296 patients.
MSM = men who have sex with men; IDU = injecting drug use; RTV = ritonavir; ABC = abacavir; IQR = interquartile range.

5.3.5 Virological response to cART: viral load less than 500 copies/mL at months six to twelve

5.3.5.1 Main analysis: missing viral load = failure

Virological response was analysed in 277 (89.9%) patients who had baseline viral loads measured of at least 500 copies/mL, of which 34 (12.3%) patients had HIV with full or intermediate resistance to at least one drug started (using the Stanford algorithm). Of these 277 patients, 22 (7.9%) had no viral load measurement in the period six to twelve months after starting cART and so were counted as virological failures in the main analysis. A total of 211 (76.2%) patients achieved virological suppression (viral load less than 500 copies/mL) at the first measurement six to twelve months after starting cART. Response rates between patients in the resistant and the susceptible groups were similar: 25 (73.5%; 95% CI: 61.6-91.2%) of 34 patients compared to 186 (76.5%; 95% CI: 71.6-82.3%) of 243 patients respectively (p=0.699).

In a univariable logistic regression model, the odds of virological suppression were lower but not significantly in the resistant group compared to the susceptible group (OR: 0.85; 95% CI: 0.38-1.93; p=0.700). After adjustment for potential confounders, namely: gender, geographical region, baseline CD4 count and viral load, date started cART, type of cART regimen and time from most recent resistance test, the findings were similar (OR: 0.68; 95% CI: 0.27-1.71; p=0.408). Table 5.5 displays these results, as well as those from sensitivity analyses. Results were similar in those starting PI- and those starting NNRTI-containing regimens; no interaction was found between type of cART regimen and resistance (p=0.222). However, the power of this analysis to detect true differences was limited by the small numbers.

5.3.5.2 Sensitivity analysis: missing = excluded

In a sensitivity analysis, patients with missing viral load measurements at six to twelve months were excluded with results were similar to those of the main analysis. Virological suppression was achieved by 83.3%: (95% CI: 73.3-99.9%) of 30 patients in the resistant group and 82.7% (95% CI: 78.2-88.0%) of 225 patients in the susceptible group (p=0.928). After adjustment for the same potentially confounding variables as in the main analysis, there was again no significant difference in the odds of virological suppression in the resistant group compared to the susceptible group (OR: 0.71; 95% CI: 0.22-2.35; p=0.580).

Table 5.5: Odds ratios and 95% confidence intervals for virological response (less than 500 copies/mL) to cART according to whether patients had HIV with full or intermediate resistance to at least one drug started ('resistant') or HIV susceptible to all drugs started ('susceptible').

	Resistant		Susceptible		Resistant versus susceptible			
	n	% VL respond	n	% VL respond	Univariable OR (95% CI)	*p*	Multivariable OR (95% CI)	*p*
Main analysis:								
Missing = failure*	34	73.5%	243	76.5%	0.85 (0.38-1.93)	0.700	0.68 (0.27-1.71)	0.408
Sensitivity analyses:								
Missing = excluded*	30	83.3%	225	82.7%	1.05 (0.38-2.91)	0.928	0.71 (0.22-2.35)	0.580
Excluding retrospective treatment data (missing = failure)*	31	74.2%	220	75.9%	0.91 (0.39-2.16)	0.835	0.83 (0.32-2.19)	0.712
Resistance interpreted by Rega Version 7.1 (missing = failure)	12	66.7%	265	76.6%	0.61 (0.18-2.10)	0.433	0.72 (0.18-2.86)	0.645
Resistance defined by Shafer *et al*. (missing = failure)	24	70.8%	253	76.7%	0.74 (0.29-1.87)	0.522	0.65 (0.23-1.82)	0.415
Endpoint viral load <50 copies/mL (missing = excluded)*	25	68.0%	206	61.2%	1.35 (0.56-3.27)	0.508	1.55 (0.52-4.62)	0.430

Notes: *Resistance interpreted by the Stanford algorithm Version 4.3.4.
Multivariable analyses adjusted for gender, EuroSIDA region, date started cART, type of cART regimen, baseline CD4 count and viral load, and time from most recent resistance test.
VL respond = virological responders; OR = odds ratio; CI = confidence interval.

5.3.5.3 Further sensitivity analyses

As shown in Table 5.5, the results from the four other sensitivity analyses carried out gave mostly similar findings to the main analysis. Analysing the alternative endpoint of a viral load of less than 50 copies/mL instead of 500 copies/mL found a result in the opposite direction to the other analyses, in that patients in the resistant group had increased odds of a successful virological response (multivariable OR: 1.55; 95% CI: 0.52-4.62; p=0.430). However this was also not statistically significant.

5.3.6 Immunological response to cART: at least 100 CD4 cells/mm^3 increase

5.3.6.1 Main analysis: missing CD4 count = failure

Immunological response was analysed in 299 (97.1%) patients with baseline CD4 counts, of which 36 (12.0%) had HIV with full or intermediate resistance to at least one drug started. Of these 299 patients, 22 (7.4%) had no CD4 count measured six to twelve months after starting cART and so were counted as immunological failures in the main analysis. In total, 130 (43.5%) patients had an immunological response (of at least 100 cells/mm^3 from baseline) at the first measurement six to twelve months after starting cART. The response rate of patients in the resistant group was significantly

higher than patients in the susceptible group: 25 (69.4%; 95% CI: 57.1-87.2%) of 36 patients compared to 119 (45.3%; 95% CI: 39.6-51.6%) of 263 patients (p=0.006).

In a univariable logistic regression model, it was found that patients in the resistant group were over two and a half times more likely to have an immunological response compared to those in the susceptible group, which was significant (OR: 2.75; 95% CI: 1.30-5.82; p=0.008). After adjustment for baseline CD4 count and viral load, time from HIV positive diagnosis, type of cART regimen and time from most recent resistance test, the results were similar (multivariable OR: 2.56; 95% CI: 1.11-5.86; p=0.027). Table 5.6 displays these results, as well as those from sensitivity analyses. No interaction was found between type of cART regimen and resistance (p=0.391), although as in the virological response analysis, the power to detect a true difference was very limited.

Table 5.6: Odds ratios and 95% confidence intervals for immunological (at least 100 CD4 cells/mm^3 increase) response to cART according to whether patients had HIV with full or intermediate resistance to at least one drug started ('resistant') or HIV susceptible to all drugs started ('susceptible').

	Resistant		Susceptible		Resistant versus susceptible			
	n	% CD4 respond	n	% CD4 respond	Univariable OR (95% CI)	*p*	Multivariable OR (95% CI)	*p*
Main analysis:								
Missing = failure*	36	69.4%	263	45.3%	2.75 (1.30-5.82)	0.008	2.56 (1.11-5.86)	0.027
Sensitivity analyses:								
Missing = excluded*	34	73.5%	243	49.0%	2.89 (1.30-6.46)	0.009	2.75 (1.16-6.51)	0.021
Excluding retrospective treatment data (missing = failure)*	33	69.7%	238	44.1%	2.91 (1.33-6.39)	0.008	3.06 (1.23-7.60)	0.016
Resistance interpreted by Rega Version 7.1 (missing = failure)	14	78.6%	285	46.7%	4.19 (1.15-15.34)	0.031	6.25 (1.50-25.99)	0.012
Resistance defined by Shafer *et al*. (missing = failure)	28	67.9%	271	46.1%	2.47 (1.08-5.64)	0.033	3.25 (1.31-8.02)	0.011
Endpoint 50% CD4 cells/mm^3 increase*	36	55.6%	263	41.8%	1.74 (0.86-3.51)	0.122	1.65 (0.73-3.73)	0.231

Notes: *Resistance interpreted by the Stanford algorithm Version 4.3.4.
Multivariable analyses adjusted for type of cART regimen, baseline CD4 count and viral load, time from HIV positive diagnosis and time from most recent resistance test.
CD4 respond = immunological responders; OR = odds ratio; CI = confidence interval.

To see if the results could be explained by the change in viral load after starting cART, the model was adjusted for this in patients with viral load measurements available at baseline and at six to twelve months. This analysis included 262 patients, of which 31 (11.8%) were in the resistant group. After adjustment for the same variables as in the main analysis as well as change in viral load, similar results were obtained (OR: 3.09;

95% CI: 1.20-7.92; *p*=0.019). To take account of variability in CD4 counts, average CD4 cell measurements at baseline (average of most recent two measurements within a year before starting cART) and during months six to twelve (average of first two measurements within this time window) were analysed. The odds of an immunological response were more similar between the resistant and susceptible groups in this analysis than in the main analysis and the multivariable OR was not significant (OR: 1.61; 95% CI: 0.74-3.53; *p*=0.232).

5.3.6.2 Sensitivity analysis: missing = excluded

Excluding patients with missing CD4 counts at six to twelve months resulted in very similar findings to those of the main analysis. An immunological response was achieved by 52.0% of 243 patients: 73.5% (95% CI: 61.6-91.2%) of 34 patients in the resistant group and 49.0% (95% CI: 43.1-55.7%) of 243 patients in the susceptible group (*p*=0.007). As in the main analysis, the odds of an immunological response in the resistant group were over two and a half times that of patients in the susceptible group (multivariable OR: 2.75; 95% CI: 1.16-6.51; *p*=0.021).

5.3.6.3 Further sensitivity analyses

The results from the other sensitivity analyses are shown in Table 5.6. The analyses excluding patients with retrospective treatment data and comparing patients with and without TDR mutations (defined by Shafer *et al.*) gave consistent results to the main analysis. Use of the Rega algorithm to interpret resistance showed the odds of an immunological response in the resistant group to be six times that of the odds in the susceptible group (multivariable OR: 6.25; 95% CI: 1.50-25.99; *p*=0.012), but with a very large confidence interval. Only 14 patients were included in the resistant group for this analysis. The alternative endpoint of a 50% or more CD4 count increase from baseline also showed higher odds of an immunological response in the resistant group but the effect was less pronounced and non-significant (multivariable OR: 1.65; 95% CI: 0.73-3.73; *p*=0.231).

5.4 Discussion

The analyses in this chapter found that the overall prevalence of TDR in chronically infected, ART-naïve patients in EuroSIDA was 11.4%. The prevalence of TDR was found to be fairly stable over time, after an initially high level in 1994-1995, and no significant differences were found in the odds of TDR between two-year time periods in a multivariable model adjusting for potentially confounding factors. Furthermore, no significant differences were found in the odds of TDR between patients infected with HIV-1 B and non-B subtypes or between Central West and East geographical regions

compared to the North region within EuroSIDA. However there were significant higher odds of TDR in the South region compared to the North.

No significant difference could be demonstrated in the odds of having a successful virological response to first-line cART in patients infected with HIV with full/intermediate resistance to at least one drug started compared to those with HIV susceptible to all drugs started, after adjustment for potentially confounding factors, although the response rate was slightly lower in the resistant group. The number of patients with resistant HIV was small so the power of the analyses to detect true differences was limited. An unexpected significantly higher immunological response rate was observed in the resistant group.

5.4.1 Interpretation of findings and previous research

5.4.1.1 Factors affecting availability of resistance test results

Amongst the 14,310 EuroSIDA patients, 3.7% had plasma samples available whilst ART-naïve that were subsequently tested retrospectively for genotypic resistance. As detailed in Chapter 2 (section 2.1.3), EuroSIDA requests that plasma samples are collected from patients every six months and shipped intermittently to the coordinating centre. Samples were then selected according to patient inclusion criteria from a number of EuroSIDA projects, including this project, and genotypic resistance testing was carried out retrospectively on viral RNA. Suspicion of having resistance was not one of the criteria and therefore these patients should not be more likely to have resistant HIV than patients whose samples were not analysed. The factors associated with having resistance test results available from a plasma sample taken whilst ART-naïve were investigated to examine how representative this subset of EuroSIDA patients was.

IDU compared to MSM, visiting a clinical centre in the East region as opposed to the North, higher or missing CD4 counts at date of enrolment compared to a CD4 count of less than 200 cells/mm^3 and a viral load at date of enrolment of more than 10,000 copies/mL compared to less than or equal to 10,000 copies/mL, were positively associated with having resistance test results available. Visiting a clinical centre in the South or Central West regions, as opposed to the North, having a prior AIDS diagnosis and positive or unknown hepatitis C status compared to negative status, were less likely to have test results available. This is likely to reflect the inclusion criteria for the EuroSIDA projects that determined which samples were selected for analysis. One particular project was 'HIV-1 subtypes and response to cART' (Chapter 4) where samples were required that were collected prior to starting cART from those suspected

of being infected with a non-B HIV-1 subtype, i.e. those of non-white ethnicity, originating from non-European countries or living in East Europe, in order to gain power for more detailed comparisons in this study. Samples were also prioritised where the patient's viral load was more than 10,000 copies/mL to increase the likelihood of the test being successful.

These results could also partly reflect the characteristics of patients who visit clinics more frequently and hence have more chance of a plasma sample being taken. For example, unknown hepatitis C status was negatively associated with having test results available; if patients visit the clinic less frequently they are less likely to be tested for hepatitis C.

5.4.1.2 Prevalence of transmitted drug resistance

Amongst 525 patients, the prevalence of TDR was found to be 11.4%. NRTI resistance was found in 9.3% of patients, NNRTI resistance in 1.0% and PI resistance in 3.0%. This overall prevalence of TDR was found to be in line with the majority of studies investigating TDR across Europe [348,435,436,439-444].

Tables 5.7 summarises the prevalence of TDR found in this analysis compared to other studies in ART-naïve patients across Europe. Most of the studies used IAS-USA figures of drug resistance mutations to define TDR, although some modified the list to exclude minor protease mutations and polymorphisms that may occur naturally. The CATCH study, similarly to EuroSIDA, analysed sequences from recently and chronically infected patients across Europe [436]. A total of 2208 sequences were analysed from patients in 19 different countries in a consistent way and an overall prevalence of 10.4% was found. NRTI resistance was found in 7.6%, NNRTI resistance in 2.9% and major PI resistance in 2.5%.

Table 5.7: Prevalence of transmitted drug resistance in ART-naïve patients across Europe as reported in this analysis compared to published research (listed in descending order of number of patients included).

Country	Recruitment period	No. of patients	Selected patients	TDR mutations (%) Any	NRTI	NNRTI	PI	Name of study	Authors, year
Europe	**1994-2005**	**525**	**Recently and chronically infected**	**11.4**	**9.3**	**1.0**	**3.0**	**EuroSIDA**	**-**
Europe	1996-2002	2208	Recently and chronically infected	10.4	7.6	2.9	2.5	CATCH	Wensing *et al*. 2005 [436]
Italy	1997 onwards	415	Chronically infected	10.1	-	-	-	I.Co.N.A.	De Luca *et al*. 2004 [348]
Italy	Up to 2000	347	Chronically infected	-	7.8	4.9	1.4	I.Co.N.A.	Perno *et al.* 2002 [453]
France	2001	363	Chronically infected	12.0	4.3	0.8	6.1	Odyssee	Descamps *et al*. 2005 [443]
France	2001-2002	303	With acute infection	14.0	10.3	3.3	4.3	PRIMO	
UK	2004-2006	239	Newly diagnosed	7.1	4.2	1.7	1.7	-	Booth *et al.* 2006 [446]
Belgium	1995	45	Newly diagnosed	26.7	15.6	17.6	4.4	-	Van Vaerenbergh *et al*. 2001 [454]
	1997	75		26.7	14.7	13.3	8.0		
	1998	111		31.5	10.8	16.5	9.9		
Switzerland	1999-2001	220	Recently infected	10.5	8.6	2.3	0.9	Swiss HIV Cohort Study	Yerly *et al*. 2004 [435]
France	1996-1997	204	With acute infection	8.8	7.8		3.9	ANRS 053/ PRIMO	Harzic *et al.* 2001 [444]
Germany	2000-2001	184	Chronically infected	14.0	10.5	2.8	2.1	-	Oette *et al*. 2004 [440]
Spain	2004	182	Newly diagnosed	-	2.2	1.1	0.5	-	Martinez-Picado 2005 [455]
UK	2000-2004	140	With acute infection	6.0	-	-	-	-	UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance 2001 [394]
Luxembourg	1992-1997	135		11.9	-	-	-	-	Fontaine *et al*. 1998 [442]
Poland	2000-2001	128	Chronically infected	51.5	51.5	-	-	-	Horban *et al*. 2002 [438]
Greece	2002-2003	101	Newly diagnosed	8.9	5.0	4.0	0	Hellenic Multi-centre study on HIV-resistance	Paraskevis *et al*. 2005 [439]
Denmark	2000	97	Newly diagnosed	2.1	2.1	0	0	-	Jorgensen *et al*. 2003 [437]
Belgium	2000	83	Recently infected	7.2	6.3	3.8	1.2	-	Derdelinckx *et al*. 2004 [452]

Notes: - denotes unknown information.
TDR = transmitted drug resistance.

Country	Recruitment period	No. of patients	Selected patients	TDR mutations (%)				Name of study	Authors, year
				Any	NRTI	NNRTI	PI		
Switzerland	1996-1998	82	With acute infection	11.0	-	-	-	-	Yerly *et al*. 1999 [352]
Slovenia	2000-2004	77	Newly diagnosed	3.9	3.9	0	0	-	Babic *et al*. 2006 [456]
UK	1994-2000	69	Recently infected	14.5	-	-	-	-	Fox *et al.* 2006 [445]
Germany	1996-1999	64	Recent seroconverters	-	9.4		4.7	German Seroconverter Study	Duwe *et al*. 2001 [457]
France	1995	12	With acute infection	-	25		0	-	Tamalet *et al*. 2000 [458]
	1996	14		-	21.5		0		
	1997	18		-	5.6		11.1		
	1998	4		-	0		0		
Italy	-	38	With acute infection	5.3	2.6	0	2.6	-	Re *et al*. 2004 [459]
	-	24	Chronically infected	8.3	8.3	0	0		
Italy	1998-2003	61	Acute and chronically infected	16.4	-	-	0	-	Torti *et al*. 2004 [460]
Spain	1997-1999	31	Acute and recent seroconverters	25.8	-	-	-	-	De Mendoza *et al*. 2002 [461]
	2000-2001	26		3.8	-	-	-		

Notes: - denotes unknown information.
TDR = transmitted drug resistance.

Other research found estimates ranging from as little as 2.1% to 51.5% [437,438]. This wide range could be due to many factors including differences in geographical location, study period, study population, study design, sampling methods, definitions of resistance, timing of the sampling and sequencing methods [337,462]. For example, the 51.5% prevalence of TDR was observed in Poland by Horban *et al.* (2002) over the period 2000-2001 [438]. Patients were all recruited in the Warsaw area and were predominantly IDUs who had a history of needle-sharing. This may have spread the same drug-resistant HIV strain across this population. The high frequency of zidovudine (ZDV) resistance mutations may be attributed to the widespread use of ZDV monotherapy in the 1990s, encouraging the selection of ZDV-resistant strains in the HIV infected population, which could then be transmitted. In addition, the study used a high sensitivity genotypic assay that may have picked up on minor resistant strains.

In this analysis, the majority of the study population (at least 81.9%) were chronically infected patients with similar findings to those found in a number of studies that also looked at chronic infections [348,436,440,443,460]. For example, in the I.Co.N.A. study, an overall prevalence of 10.1% TDR was observed in 415 patients enrolled from 1997 onwards in Italy, using the 2003 IAS-USA figures of drug resistance mutations to define TDR [348]. In 363 patients enrolled in the French Odyssee study in 2000, TDR prevalence was found to be 12.0%, also using the 2003 IAS-USA list of mutations [443]. Prevalence of TDR may be lower in chronically infected patients compared to newly infected as the resistant virus may revert back to wild-type due to its reduced fitness [433].

5.4.1.3 Factors associated with detection of transmitted drug resistance

The prevalence of TDR remained fairly stable over the years 1996-2004 in this analysis, after an initially high prevalence in 1994-1995. In a multivariable analysis adjusting for HIV-1 subtype, geographical region, hepatitis C status and viral load at the date of plasma sample analysed, there appeared to be no significant differences in any two-year period compared to 1996-1997. Other research has reported conflicting trends over time. Increases in TDR over calendar time up to the year 2003 have been observed in a number of studies [388-393,447]. In a Californian study by Grant *et al.* (2002), NNRTI resistance rose from 0% in 1996-1997 to 13.2% in 2000-2001, and PI resistance from 2.5% to 7.7% in these same periods [392]. No increase was observed in NRTI resistance. Cane *et al.* (2005) reported an increasing prevalence of resistance in the UK, over the years 1996-2003 in both high and medium resistance as defined by the Stanford algorithm [389]. In 1996-1997, the prevalence of high resistance to any drug was 11.0%, compared to 19.2% in 2002-2003. This trend could be explained by

an increase in resistant strains in the HIV infected population following the increasing availability of ART.

However, a study carried out in Madrid showed a decrease from 25.8% in 1997-1999 to 3.8% in 2000-2001 in a sample of 57 acute or recent HIV seroconverters [461]. A decrease in TDR was also observed in the UK in 2004, which may be due to an increased use of successful initial regimens that achieve virological suppression. This decreases the likelihood of resistant strains developing and therefore the chance of them being transmitted [394]. Furthermore, Yerly *et al.* (2004) found evidence that drug-resistant HIV, especially multidrug-resistant and HIV with the M184IV mutation, has a substantially reduced transmission capacity compared to wild-type [435]. Therefore effective treatment in chronically infected patients may be reducing the transmission of resistant HIV in recently infected patients.

A slightly higher prevalence of TDR was observed in patients infected with HIV-1 subtype B compared to non-B subtypes in this analysis (12.5% versus 6.5%; p=0.096) but this difference was not statistically significant. In the multivariable analysis, no significant difference was found in the odds of detection of TDR between A and B subtypes and a borderline significantly lower odds was found in patients infected with a subtype other than A or B, compared to B (p=0.066). Due to the small number of non-B subtypes included in the analyses (n=93), there may not have been enough power to detect a difference between HIV-1 subtypes, if it truly existed. Jayaraman *et al*. (2006) also found a non-significant higher prevalence of TDR in HIV-1 subtype B compared to non-B (8.1% versus 3.0%; p=0.31) in 715 individuals [447]. Amongst 77 individuals in a Slovenian study by Babic *et al.* (2005), only 3 (4.8%) of 61 patients infected with HIV-1 subtype B had TDR compared to none of 12 patients infected with a non-B subtype [456]. The CATCH study, which had a larger study population found a significantly higher prevalence of TDR in 1431 patients infected with HIV-1 subtype B compared to 673 infected with a non-B virus (12.9% versus 4.8%; p<0.001) and this difference remained in a multivariable analysis [436].

A significant difference was found in the odds of detecting TDR in patients from the South region compared to the North region after adjustment for calendar time, HIV-1 subtype, hepatitis C status and viral load at the date of the plasma sample analysed. Epidemiological features such as drug exposure, adherence and treatment response at the population level may be responsible for this. It is impossible to compare the findings from other research across regions of Europe because of the many differences in study periods, designs, sampling and sequencing methods, and definitions of

resistance, as already mentioned. However, the CATCH study collected data from countries all across Europe in a consistent way and showed some variation in the rate of TDR from 0% (0 out of 8 patients) in Finland to 23% (14 out of 62 patients) in Germany [436].

5.4.1.4 Virological response to cART

The results of the analyses investigating initial virological response to cART (a viral load less than 500 copies/mL at the first measurement six to twelve months after initiation of cART) were unable to show a significant difference in the odds of a virological response between patients with HIV-1 with full or intermediate resistance to at least one drug started compared to those fully susceptible, after adjustment for potentially confounding factors. Due to the relatively small number of patients in the resistant group, the power of this analysis to detect a true difference was restricted and so more data are needed to draw a firm conclusion. Although the finding was not significant, an inferior virological response to cART in patients with resistant HIV was observed, which supported results found in two US studies studies investigating time to virological suppression after initiation of therapy in recently infected patients [392,393]. Three European studies also evaluated virological response in both chronically infected patients and patients with primary HIV infection and found that predicted virus susceptibility using genotypic sensitivity score interpretation systems was significantly associated with time to virological success [348,448,452]. A further European study could not find any difference in mean change in viral load or CD4 count at months six and twelve after starting therapy between patients with TDR and those without [458].

In the sensitivity analysis investigating the alternative virological endpoint of a viral load less than 50 copies/mL instead of 500 copies/mL, the odds of a virological response were higher in the resistant group than in the susceptible group. This result was also not significant and similarly to the results from the main analysis, could be due to chance.

5.4.1.5 Immunological response to cART

Investigating initial immunological response to cART (a 100 CD4 cells/mm^3 increase or more from baseline at the first measurement six to twelve months after initiation of cART) showed that after adjustment for potentially confounding factors, there were significantly increased odds of an immunological response in patients with HIV-1 with full or intermediate resistance to at least one drug started compared to those fully susceptible. This remained after adjustment for change in viral load from baseline. However it was less pronounced (and non-significant) in a sensitivity analysis looking

at the alternative endpoint of a CD4 count increase of 50% or more from baseline. Fewer patients with resistant HIV achieved a 50% increase compared to a 100 cells/mm^3 increase, which narrowed the difference between resistant and susceptible groups. Therefore, although the proportion of immunological responders in the susceptible group was smaller, the CD4 increases experienced in this group were larger and mostly over 100 cells/mm^3.

Studies in the UK found that patients with TDR had higher rates of CD4 cell decline before starting ART [445,463], however the immunological response following initiation of cART in these patients is unknown. There is some evidence that patients who develop NNRTI and PI resistance on first-line therapy and experience virological failure will have better CD4 cell increases than non-responders without mutations. This may be explained by a reduced viral fitness in resistant strains, which reduces immunological deterioration [464]. An analysis of nearly 2000 patients on ART found little evidence of differences in CD4 slope for a given viral load greater than 500 copies/mL according to the presence of resistance, with the exception of certain NNRTI mutations that were found to be associated with greater CD4 count declines, but with large confidence intervals around the estimates [465].

The observed superior immunological response in patients with resistant HIV in this analysis may be due to chance as there were few patients in this group. It may also be explained by the natural variability in CD4 counts. To try to take this into account, average CD4 cell measurements at baseline and in months six to twelve were analysed, which still resulted in higher odds of immunological response in the resistant group compared to the susceptible group but the difference was smaller and not statistically significant. Not all patients had CD4 counts measured frequently enough to be able to calculate average measurements, therefore variability could explain the results. All known, measured factors found to be potentially confounding the response were adjusted for in multivariable analyses. However, as the patients were from an observational study, unmeasured or unknown confounding variables may have biased the findings [209].

5.4.2 Limitations of analysis

The main limitation of the analyses in this chapter was the limited statistical power due to only a small number of patients having TDR. This made it difficult to detect true differences in response to therapy and more data are required to make conclusions with any certainty.

A further limitation of this analysis was that the average time from HIV positive diagnosis to earliest resistance test was more than three years. Although EuroSIDA aims to collect all prior treatment data, when a patient is enrolled, it cannot be ruled out that errors with dates may have occurred, which could potentially mean undisclosed drug exposure. To try and ensure only truly ART-naïve patients were included, plasma samples were required to be dated at least a month before starting ART. If the patient had more than one plasma sample available before starting therapy that had been analysed for genotypic resistance, results were cumulated to obtain the best estimate of the extent of mutated virus populations present in the individual [466]. As routine assays can only detect mutations in the dominant virus, this gives a more conservative estimate of prevalence as mutations found in later tests are likely to have been present at transmission but were just not detected.

The centres involved in EuroSIDA tend to be highly specialised and consequently may have more clinical experience with HIV and earlier access to new treatments than centres not included, therefore the cohort may not be completely representative of European clinics in general. However as patients are enrolled consecutively, this should capture a broad representation of patients regularly seen and because of the large numbers of clinics in the study, this may be more representative than any one clinic cohort.

5.4.3 Implications of findings

The observed finding that virological response rate was not significantly different according to whether or not patients were infected with resistant HIV implies that resistance testing in ART-naïve patients may not be necessary. However as already mentioned, the power to detect true differences was limited and these results should be treated with caution. Although a significantly higher immunological response rate was observed in patients with resistant HIV, which is the opposite to what was expected, i.e. that drug resistance would result in an inferior response to cART, this result may be explained by variability in CD4 counts or unmeasured confounders.

5.4.4 Further research

These analyses would benefit from a larger number of patients to achieve the statistical power to detect true differences. As the EuroSIDA genotypic resistance database continues to expand, more patients may be available who meet the inclusion criteria. However, as resistance testing in ART-naïve patients becomes more widespread, more patients will receive treatment guided by test results and therefore the number of patients starting treatment with resistance to one or more drugs in their regimen may

not increase greatly. As mentioned in Chapter 4 (section 4.4.4), collaborations of studies can be ideal for expanding datasets in order to be able to address certain research questions. The ongoing collaboration known as EuroCOORD (The European Coordinating Committee for the Integration of Ongoing Coordination Actions Related to Clinical, Virological and Epidemiological HIV Research), integrating five projects including EuroSIDA, may provide a suitable study population for this and is planning to investigate TDR. The disadvantages of collaboration studies are the variability across projects in factors such as the type of data collected, the frequency of data updates and the quality of the data collected.

If a larger dataset was available, another aspect that could be investigated further is to establish whether or not there is an interaction between TDR and cART regimen started. Due to lack of power, it was not possible to look at this in detail in this analysis. A significant interaction would imply that patients with TDR might do better on certain regimens than on others. An increased number of patients could provide the power to stratify analyses by type of TDR, i.e. NRTI, NNRTI or PI-associated or type of cART regimen.

5.4.5 Conclusions

The patients included in this study were selected based on availability of plasma samples whilst ART-naïve and were not selected based on suspicion of drug resistance. The choice of samples analysed for genotypic resistance reflect the inclusion criteria for various studies in EuroSIDA and so may not be representative of the EuroSIDA cohort as a whole. The prevalence of TDR was found to be in line with many other studies from across Europe and there was no evidence of significant differences in prevalence over calendar time or between HIV-1 subtypes after adjustment for potentially confounding variables. There was some evidence of significantly higher odds of TDR in the South geographical region compared to the North, which may be due to drug exposure and adherence levels at the population level.

There was no evidence of a significant difference in the odds of achieving a successful virological response to first-line cART in patients infected with HIV with full/intermediate resistance to at least one drug started compared to those with HIV susceptible to all drugs started, after adjustment for potentially confounding factors, although the response rate was slightly lower in the resistant group. A significantly higher immunological response rate was observed in the resistant group, which may be explained by a reduced viral fitness in drug-resistant strains decreasing CD4 cell

depletion but could be a result of the natural variability in the data. The number of patients with resistant HIV was small so the power of the analyses to detect true differences was limited. This has identified the need for a larger dataset to be able to draw conclusions with more certainty, which could arise from a collaboration of projects and the pooling of resources.

A manuscript of this analysis was published by the Journal of Acquired Immune Deficiency Syndrome in July 2008 and can be found in Appendix VIII.

Chapter 6. Comparison of genotypic resistance profiles and virological response between patients starting nevirapine and efavirenz

6.1 Introduction

As detailed in Chapter 1 (section 1.7.4), cART regimens containing two NRTIs and either an NNRTI or a ritonavir (RTV)-boosted PI are currently recommended by the IAS-USA Panel and the Department of Health and Human Services (DHHS) Panel as first-line regimens due to their frequently observed efficacy and convenience [40,41]. Regimens containing the NNRTI, efavirenz (EFV), have become the standard-of-care comparator in clinical trials and have consistently proven to be an optimal choice for achieving viral suppression [185,290,291,467]. The other main NNRTI currently in use is nevirapine (NVP), which is recommended for pregnant women and can be used as an alternative for men and women with low pre-treatment CD4 counts and for patients who cannot tolerate the central nervous system toxicity of EFV [40,41]. For patients with high pre-treatment CD4 counts, there is evidence of an increased risk of serious symptomatic hepatic events associated with NVP use (in men with CD4 counts more than 400 cells/mm^3 and in women with CD4 counts more than 250 cells/mm^3) therefore it should only be used for these patients if the benefits outweigh the risks [468,469].

6.1.1 Use of NNRTIs in resource-limited settings

To assist in the scale-up of ART in resource-limited settings, in 2002, the WHO published treatment guidelines to help develop standardised ART programmes to reach as many people in need of therapy as possible [470]. They recommended that developing countries should select a first-line regimen consisting of two NRTIs plus either an NNRTI, a PI or abacavir (ABC). Due to the cost and risk of hypersensitivity reaction, triple NRTI regimens containing ABC were almost never selected. Cost was also the main reason for PI-containing regimens being kept as secondary options [471]. NNRTIs proved to be a popular selection due to their low cost, availability of fixed-dose combinations, lack of requirement for a cold chain (storage and transportation of the drug at the correct temperature to maintain potency) and drug availability.

In 2006, the WHO carried out a survey in resource-limited countries to investigate the uptake of ART and predict future demand. Across 23 countries that returned the questionnaires (representing 53% of relevant patients in developing countries), the majority of patients receiving first-line regimens started an NVP-based regimen; 61% received stavudine (d4T) plus lamivudine (3TC) plus NVP and 16% received

zidovudine (ZDV) plus 3TC plus NVP [472]. A further 17% received an EFV-based regimen; 9% received ZDV plus 3TC plus EFV and 8% received d4T plus 3TC plus EFV. Even though the price of EFV has dropped substantially in the past two years, NVP remains the much cheaper option, which explains the widespread uptake of this drug in ART programmes in many developing countries [473].

6.1.2 Nevirapine versus efavirenz

A number of studies have compared the efficacy of these two NNRTIs. Virological outcomes of NNRTI-based regimens containing either NVP or EFV were compared in patients mostly experienced in NRTIs and PIs in a previous EuroSIDA analysis by Phillips *et al.* (2001) [300]. This analysis showed that patients starting NVP were twice as likely to experience virological failure than patients starting EFV (relative hazards (RH): 0.49; 95% CI: 0.33-0.74; p<0.001), after adjustment for baseline characteristics including CD4 count and viral load, previous ART and number of drugs in the regimen. The finding was in line with results from a number of other observational studies [301-304]. For example, the I.Co.N.A. study showed the RH of virological failure to be 2.08 (95% CI: 1.37-3.15; p<0.001) for NVP compared to EFV [303]. Also, a recent analysis presented at CROI 2007 (14th Conference on Retroviruses and Opportunistic Infections) showed that South African patients starting a NVP-based regimen were 43% more likely to experience virological failure than those starting an EFV-based one [301].

Although this may have reflected differences in the effectiveness of the two drugs in this setting, as the patients were not randomised to starting either drug there may have been unmeasured confounding variables that biased the results. The 2NN large-scale randomised clinical trial making a similar comparison in ART-naïve patients did not find a significant difference (at the 5% level) between NVP and EFV, but found slightly less treatment failure in those starting EFV compared to those starting NVP (38%; 95% CI: 33-43%; and 44%; 95% CI: 39-49% respectively; p=0.091) [299]. However, the study was powered to investigate equivalence of the two drugs in treatment failure (assumed if the 95% CI of the difference was within the 10% of zero) and the results did not demonstrate this.

A number of other studies also did not find a significant difference between the outcomes of patients starting the two NNRTIs, including the NNRTI substudy of the FIRST-CPCRA 058 trial, a randomised open-label pilot study in Spain and observational, non-randomised cohort studies in Italy, India and Thailand [474-478]. Although randomised controlled trials have not found a significant difference in

virological outcome between patients starting the two drugs, the reasons for the inferior outcome in patients starting NVP compared to EFV found in observational studies remain unclear.

6.1.3 NNRTI resistance

In contrast to the patients included in 2NN, most of the patients in the previous EuroSIDA analysis had already virologically failed other antiretroviral drugs, raising the possibility that the differences in outcome could be explained by differences in HIV ART resistance to other drugs used as part of the NNRTI-containing regimen. The subsequent development of the EuroSIDA genotypic resistance database has allowed a re-investigation of this question to assess whether potential differences in prevalence of resistance to both the NNRTI component of the regimen and the NRTI backbone in the two groups could be a source of bias. This chapter covers the findings from this analysis.

Drug resistance is associated with virological failure in patients undergoing treatment [16,317,318]. NNRTIs have a low genetic barrier for resistance and only one HIV RNA point mutation is needed for high-level NNRTI resistance to develop [40,479]. A recent study by Harrigan *et al.* (2007) showed that certain mutations might cause intermediate phenotypic resistance: A98G and V179D for NVP resistance, and Y181C and V179D for EFV resistance [480]. There are also studies that have demonstrated a role for mutations in another region of HIV RNA, the connection domain (residues 316-427), in conferring resistance to NNRTIs [481,482].

Conversely, there is evidence that some HIV RNA mutations in the RT gene may encourage increased susceptibility (hypersusceptibility) to NNRTIs [481,482]. For example, the M41L, M184V, L210W and T215Y mutations were found to be associated with a better virological outcome in patients treated with EFV-containing regimens [483].

6.1.4 Motivation and aims for chapter

The question of whether the two NNRTIs are equally effective at achieving virological suppression is very important as although EFV is proven to be a reliable option and is recommended to be used in first-line therapy, there are circumstances where NVP is preferred, e.g. if the patient is pregnant or has a low CD4 count. NVP is also extensively used in the developing world due to its convenience and low cost. The main aim of this chapter was therefore to re-investigate virological outcomes in patients starting NVP- and EFV-containing regimens for the first time in EuroSIDA. Using the

new information collected in the genotypic resistance database, it was possible to adjust the analyses for ART resistance present at initiation of treatment to see if this could explain the previous finding of an inferior response in patients starting NVP. A further aim was to look at the prevalence of ART resistance at time of virological failure and compare resistance profiles between the two groups.

6.2 Methods

6.2.1 Inclusion criteria

The EuroSIDA dataset used for the analyses in this chapter was the update completed in July 2007, including data on 14,282 patients with follow-up to (median date of last visit) July 2006. Inclusion criteria for the current analyses were as in the previous EuroSIDA analysis [300]. All patients were required to be under follow-up at or after July 1997, the date that EFV was first used in patients in the study. They were required to have started a regimen containing either NVP or EFV (not both) after July 1997, with no previous NNRTI experience and with a viral load and CD4 count measured within six months before starting the regimen. They were also required to have at least two viral load measurements after the date of starting the NNRTI.

Finally, to investigate the effect of ART resistance present at the time of treatment initiation, patients needed to have genotypic resistance test results available from a test performed on a blood plasma sample taken within one year before starting the NNRTI regimen. The majority of these tests were performed retrospectively using sequence analysis of viral RNA extracted from the plasma samples that were stored in the central EuroSIDA laboratories. Some resistance test results were obtained from tests carried out at the clinical sites. The methods by which this information is collected in EuroSIDA were previously detailed in Chapter 2 (section 2.1.3).

6.2.2 Statistical methods

6.2.2.1 Definitions

Baseline was defined as the date of starting the NNRTI-containing regimen. Genotypic resistance profiles were described at baseline (most recent resistance test results within a year before treatment initiation) and at time of virological failure (most recent test results at least two weeks after treatment initiation and up to one year after time of virological failure as defined below). At time of failure, resistance profiles were also determined in a subset of patients who were still receiving an NNRTI at the time of the test. In addition, as some patients had more than one set of genotypic resistance test

results available, resistance profiles were examined where all previous test results were cumulated.

Genotypic resistance was interpreted using both the IAS-USA 2006 figures of HIV-1 drug resistance mutations [484] with resistance defined as at least one NRTI, NNRTI or major PI mutation, and using three algorithms that produce genotypic sensitivity scores to measure sensitivity to the drugs in the regimen: Rega Version 7.1 [349], ANRS July 2006 [350] and Stanford University (HIVdb) Version 4.2.9 [351].

6.2.2.2 Time to virological failure following initiation of NNRTI regimen

Time to virological failure following initiation of an NNRTI-containing regimen was investigated using Kaplan-Meier methodology and Cox proportional hazards models, with virological failure defined as two consecutive viral loads more than 500 copies/mL after starting the regimen. If the baseline viral load was more than 500 copies/mL, these values were required to have been measured at least six months after initiation. Time of virological failure was defined as the first of these two measurements. For patients who did not experience virological failure, the follow-up time was right censored at the time of the penultimate available viral load measurement.

A multivariable model was developed to compare patients starting NVP and those starting EFV, stratified by the clinical centre in which patients were seen and adjusting for factors that could potentially affect the outcome. These were chosen *a priori* in the research paper by Phillips *et al*. (2001) [300] based on experience from previous analyses. Additional potential confounders were investigated, including demographics and use of specific drugs, as was stratification by the prior number of drugs used, geographical region (defined in Chapter 2, section 2.1.5) and calendar year of starting the regimen. Furthermore, the effect of baseline genotypic drug resistance was analysed.

6.2.2.3 Sensitivity analyses

Sensitivity analyses were carried out using left censoring at the time of enrolment into EuroSIDA for those who were enrolled after starting the NNRTI-containing regimen and using right censoring at time of discontinuation of the NNRTI for those who discontinued. Analyses were also repeated in a subset of patients who were ART-naïve at baseline and in a subset of only the patients who had genotypic resistance tests carried out on stored plasma samples in the central EuroSIDA laboratories as opposed to at the clinical sites.

Multivariable linear regression models were also used to analyse changes in viral load and CD4 count between baseline and the last measurement six to twelve months following treatment initiation. Patients with no measurements during this time were excluded and for undetectable viral loads, the value taken was the level of detection minus one (e.g. for undetectable viral load less than 50 copies/mL, viral load was taken to be 49).

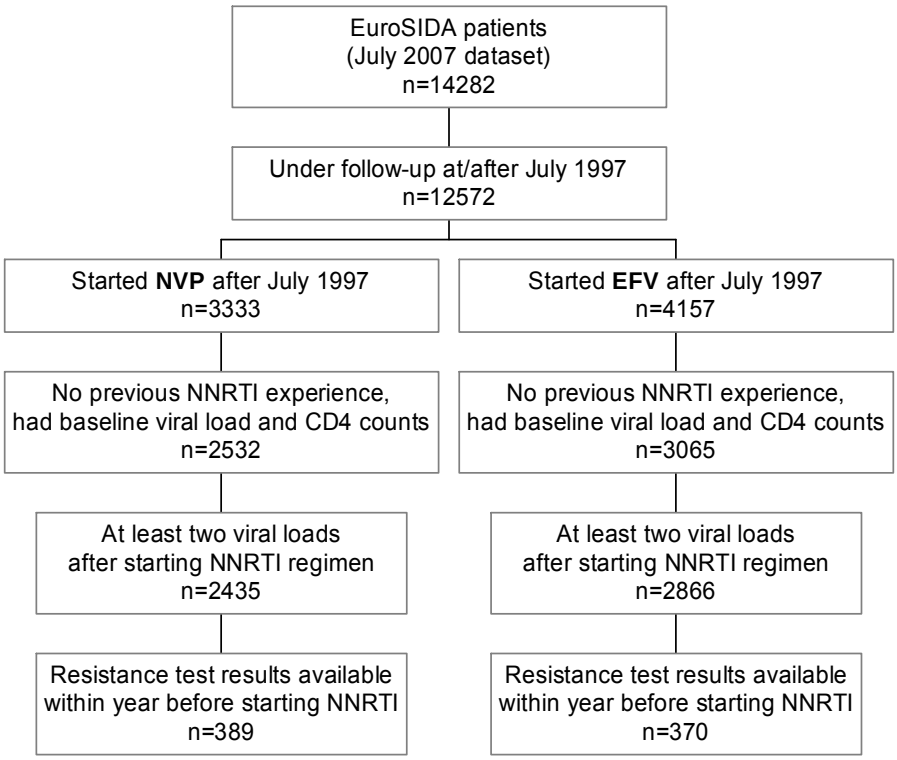
Finally, a multivariable logistic regression model was also developed to investigate virological success defined as a viral load less than 50 copies/mL at the first measurement six to twelve months after initiation of the regimen. Patients were required to have this measured using an assay with a level of detection as low as 50 copies/mL and so any patients with missing values were excluded. Patients were also required to have a baseline viral load of at least 50 copies/mL.

6.3 Results

6.3.1 Patient numbers

A total of 5301 NNRTI-naïve patients fulfilled the original inclusion criteria, of whom 2435 (45.9%) started NVP and 2866 (54.1%) started EFV. Baseline genotypic resistance test results were available for 759 (14.3%) patients. Amongst these, 618 (81.4%) patients had stored plasma samples taken within a year before starting treatment, from which HIV RNA was subsequently tested for genotypic resistance in the central EuroSIDA laboratories. The remaining 141 (18.6%) patients had resistance tests carried out at the clinical sites. Figure 6.1 displays the subsets of patients investigated in these analyses.

Figure 6.1: Patient numbers in analyses according to inclusion criteria.



6.3.2 Patient characteristics at date of NNRTI initiation

6.3.2.1 Resistance test results available versus not

Table 6.1 shows a comparison of the patient characteristics at the date of NNRTI initiation between the 759 patients with resistance test results available (either from retrospective analysis of samples or resistance tests performed at clinical sites) and the 4542 patients who did not. Patients with resistance tests on average started their regimens earlier (median: May 1999 versus August 2000; $p<0.001$), had lower baseline CD4 counts (median: 237 versus 321 cells/mm^3; $p<0.001$) and higher baseline viral loads (median: 4.3 versus 3.6 $\log_{10}$copies/mL; $p<0.001$). Less patients with resistance tests were ART-naïve at the start of the regimen (13.0% versus 18.6%; $p<0.001$) and slightly more started NVP (51.3% versus 45.1%; $p=0.002$).

Table 6.1: Patient characteristics at start of NNRTI-containing regimen according to whether or not baseline genotypic resistance test results were available.

	Total		Resistance test results available		Resistance test results not available		*p*
n %							
All	5301	100.0	759	14.3	4542	85.7	-
Male	4042	76.2	612	80.6	3430	75.5	0.002
HIV exposure group							<0.001
MSM	2384	45.0	1992	43.9	392	51.6	-
IDU	1049	19.8	910	20.0	139	18.3	-
Heterosexual	1496	28.2	1337	29.4	159	20.9	-
Other	372	7.0	303	6.7	69	9.1	-
Previous AIDS	1756	33.1	297	39.1	1459	32.1	<0.001
ART-naïve	919	17.8	97	13.0	822	18.6	<0.001
Started NVP instead of EFV	2435	45.9	389	51.3	2046	45.1	0.002
Median (IQR)							
Date started NNRTI	Jun 00	(Feb 99-Mar 02)	May 99	(Jun 98-Feb 01)	Aug 00	(Apr 99-Jul 02)	<0.001
Age (years)	40	(35-47)	40	(35-47)	40	(34-47)	0.138
CD4 count							
Baseline	304	(179-480)	237	(140-365)	321	(186-498)	<0.001
Nadir	148	(58-245)	99	(30-197)	155	(63-251)	<0.001
Viral load (log_{10}copies/mL)							
Baseline	3.8	(2.2-4.8)	4.3	(3.6-4.9)	3.6	(1.9-4.7)	<0.001
Max ever	4.8	(4.1-5.4)	5.1	(4.6-5.6)	4.8	(4.0-5.3)	<0.001

Notes: *P* values obtained from Chi-squared, Fisher's Exact and Kruskal-Wallis tests.
MSM = men who have sex with men; IDU = injecting drug use; NVP = nevirapine; EFV = efavirenz; IQR = interquartile range.

6.3.2.2 Nevirapine versus efavirenz

Table 6.2 displays the patient characteristics at the date of initiation of the NNRTI-containing regimen in the 759 patients with baseline resistance test results available. A total of 389 (51.3%) patients started NVP and 370 (48.7%) started EFV for the first time. NVP patients on average started their regimens earlier than EFV patients (median: August 1998 versus May 2000; $p<0.001$) and had resistance test results available earlier (median: May 1998 versus December 1999; $p<0.001$).

Table 6.2: Patient characteristics at start of NNRTI-containing regimen in patients with baseline resistance test results according to whether they started nevirapine or efavirenz.

	Total		NVP		EFV		*p*
n %							
All	759	100.0	389	51.3	370	48.7	-
Male	612	80.6	323	83.0	289	78.1	0.086
HIV exposure group							0.277
MSM	392	51.6	210	54.0	182	49.2	-
IDU	139	18.3	72	18.5	67	18.1	-
Heterosexual	159	20.9	70	18.0	89	24.1	-
Other	69	9.1	37	9.5	32	8.6	-
Previous AIDS	297	39.1	158	40.6	139	37.6	0.390
HIV-1 subtype identified	707	93.1	365	93.8	342	92.4	0.466
B*	641	90.7	333	91.2	308	90.1	0.592
Non-B*	66	9.3	32	8.8	34	9.9	-
Median (IQR)							
Date started NNRTI	May 99	(Jun 98-Feb 01)	Aug 98	(Feb 98-Oct 99)	May 00	(Mar 99-Apr 02)	<0.001
Date of plasma sample	Dec 98	(Jan 98-Oct 00)	May 98	(Sep 97-Apr 99)	Dec 99	(Aug 98-Dec 01)	<0.001
Age (years)	40	(35-47)	40	(34-47)	41	(36-47)	0.459
CD4 count (cells/mm^3)							
Baseline	237	(140-365)	230	(131-349)	240	(146-377)	0.262
Nadir	99	(30-197)	102	(30-204)	98	(30-188)	0.376
Viral load (log_{10}copies/mL)							
Baseline	4.3	(3.6-4.9)	4.3	(3.7-4.9)	4.3	(3.5-4.9)	0.566
Max ever	5.1	(4.6-5.6)	5.1	(4.6-5.6)	5.1	(4.6-5.5)	0.970

Notes: *P* values obtained from Chi-squared, Fisher's Exact and Kruskal-Wallis tests.
*Percentage of HIV-1 B/non-B subtypes amongst those with subtype identified.
MSM = men who have sex with men; IDU = injecting drug use; NVP = nevirapine; EFV = efavirenz; IQR = interquartile range.

Table 6.3 gives details of the antiretroviral drugs started as part of the NNRTI-containing regimen, as well as prior experience of ART. The majority of patients started three or four drugs in the regimen (86.8%). In both the NVP and EFV groups, d4T and 3TC were the most commonly used NRTIs in the regimens. More patients in the NVP group started a PI as well as the NNRTI (59.6% versus 50.8%; p=0.015) and less were ART-naïve prior to starting the regimen (9.5% versus 16.5%; p=0.004). Patients starting NVP were more likely to have previous experience of NRTIs than patients starting EFV (90.5% versus 83.2%; p=0.003). Previous use of PIs was similar between the groups (76.6% versus 75.4%; p=0.698).

Table 6.3: Use of antiretroviral drugs in patients with baseline resistance test results according to whether they started nevirapine or efavirenz.

	Total		NVP		EFV		*p*
All	759	100.0	389	51.3	370	48.7	-
Antiretroviral drugs in regimen, n %							
Total drugs in regimen							0.178
1-2	37	4.9	24	6.2	13	3.5	-
3-4	659	86.8	336	86.4	323	87.3	-
≥ 5	63	8.3	29	7.5	34	9.2	-
NRTI(s) in regimen	728	95.9	372	95.6	356	96.2	0.683
ZDV	157	20.7	76	19.5	81	21.9	0.423
ddI	256	33.7	134	34.4	122	33.0	0.668
d4T	357	47.0	219	56.3	138	37.3	<0.001
3TC	378	49.8	189	48.6	189	51.1	0.492
ABC	167	22.0	44	11.3	123	33.2	<0.001
PI(s) in regimen	420	55.3	232	59.6	188	50.8	0.015
IDV	93	12.3	47	12.1	46	12.4	0.883
RTV	184	24.2	80	20.6	104	28.1	0.015
NFV	181	23.8	111	28.5	70	18.9	0.002
ART use prior to start of regimen, n %							
ART-naïve	98	12.9	37	9.5	61	16.5	0.004
Previous NRTI use	660	87.0	352	90.5	308	83.2	0.003
ZDV	630	83.0	336	86.4	294	79.5	0.011
ddI	379	49.9	198	50.9	181	48.9	0.585
d4T	491	64.7	254	65.3	237	64.1	0.721
3TC	611	80.5	322	82.8	289	78.1	0.105
ddC	260	34.3	158	40.6	102	27.6	<0.001
Previous PI use	577	76.0	298	76.6	279	75.4	0.698
IDV	399	52.6	207	53.2	192	51.9	0.716
RTV	314	41.4	145	37.3	169	45.7	0.019
SQV (hard gel)	188	24.8	70	18.0	118	31.9	<0.001
NFV	294	38.7	158	40.6	136	36.8	0.275

Notes: *P* values obtained from Chi-squared, Fisher's Exact and Kruskal-Wallis tests.
Specific NRTIs/PIs only shown where proportion of patients is more than 10%.
NVP = nevirapine; EFV = efavirenz; ZDV = zidovudine; ddI = didanosine; d4T = stavudine;
3TC = lamivudine; ABC = abacavir; ddC = zalcitabine; IDV = indinavir; RTV = ritonavir; NFV = nelfinavir;
SQV = saquinavir.

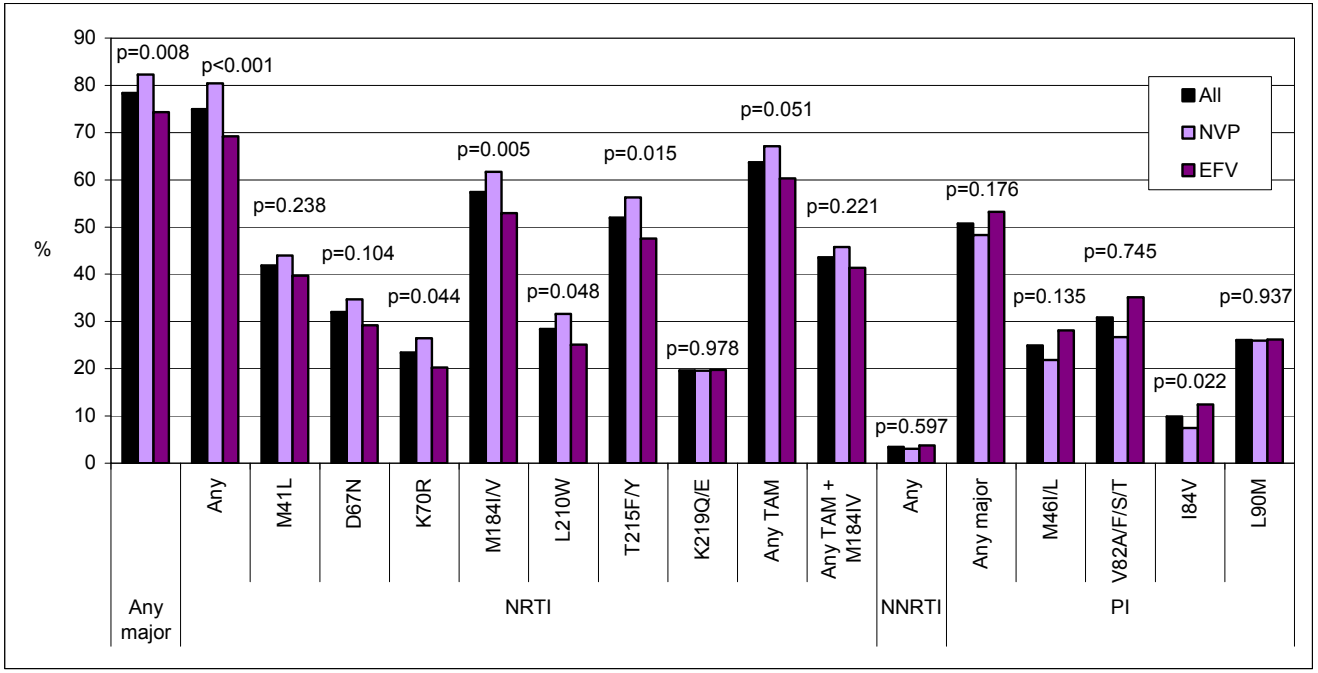
6.3.3 Baseline genotypic HIV drug resistance

6.3.3.1 Prevalence of baseline IAS-USA resistance mutations

Figure 6.2 displays IAS-USA viral mutations associated with drug resistance detected at the start of the NNRTI-containing regimens. In general, there were similar proportions of patients with resistant HIV in those starting NVP and those starting EFV.

Figure 6.2: IAS-USA drug resistance mutations detected at start of NNRTI-containing regimen according to whether patients started nevirapine or efavirenz.

Note: *P* values obtained from Chi-squared tests comparing patients starting nevirapine (NVP) and efavirenz (EFV).



Overall, 595 (78.4%) had at least one major mutation (82.3% NVP versus 74.3% EFV; *p*=0.008). Of these, 569 (75.0%) had at least one NRTI resistance mutation (80.5% NVP versus 69.2% EFV; *p*<0.001), 26 (3.4%) had an NNRTI resistance mutation (3.1% NVP versus 3.8% EFV; *p*=0.597) and 385 (50.7%) had a major PI resistance mutation (48.3% NVP versus 53.2% EFV; *p*=0.176). NVP patients had a higher prevalence of the M184I/V (61.7% versus 53.0%), the L210W (31.6% versus 25.1%) and the T215F/Y (56.3% versus 47.6%) mutations than EFV patients. They also had a lower prevalence of the I84V mutation (7.5% versus 12.4%). There were no differences in prevalence of the A98G, V179D or Y181C mutations, which may confer intermediate phenotypic resistance.

6.3.3.2 Baseline resistance using interpretation algorithms

Using the Rega algorithm to interpret resistance, 460 (64.3%) patients had HIV with resistance (full or intermediate) to at least one drug in the regimens they were starting: 256 (68.6%) NVP patients and 204 (59.5%) EFV patients (*p*=0.011). A total of 27 (3.6%) patients had NNRTI resistance: 13 (3.3%) NVP patients (11 with full resistance, 2 with intermediate) versus 14 (3.8%) EFV patients (10 with full, 4 with intermediate) (*p*=0.743). Median genotypic sensitivity scores calculated according to the Rega algorithm were 2.5 (IQR: 1.5-3.0) for patients starting NVP and 3.0 (IQR: 2.0-3.0) for patients starting EFV (*p*<0.001).

Results varied slightly according to which algorithm was used. Overall 429 (56.5%) patients were found to have full or intermediate resistance according to ANRS and 550 (72.5%) according to Stanford. A similar level of NNRTI resistance was found using ANRS: 27 (3.6%) patients (all with full resistance), but a higher level was found using the Stanford algorithm: 66 (8.7%) patients (24 with full, 42 with intermediate). Both showed similar levels of NNRTI resistance between patients starting NVP and EFV.

6.3.4 Virological failure after starting NNRTI regimen: time to two consecutive viral loads greater than 500 copies/mL

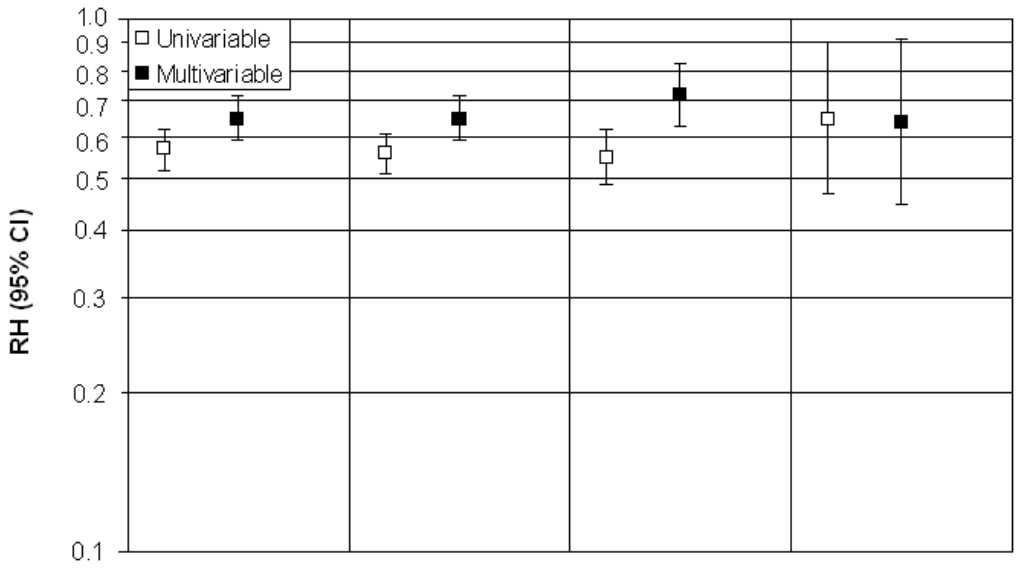
6.3.4.1 Main analysis in full set of 5301 patients

Virological failure was first analysed in the 5301 NNRTI-naïve patients who fulfilled the original inclusion criteria regardless of whether baseline resistance test results were available. A total of 2319 (43.7%) experienced virological failure: 1373 (56.4%; 95% CI: 54.5-58.4%) of the 2435 patients starting NVP and 946 (33.0%; 95% CI: 31.3-34.8%) of the 2866 starting EFV (*p*<0.001). Of these 2319 virological failures, 517 (22.3%) started the regimen with a baseline viral load of less than or equal to 500 copies/mL (22.3% NVP versus 22.3% EFV; *p*=0.992). Median times between viral load

measurements after starting the NNRTI-containing regimen were similar for NVP and EFV (3.7 months (IQR: 2.9-5.0 months) and 3.6 months (IQR: 2.8-5.1 months) respectively; p=0.551) therefore it was unlikely that frequency of measurements was a source of bias explaining these differences.

Using a Cox proportional hazards model stratified by clinical centre and after adjustment for number of previous NRTIs and PIs, previous AIDS diagnosis, year started NNRTI, CD4 count (baseline and nadir), viral load (baseline and maximum ever) and number of NRTIs and PIs in the regimen, it was found that patients starting EFV were 35% less likely to virologically fail their regimen than those starting NVP, which was highly significant (RH: 0.65; 95% CI: 0.59-0.72; p<0.001). Figure 6.3 displays univariable and multivariable results.

Figure 6.3: Relative hazards and 95% confidence intervals of virological failure (two viral loads greater than 500 copies/mL) following initiation of an efavirenz-containing regimen compared to a nevirapine-containing regimen.



No. of patients included in each analysis:	**All**	**Left censored**	**Right censored at NNRTI discontinuation**	**ART-naïve**
NVP patients	2435	2244	2435	330
% vl failure	56.4	54.4	24.1	33.9
EFV patients	2866	2705	2866	617
% vl failure	33.0	31.7	15.8	17.0

Notes: Multivariable analyses adjusted for number of previous NRTIs and PIs, previous AIDS diagnosis, year started NNRTI, baseline CD4 count, CD4 nadir, baseline viral load, maximum viral load ever and number of NRTIs and PIs in the regimen.
vl = virological; RH = relative hazards; NVP = nevirapine; EFV = efavirenz.

6.3.4.2 Sensitivity analyses in 5301 patients

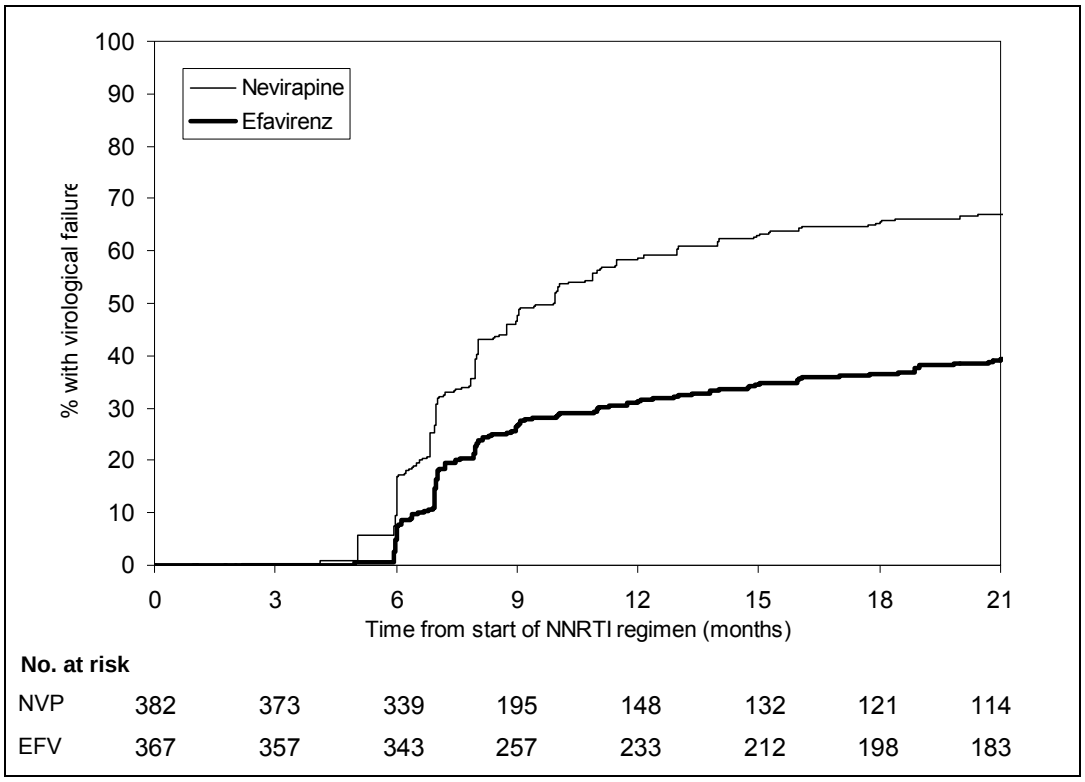
A total of 1280 (24.2%) patients started the NNRTI-containing regimen before enrolment into EuroSIDA and so were left censored in a sensitivity analysis. Of these, 352 patients were enrolled either after virological failure or after their penultimate viral loads and were therefore excluded from the analysis. This left 4949 (93.4%) patients (2244 starting NVP and 2705 starting EFV), of which 2078 (42.0%) experienced virological failure (1221 (54.4%) of those starting NVP and 857 (31.7%) starting EFV).

In a further sensitivity analysis, additional right censoring was applied to 1954 (36.9%) patients who discontinued the NNRTI before virological failure or their penultimate viral load measurement. A total of 1040 patients discontinued before virological failure and therefore were not counted as virological failures in this analysis. This left 1279 patients who experienced virological failure (588 (24.1%) of those starting NVP and 452 (15.8%) of those starting EFV). The results from both these analyses, in addition to repeating the analysis in a subset of 947 (17.9%) ART-naïve patients, gave consistent findings to those of the main analysis.

6.3.4.3 Main analysis in subset of 759 patients with baseline resistance test results

This analysis was then repeated in the subset of 759 patients with baseline resistance test results available. A total of 287 (73.8%; 95% CI: 69.7-78.4%) of the 389 NVP patients and 168 (45.4%; 40.6-50.7%) of the 370 EFV patients experienced virological failure (p<0.001). Of these 455 virological failures, 24 (5.3%) started the regimen with a baseline viral load of less than or equal to 500 copies/mL (6.6% NVP versus 3.0% EFV; p=0.093). The median times between viral load measurements after starting the regimen were similar (3.0 months (IQR: 2.0-4.1 months) for NVP and 3.0 months (IQR: 2.3-4.3 months) for EFV; p=0.601) therefore it was unlikely that frequency of measurements was a source of bias explaining these differences. In particular, in those who experienced virological failure, the median time between the two consecutive measurements more than 500 copies/mL was 2 months (95% CI: 1-4 months) in the NVP group and 2 months (95% CI: 2-4 months) in the EFV group (p=0.748). Figure 6.4 displays a Kaplan-Meier plot of the percentage of patients with virological failure by time from start of the NNRTI-containing regimen.

Figure 6.4: Kaplan-Meier estimate of the percentage of patients with baseline resistance test results available and with virological failure (two viral loads greater than 500 copies/mL) by time from start of NNRTI regimen, according to use of nevirapine or efavirenz.



Notes: NVP = nevirapine; EFV = efavirenz.

A total of 275 (36.2%) patients discontinued the NNRTI they started on within the first year of the regimen. NVP was discontinued more than EFV (40.1% versus 32.2%; *p*=0.023). Reasons for discontinuation were specified in 202 (73.5%) patients. Treatment failure was the reason given for 24.4% of discontinuations (29.5% NVP versus 17.7% EFV). Hypersensitivity reaction accounted for a further 8.7% (13.5% NVP versus 2.5% EFV), toxicity predominantly in the nervous system accounted for 6.9% (2.6% NVP versus 12.6% EFV) and patient/physician choice for 19.6% (12.2% NVP versus 29.4% EFV). The remaining reasons included abnormal fat redistribution, concern over cardiovascular disease, toxicities in the gastro-intestinal tract, abdomen and liver, and other toxicities not specified. Of the 156 patients discontinuing NVP during the first year, 7.7% switched to EFV and 17.3% switched to a PI-based regimen within a month of stopping. Of the 119 patients discontinuing EFV, 16.8% switched to NVP and 15.1% switched to a PI within a month.

A univariable Cox proportional hazards model showed that patients starting EFV were 51% less likely to virologically fail their regimen than those starting NVP (RH: 0.49;

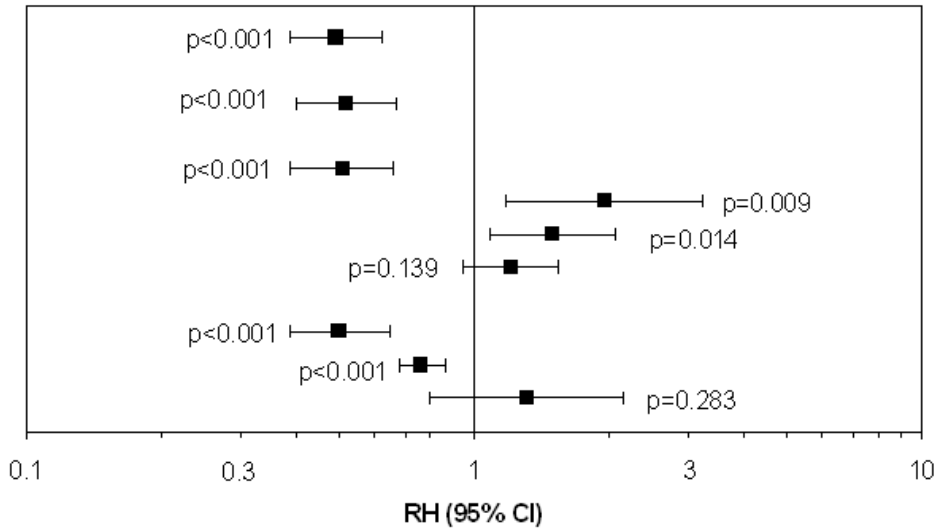
95% CI: 0.39-0.62; p<0.001). After adjustment for the same variables as mentioned above, the result remained similar (RH: 0.52; 95% CI: 0.40-0.67; p<0.001). Figure 6.5 displays these results. Other potential confounding variables were investigated: gender, age, HIV exposure group, ethnicity, hepatitis B/C coinfection status, time from CD4 nadir, time from HIV positive diagnosis, HIV-1 subtype, specific drugs in the regimen and duration of previous use of drugs. Adjustment for these also did not affect the findings. Results were similar when stratifying by the prior number of drugs taken (RH: 0.60; 95% CI: 0.49-0.75; p<0.001), region of Europe (RH: 0.59; 95% CI: 0.47-0.73; p<0.001) and by calendar year of starting the regimen (RH: 0.56; 95% CI: 0.45-0.70; p<0.001).

Patients with HIV with baseline NNRTI resistance mutations (defined according to IAS-USA) were twice as likely to experience virological failure than those without NNRTI mutations (multivariable RH: 1.96; 95% CI: 1.18-3.24; p=0.009), those with NRTI-resistant HIV were 49% more likely (multivariable RH: 1.49; 95% CI: 1.09-2.06; p=0.014) and those with PI-resistant HIV were 21% more likely (multivariable RH: 1.21; 95% CI: 0.94-1.54; p=0.139). However, adjustment of the multivariable model for this information did not explain the difference in virological failure between EFV and NVP (RH: 0.51; 95% CI: 0.39-0.66; p<0.001). Figure 6.5 displays these results.

An alternative model adjusting for the Rega genotypic sensitivity score, i.e. number of active drugs in the regimen excluding the NNRTI, as well as for NNRTI resistance, was also developed. It was found that for each increase of one active drug (NRTI or PI) in the regimen, there was a 24% decreased chance of virological failure (multivariable RH: 0.76; 95% CI: 0.68-0.86; p<0.001). There was no significant difference between patients with NNRTI-resistant HIV and those without (multivariable RH: 1.31; 95% CI: 0.80-2.15; p=0.283). This model also did not result in a change in the RH of virological failure in patients starting EFV compared to NVP (RH: 0.50; 95% CI: 0.39-0.65; p<0.001) (Figure 6.5). Using the ANRS and Stanford algorithms instead of Rega gave similar findings and the RH for EFV versus NVP remained the same for both (RH: 0.51; 95% CI: 0.39-0.66; p<0.001; and RH: 0.50; 95% CI: 0.39-0.65; p<0.001; respectively).

Figure 6.5: Relative hazards and 95% confidence intervals of virological failure (two viral loads greater than 500 copies/mL) following initiation of an NNRTI-containing regimen.

Univariable	EFV versus NVP
Multivariable	EFV versus NVP
Multivariable adjusted for IAS-USA resistance	EFV versus NVP NNRTI resistance versus none NRTI resistance versus none PI resistance versus none
Multivariable adjusted for Rega algorithm resistance	EFV versus NVP No. active non-NNRTI drugs (per extra drug) NNRTI resistance versus none



Notes: Multivariable analyses adjusted for number of previous NRTIs and PIs, previous AIDS diagnosis, year started NNRTI, baseline CD4 count, CD4 nadir, baseline viral load, maximum viral load ever and number of NRTIs and PIs in the regimen.
NVP = nevirapine; EFV = efavirenz; RH = relative hazards; CI = confidence interval.

6.3.4.4 Sensitivity analyses in 759 patients

Sensitivity analyses using the multivariable model adjusting for the Rega score and NNRTI resistance were carried out on the 759 patients with resistance test results available. A total of 26 (3.4%) patients were left censored at the date of enrolment into EuroSIDA, giving a multivariable RH (EFV versus NVP) of 0.51 (95% CI: 0.39-0.65; p<0.001). A total of 291 (38.3%) patients discontinued the NNRTI they started (at any time) and so were right censored at the time of discontinuation. This gave a multivariable RH of 0.62 (95% CI: 0.44-0.88; p=0.007). Taking only 618 patients whose resistance test results came from retrospective analysis of prospectively stored plasma samples in the central EuroSIDA laboratories resulted in a multivariable RH of 0.53 (95% CI: 0.41-0.71; p<0.001). It was found that patients whose results came from tests carried out in the clinical centres were almost three times as likely to start EFV than NVP in a logistic regression analysis after adjusting for other explanatory variables (OR: 2.73; 95% CI: 1.77-4.21; p<0.001). Finally in a subset of 98 patients who were previously ART-naïve, the multivariable RH was 0.22 (95% CI: 0.04-1.18; p=0.215).

6.3.5 Response to NNRTI regimen: change in viral load and CD4 count at months six to twelve

Linear regression was then used to analyse change in viral load and CD4 count from baseline to the last measurement six to twelve months after starting the regimen. A total of 711 (93.7%) of the 759 patients had viral loads measured during this time and those on EFV-containing regimens had a viral load reduction on average 0.65 $\log_{10}$copies/mL greater than those on NVP-containing regimens (p<0.001) after adjustment for the same variables as in the main analysis. CD4 counts were also measured in 711 (93.7%) patients during this six month period and after adjustment, those in the EFV group had a CD4 count increase of on average 26 cells/mm^3 higher than those in the NVP group (p=0.042). After adjustment for the change in viral load, changes in CD4 count were similar between the two groups: 9 cells/mm^3 higher in the EFV group compared to the NVP group (p=0.474).

6.3.6 Virological response to NNRTI regimen: viral load less than 50 copies/mL at months six to twelve

Finally logistic regression was used to compare virological success rates between those starting NVP and EFV. A total of 632 patients were included who had their first viral load in the six to twelve month period after starting the NNRTI-containing regimen measured using an assay with a lower limit of detection of 50 copies/mL and also had a baseline viral load measurement of at least 50 copies/mL. Virological suppression (less than 50 copies/mL at six to twelve months) was achieved by 72 (22.5%; 95% CI:

18.2-27.4%) of 320 NVP patients and 178 (57.1%; 95% CI: 51.9-62.9%) of 312 EFV patients (p<0.001). A multivariable logistic regression model adjusting for the same variables as above, found that patients starting EFV were almost four times as likely to achieve virological suppression than those starting NVP (multivariable OR: 3.65; 95% CI: 2.37-5.62; p<0.001).

6.3.7 Genotypic HIV drug resistance at time of virological failure

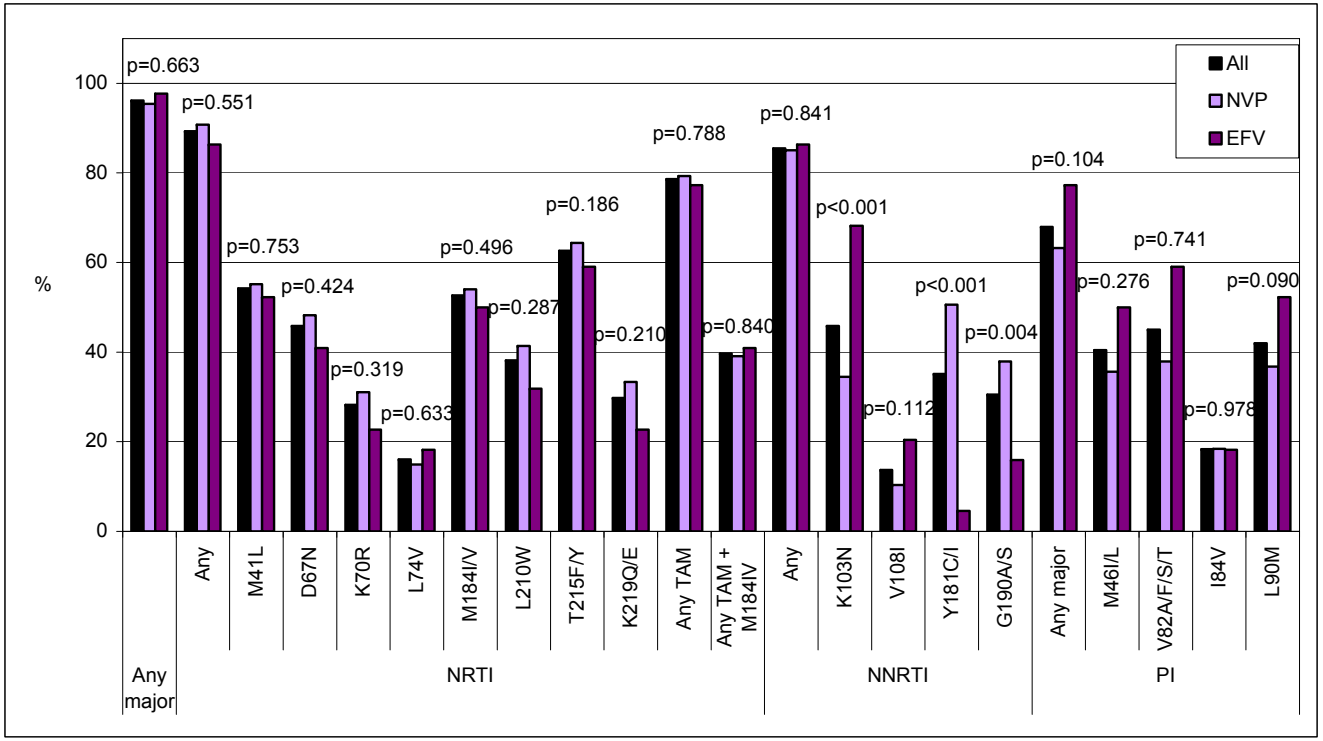
6.3.7.1 Prevalence of IAS-USA resistance mutations at time of failure

In total 131 (28.8%) of the 455 patients who experienced virological failure (amongst the 759 with a baseline resistance test) were still taking an NNRTI and had resistance test results available at the time of failure. Prevalence of resistance was found to be similar between the NVP and EFV groups (95.4% NVP versus 97.7% EFV; p=0.663). Overall, 117 (89.3%) patients had NRTI resistance, 112 (85.5%) had NNRTI resistance and 89 (67.9%) had major PI resistance (Figure 6.6). The Y181C mutation was the most frequently observed in the NVP group (47.1%) and the K103N mutation in the EFV group (68.2%).

6.3.7.2 New IAS-USA resistance mutations at time of failure compared to baseline

A comparison with baseline results showed that between baseline and virological failure times, 55 (42.0%) patients developed a new NRTI resistance mutation (39.1% NVP versus 47.7% EFV; p=0.344), 111 (84.7%) developed a new NNRTI resistance mutation (83.9% NVP versus 86.4% EFV; p=0.712) and 36 (27.5%) developed a new PI resistance mutation (28.7% NVP versus 25.0% EFV; p=0.651). The most common new NNRTI resistance mutations that developed were K103N (34.5% NVP versus 65.9% EFV), V108I (10.3% NVP versus 20.5% EFV), Y181C (46.0% NVP versus 4.6% EFV) and G190A (35.7% NVP versus 9.1% EFV). Logistic regression models were used to investigate whether the differences in development of mutations between those starting NVP and EFV were affected by the factors in the main analysis. After adjustment for these factors including Rega score and NNRTI resistance, the significant differences in the detection of K103N, Y181C and G190A remained present between the two groups.

Figure 6.6: IAS-USA drug resistance mutations detected at time of virological failure following initiation of NNRTI-containing regimen in patients with resistance test results available whilst still on NNRTI, according to whether patients started nevirapine or efavirenz.



Note: *P* values based on comparison between patients starting nevirapine (NVP) and efavirenz (EFV).

6.4 Discussion

The results from these analyses found that amongst 759 mostly NRTI/PI-experienced but NNRTI-naïve patients starting an NNRTI-containing regimen for the first time with a baseline genotypic resistance test result available, those starting EFV had a 50% reduced risk of virological failure compared to those starting NVP. This was adjusted for demographics, previous ART, baseline NNRTI resistance and the predicted susceptibility to the other drugs included in the regimen. NNRTI-resistant HIV was detected in 3.4% of patients at baseline with similar levels in both NVP and EFV groups. Out of 131 patients still on an NNRTI and with resistance test results available at time of virological failure, NNRTI resistance was detected in 85.5% of patients and was similar between the groups, however different resistance profiles emerged. After adjustment for the same factors as in the main analysis, the K103N mutation was more prevalent in those who failed on EFV and the Y181C and G190A mutations were more prevalent in those who failed on NVP.

6.4.1 Interpretation of findings and previous research

6.4.1.1 EuroSIDA

The main findings match those from the previous EuroSIDA analysis, which found that patients starting EFV (n=739) had a 43% reduced risk of virological failure than those starting NVP (n=1174) [300]. At the time of that analysis, drug resistance data were not available therefore it was unknown as to whether baseline resistance profiles could explain the difference in virological outcome observed. The findings in this chapter suggest that although patients with at least one IAS-USA NNRTI resistance mutation at baseline or a lower number of fully active drugs in the regimen (as defined by the Rega, ANRS or Stanford algorithms) were more likely to experience virological failure, neither of these factors confounds the association between the risk of virological failure and the use of NVP or EFV. This result was consistent in a number of sensitivity analyses with the exception of a non-significant difference found between groups in the subset of 98 ART-naïve patients. However, the results remained in favour of EFV and as the number of patients included was much lower, the power of the analyses to detect true differences was reduced. Conversely, this could also reflect that possible differences in intrinsic virological efficacy between the two NNRTIs are less pronounced in patients harbouring fully susceptible virus.

6.4.1.2 Other cohort studies

The results also match findings from other observational cohort studies, which all considered many confounding factors but did not take into account baseline NNRTI resistance and so could not rule this out as potentially biasing the results [302-304].

Matthews *et al.* (2002) included 888 ART-naïve patients from two large London clinics, who started a cART regimen containing a PI, NVP or EFV and found a better initial virological response, measured as time to virological suppression less than 500 copies/mL, for EFV compared to either of the alternatives [302]. The I.Co.N.A. study (n=694) found that patients starting NVP were twice as likely to experience virological failure (two consecutive measurements more than 500 copies/mL) than those starting EFV [303] and a US cohort study by Keiser *et al.* (2002) looking at 1078 ART-naïve patients found a 50% increased risk of virological failure (two consecutive measurements more than 400 copies/mL) for NVP versus EFV [304].

Some other observational studies found no difference in response to NNRTI-based regimens. A retrospective observational cohort study found no significant difference in time to virological or immunological response between ART-naïve patients starting NVP and EFV but with small patient numbers (n=53) [476]. Another prospective observational survey compared 287 patients treated with EFV to 258 treated with NVP over an 18-month period. This also found comparable virological and immunological responses, however in a subset of 154 ART-naïve patients, those treated with EFV showed a virological advantage at three to twelve months [477]. An Indian cohort of 1111 ART-naïve patients found equivalent CD4 cell increases between patients starting two NRTIs plus either NVP or EFV [475].

Table 6.4 summarises the findings of previous research from both observational studies and clinical trials.

6.4.1.3 Randomised clinical trials

The question was also addressed in a large randomised clinical trial, the 2NN study [299]. A composite measurement of treatment failure, including virological failure, disease progression and therapy change was investigated in 1216 ART-naïve patients starting NVP once daily, NVP twice daily, EFV, or NVP plus EFV. In the primary analysis, a comparison of NVP twice daily (n=387) versus EFV (n=400) found a non-significant but slightly lower proportion of treatment failure at week 48 in those starting EFV (37.8%; 95% CI: 33.0-42.7%) compared to those starting NVP (43.7%; 95% CI: 38.7-48.8%; p=0.091). However, a sensitivity analysis excluding 28 patients who were randomised to either of these groups but who never started their treatment found a significant difference between them (7.7%; 95% CI: 0.8-14.6%; p=0.030). Furthermore, the study was powered to investigate equivalence of the two drugs but failed to demonstrate this.

Table 6.4: Previous research investigating virological response to nevirapine-containing versus efavirenz-containing regimens.

Authors, year	Type of study (name of study)	Patient numbers on each NNRTI	Main outcomes	Conclusions
Phillips *et al.* 2001 [300]	Observational cohort (EuroSIDA)	1174 NVP, 739 EFV (NNRTI-naïve)	Time to virological failure defined as two consecutive values > 500 copies/mL after starting the regimen (> 6 months after start of the regimen if baseline viral load was > 500 copies/mL).	Patients starting EFV had a significantly reduced risk of virological failure than those starting NVP in multivariable analysis.
Matthews *et al.* 2002 [302]	Observational cohort	237 NVP, 167 EFV, 484 PIs (ART-naïve)	Analysis 1: Time to first undetectable viral load (< 500 copies/mL) after cART initiation. Analysis 2: Composite failure endpoint – failed to achieve viral load < 500 copies/mL within 6 months or who achieved this but then experienced viral load rebound (two consecutive viral load measurements of > 500 copies/mL), both before 6 months.	Patients starting EFV were significantly more likely to achieve an undetectable viral load and had a significantly reduced risk of virological failure than those starting NVP in multivariable analyses.
Cozzi-Lepri *et al.* 2002 [303]	Observational cohort (I.Co.N.A.)	460 NVP, 234 EFV (NNRTI-naïve)	Time to virological failure defined as two consecutive values > 500 copies/mL after starting the regimen (> 6 months after start of the regimen if baseline viral load was > 500 copies/mL).	Patients starting EFV had a significantly reduced risk of virological failure than those starting NVP in multivariable analysis.
Núñez *et al.* 2002 [478]	Randomised pilot study	36 NVP, 31 EFV (ART-naïve)	Endpoint: viral load < 50 copies/mL 48 weeks after starting cART regimen.	No significant difference in virological response between patients starting the two NNRTIs was found.
Keiser *et al.* 2002 [304]	Observational cohort	523 NVP, 555 EFV (ART-naïve)	Time to virological failure defined as two consecutive counts of viral load > 400 copies/mL in those who previously had undetectable viral load or the failure to achieve viral load < 400 copies/mL.	Patients starting EFV had a significantly reduced risk of virological failure than those starting NVP in multivariable analysis.
Manfredi *et al.* 2004 [477]	Observational cohort	258 NVP, 287 EFV (154 ART-naïve, 298 ART-experienced but NNRTI-naïve, 103 on salvage regimens)	Mean decrease in viral load at 3, 6, 9, 12, 15 and 18 months after starting cART regimen.	Comparable efficacy between NVP and EFV in ART-experienced patients and patients on salvage therapy. Increased drop in viral load in ART-naïve patients starting EFV over NVP between months 3-12.

Notes: NVP = nevirapine; EFV = efavirenz.

Authors, year	Type of study (name of study)	Patient numbers on each NNRTI	Main outcomes	Conclusions
Manosuthi *et al.* 2004 [476]	Observational cohort	24 NVP, 29 EFV (ART-naïve with advanced infection)	Time to virological success defined as undetectable viral load < 400 copies/mL after cART initiation.	No significant difference in virological success rates was found between patients starting NVP and EFV in multivariable analysis.
Van den Berg-Wolf *et al.* 2006 [474]	Randomised substudy of clinical trial (FIRST-CPCRA 058)	117 NVP, 111 EFV (ART-naïve)	Endpoint: viral load < 50 copies/mL 8 months after starting regimen or death.	No significant difference in virological response between patients starting the two NNRTIs was found.
Nachega *et al.* 2007 [301]	Observational cohort	999 NVP, 1822 EFV	Endpoint: sustained viral load < 400 copies/mL throughout follow-up.	Patients starting EFV were more likely to achieve 100% suppression than those starting NVP in multivariable analysis.
Van Leth *et al.* 2004 [299]	Randomised clinical trial (2NN)	220 NVP once daily, 387 NVP twice daily, 400 EFV, 111 EFV plus NVP (ART-naïve)	Composite treatment failure endpoint: a decline of < 1 $\log_{10}$ copies/mL in viral load within first 12 weeks or two consecutive measurements ≥ 50 copies per mL from week 24 onwards, CDC grade C event from 8 weeks onwards or death, and therapy change.	No significant difference in treatment failure between patients receiving NVP twice daily and patients receiving EFV was found.

Notes: NVP = nevirapine; EFV = efavirenz.

A substudy of the FIRST-CPCRA 058 trial randomised patients to receiving NVP or EFV and compared them in terms of rate of virological failure (more than 50 copies after eight months or death) in an intention-to-treat analysis. No significant difference between NVP and EFV in the 228 ART-naïve patients was found (42.8 and 41.2 per 100 person years respectively; p=0.590) but virological failure on NVP was associated with more drug resistance [474]. Recently, the group reported that using an ultra-deep genetic sequencing method (which can detect minor HIV populations at 1-3% levels), as opposed to standard methods, could detect low abundant drug-resistant HIV. It was shown that patients with low abundant NNRTI-resistant variants were at significantly greater risk of virological failure than those without [485].

A Spanish pilot randomised study in 67 ART-naïve patients also found no significant difference in virological response but acknowledged that a much larger sample size is needed to detect any true difference [478].

6.4.2 Limitations

Although a large number of known, measured confounding variables were considered for this analysis, including the new information EuroSIDA has collected on genotypic drug resistance, as the data were from an observational study as opposed to from a randomised clinical trial, there may still be some unmeasured or unknown variables that may have biased the comparison, including neuropsychological issues that are not collected in routine clinical care. For example, patients suffering from depression may be less adherent to their regimens resulting in a poorer virological response rate [486] and may also be more likely to receive NVP due to the neurological side effects of EFV [40,41]. EuroSIDA has recently (in January 2008) started collecting data on patient adherence based on any comments on the patient notes that may indicate their level of adherence: '<70%' ('poor', 'inadequate', 'not good' or 'intermittent'), 'anything in between' or '>95%' ('perfect', 'full', 'excellent'). However, at present this information is scarce and no patients included in this analysis had it available. Differences in toxicities between the two drugs may have influenced adherence and it was found that in patients discontinuing either NNRTI, there were different reasons for discontinuation between NVP and EFV groups.

Specific drug resistance mutations do not necessarily occur independently from each other and so comparisons between patients starting NVP and EFV carry an increased risk that significant results could be due to chance. Adjustments can be made to the p values (such as the Bonferroni correction) to compensate for multiple testing. These

comparisons were made in this chapter to report the proportions of each mutation and conclusions were not drawn from the *p* values.

6.4.3 Implications of findings

6.4.3.1 Nevirapine versus efavirenz

As both EFV and NVP are two of the most widely used drugs across the world, it is important to conduct more randomised trials comparing the virological efficacy of the two drugs. If evidence is found that NVP is inferior to EFV, then this implies that developing countries (where NVP is commonly used [473]) are using a second-rate drug. Although the introduction of the alternative NNRTI etravirine (TMC-125) will become a useful alternative option in clinical care, especially in the management of failure patients, access to this drug will be limited worldwide for some time yet.

6.4.3.2 NNRTI resistance

In this analysis, similar high levels of NNRTI resistance were found in patients treated with NVP and EFV by the time of virological failure suggesting that the drugs emitted selection pressure, which indicates that patients had actually adhered to their regimens. The Y181C mutation was the most frequently observed in plasma samples from patients who failed a NVP-based regimen (46.0%) and the K103N mutation in samples from patients who failed an EFV-based regimen (51.4%), which supports other research [487-489]. It has implications for future therapy options as the K103N mutation has been linked to high-level cross-resistance to first-line EFV and NVP but there is evidence that it does not cause cross-resistance to TMC-125 and that Y181C alone only confers very limited resistance to TMC-125 when it is not accompanied by any other NNRTI related mutation [490-492].

However another bias for explaining the differences observed between NVP and EFV that cannot be ruled out is the possible impact of mutations in the connection domain (C-domain, residues 316-427) of HIV RNA. It has recently been demonstrated that N348I decreases susceptibility to NVP (7.4 fold) and EFV (2.5 fold) and enhances NNRTI resistance in the context of K103N [481]. Mainly N348I confers decreased susceptibility to ZDV and NVP and is more likely to be selected with ZDV and NVP treatment. Another mutation in the C-domain, E399G, has also been shown to slightly increase EFV resistance (3.6 fold) and significantly reduce viral replication capacity when associated with other mutations in the NNRTI binding pocket (L100I, V106I, V179D and F227C) [482]. However, the clinical impact of the C-domain mutations has not been assessed at the clinical level, therefore until these results are available, C-

domain mutations cannot be ruled out as a possible explanation for the differences observed in this study and the discordances seen between different trials.

6.4.4 Further research

As already mentioned, another potential confounder not addressed to date is the existence of resistance mutations in the connection domain of HIV RNA (C-domain, residues 316-427). An investigation to determine the effect of pre-treatment mutations in both the connection and RNAseH reverse transcriptase domains on the virological outcome of first-line NNRTI-based cART has been proposed in EuroSIDA (the Connect project). As more plasma samples become available, this study also proposes to look at the effect of on-treatment suboptimal plasma NNRTI levels.

It would be useful to focus the analysis on ART-naïve patients only, as most future clinical use of these drugs will be in this group. This may be possible in future updates of the dataset, however at present, there are few ART-naïve patients in the EuroSIDA dataset who fit the inclusion criteria of these analyses.

6.4.5 Conclusions

In this analysis, it was found that NVP was associated with an inferior virological outcome compared to EFV in NNRTI-naïve (but generally NRTI/PI experienced) patients confirming findings previously reported from EuroSIDA. This difference does not appear to be explained by baseline drug resistance or number of active drugs in the regimen but could be linked to the different resistance profiles emerging over the course of treatment in those starting NVP compared to EFV. However this is not a randomised comparison and the observational studies that have found similar findings may all have similar biases that could explain the results. As results suggesting a difference in the efficacy of the two drugs consistently point towards an inferior virological response in NVP patients and as both drugs are widely used worldwide, it is important for more randomised trials to be carried out in both ART-naïve and NRTI/PI experienced patients to help to draw a reliable conclusion.

A manuscript of this analysis was published by AIDS in January 2008 and can be found in Appendix IX.

Chapter 7. Toxicity of antiretroviral therapy: safety of abacavir

7.1 Introduction

This thesis has so far examined the factors affecting virological and immunological response to therapy by investigation of different subsets of EuroSIDA patients. A further response to ART that patients may experience is toxicity that can be potentially life threatening. Toxicity is a major reason for non-adherence to ART, which can in turn lead to virological and immunological failure [191-195,197], and may also result in treatment discontinuation, switches or interruptions [204,493]. This chapter focuses on the reasons for discontinuation of one NRTI, abacavir (ABC).

7.1.1 Toxicities of antiretroviral drugs

In Chapter 1 (section 1.6), the different classes of antiretroviral drugs available and their associated toxicities were discussed. Toxicities can be mild to severe in intensity, sometimes even fatal and some are drug- or class-specific. Early adverse effects (occurring within the first three to six months of treatment) tend to be mild to moderate conditions, such as headaches, nausea, diarrhoea and insomnia, which can be managed with advice and palliative drugs [215,216]. As treatment options are limited by the number of drugs available, it is beneficial to preserve options for as long as possible therefore drugs should only be discontinued or switched if the side effects cannot be controlled or lead to a reduced quality of life or lack of adherence. One of the exceptions is hypersensitivity reaction (HSR), which usually occurs within the first six weeks and is life threatening if the drug responsible is not discontinued [234,494].

Later complications after long-term ART exposure occur much less frequently but can lead to permanent disability, stigmatising body changes or death. Examples of long-term adverse events associated with antiretroviral use include lactic acidosis, hepatotoxicity, peripheral neuropathy and lipodystrophy [223,224,231,241,495]. These conditions are likely to result in treatment modification or interruption.

Some individuals may be more susceptible to adverse drug effects than others. For example, patients who are co-infected with hepatitis B or C have an increased risk of hepatotoxicity (liver damage) after starting ART compared with those who are not [226,227,230]. Other factors that may increase risk of adverse effects are the concurrent use of other medicines that can also have toxic effects, certain individual conditions such as alcoholism, and use of combinations of drugs that may interact with

each other to increase dose related toxicities, e.g. use of tenofovir (TDF) with didanosine (ddI) leads to increased plasma concentrations of ddI, which increases the risk of ddI-associated pancreatitis [40,496].

Although the main aim of ART is to achieve and maintain virological suppression, enabling recovery of the immune system, it is important that the regimen is selected not only for its efficacy, but also for its safety based on knowledge of the patient's conditions, concomitant medications and history of drug intolerance. It is recommended by current guidelines that ART should be changed if toxicities or intolerance develop that cannot be managed or treated. If one drug can be identified that is likely to be causing the toxic effect, it is virologically safe to switch only that drug to another within the same drug class. If acute toxicities occur such as rash, hepatic dysfunction and fever, then it is recommended that all drugs be stopped [40,41].

7.1.2 Abacavir

Abacavir (ABC) was first approved for use as an anti-HIV drug by the US Food and Drug Administration (FDA) in 1998 [220], followed by the European Medicines Agency (EMEA) in 1999 [216]. Treatment guidelines recommend the use of ABC as an alternative option rather than a preferred option because of the risk of HSR [40-42]. For those starting ABC as part of a first-line regimen, it is suggested that ABC be taken as part of an NRTI backbone for efavirenz (EFV), nevirapine (NVP), atazanavir (ATV), fosamprenavir (fAPV), fosamprenavir/ritonavir (fAPV/r), indinavir/ritonavir (IDV/r), lopinavir/ritonavir (LPV/r), nelfinavir (NFV), or saquinavir/ritonavir (SQV/r) with either lamivudine (3TC), which is co-formulated under the brand names Epzicom (in the USA) or Kivexa (in Europe), or with emtricitabine (FTC). Only in cases where there are concerns over toxicities, drug interactions or complexity of regimen, ABC may be used in a first-line triple NRTI regimen, which has the advantages of fewer drug interactions, low pill burden as it can be taken in a twice-daily fixed-dose combination pill (Trizivir, also containing zidovudine (ZDV) and 3TC) and avoidance of the side effects associated with PIs and NNRTIs [40-42]. However, randomised clinical trial data suggests that this regimen may be virologically inferior to an EFV-based regimen [185] and to an IDV-based regimen in patients with high baseline viral loads [307], therefore this regimen is only recommended in occasional circumstances.

The adverse effects of ABC are commonly appetite loss, headaches, malaise, nausea, vomiting and diarrhoea, which often improve within a few weeks of taking the drug [215,216]. As detailed in Chapter 1 (section 1.6.2.1), potentially fatal toxic effects that have been reported in patients taking NRTIs, including ABC, are lactic acidosis and

severe liver problems. Other complications include lipodystrophy, increased cholesterol and triglycerides in the blood and diabetes. However, the most dangerous potential side effect of taking ABC is HSR, which is detailed below.

7.1.3 Hypersensitivity reaction

HSR occurs in approximately 4-8% of patients starting the drug [40,41,234-236]. The majority of cases occur within six weeks of starting the drug with a median time to onset of 11 days [235,497], although symptoms have been reported up to 318 days after initiation [497]. HSR is characterised by fever, rash, neurologic symptoms (fatigue, malaise, headache), gastro-intestinal symptoms (nausea, vomiting, diarrhoea), musculoskeletal symptoms (myalgias, body aches) and respiratory symptoms (coughing, wheezing, pharyngitis) [234-236,495]. If the drug is not discontinued immediately there is the risk of death [40,495,498] and potentially fatal symptoms may reoccur within hours if the drug is restarted [499,500]. However, as patients are advised to stop immediately if symptoms arise, reported incidence of fatal HSR is fortunately rare. A recent study reported no HSR related deaths in over 11,500 patients exposed to either Ziagen (ABC as a single tablet) or Trizivir [501]. The study also found no differences in the frequency of ABC HSR between patients starting either formulation.

The HLA (human leukocyte antigen) genes in human DNA regulate the immune response to foreign antigens. In particular, a genetic polymorphism in the HLA-B region may be linked to ABC metabolism [502]. The allele HLA-B*5701 has been found to be strongly associated with ABC related HSR and is more prevalent in Caucasian individuals, which could account for differences in observed rates of HSR between ethnic groups [502-506]. In particular, the presence of HLA-B*5701, HLA-DR7 and HLA-DQ3 was found to have a positive predictive value of 100% and absence of this combination had a negative predictive value of 97% [507]. Prospective genetic screening for HLA-B*5701 prior to prescribing ABC has been shown to dramatically reduce the incidence of ABC related HSR [503,505,506,508-512]. Despite the fact that the test has been found to be highly predictive, its implementation in clinical practice has been impeded by the fact that high-resolution HLA genotyping is expensive, performed in specialised immunology laboratories and has long turnaround times [511]. Cost-effective, rapid and sensitive molecular tests have more recently been developed that can easily be incorporated into routine patient management and may remove the barriers to widespread use of genetic screening prior to ABC prescription [506,512].

In clinical practice, diagnosis of HSR may be difficult as it includes a combination of non-specific symptoms. Patients who exhibit symptoms consistent with HSR may be given a skin patch test to immunologically confirm whether or not they are a true HSR case. To date, 100% of patch test positive patients identified worldwide have been HLA-B*5701 positive [509,513]. Recent findings from the PREDICT-1 study provided evidence that the presence of HLA-B*5701 was a necessary condition for ABC HSR and that withholding the drug from these patients would eliminate the reaction [506]. It also highlighted the problem of clinical over-diagnosis of HSR, which results from physicians not being able to risk missing any potential true cases of HSR. Further factors that have been linked to an increased risk of HSR include female gender, higher CD8 cell count at initiation of ABC (CD8 cells are another type of T lymphocyte with a CD8 receptor) and ABC use during primary HIV infection. Factors linked to a decreased risk are previous treatment experience and more advanced disease [494,514-516].

Other antiretroviral drugs have also been linked to HSR in a small number of patients: ZDV [517], zalcitabine (ddC) [518], delavirdine (DLV) [519], EFV [520], fAPV [521] and in particular NVP [522,523]. Risk of mortality through NVP hypersensitivity is increased by hepatitis C co-infection [523]. In addition there have been cases of HSR following initiation of the antimicrobial drugs cotrimoxazole [524], dapsone [525] and sulfadiazine [526], which are used to prevent and treat HIV related opportunistic infections such as *Pneumocystis jiroveci* pneumonia and toxoplasmosis.

7.1.4 Motivation and aims for chapter

EuroSIDA collects prospective longitudinal data from a large, heterogeneous population in centres across Europe, which provides the opportunity to compare incidence of ABC related HSR across different patient subsets. The aims of these analyses were to investigate the incidence of ABC discontinuation, particularly as a result of HSR, according to: the line of therapy within which ABC was received (first, second, third or subsequent regimens), geographical region, calendar time, and co-formulation of ABC (as part of Kivexa or as either the single formulation, Ziagen, or as part of Trizivir). Incidence of HSR within these patient subsets has not previously been evaluated in a large cohort study and is important to identify patients most at risk and to monitor trends. The rate of death associated with ABC HSR was also determined.

7.2 Methods

7.2.1 Inclusion criteria

The EuroSIDA dataset used for these analyses, as in Chapter 5, was the update completed in February 2008, providing information on 14,310 patients with follow-up to (median date of last visit) January 2007. The inclusion criteria required patients to have initiated Ziagen, Trizivir or Kivexa for the first time after January 1999 (the date at which EuroSIDA started collecting data on reasons for discontinuation of therapy) during prospective follow-up, i.e. those who started ABC after enrolment into EuroSIDA and before the date of their last visit at the clinical centre.

7.2.2 Statistical methods

7.2.2.1 Definitions

The EuroSIDA forms provide a list of reasons for discontinuation of antiretroviral drugs from which sites are asked to indicate the most appropriate based on physician assessment, with just one reason chosen per drug stopped (see follow-up form in Appendix IV). Only the first ABC discontinuation was considered and baseline was defined as the date of first starting the drug.

7.2.2.2 Incidence of discontinuation of ABC

Incidence of ABC discontinuation was assessed using a person-year analysis with person-years of follow-up (PYFU) defined from the date of starting ABC to the earliest of the last follow-up visit, death or until ABC was discontinued for any reason. The incidence of ABC discontinuation due to HSR (as reported on the EuroSIDA forms) was assessed in the three months after initiating ABC treatment with follow-up time to the earliest of three months, death or ABC discontinuation.

Univariable Poisson regression models were used to identify factors that could potentially affect the incidence of HSR related discontinuation within three months of starting ABC. Factors investigated were: gender, age, HIV exposure group (defined as ‘men who have sex with men (MSM)’, ‘injecting drug use (IDU)’, ‘heterosexual’ or ‘other’), ethnicity (defined as ‘white’ or ‘other’), baseline viral load, CD4 count (baseline and nadir), hepatitis B/C coinfection status, prior AIDS diagnosis, whether or not patients were ART-naïve at baseline and concurrent use of other drugs that may cause HSR. The potential interactions between hepatitis C status and both HIV exposure group and NVP use were also investigated. A multivariable Poisson regression model was then developed adjusting for the factors found to be significant (p<0.1) in the univariable models and also including the following factors: whether patients started

ABC as part of a first-line regimen (containing at least one PI, one NNRTI or ABC), second-line regimen (containing a new PI, a new NNRTI or ABC and started at least one month after initiation of the first), third-line or subsequent regimen; geographical region the patients were seen in (defined as 'South', 'Central West', 'North' and 'East', as described in Chapter 2, section 2.1.5); and calendar time of starting ABC, which was divided into two-year periods ('1999-2000', '2001-2002', '2003-2004' and '2005' onwards). A further factor analysed in a separate model was whether a patient started ABC for the first time as part of the co-formulation Kivexa, or whether they started either ABC as Ziagen or as part of Trizivir. As Kivexa was first introduced in January 2004, only patients who started ABC after this time were included in this particular analysis.

7.2.2.3 Rate of deaths

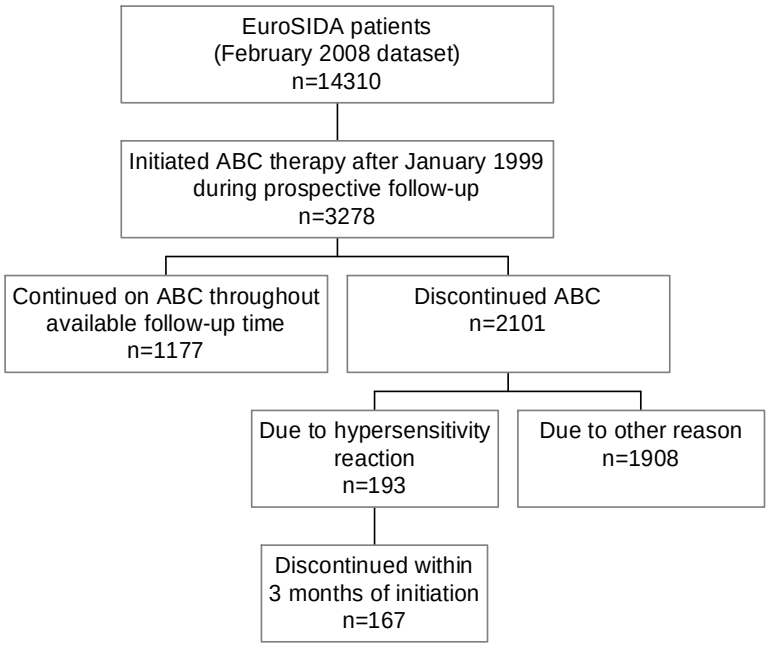
As mentioned in Chapter 2 (section 2.1.2), EuroSIDA requests that all deaths are recorded on the follow-up forms with cause of death chosen from a list provided (see follow-up form in Appendix IV). A fatal case of HSR was defined as a death that occurred either on ABC therapy or within one month of discontinuing ABC with the cause of death stated as HSR, or as a death that occurred within one month of discontinuing ABC with the reason for discontinuation stated as HSR. Incidence of HSR related death was assessed during the follow-up time from ABC initiation to the earliest of the last follow-up visit, death or if ABC was discontinued, one month after discontinuation (for any reason). Non-HSR related deaths that occurred whilst on ABC or within one month of stopping the drug were investigated further.

7.3 Results

7.3.1 Patient numbers

A total of 3278 (22.9%) patients fulfilled the inclusion criteria with a median follow-up time of 1.4 years (range: 0.0-8.5) and a total follow-up time of 6803 person-years from the date of ABC initiation. Amongst these patients who started ABC, there were 2101 (64.1%) discontinuations. The remaining 1177 (35.9%) patients continued on ABC therapy to the end of their available follow-up time. A total of 193 (9.2%) of ABC discontinuations were reported to be associated with HSR, of which 167 (86.5%) had reported HSR within three months of initiation, accounting for 5.1% of all patients starting ABC therapy. Figure 7.1 displays the subsets of patients investigated in these analyses.

Figure 7.1: Patient numbers in analyses according to inclusion criteria.



7.3.2 Patient characteristics at date of abacavir initiation

Table 7.1 shows a comparison of the patient characteristics at date of ABC initiation between the 1177 patients who continued on ABC therapy in the available follow-up time, the 193 patients who discontinued ABC due to HSR and 1908 patients who discontinued due to a reason not reported as HSR. Patients who continued ABC started treatment most recently, followed by those who discontinued due to HSR and then those who discontinued for another reason (medians: June 2004, June 2001 and January 2001 respectively; $p<0.001$). More patients who discontinued ABC were IDUs ($p<0.001$), had lower baseline CD4 counts ($p<0.001$) and higher baseline viral loads ($p<0.001$). Slightly more patients who discontinued ABC due to HSR were Caucasian (95.3%; $p=0.011$), were hepatitis C positive (28.0%; $p<0.001$) and were ART-naïve (5.7%; $p=0.007$). They were also more likely to have started NVP at the same time as ABC (7.8%; $p=0.042$). There were similar proportions of patients across the three groups starting other drugs that have been linked to HSR, i.e. EFV, APV and cotrimoxazole.

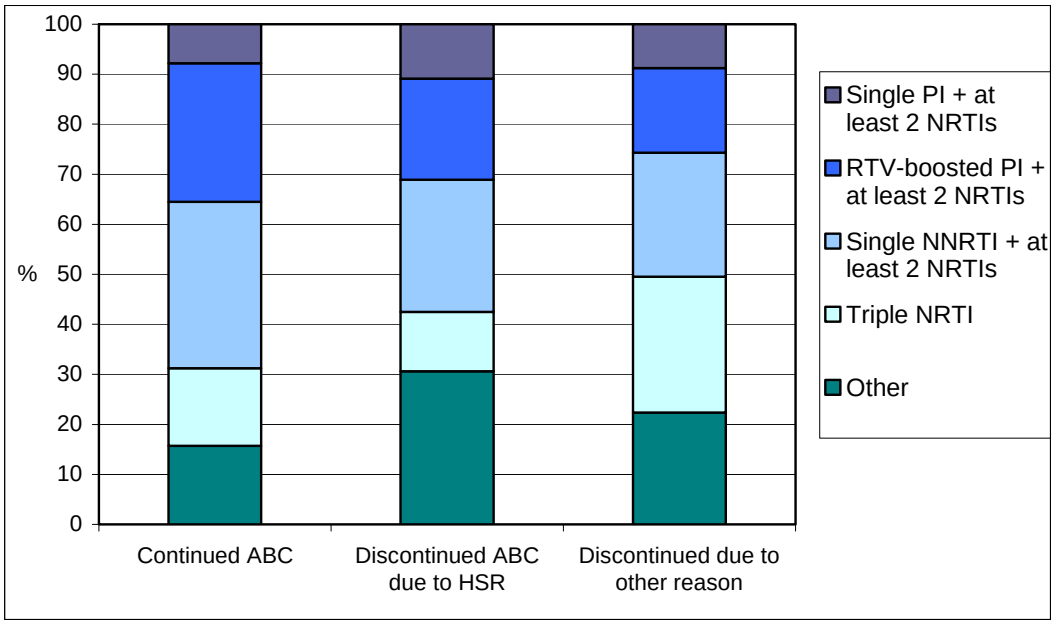
Table 7.1: Patient characteristics at start of abacavir therapy according to continuation of abacavir therapy, discontinuation of abacavir due to hypersensitivity reaction or discontinuation of abacavir due to another reason.

		Total		Continuation of ABC		Discontinuation due to HSR		Discontinuation due to other reason		*p*
All n. %		3278	100.0	1177	35.9	193	5.9	1908	58.2	-
Male		2539	77.5	937	79.6	141	73.1	1461	76.6	0.047
	MSM	1507	46.0	591	50.2	80	41.5	836	43.8	<0.001
HIV exposure group	IDU	672	20.5	192	16.3	51	26.4	429	22.5	-
	Heterosexual	861	26.3	306	26.0	48	24.9	507	26.6	-
	Other	238	7.3	88	7.5	14	7.3	136	7.1	-
White ethnicity		3026	92.3	1066	90.5	184	95.3	1776	93.1	0.011
Previous AIDS		1197	36.5	412	35.0	59	30.6	726	38.1	0.049
	Negative	2561	78.1	976	82.9	143	74.1	1442	75.6	<0.001
Hepatitis B status	Positive	206	6.3	58	4.9	14	7.0	134	7.0	-
	Unknown	511	15.6	143	12.2	36	18.7	332	17.4	-
	Negative	1950	59.5	785	66.7	103	53.4	1062	59.5	<0.001
Hepatitis C status	Positive	717	21.9	228	19.4	54	28.0	435	22.8	-
	Unknown	611	18.6	164	13.9	36	18.7	411	21.5	-
	NVP	183	5.6	51	4.3	15	7.8	117	6.1	0.042
Concurrent use of drugs	EFV	511	15.6	165	14.0	33	17.1	313	16.4	0.173
that may cause HSR	APV	108	3.3	32	2.7	3	1.6	73	3.8	0.093
	Cotrimoxazole	42	1.3	14	1.2	3	1.6	25	1.3	0.903
ART-naïve at start of ABC		96	2.9	42	3.6	11	5.7	43	2.3	0.007
Median (IQR)										
Date started ABC		Oct 01	(Apr 00-Jun 04)	Jun 04	(Jul 01-Feb 06)	Jun 01	(Jan 00-Feb 06)	Jan 01	(Jan 00-Sep 02)	<0.001
Age (years)		41	(37-48)	42	(37-49)	41	(37-48)	41	(36-48)	0.005
CD4 count (cells/mm^3)	Baseline[(1)]	357	(211-544)	394	(239-586)	327	(226-480)	336	(190-520)	<0.001
	Nadir[(2)]	127	(50-215)	134	(51-218)	136	(63-200)	121	(46-214)	0.114
Viral load (log_{10}copies/mL)	Baseline[(3)]	2.8	(1.7-4.4)	2.1	(1.7-4.0)	3.1	(1.7-4.4)	3.3	(1.7-4.6)	<0.001

Notes: *P* values obtained from Chi-squared, Fisher's Exact and Kruskal-Wallis tests.
Median CD4 counts and viral loads based on measurements from [(1)]3138 patients, [(2)]3276 patients, [(3)]3090 patients.
ABC = abacavir; HSR = hypersensitivity reaction; MSM = men who have sex with men; IDU = injecting drug use; NVP = nevirapine; EFV = efavirenz; APV = amprenavir; IQR = interquartile range.

Figure 7.2 displays the types of ABC-containing regimens started in the three groups. Patients who continued on ABC therapy throughout their follow-up time were more likely to have started it as part of an NRTI backbone for a PI, RTV-boosted PI or NNRTI regimen. More patients who discontinued ABC due to a reason other than HSR started the drug in a triple NRTI regimen. The patients who discontinued ABC due to HSR were more likely to have started a more non-conventional regimen and had the smallest proportion of triple NRTI regimens. Overall there was a significant difference in the type of regimen started between the three groups (p<0.001).

Figure 7.2: Type of abacavir-containing regimen according to continuation of therapy, discontinuation due to hypersensitivity reaction or discontinuation due to another reason.



Note: ABC = abacavir; HSR = hypersensitivity reaction; RTV = ritonavir.

7.3.3 Reasons for discontinuation of abacavir

For patients who discontinued ABC, the reasons for discontinuation as reported on the EuroSIDA follow-up forms and the median duration of ABC therapy from initiation to the time when the drug was discontinued according to reason, are displayed in Table 7.2. As expected from the literature, HSR was experienced very soon after starting ABC with a median time to HSR related discontinuation of 1.0 month (IQR: 0.3-1.2 months). A further 344 (16.4%) discontinued due to treatment failure with median time on ABC of 16.0 months (IQR: 8.4-29.0 months). Other reasons for ABC discontinuation reported in 317 (15.1%) patients were clinical fat abnormalities, dyslipidaemia, toxicities and structured treatment interruption. A further 285 (13.5%) patients had other known causes, not specified in the list. Finally, 607 (28.9%) had the reason for ABC

discontinuation as patient or physician's choice and 357 (17.0%) had an unknown reason. As the median times to discontinuation for these groups were over a year (13.0 months (IQR: 4.2-32.5 months) and 18.0 months (IQR: 5.3-40.6 months) respectively), it is unlikely that these patients experienced HSR.

Table 7.2: Reasons for discontinuation of abacavir.

Reason for discontinuation	Patients		Duration of ABC therapy at discontinuation (months), median (IQR)
	n	%	
All	2101	64.1	12.1 (3.0-29.4)
Hypersensitivity reaction	193	9.2	1.0 (0.3-1.2)
Treatment failure (virological, immunological and/or clinical failure)	344	16.7	16.0 (8.4-29.0)
Clinical fat abnormalities	45	2.1	31.7 (16.2-45.1)
Dyslipidaemia	9	0.4	28.1 (15.7-33.3)
Toxicity, predominantly from abdomen/G-I tract	102	4.9	3.6 (1.1-12.0)
Toxicity, predominantly from nervous system	24	1.1	4.6 (2.0-23.3)
Toxicity, predominantly from kidneys	4	0.2	22.4 (15.6-29.2)
Toxicity, predominantly from the endocrine system	5	0.2	10.0 (10.0-10.7)
Toxicity, haematological	10	0.5	6.5 (3.1-19.0)
Toxicity, hyperlactataemia or lactic acidosis	3	0.1	7.0 (0.5-9.0)
Toxicity, not mentioned above	74	3.5	3.0 (1.0-8.0)
Patient's wish, not specified above	291	13.9	8.5 (2.5-18.6)
Physician's decision, not specified above	316	15.0	20.7 (9.1-44.7)
Structured treatment interruption	41	2.0	16.9 (8.2-33.0)
Other causes, not specified above	283	13.5	19.0 (7.3-38.0)
Unknown	357	17.0	18.0 (5.3-40.6)

Notes: ABC = abacavir; G-I = gastro-intestinal; IQR = interquartile range.

7.3.4 Incidence of abacavir discontinuation and hypersensitivity reaction

7.3.4.1 Overall incidence of abacavir hypersensitivity reaction

The incidence of ABC discontinuation for any reason (including HSR) was 30.9 (95% CI: 29.6-32.2) per 100 PYFU, and for HSR specifically was 2.8 (95% CI: 2.4-3.2) per 100 PYFU. The incidence of ABC discontinuation due to HSR within three months was 22.1 (95% CI: 18.7-25.4) per 100 PYFU during 757 PYFU.

7.3.4.2 Incidence according to the line of therapy in which abacavir was received

A total of 252 (7.7%) patients started ABC as part of a first-line regimen, 952 (29.0%) started it as part of a second regimen, 1081 (33.0%) as part of a third and 993 (30.3%) as part of a fourth or subsequent regimen. The highest incidence of ABC discontinuation for any reason was in those who started ABC as part of a fourth/subsequent regimen (p=0.002). Incidence of HSR related discontinuation within the first three months was highest in those who started ABC as part of a first-line regimen (p=0.002). Table 7.3 displays the incidences of ABC discontinuation due to any reason and due to HSR only, according to the line of therapy within which ABC was received, as well as the proportions of patients in each group.

Table 7.3: Abacavir discontinuation due to any reason or due to hypersensitivity reaction within three months according to the line of therapy within which abacavir was received.

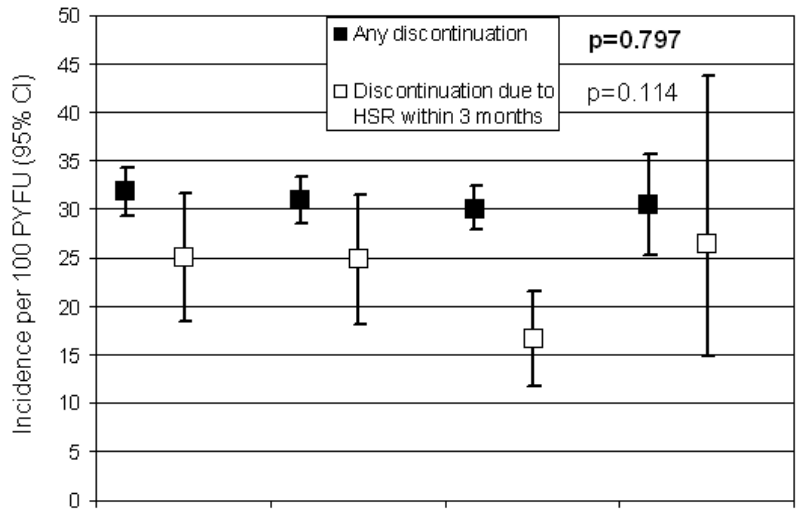
Number of patients who started ABC	Discontinued ABC due to *any reason* n (%)	Discontinued due to *HSR in 3 months*:		*Any* discontinuation: incidence per 100 PYFU (95% CI)	Total PYFU	Discontinuation due to *HSR in 3 months*: incidence per 100 PYFU (95% CI)	PYFU up to 3 months
		of all those who discontinued n (%)	of all those started ABC, %				
Overall: n=3278	2101 (64.1)	167 (7.9)	5.1	30.9 (29.6-32.2)	6803	22.1 (18.7-25.4)	757
First-line: n=252	171 (67.9)	27 (15.8)	10.7	32.3 (27.5-37.2)	529	48.8 (30.4-67.2)	55
Second-line: n=952	585 (61.5)	44 (7.5)	4.6	27.4 (25.1-29.6)	2138	19.9 (14.0-25.7)	222
Third-line: n=1081	714 (66.1)	52 (7.3)	4.8	31.5 (29.2-33.8)	2266	21.0 (15.3-26.7)	248
Fourth-line/later: n=993	631 (63.5)	44 (7.0)	4.4	33.7 (31.1-36.4)	1870	19.0 (13.4-24.6)	232
p value	0.095[(1)]	0.001[(1)]	<0.001[(1)]	0.002[(2)]	-	0.002[(2)]	-

Notes: *P* values obtained from [(1)]Chi-squared tests and [(2)]univariable Poisson regression models.
ABC = abacavir; HSR = hypersensitivity reaction; PYFU = person-years of follow-up; CI = confidence interval.

7.3.4.3 Incidence according to geographical region within EuroSIDA

A total of 929 (28.3%) patients were from the South region, 938 (28.6%) were from the Central West, 1164 (35.5%) were from the North and 247 (7.5%) were from the East. Overall there was no significant difference in incidence of discontinuation due to any reason and incidence of discontinuation due to HSR between regions (p=0.797 and p=0.114 respectively), however the North region appeared to have a lower incidence of HSR related discontinuation than the other regions. Results are shown in Figure 7.3.

Figure 7.3: Abacavir discontinuation due to any reason or due to hypersensitivity reaction within three months according to geographical region within EuroSIDA.



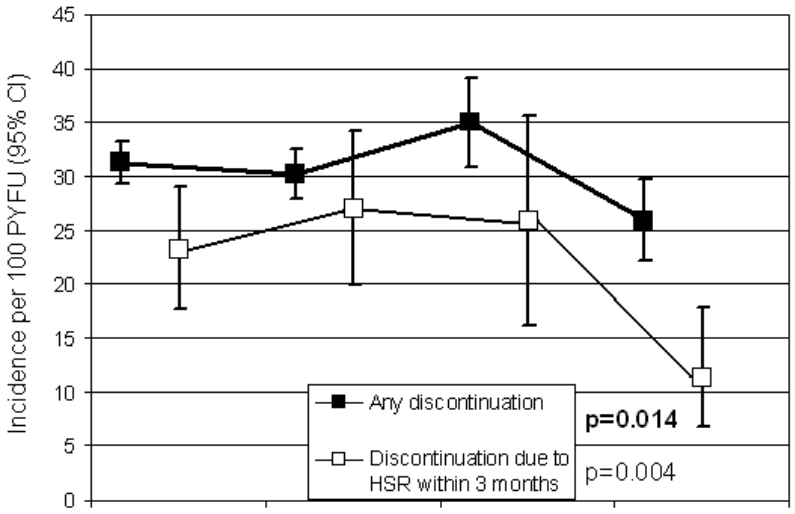
	South	Central West	North	East	*p*
n	929	938	1164	247	-
Discontinued due to any reason, n (%)	593 (63.8)	659 (70.3)	715 (61.4)	134 (54.3)	<0.001
Discontinued due to HSR:					
of all discontinued, n (%)	54 (9.1)	53 (8.0)	45 (6.3)	15 (11.2)	0.127
of all started, %	5.8	5.7	3.9	6.1	0.126
Total PYFU	1861	2130	2372	440	-
PYFU up to 3 months	216	214	271	57	-

Notes: *P* values obtained from Chi-squared tests and univariable Poisson regression models.
HSR = hypersensitivity reaction; PYFU = person-years of follow-up; CI = confidence interval.

7.3.4.4 Incidence according to date of initiation of abacavir

A total of 1245 (38.0%) patients started ABC in 1999-2000, 863 (26.3%) in 2001-2002, 450 (13.7%) in 2003-2004 and 720 (22.0%) in 2005 or later. Incidence of ABC discontinuation for any reason was similar over the time periods 1999-2000 and 2001-2002, followed by a slight increase in the years 2003-2004 and a drop in 2005 onwards, giving an overall significant difference (*p*=0.014). Incidence of ABC discontinuation due to HSR within the first three months of ABC treatment remained similar over the years until 2005 onwards when a sharp decrease to 11.4 cases per 100 PYFU during 166 PYFU was observed, also giving an overall significant difference (*p*=0.004), shown in Figure 7.4.

Figure 7.4: Abacavir discontinuation due to any reason or due to hypersensitivity reaction within three months according to date of initiation of abacavir.



	1999-2000	2001-2002	2003-2004	2005 onwards	*p*
n	1245	863	450	720	-
Discontinued due to any reason, n (%)	1012 (81.3)	631 (73.1)	282 (62.7)	176 (24.4)	<0.001
Discontinued due to HSR:					
of all discontinued, n (%)	67 (6.6)	54 (8.6)	27 (9.6)	19 (10.8)	0.126
of all started, %	5.4	6.3	6.0	2.6	0.006
Total PYFU	3169	2010	736	424	-
PYFU up to 3 months	287	200	104	135	-

Notes: *P* values obtained from Chi-squared tests and univariable Poisson regression models
HSR = hypersensitivity reaction; PYFU = person-years of follow-up; CI = confidence interval.

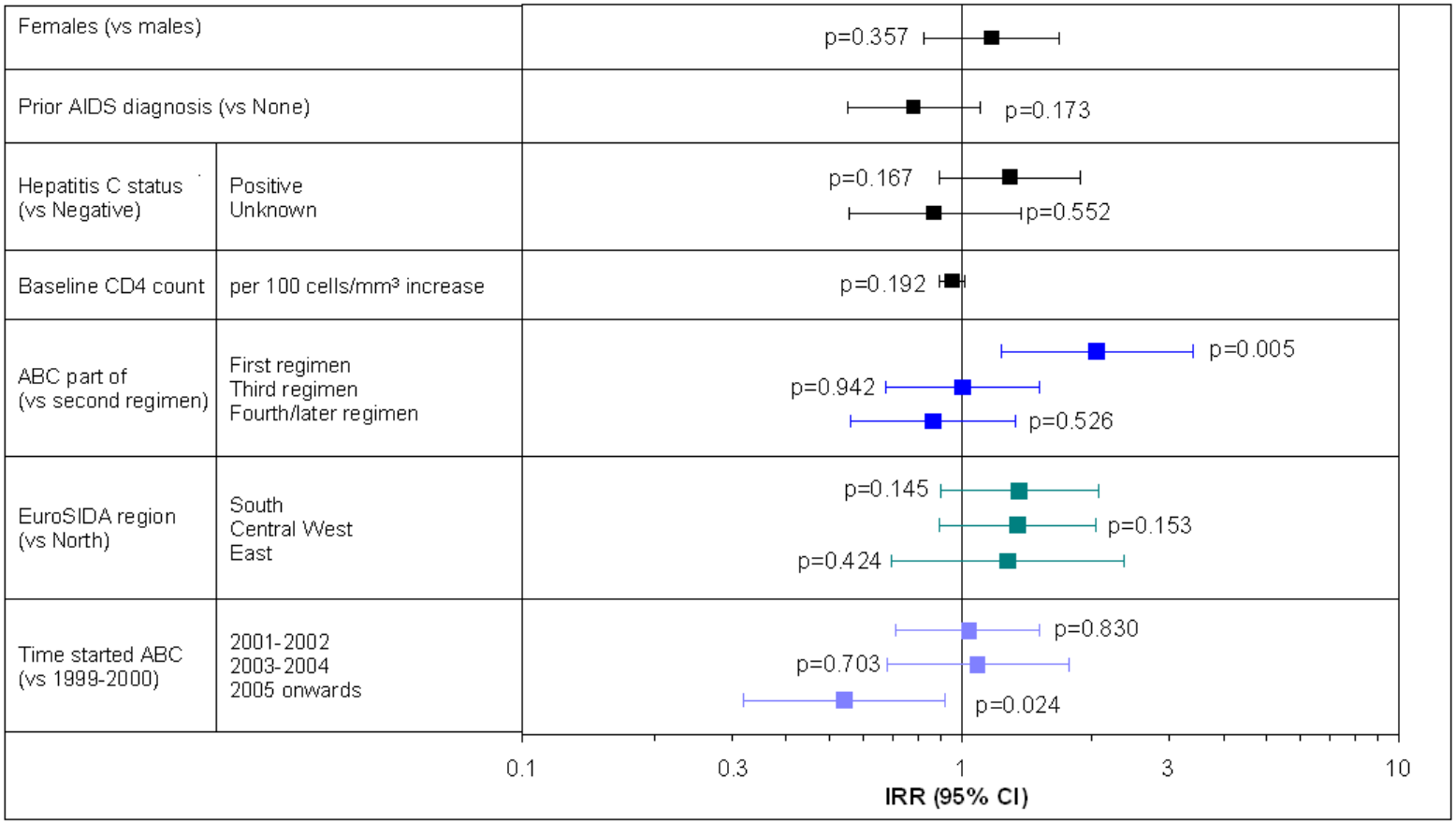
7.3.4.5 Incidence according to formulation of abacavir

A total of 928 (28.3%) previously ABC-naïve patients started ABC from January 2004 onwards, the date when Kivexa was first introduced into clinical practice. Of these, 342 (36.9%) patients started Kivexa. Overall, 305 (32.9%) patients discontinued ABC, of which 27 (8.9%) discontinuations occurred within three months and were associated with HSR. Incidence of discontinuation due to any reason was significantly higher in the non-Kivexa group: 38.6 (95% CI: 33.9-43.3) cases per 100 PYFU during 676 PYFU compared to 13.1 (95% CI: 9.3-17.0) cases per 100 PYFU during 335 PYFU in the Kivexa group (p<0.001). There was also a significant difference in incidence of discontinuation due to HSR within three months: 16.2 (95% CI: 9.4-23.0) cases per 100 PYFU during 136 PYFU in the non-Kivexa group, compared to 6.3 (95% CI: 2.0-14.6) cases per 100 PYFU during 80 PYFU in the Kivexa group (p=0.036).

7.3.4.6 Multivariable incidence rate ratios

In univariable models, female gender, positive hepatitis C coinfection status, lack of prior AIDS diagnosis and a lower baseline CD4 cell count were significantly associated with incidence of ABC discontinuation due to HSR within three months. The concurrent use of drugs such as NVP, EFV, APV and cotrimoxazole that can cause HSR, started at the same time as ABC, were investigated to check that symptoms arising from one of these drugs had not been misdiagnosed as ABC HSR. However none were found to be significantly associated with HSR related ABC discontinuation. Ethnicity was also not found to be associated with this, although the incidence rate was lower in those with non-white ethnicity compared to those with white ethnicity as expected from the literature, with an incidence rate ratio (IRR) of 0.66 (95% CI: 0.34-1.30; p=0.230). A multivariable model was then developed that contained the above factors significantly associated with HSR in univariable analyses (p<0.1), as well as whether ABC was a component of a first, second, third or subsequent regimen, geographical region and calendar time when ABC was started. Figure 7.5 displays the adjusted incidence rate ratios (IRRs) and 95% CIs from this model.

Figure 7.5: Multivariable incidence rate ratios and 95% confidence intervals of abacavir discontinuation due to hypersensitivity reaction within three months.



Note: ABC = abacavir; IRR = incidence rate ratio; CI = confidence interval;

This multivariable model provided evidence that the incidence of ABC discontinuation due to HSR amongst patients starting ABC as part of a first-line regimen was twice that observed amongst those starting ABC in a second-line regimen (IRR: 2.04; 95% CI: 1.24-3.38; *p*=0.005). Incidence of HSR was similar across regions although was higher in all regions compared to the North. There was also evidence that amongst patients starting ABC in 2005 onwards, the incidence of HSR related discontinuation was almost half that observed amongst patients starting ABC in 1999-2000 (IRR: 0.54; 95% CI: 0.32-0.92; *p*=0.024).

A multivariable model was developed containing the same variables as before, but with date started ABC adjusted for instead of time period, and with the addition of whether patients received Kivexa or Ziagen/Trizivir. After adjustment, there was evidence of a significantly lower incidence of ABC discontinuation due to HSR within three months in those starting Kivexa compared to those starting Ziagen/Trizivir (IRR: 0.33; 95% CI: 0.13-0.88; *p*=0.027).

7.3.5 Rate of death associated with abacavir hypersensitivity reaction

There were no fatal cases of HSR registered during 6969 PYFU; no patients died on ABC therapy or within a month of stopping the drug with HSR recorded as the cause of death, and no patients discontinued ABC therapy due to HSR and died within one month of discontinuation. A total of 111 (3.4% of all those who started ABC) patients died on ABC therapy or within one month of a non-HSR related discontinuation. Of these, 55 had specific non-HSR related causes of death recorded and of the remaining 56 patients, 33 had a previous AIDS diagnosis and 36 had extensive treatment experience and had received at least two or three PI/NNRTI-containing regimens prior to the ABC regimen. Amongst these 111 patients, 15 died within three months of starting ABC. The investigators classified 13 of these 15 deaths as being due to specific and mainly AIDS related causes, rather than as a result of HSR. The two remaining patients had unknown causes of death but both died with low CD4 counts of 106 cells/mm^3 (measured two months before date of death) and 120 cells/mm^3 (measured 16 days before date of death). The characteristics of all 111 patients who died are summarised in Table 7.4, as well as characteristics of the subgroup of 15 patients who had received less than three months of ABC therapy at the time of death.

Table 7.4: Characteristics at time of death of patients who died on abacavir therapy or within one month after abacavir discontinuation.

		All	ABC treatment < 3 months
n (%)		111 (3.4)*	15 (0.5)*
Cause of death:	AIDS related	24 (21.6)	6 (40.0)
	Cardiovascular disease	15 (13.5)	2 (13.3)
	Liver failure/hepatitis	10 (9.0)	1 (6.7)
	Lactic acidosis	2 (1.8)	0 (0.0)
	Suicide	2 (1.8)	0 (0.0)
	Renal failure	1 (0.9)	0 (0.0)
	Pancreatitis	1 (0.9)	0 (0.0)
	Other	36 (32.4)	4 (26.7)
	Unknown	20 (18.0)	2 (13.3)
Prior AIDS		62 (55.9)	9 (60.0)
ABC started as part of:	First PI/NNRTI/ABC regimen	9 (8.1)	1 (6.7)
	Second regimen after starting first	31 (27.9)	4 (26.7)
	Third regimen	38 (34.2)	6 (40.0)
	Fourth or later regimen	33 (29.7)	4 (26.7)
No. drugs in regimen (excluding ABC)	1-2	4 (3.6)	2 (13.3)
	3	48 (43.2)	4 (26.7)
	4-5	51 (45.9)	9 (60.0)
Median (IQR)			
Date of death		Oct 02 (Jan 01-Sep 04)	May 00 (Nov 99-Jan 02)
Duration on ABC (months)		18 (8-37)	1 (0-2)
CD4 count (cells/mm^3)		227 (84-361)	90 (33-175)
Viral load (log10 copies/mL)		2.3 (1.7-4.4)	4.6 (2.7-5.3)

Notes: *Proportion of 3278 patients who started abacavir.
ABC = abacavir; IQR = interquartile range.

7.4 Discussion

The results from these analyses found that amongst 3278 HIV-1 infected patients who started ABC across Europe, the incidence rate of ABC discontinuation for any reason (including HSR) was 30.9 (95% CI: 29.6-32.2) per 100 PYFU. The incidence of ABC discontinuation due to HSR within three months was 22.1 (18.7-25.4) per 100 PYFU and this occurred in 5.1% of patients who started the drug.

HSR was the most common named toxicity reported in patients who discontinued ABC, although the main reason for discontinuation was treatment failure. The main findings were that in a multivariable model adjusting for potentially confounding factors, there were significantly higher incidence rates of HSR (within three months) when ABC was started in a first-line regimen compared to a second-line regimen and in the time period 2005 onwards compared to earlier years. The geographical region that patients were seen in was not associated with HSR incidence after adjustment. Patients starting Kivexa compared to those starting Ziagen or Trizivir were observed to have a lower incidence of ABC discontinuation due to HSR but the person-years available in the

subgroup of patients starting Kivexa were limited. Finally, there were no deaths that were attributed to HSR in the one-month period after discontinuation of the drug.

7.4.1 Interpretation of findings and previous research

The overall incidence of HSR in patients starting ABC found in this dataset was in line with the 4-8% found in other studies [40,41,234-236]. HSR was assessed by physicians in each clinical centre based on the interpretation of clinical symptoms. It is unknown as to whether or not the results of skin patch tests were used as well. Patch tests appear to correlate well with immunological and genetic testing and may be useful in defining true HSR, however their use in diagnosis remains experimental at present [509,512,513]. The incidences reported in this analysis therefore indicate a practical "real life" approach to the diagnosis of HSR and possibly reflect clinical diagnosis rather than true HSR. Recent discussion at the 4th IAS Conference 2007, Sydney, Australia, identified the difficulties in diagnosing HSR correctly and the consequences of misclassification in studies [527].

7.4.1.1 Incidence of hypersensitivity reaction according to the line of therapy

In this analysis, the adjusted HSR incidence rate was found to be twice as high when ABC was started as part of a first-line regimen compared to a second-line regimen. This supports research by Symonds *et al.* (2002) that showed a 42% decreased odds of HSR in patients with prior treatment experience in a multivariable model [516]. Patients starting therapy for the first time may be more likely to be clinically diagnosed with suspected HSR, which may not reflect the incidence of true HSR cases in this group. New patients may be more likely to report all symptoms experienced due to anxiety about starting treatment whereas more experienced patients may not mention the more minor symptoms. Physicians may also be more cautious with new patients who have no previous history of ART. This may result in physicians misdiagnosing HSR more often in patients starting first-line therapy to ensure that no true HSR cases are missed.

HLA-B*5701 is linked to ABC related HSR in many studies [502-506]. The HLA human genes help in the body's immune defence by allowing CD8 cells to recognise cells infected by pathogens [528]. It is also well-established that HLA-B*5701 is associated with long-term non-progression of HIV [528-530]. HIV infected individuals who do not experience disease progression may start therapy later and hence this allele may be under-represented in the groups of patients starting ABC as part of a second-, third- or fourth-line regimen. This could be another explanation for the increased incidence of HSR in the group starting ABC in a first-line regimen. HLA-

B*5701 is more often found in Caucasian patients than in non-Caucasians and therefore ethnicity could be a marker for the presence of this allele [502,504,505]. The frequency has been found to be 5-8% in Caucasians, 4-7% in Hispanics, less than 1% in Asians and rare in Sub-Saharan Africans [531,532]. However the analyses in this chapter found no significant association between white ethnicity and risk of HSR and so ethnicity was ruled out as a potential confounder in this dataset. This may be due to limited power as there were very few non-Caucasians included in the study population. Just 7.7% of patients who met the inclusion criteria were non-Caucasian.

7.4.1.2 Incidence of hypersensitivity reaction according to geographical region

There was no evidence of a significant regional difference in ABC discontinuation due to HSR within the first three months in this analysis. The frequency of HLA-B*5701 is known to vary across different populations and differences in frequency have been found across Europe [511,531,532]. Therefore, a possible explanation for this finding is that in areas of low prevalence of the allele, there may be more cases of clinical over-diagnosis of suspected HSR resulting in an overall similar incidence of ABC HSR between regions.

7.4.1.3 Incidence of hypersensitivity reaction according to date started abacavir

A significantly higher incidence rate of HSR was found in earlier years compared to the time period 2005 onwards. A reason for this could be that more clinics are using prospective genetic screening, which has been shown to effectively reduce the incidence of HSR by preventing the prescription of ABC to those at high-risk [503,505,506,508-512]. A study looking at an ethically mixed French HIV infected population showed that introducing prospective screening reduced the incidence of ABC related HSR from 12% to 0% [505]. Rauch *et al.* (2006) also found a significant drop in the incidence of true HSR (confirmed by skin patch tests) in 260 ABC-naïve patients from the Western Australian HIV Cohort Study, from 8% before the introduction of screening, to 2% after its implementation [508]. The development of low-cost, rapid, accurate tests may have allowed more widespread use of genetic screening in more recent years [533,534]. Another reason for the decrease observed in 2005 could be that as HSR symptoms are now better documented, HSR may be more recognisable and therefore correctly identified more frequently, reducing the chance of over-diagnosis. If symptoms were misclassified as HSR in earlier years, this could explain the decrease in recent years.

7.4.1.4 Incidence of hypersensitivity reaction according to formulation of abacavir

A decreased incidence of ABC discontinuation due to HSR within three months was observed in patients starting Kivexa compared to those starting Ziagen or Trizivir. The Trizivir Epidemiology Study found no differences in the frequency of ABC HSR between patients starting either Ziagen or Trizivir [501]. However, to date there have been no studies that have compared the incidence of HSR between patients starting Kivexa and other formulations of ABC. In this analysis, all known, measured variables that potentially may have confounded the results were investigated but a difference remained after adjustment. This may be due to the relatively small number of person-years available for the Kivexa group and the small number of events during this time and so these results should be treated with caution.

7.4.1.5 Hypersensitivity reaction related deaths

Severe ABC related HSRs, which can lead to death, are rare but have been reported in a few case studies [498-500]. In this analysis, there were no reports of patients who discontinued ABC due to HSR and then died within one month. Patients who died on ABC therapy or within a month after stopping due to a reason not recorded as HSR mostly had advanced disease with known non-HSR causes of death. Of patients who died within three months of starting ABC, only two had unknown causes of death. However as recent CD4 counts at time of death were low for these patients, it is likely that immunodeficiency was the cause of death. This suggests that any patients with severe reactions to ABC were taken off the drug promptly to prevent fatality.

7.4.2 Limitations

The main limitation of the analyses in this chapter was that the incidence rates can only reflect clinical diagnosis of HSR, rather than cases confirmed to be immunologically "true". However, it is interesting to observe these trends across the different patient subsets defined in this chapter, to identify patients more likely to be given a diagnosis of HSR. No standardised guidelines were used to define HSR and cases of HSR were not reviewed centrally, therefore reporting bias between centres cannot be ruled out. Some centres may be more likely to be cautious about stopping ABC treatment if they suspect HSR than others, which may be a source of confounding. Furthermore, the follow-up forms only collect one reason for discontinuation per drug. As some patients may experience more than one toxicity, it is possible that HSR is not always reported. The forms also allow 'patient's wish' and 'physician's decision' as reasons for discontinuation, which could result in misclassification.

Another limitation is that EuroSIDA does not collect information on genetic screening. Thus, it is unknown as to whether or not patients were tested for HLA-B*5701 and prescribed ABC according to results. There is also no information on CD8 cell count, which was identified by Easterbrook *et al.* (2003) as a factor associated with HSR (i.e. the higher the baseline CD8 count, the higher the risk of HSR) [494].

Although it is assumed in this study that all deaths are recorded on the follow-up forms, it is possible that some were missed. Causes of death could also be misclassified or coded differently in different centres. The introduction of the CoDe ("Coding of Death in HIV") project in cohort studies (details at www.cphiv.dk) has helped to standardise the approach to collecting and reviewing causes of death.

7.4.3 Implications of findings

The results of these analyses identified that HSR diagnoses occurred more frequently in patients starting ABC in a first-line regimen than in later regimens, which may not necessarily reflect the situation when looking at true cases of HSR. Clinical over-diagnosis may prevent patients who would benefit from ABC use from continuing to take the drug but is preferable to the risk of fatality from HSR that accompanies continued use. The wider use of prospective genetic screening may allow physicians to assess individual patients' risk of HSR more accurately. The decrease in incidence of ABC related HSR observed in recent years could indicate that this is becoming the case and that with a greater awareness of the factors that are associated with this reaction, doctors are able to avoid prescribing ABC to those most at risk.

7.4.4 Further research

Further data that would be useful to collect is whether or not genetic screening was used prior to prescribing ABC, whether or not skin patch tests were used to confirm HSR and all reasons for discontinuation per drug, rather than just one reason. This would establish more clearly the extent of true HSR and whether or not physicians had prior knowledge of genetic factors that would affect the risk of HSR. It would be helpful to have more details about patients who discontinued ABC due to patient or physician choice as symptoms experienced may have led the patient or physician to decide to stop the drug; these symptoms may have been early signs of HSR.

As the incidence of HSR related discontinuation of ABC only decreased in 2005 onwards in these analyses and was observed to be fairly stable over calendar time before then, it would be interesting to continue to monitor HSR incidence over the next few years to see if the incidence rate continues to drop. It would also be interesting to

investigate whether these trends are observed in each geographical region, which would require a larger study population.

Further analyses could also compare the short and long-term reasons for discontinuation of other drugs to ABC and look at the factors affecting this. Some patients may be more likely to stop any regimen in the first three months and the factors associated with this may not be ABC-specific.

7.4.5 Conclusions

Incidence of ABC discontinuation due to HSR appears to be higher in patients starting ABC as part of first-line therapy, which may be explained by increased clinical over-diagnosis. It has decreased in recent years, suggesting that prospective genetic screening, improved patient care and awareness of the symptoms of HSR may have prevented the use of this drug for high-risk patients. There appears to be a similar rate of ABC discontinuation due to HSR across Europe. Patients starting the co-formulation drug, Kivexa, which was introduced in January 2004, appear to have a decreased risk to those starting ABC as the single tablet, Ziagen, or as part of Trizivir, however limited data are available for the use of Kivexa and so results remain preliminary. There were no reported deaths due to ABC HSR in this analysis.

A manuscript of this analysis was published by Antiviral Therapy in July 2008 and can be found in Appendix X.

Chapter 8. Conclusions

Since combination antiretroviral therapy (cART) was introduced into clinical practice across North America and Western Europe in 1995-1996, mortality and morbidity rates in HIV infected individuals have been reduced significantly [118-121,139,140,179]. Antiretroviral drugs inhibit HIV from successful replication, which protects the immune system from further damage and allows restoration of the key target host cells, CD4 cells [40,41,182-186]. This protects the body from opportunistic infections that can lead to AIDS or death [187].

Factors strongly associated with a successful response to therapy include a potent and tolerable regimen [180,181,185,202-205,290], good adherence (more than 95%) [192] and low levels of HIV drug resistance [16,318,319]. The aims of this thesis were to investigate specific factors potentially associated with virological and immunological responses to cART, including HIV drug resistance and tolerability of a specific drug, in a large observational cohort study of HIV-1 infected individuals.

8.1 Summary of main findings

8.1.1 Virological response to first-line combination antiretroviral therapy across geographical regions and over calendar time

In Chapter 3, differences in virological response rate were analysed across geographical regions within calendar time periods, corresponding to when patients started a cART regimen for the first time, and also over calendar time within each region. Multivariable analyses were carried out on ART-naïve patients to eliminate patients with a history of ART use who may have accumulated HIV drug resistance that would confound the results. As it is now common for patients to start cART for the first time from ART-naïve, these results are widely applicable to newly diagnosed HIV infected individuals across Europe.

One of the purposes of these analyses was to explore whether or not patients starting therapy for the first time in different regions had similar chances of responding successfully and to see if this had been the case in different time periods. Another was to monitor trends in virological response over time within each region to see if improvements in patient care and management were reflected in response rates throughout Europe. Analyses were adjusted for type of cART regimen to see if there was an additional effect of calendar time or region after accounting for differences in regimen.

The results showed some variation between regions of EuroSIDA in virological response to first-line cART. This was most apparent in early-cART years (1996-1997) when the East region had a comparatively inferior response rate and Central West had the highest rate. In mid-cART (1998-1999) and late-cART (2000-2004) years, virological response rate in the East region was still generally lower than other regions, however response rates were more similar across all regions than in the earlier years. This suggests that the standard of patient care, toxicity management, patient information and availability of new potent antiretroviral drugs have become more uniform across Europe since the introduction of cART and fits in with previous EuroSIDA findings that showed a decrease in regional variation of the type of cART regimen received over calendar time [383].

Another finding was that there were significant improvements in virological response in all EuroSIDA regions, but especially in East Europe, where a large-scale HIV epidemic has been growing rapidly since the mid-1990s [83]. These improvements may be explained by better patient adherence to their treatment due to improved toxicity management and clinical support, and increased access to new antiretroviral drugs. Previous research has also found improvements in virological response over calendar time [379-382]. These increases in virological response observed in ART-naïve patients suggest that transmitted HIV drug resistance has not yet had a clinically significant impact and may even be decreasing in the population. Recent data have suggested this is the case in the UK [394]. This question was further researched in Chapter 5 of this thesis and no significant trends over time were observed.

These results highlight the key role of a study like EuroSIDA to monitor trends and potentially signal variability in type of care and treatment outcomes across Europe.

8.1.2 HIV-1 subtypes and virological and immunological response to combination antiretroviral therapy

HIV-1 is classified into a number of genetically distinct subtypes, A-D, F-H, J and K, as well as circulating recombinant forms including the most common CRF01_AE and CRF02_AG strains [344,355,399,401]. Subtype B is largely predominant across North America and Western Europe. As antiretroviral drugs have historically been designed based on biological and clinical trial data from these regions, it is uncertain as to whether patients infected with strains more prevalent elsewhere in the world will respond as successfully as those infected with subtype B.

Chapter 4 investigated the prevalence of HIV-1 subtypes in EuroSIDA patients who had the information available and found that of the patients selected for inclusion in these analyses, the majority (86.5%) were infected with subtype B as expected. Subtypes A, C, CRF01_AE and CRF02_AG were the next most prevalent strains. Virological and immunological response to cART were compared between patients infected with B and non-B subtypes in multivariable analyses and no significant differences were found, which supports findings from previous research [422-429]. However, the power to detect true differences was limited due to the small number of patients infected with a non-B virus. Comparison of A, C and 'other' subtypes with B subtype showed slightly higher odds of a virological response in patients infected with C or 'other' and borderline significantly lower odds in those infected with A, which could explain the overall result of no difference between B and non-B subtypes. It is recognised that a larger sample size is needed in order to gain the power to draw firm conclusions, which could be obtained by combining data from a collaboration of studies. With a larger dataset, it may be possible to analyse specific non-B subtypes in more detail.

If the finding of no significant difference in response to cART between HIV-1 B and non-B is to be taken as true, this suggests that the genetic distinctions and also differences observed in resistance development between subtypes [399,406,411,413-417] do not seem to have a clinically significant impact on overall response rates. Results from studies such as this have important implications for the resource-limited setting where the majority of patients have non-B HIV-1 subtypes and are receiving the same antiretroviral drugs as patients treated in the Western world.

8.1.3 Transmitted drug-resistant HIV-1 and association with virological and immunological response to first-line combination antiretroviral therapy

Transmitted drug resistance (TDR) is a potential problem for patients starting therapy for the first time as it could hinder the chances of successful viral suppression. In Chapter 5, the prevalence of TDR in ART-naïve EuroSIDA patients was investigated across different patient subsets. Overall 11.4% of patients with genotypic resistance test results available from tests performed retrospectively, on plasma samples stored prospectively whilst ART-naïve, were infected with a resistant strain. This was in line with the estimates reported by other European studies [348,436,440,443,460]. No evidence was found to support a significant difference in the odds of detecting TDR over calendar time, between HIV-1 subtypes B versus non-B, or between Central West and East geographical regions compared to the North region within EuroSIDA, in

multivariable analyses. Significant higher odds of TDR were found in the South region compared to the North.

Previous research showed conflicting findings in whether or not TDR was on the increase or decline [388-394,447,461]. The increase observed in a number of studies could be explained by the wider accessibility to ART leading to an increase in the selection of drug-resistant strains. It could also be due to increasing high-risk behaviour in patients on treatment [388-393]. However the most recent data from the UK showed a decrease that may be explained by improved virological control in patients on therapy, management of treatment failure and the fact that the most infectious HIV infected people are not receiving ART [394].

Virological and immunological response to first-line cART was compared between patients infected with HIV with full/intermediate resistance to at least one drug started and those with HIV susceptible to all drugs started in multivariable analyses. No significant difference was found in the odds of virological suppression, although there was a non-significant trend towards decreased odds in patients with resistant HIV. The analyses had limited power due to the relatively small number of patients in this group. Significantly increased odds of immunological response were found in patients with TDR, which could be explained by a reduced viral fitness in resistant strains that may reduce immunological deterioration [464], but could likely be a result of the natural variability in the data. As with the analyses in Chapter 4, these analyses would benefit from a larger dataset. The collaboration of studies known as EuroCOORD is planning to investigate TDR in the near future.

8.1.4 Comparison of genotypic resistance profiles and virological response between patients starting nevirapine and efavirenz

At present, the two most commonly used NNRTIs in clinical practice across the world are nevirapine (NVP) and efavirenz (EFV). Given their high potency and low cost these are the drugs recommended by the World Health Organisation for use in first-line ART in resource-limited settings [471,473]. Previous findings from a number of cohort studies, including a previous EuroSIDA analysis of mostly NRTI/PI-experienced patients, have highlighted an inferior virological response in patients starting NVP for the first time compared to EFV [300-304]. In contrast, a large randomised clinical trial of ART-naïve patients (known as 2NN) found no significant difference in treatment failure in their main analysis, although the study failed to demonstrate equivalence after being powered to investigate this. Also, results from a sensitivity analysis excluding a small number of patients who were randomised to either of the groups, but who never

started their treatment, did find results supporting those from the observational studies [299].

As at the time of the previous EuroSIDA analysis, resistance data were not available, it was uncertain as to whether or not a different prevalence of pre-existing resistance in patients starting NVP or EFV may have confounded the comparison. In Chapter 6, the analysis was repeated taking into account the new genotypic resistance data from tests performed retrospectively on stored plasma samples. The results were found to be consistent with previous findings showing that those starting EFV had a 50% reduced risk of virological failure compared to those starting NVP. In addition to controlling for demographics, previous ART and baseline NNRTI resistance, this more recent analysis was also adjusted for the predicted susceptibility to the drugs included in the regimen besides NVP and EFV. NNRTI-resistant HIV was detected in 3.4% of patients at baseline with similar levels in both NVP and EFV groups.

Different resistance profiles emerged over the course of treatment in the two groups. NNRTI resistance was detected in the majority (85.5%) of patients at time of virological failure and was similar between the groups. Resistance profiles were compared in those who were still on an NNRTI at time of virological failure and with resistance test results available. The K103N mutation was found to be more prevalent in those who failed on EFV and the Y181C and G190A mutations were more prevalent in those who failed on NVP, consistent with previous research [487-489].

Since this was not a randomised comparison, there may still be unmeasured confounding variables that may have biased the results. These may be common to all the observational studies that found similar results, therefore this highlights the need for more randomised trials to compare the virological efficacy of NNRTIs in both NRTI/PI experienced patients as well as ART-naïve patients. Although the 2NN randomised study concluded that there was no significant difference in the risk of treatment failure between patients starting NVP and EFV, the risk was higher in those starting NVP and it could be argued that this was of borderline statistical significance (significantly higher at the 10% level although not at the 5% level) and should be investigated further.

As far as future prospectives in the observational setting are concerned, there is a plan to extend the sequencing to other regions of HIV RNA to definitely rule out the possible confounding due to a different prevalence of pre-existing resistance not detected by standard sequencing of the RT region (the Connect project within EuroSIDA).

8.1.5 Toxicity of antiretroviral therapy: safety of abacavir

As briefly mentioned above, access to tolerable treatment is one of the key components for therapy success as toxicities are a major reason for non-adherence and may also result in treatment discontinuation, switches or interruptions [197,204,493]. Chapter 7 investigated a toxicological response to therapy, focusing on the reasons for discontinuation of one NRTI, abacavir (ABC), in particular the potentially fatal adverse effect, hypersensitivity reaction (HSR). Incidence of discontinuation and specifically discontinuation due to HSR was compared across different patient subsets, which is important to identify patients most at risk and to monitor trends in a large heterogeneous population across Europe.

Amongst patients who started ABC, HSR was the most commonly named toxicity reported in patients who discontinued. It was found that 5.1% discontinued due to HSR within three months of initiation in line with other studies [40,41,234-236]. Incidence of ABC discontinuation due to HSR was found to be significantly higher in patients starting ABC as part of first-line therapy than in those starting the drug in a second-line regimen, which may be explained by increased clinical over-diagnosis and not necessarily true cases of HSR. The HLA-B*5701 allele, which has been linked to ABC related HSR in many studies [502,503,507,508], is also associated with long-term non-progression of HIV [528-530]. Therefore, genetically susceptible patients carrying this allele may have been under-represented in the groups starting ABC in second/third/fourth line regimens as they may start therapy later.

Incidence decreased in recent years, suggesting that prospective genetic screening for the HLA-B*5701 haplotype, improved patient care and awareness of HSR may have prevented the use of this drug for high-risk patients. Similar rates of discontinuation of ABC due to HSR were observed across EuroSIDA regions. Patients starting the co-formulation drug, Kivexa (introduced in January 2004), appear to have a decreased risk to those starting ABC as the single tablet, Ziagen, or as part of Trizivir, however limited data are available for the use of Kivexa and so results remain preliminary. There were no reported deaths due to ABC HSR in this analysis, suggesting that any patients with severe reactions were taken off the drug early enough to prevent fatality.

Recently the use of ABC has been also associated with an increased risk of myocardial infarction in the D:A:D study [533]. These findings were unexpected and the biological mechanisms by which the risk is increased are at present unknown. The US Food and Drug Administration (FDA) and European Medicines Agency (EMEA) are both reviewing the safety data on ABC in relation to myocardial infarction [534].

8.2 Main limitations of analyses

The research questions addressed in this thesis were investigated using data from a large prospective observational cohort, which carries the general limitations of all observational studies. Analyses are adjusted to take into account potentially confounding factors that may bias the results, however as previously discussed in Chapter 1 (section 1.6.1) there may be unmeasured or unknown factors that cannot be adjusted for and so bias cannot be ruled out, only minimised. Randomised controlled trials are the gold standard for comparisons of drug interventions, as factors that may affect the outcome should be balanced between groups resulting in unbiased comparisons. Chapter 6 comparing NVP and EFV would benefit from trial data to address this question. However, it is not possible to conduct trials to compare naturally occurring subsets of patients, e.g. patients infected with different HIV-1 subtypes, and so for this type of research question, observational study data are required. The benefits of observational studies are the longer follow-up time collected for patients, the lack of strict inclusion/exclusion criteria that can result in quite a specialised selection of patients, and the opportunity to study long-term or rare effects of treatment.

One of the main limitations throughout this thesis was the lack of statistical power for certain analyses due to limited numbers of patients in certain subsets. In Chapter 3, there were few EuroSIDA patients starting cART in 1996-1997 in East Europe due to the fact that EuroSIDA started collecting data from East European countries later than the rest of Europe. The small number of non-B HIV-1 subtypes limited the analyses in Chapter 4 and there were few patients starting cART with TDR in Chapter 5. Collaborative studies are a good way to pool data in order to maximise power, although a disadvantage is the variability across the different datasets in frequency and quality of data collection, as well as the specific information collected.

A further question when interpreting the results is how representative the patients included in EuroSIDA are of the general European population. Although many clinics across Europe are represented in EuroSIDA, those not included may differ in terms of clinician experience, treatment decisions and availability of ART. Patients were entered into the study consecutively within the seven cohorts within EuroSIDA, which should give a good representation of patients attending each clinic. Due to the large number of clinics, it could be argued that the sample is broadly representative of centres across Europe and more so than any one clinic cohort.

A further general limitation of the dataset analysed in this thesis is the fact that there are missing values. Data can be missing for a variety of unknown reasons and it is

possible that if merely excluded, this could bias the results. EuroSIDA is a prospective observational study but attempts to collect complete treatment histories when a patient is enrolled. Retrospective collection of data is likely to result in missing values. Patients may be too ill to travel to a clinic for a follow-up visit, which could mean that patients not responding to treatment are excluded from analyses, when there may be a common reason as to why they are not responding. Two common methods of dealing with missing viral loads or CD4 counts were considered throughout, i.e. excluding the patients with missing values or counting the missing values as treatment failures. Conclusions were drawn having evaluated the results based on the main analysis as well as sensitivity analyses to allow for potential biases in the choice of statistical method.

8.3 Overall implications of findings

This thesis investigated trends in response to ART to identify factors that could affect a patient's chance of a successful response. This is useful for many reasons. Improvements in virological response, the lack of an increase in transmitted drug resistance and the decrease in the incidence of the potentially fatal toxicity of ABC HSR over calendar time, indicate that treatment strategies, physician knowledge and awareness of the effects of treatment, clinical support and toxicity management have all contributed to the success of ART and the increasing standard of HIV patient care across Europe. Genetic variability of the virus has not yet proved to be a significant factor in the chance of responding to treatment, however it is recognised that this needs to continue to be monitored as studies investigating this have been fairly small-scale or lacking in the power to make statistical comparisons. There appears to be evidence of a difference between the two main NNRTIs used worldwide, which if true, could potentially have major implications for treatment policy. At present, NVP is a popular choice for first-line therapy in developing countries due to its low cost, availability and ease of taking. Results from EuroSIDA, as well as a number of other studies, suggest that this could be a second-rate treatment compared with EFV. However the studies may be biased by unmeasured or unknown confounding factors.

8.4 Further research

This thesis covers issues that are currently of interest in HIV research, namely, recent trends in response to cART, the genetic variability of the virus including drug resistance, a comparison of two frequently used antiretroviral drugs and an adverse effect of another drug commonly prescribed in clinical practice. Over the next few years, it is important to continue to monitor trends in response to treatment and in HIV

drug resistance, and observational cohort studies present the ideal setting to study a large heterogeneous population over a long period of time. This thesis has identified the need for more randomised controlled trials to compare the two most widely used NNRTIs, NVP and EFV. The newer NNRTIs such as etravirine (TMC-125) and others currently being developed or in the trial stage, may prove to be useful alternative options in the future. During the preparation of this thesis, a number of new drugs have been approved by the FDA and the EMEA, including TMC-125, a PI (darunavir), an entry inhibitor (maraviroc), as well as a drug from a new drug class (an integrase inhibitor, raltegravir). There are also further new drug classes in the experimental stage [215], which has provided hope for patients on salvage therapy who have extensive ART experience and possibly multi-drug resistance. Monitoring the response to therapy, including any adverse reactions, over the coming years in patients starting these new drugs is essential to guide physicians in terms of appropriate prescription of treatment.

Understanding more about the genetic diversity of the virus is important to ensure that the drugs being developed have universal efficacy. Travel and migration are on the increase, which means that different strains of the virus are spreading worldwide. There is still a lot to learn about drug resistance and the combinations of genetic mutations that may predict whether or not a patient will have a successful response to treatment. The genetic interpretation systems currently in use are constantly updated based on the latest research.

This thesis concentrated on virological and immunological endpoints. Ideally, it is clinical endpoints (AIDS and death) that are ultimately important and should be studied. Realistically, as treatment and patient management are improving all the time and keeping infected individuals alive for longer, they are fortunately becoming increasingly rare, making it difficult to study these endpoints. With a much larger dataset, it may be possible to investigate them in some analyses.

As discussed above, one of the main limitations of some of the analyses in this thesis was the lack of statistical power to detect true differences between groups of interest. This thesis would benefit from further HIV RNA sequence data in order to evaluate HIV drug resistance and to determine subtype. This is an area that is being expanded in EuroSIDA and in the future, these analyses may be repeated in order to draw conclusions with more certainty. At present, EuroSIDA is also recruiting patients for an eighth cohort, which will provide an additional 2500 patients, including 1250 in East European countries. It also provides data for the D:A:D study and is a part of the

ongoing collaborative studies, EuroCOORD (The European Coordinating Committee for the Integration of Ongoing Coordination Actions Related to Clinical, Virological and Epidemiological HIV Research), COHERE (Collaboration of Observational HIV Epidemiological Research Europe) and the ART Cohort Collaboration.

8.5 Concluding remarks

This thesis has compared a variety of different responses to antiretroviral therapy across subsets of a large heterogeneous HIV infected population across Europe (and a minority from Israel and Argentina). The main aim was to study a number of factors potentially associated with outcomes of therapy that have not been previously investigated in a large prospective cohort study with such a substantial amount of data collected, including analyses based on the link between the clinical data and the increasingly growing EuroSIDA genotypic resistance database. The continued expansion of EuroSIDA will allow more powerful analyses in the future with stricter inclusion criteria and will permit further stratifications of variables of interest for more detailed analyses. It is hoped that these findings will contribute to research in monitoring trends in response to therapy and help to provide insight into association with the vast genetic variability of the virus.

Appendix I. International AIDS Society (IAS)-USA. Update of the drug resistance mutations in HIV-1: Spring 2008

Reprinted with permission from the International AIDS Society–USA. Johnson VA, Brun-Vézinet F, Clotet B, et al. Update of the drug resistance mutations in HIV-1: Spring 2008. Topics in HIV Medicine. 2008; ***16****(1): 62-68. ©2008, IAS-USA. Updated information [add: "(and thorough explanatory notes)" if the user notes are not reprinted] is available at www.iasusa.org.*

Update of the Drug Resistance Mutations in HIV-1: Spring 2008

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This Spring 2008 version of the International AIDS Society–USA (IAS-USA) Drug Resistance Mutations Figures updates the figures published in this journal in August/September 2007.[1] The authors comprise the IAS-USA Drug Resistance Mutations Group, an independent, volunteer panel of experts charged with the goal of delivering accurate, unbiased, and evidence-based information on these mutations to HIV clinical practitioners. As for all IAS-USA panels, a rotation procedure is in place whereby 1 or 2 panel members periodically step down from panel participation and new members join. These rotations are designed to ensure that all IAS-USA expert panels remain diverse in member affiliations and areas of expertise.

The figures are designed for practitioners to use in identifying key mutations associated with viral resistance to antiretroviral drugs and in making therapeutic decisions. Updates are posted periodically at www.iasusa.org. Care should be taken if using this list of mutations in surveillance or epidemiologic studies of transmission of drug-resistant virus. Some amino acid substitutions, particularly minor mutations, represent polymorphisms that in isolation may not reflect prior drug selective pressure or reduced drug susceptibility.

The mutations listed have been identified by 1 or more of the following criteria: (1) in vitro passage experiments or validation of contribution to resistance by using site-directed mutagenesis; (2) susceptibility testing of laboratory or clinical isolates; (3) genetic sequencing of viruses from patients in whom the drug is failing; (4) correlation studies between genotype at baseline and virologic response in patients exposed to the drug. The group reviews data that have been published or have been presented at a scientific conference.

Drugs that have been approved by the US Food and Drug Administration (FDA) as well as any drugs available in expanded access programs are included. They are listed in alphabetic order by drug class. User notes provide additional information as necessary. Although the Drug Resistance Mutations Group works to maintain a complete and current list of these mutations, it cannot be assumed that the list presented here is exhaustive. Readers are encouraged to consult the literature and experts in the field for clarification or more information about specific mutations and their clinical impact.

In the context of making clinical decisions regarding antiretroviral therapy, evaluating the results of HIV genotypic testing includes: (1) assessing whether the pattern or absence of a pattern in the mutations is consistent with the patient's antiretroviral therapy history; (2) recognizing that in the absence of drug (selection pressure), resistant strains may be present at levels below the limit of detection of the test (analyzing stored samples, collected under selection pressure, could be useful in this setting); and (3) recognizing that virologic failure of the first regimen typically involves HIV-1 isolates with resistance to only 1 or 2 of the drugs in the regimen (in this setting, resistance most commonly develops to lamivudine or the nonnucleoside analogue reverse transcriptase inhibitors [NNRTIs]). The absence of detectable viral resistance after treatment failure may result from any combination of the following factors: the presence of drug-resistant minority viral populations, nonadherence to medications, laboratory error, drug-drug interactions leading to subtherapeutic drug levels, and possibly compartmental issues, indicating that drugs may not reach optimal levels in specific cellular or tissue reservoirs.

Revisions to the Figures for the Spring 2008 Update

In addition to minor formatting and color alterations, revisions to the figures include removal of the "expanded access" indication for etravirine because the drug was approved by the US FDA in early 2008. A new etravirine mutation, V179T, has been added to the figure bar, and user note 13 has been revised to reflect new information concerning etravirine mutations. Also, the expanded access indication for raltegravir has been removed because the drug was approved by the US FDA in late 2007.

Comments?

The IAS-USA Drug Resistance Mutations Group welcomes comments on the mutations figures and user notes.

(continued, page 67)

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MUTATIONS IN THE REVERSE TRANSCRIPTASE GENE ASSOCIATED WITH RESISTANCE TO REVERSE TRANSCRIPTASE INHIBITORS

Nucleoside and Nucleotide Analogue Reverse Transcriptase Inhibitors (nRTIs)[1]

Multi-nRTI Resistance: 69 Insertion Complex[2] (affects all nRTIs currently approved by the US FDA)

M	A	▼	K	L	T	K
41	**62**	**69**	**70**	**210**	**215**	**219**
L	V	Insert	R	W	Y F	Q E

Multi-nRTI Resistance: 151 Complex[3] (affects all nRTIs currently approved by the US FDA except tenofovir)

A	V	F	F	Q
62	**75**	**77**	**116**	**151**
V	I	L	Y	M

Multi-nRTI Resistance: Thymidine Analogue-associated Mutations[4,5] (TAMs; affect all nRTIs currently approved by the US FDA)

M	D	K	L	T	K
41	**67**	**70**	**210**	**215**	**219**
L	N	R	W	Y F	Q E

Abacavir[6]

K	L	Y	M
65	**74**	**115**	**184**
R	V	F	V

Didanosine[7,8]

K	L
65	**74**
R	V

Emtricitabine

K	M
65	**184**
R	V I

Lamivudine

K	M
65	**184**
R	V I

Stavudine[4,5,9,10]

M	D	K	L	T	K
41	**67**	**70**	**210**	**215**	**219**
L	N	R	W	Y F	Q E

Tenofovir[11]

K	K
65	**70**
R	E

Zidovudine[4,5,9,10]

M	D	K	L	T	K
41	**67**	**70**	**210**	**215**	**219**
L	N	R	W	Y F	Q E

Nonnucleoside Analogue Reverse Transcriptase Inhibitors (NNRTIs)[1,12]

Efavirenz

L	K	V	V	Y	Y	G	P
100	**103**	**106**	**108**	**181**	**188**	**190**	**225**
I	N	M	I	C I	L	S A	H

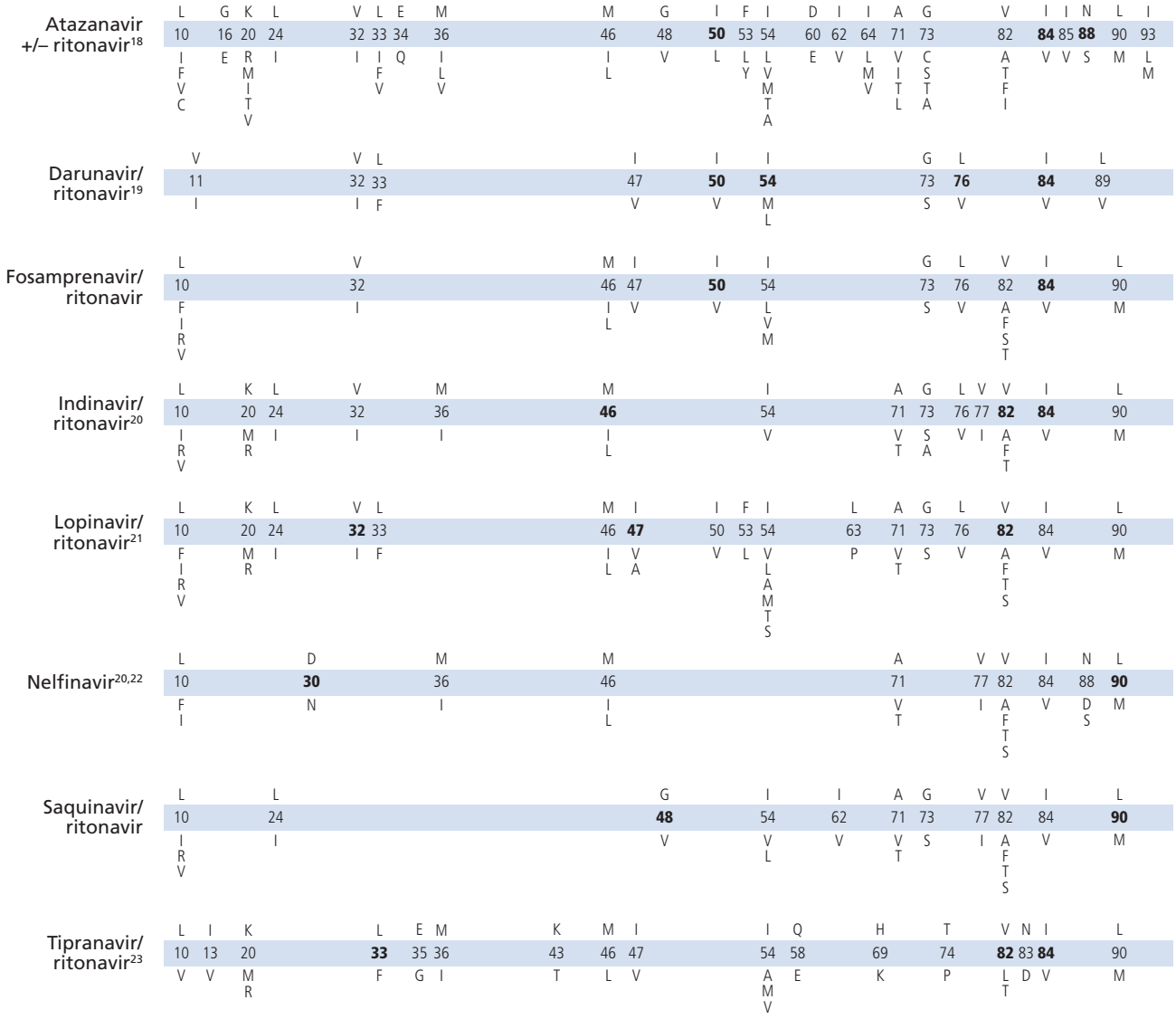
Etravirine[13]

V	A	L	K	V	V	Y	G
90	98	100	101	106	179	181	190
I	G	I	E P	I	D F T	C I V	S A

Nevirapine

L	K	V	V	Y	Y	G
100	**103**	**106**	**108**	**181**	**188**	**190**
I	N	A M	I	C I	C L H	A

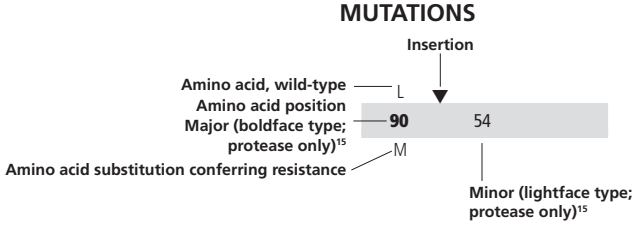
MUTATIONS IN THE PROTEASE GENE ASSOCIATED WITH RESISTANCE TO PROTEASE INHIBITORS[14,15,16,17]



MUTATIONS IN THE ENVELOPE GENE ASSOCIATED WITH RESISTANCE TO ENTRY INHIBITORS

MUTATIONS IN THE INTEGRASE GENE ASSOCIATED WITH RESISTANCE TO INTEGRASE INHIBITORS

Amino acid abbreviations: A, alanine; C, cysteine; D, aspartate; E, glutamate; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.



The International AIDS Society–USA (IAS–USA) Drug Resistance Mutations Group reviews new data on HIV drug resistance to maintain a current list of mutations associated with clinical resistance to HIV. This list includes mutations that may contribute to a reduced virologic response to a drug.

The mutations listed have been identified by 1 or more of the following criteria: (1) in vitro passage experiments or validation of contribution to resistance by using site-directed mutagenesis; (2) susceptibility testing of laboratory or clinical isolates; (3) genetic sequencing of viruses from patients in whom the drug is failing; (4) correlation studies between genotype at baseline and virologic response in patients exposed to the drug. In addition, the group reviews only data that have been published or have been presented at a scientific conference. Drugs that have been approved by the US Food and Drug Administration (US FDA) as well as any drugs available in expanded access programs are included (listed in alphabetical order by drug class). User notes provide additional information as necessary. Although the Drug Resistance Mutations Group works to maintain a complete and current list of these mutations, it cannot be assumed that the list presented here is exhaustive. Readers are encouraged to consult the literature and experts in the field for clarification or more information about specific mutations and their clinical impact.

User Notes

1. Numerous nucleoside (or nucleotide) analogue reverse transcriptase inhibitor (nRTI) mutations, such as the M41L, L210W, and T215Y mutations, may lead to viral hypersusceptibility to the nonnucleoside analogue reverse transcriptase inhibitors (NNRTIs) in nRTI-treated individuals. The presence of these mutations may improve subsequent virologic response to NNRTI-containing regimens in NNRTI treatment-naive individuals (Shulman et al, *AIDS,* 2004; Demeter et al, 11th CROI, 2004; Haubrich et al, *AIDS,* 2002; Tozzi, *J Infect Dis,* 2004; Katzenstein et al, *AIDS,* 2003). NNRTI hypersusceptibility can be conferred by 2 distinct phenotypes: increased enzyme susceptibility to NNRTI (eg, V118I/T215Y) or decreased virion-associated levels of reverse transcriptase (eg, H208Y/T215Y and V118I/H208Y/T215Y). The viruses that contained less reverse transcriptase replicated less efficiently than those with wild-type levels of reverse transcriptase. (Clark et al, *Antivir Ther,* 2006). The clinical relevance of all these mutations has not been assessed.

2. The 69 insertion complex consists of a substitution at codon 69 (typically T69S) and an insertion of 2 or more amino acids (S-S, S-A, S-G, or others). The 69 insertion complex is associated with resistance to all nRTIs currently approved by the US FDA when present with 1 or more thymidine analogue-associated mutations (TAMs) at codons 41, 210, or 215 (Miller et al, *J Infect Dis,* 2004). Some other amino acid changes from the wild-type T at codon 69 without the insertion may also be associated with broad nRTI resistance.

3. Tenofovir retains activity against the Q151M complex of mutations (Miller et al, *J Infect Dis,* 2004).

4. Multi-nRTI resistance mutations, also known as nucleoside analogue-associated mutations (NAMs), are associated with resistance to numerous nRTIs. The M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E are known as TAMs. TAMs are a subset of NAMs that are selected by the thymidine analogues zidovudine and stavudine and are associated with cross-resistance to all nRTIs currently approved by the US FDA (Larder et al, *Science,* 1989; Kellam et al, *Proc Natl Acad Sci USA,* 1992; Calvez et al, *Antivir Ther,* 2002; Kuritzkes et al, *J Acquir Immune Defic Syndr,* 2004). Mutations at the C-terminal reverse transcriptase domains (amino acids 293–560) outside of regions depicted on the figure bars may prove to be important for HIV drug resistance. Mutations in the connection (A371V) and RNase H (Q509L) domains of reverse transcriptase are coselected on the same genome as TAMs and increase significantly zidovudine resistance when combined with TAMs. They also increase, although to a much lesser extent, cross-resistance to lamivudine, abacavir, and tenofovir but not to stavudine or didanosine (Brehm et al, *Antivir Ther,* 2006). When the polymerase domain contains TAMs, mutations in the connection domain (E312Q, G335C/D, N348I, A360I/V, V365I, and A376S) increase resistance to zidovudine from 11-fold to as much as 536-fold over wild-type reverse transcriptase (Nikolenko et al, *Proc Natl Acad Sci USA,* 2007). Three mutations (N348I, T369I, and E399D) in the reverse transcriptase C-terminus are associated with the increased resistance to zidovudine and to NNRTIs. Mutations at this level could modulate NNRTI resistance by affecting dimerization of p66/p51 heterodimers (Gupta et al, *Antivir Ther,* 2006). Since the clinical relevance of these mutations has not been demonstrated, they are not depicted on the figure bars.

5. The E44D and the V118I mutations increase the level of resistance to zidovudine and stavudine in the setting of TAMs, and correspondingly increase cross-resistance to the other nRTIs. The significance of E44D or V118I when each occurs in isolation is unknown (Romano et al, *J Infect Dis,* 2002; Walter et al, *Antimicrob Agents Chemother,* 2002; Girouard et al, *Antivir Ther,* 2002).

6. The M184V mutation alone does not appear to be associated with a reduced virologic response to abacavir in vivo (Harrigan et al, *J Infect Dis,* 2000; Lanier et al, *Antivir Ther,* 2004). When present with 2 or 3 TAMs, M184V contributes to reduced susceptibility to abacavir and is associated with impaired virologic response in vivo (Lanier et al, *Antivir Ther,* 2004). The M184V plus 4 or more TAMs resulted in no virologic response to abacavir in vivo (Lanier et al, *Antivir Ther,* 2004).

7. The K65R mutation may be selected by didanosine and is associated in vitro with decreased susceptibility to the drug (Winters et al, *Antimicrob Agents Chemother,* 1997). The impact of the K65R mutation in vivo is unclear.

8. The presence of 3 of the following—M41L, D67N, L210W, T215Y/F, and K219Q/E—has been associated with resistance to didanosine (Marcelin et al, *Antimicrob Agents Chemother,* 2005). The K70R and M184V mutations are not associated with a decreased virologic response to didanosine in vivo (Molina et al, *J Infect Dis,* 2005).

9. The presence of the M184V mutation appears to delay or prevent emergence of TAMs (Kuritzkes et al, *AIDS,* 1996). This effect may be overcome by an accumulation of TAMs or other mutations. The clinical significance of this effect of M184V is not known.

10. The T215A/C/D/E/G/H/I/L/N/S/V substitutions are revertant mutations at codon 215, conferring increased risk of virologic failure of zidovudine or stavudine in antiretroviral-naive patients (Riva et al, *Antivir Ther,* 2002; Chappey et al, *Antivir Ther,* 2003; Violin et al, *AIDS,* 2004). In vitro studies and preliminary clinical studies suggest that the T215Y mutant may emerge quickly from one of these mutations in the presence of zidovudine or stavudine (Garcia-Lerma et al, *J Virol,* 2004; Lanier et al, *Antivir Ther,* 2002; Riva et al, *Antivir Ther,* 2002).

11. The K65R mutation is associated with a reduced virologic response to tenofovir

in vivo (Miller et al, *J Infect Dis,* 2004). A reduced response occurs in the presence of 3 or more TAMs inclusive of either M41L or L210W (Miller et al, *J Infect Dis,* 2004). Slightly increased treatment responses to tenofovir in vivo were observed if M184V was present (Miller et al, *J Infect Dis,* 2004).

12. The long-term virologic response to sequential NNRTI use is poor, particularly when 2 or more mutations are present (Antinori et al, *AIDS Res Hum Retroviruses,* 2002; Lecossier et al, *J Acquir Immune Defic Syndr,* 2005). The K103N or Y188L mutation alone prevents the clinical utility of **efavirenz and nevirapine** (Antinori et al, *AIDS Res Human Retroviruses,* 2002). The V106M mutation is more common in HIV-1 subtype C than in subtype B, and confers cross-resistance to all currently approved NNRTIs (Brenner et al, *AIDS,* 2003; Cane et al, *J Clin Microbiol,* 2001).

13. Virologic response was seen in clinical trials despite the presence of single mutations. The impact of most mutations depends on the simultaneous presence of Y181C; Y181C has impact only when present with 1 or more of these mutations (Vingerhoets et al, *Antivir Ther,* 2007). The presence of V179D/F/T, Y181V, or G190S at study baseline was associated with a decreased virologic response to etravirine (etravirine package insert). The presence of 3 or more baseline mutations (V90I, A98G, L100I, K101E/P, V106I, V179D/F, Y181C/I/V, G190A/S) resulted in a reduced virologic response to etravirine that was similar to placebo (Picchio et al, CROI, 2008). However, the presence of K103N does not affect etravirine response (etravirine package insert). Correlations between detection of etravirine mutations and subsequent virologic response will likely undergo revision with the accumulation of more phenotypic susceptibility data and genotypic results in treatment-experienced individuals.

14. The same mutations usually emerge whether or not PIs are boosted with low-dose ritonavir, although the relative frequency of mutations may differ. Data on the selection of mutations in antiretroviral-naive patients in whom a boosted PI is failing are very limited. Numerous mutations are often necessary to significantly impact virologic response to a boosted PI. Although numbers vary for the different drugs, 3 or more mutations are often required.

15. Resistance mutations in the protease gene are classified as either "major" or "minor," if data are available.

Major mutations in the protease gene are defined in general either as those selected first in the presence of the drug; or those shown at the biochemical or virologic level to lead to an alteration in drug binding or an inhibition of viral activity or viral replication. Major mutations have an effect on drug susceptibility phenotype. In general, these mutations tend to be the primary contact residues for drug binding.

Minor mutations generally emerge later than major mutations and by themselves do not have a significant effect on phenotype. In some cases, their effect may be to improve replicative fitness of the virus containing major mutations. However, some minor mutations are present as common polymorphic changes in HIV-1 nonsubtype B clades, such as K20I/R and M36I in protease.

16. Ritonavir is not listed separately as it is currently used at therapeutic doses as a pharmacologic booster of other PIs. At higher doses tested previously in humans, ritonavir administered as monotherapy produces mutations similar to those produced by indinavir (Molla, *Nature Med,* 1996).

17. HIV-1 Gag cleavage site changes can cause PI resistance in vitro. It has been observed that mutations in the N-terminal part of *gag* (MA: E40K; L75R; K113E and CA: M200I; A224A/V), outside the cleavage site, contribute directly to PI resistance by enhancing the overall Gag processing by wild-type protease (Nijhuis et al, *PLoS Med,* 2007). The clinical relevance of these mutations has not been assessed.

18. In most patients in whom an atazanavir/ritonavir-containing regimen was failing virologically, accumulations of the following 13 mutations were found (L10F/I/V, G16E, L33F/I/V, M46I/L, I54L/V/M/T, D60E, I62V, A71I/T/L, V82A/T, I84V, I85V, L90M, and I93L). Seven mutations were retained in an atazanavir score (L10F/I/V, G16E, L33F/I/V, M46I/L, D60E, I84V, I85V); the presence of 3 or more of these mutations predicts a reduced virologic response at 3 months, particularly when L90M was present (Vora et al, *AIDS,* 2006; http://www.hivfrenchresistance.org/2006/tab2.html). A different report (Bertoli et al, *Antivir Ther,* 2006) found that the presence of 0, 1, 2, or greater than or equal to 3 of the following mutations was associated with 92%, 93%, 75%, and 0% virologic response to atazanavir/ritonavir: L10C/I/V, V32I, E34Q, M46I/L, F53L, I54A/M/V, V82A/F/I/T, I84V; presence of I15E/G/L/V, H69K/M/N/Q/R/T/Y, and I72M/T/V improved the chances of response. For unboosted atazanavir, the presence of 0, 1, 2, or greater than or equal to 3 of the following mutations was associated with 83%, 67%, 6%, and 0% response rates: G16E, V32I, K20I/M/R/T/V, L33F/I/V, F53L/Y, I64L/M/V, A71I/T/V, I85V, I93L/M.

19. Darunavir (formerly TMC-114), boosted with ritonavir, was approved by the US FDA in June 2006. Resistance data are therefore still preliminary and limited. HIV RNA response to boosted darunavir correlated with baseline susceptibility and the presence of multiple specific PI mutations. Reductions in response were associated with increasing numbers of the mutations indicated in the bar. Some of these mutations appear to have a greater effect on susceptibility than others (eg, I50V versus V11I). Further study and analysis in other populations are required to refine and validate these findings.

20. The mutations depicted on the chart bar cannot be considered to be comprehensive since little relevant research has been reported in recent years to update the resistance and cross-resistance patterns for this drug.

21. In PI-experienced patients, the accumulation of 6 or more of the mutations indicated on the bar is associated with a reduced virologic response to lopinavir/ritonavir (Masquelier et al, *Antimicrob Agents Chemother,* 2002; Kempf et al, *J Virol,* 2001). The product information states that accumulation of 7 or 8 mutations confers resistance to the drug. In contrast, in those in whom lopinavir/ritonavir is their first PI used, resistance to this drug at the time of virologic rebound is rare. However, there is emerging evidence that specific mutations, most notably I47A (and possibly I47V) and V32I are associated with high-level resistance (Mo et al, *J Virol,* 2005; Friend et al, *AIDS,* 2004; Kagan et al, *Protein Sci,* 2005).

22. In some nonsubtype-B HIV-1, D30N is selected less frequently than other PI mutations (Gonzalez et al, *Antivir Ther,* 2004).

23. Accumulation of more than 2 mutations at positions 33, 82, 84, and 90 correlates with reduced virologic response to tipranavir/ritonavir, although an independent role for L90M was not found. Detailed analyses of data from phase II and III trials in PI-experienced patients identified mutations associated with reduced susceptibility or virologic response. These include: L10V, I13V, K20M/R, L33F, E35G, M36I, K43T, M46L,

I47V, I54A/M/V, Q58E, H69K, T74P, V82L/T, N83D, and I84V. Accumulation of these mutations is associated with reduced response. Subsequent genotype-phenotype and genotype-virologic response analyses determined some mutations have a greater effect than others (eg, I84V versus I54M). Refinement and clinical validation of these findings are pending (Baxter et al, J Virol, 2006; Mayers et al, *Antivir Ther,* 2004; Hall et al, *Antivir Ther,* 2003; McCallister et al, *Antivir Ther,* 2003; Parkin et al, CROI, 2006; Bacheler et al, European HIV Drug Resistance Workshop, 2006).

24. Although resistance to enfuvirtide is associated primarily with mutations in the first heptad repeat (HR1) region of the gp41 envelope gene, viruses that are wild type in the depicted HR1 region vary 500-fold in susceptibility. Such pretreatment susceptibility differences were not associated with differences in clinical responses (Labrosse et al, *J Virol,* 2003). Furthermore, mutations or polymorphisms in other regions in the envelope (eg, the HR2 region or those yet to be identified) as well as coreceptor usage and density may affect susceptibility to enfuvirtide (Reeves et al, *Proc Natl Acad Sci USA,* 2002; Reeves et al, *J Virol,* 2004; Xu et al, *Antimicrob Agents Chemother,* 2005). Thus, testing to detect only the depicted HR1 mutations may not be adequate for clinical management of suspected failure (Reeves et al, *J Virol,* 2004; Menzo et al, *Antimicrob Agents Chemother,* 2004; Poveda et al, *J Med Virol,* 2004; Sista et al, *AIDS,* 2004; Su, *Antivir Ther,* 2004).

25. Maraviroc activity is limited to patients with only CCR5 (R5)-using virus detectable; CXCR4 (X4)-CCR5 mixed tropic viruses and X4-using viruses do not respond to maraviroc treatment. Some cases of virologic failure during maraviroc therapy are associated with outgrowth of X4 virus that pre-exists as a minority population below the level of assay detection. Mutations in the HIV-1 gp120 molecule that allow the virus to bind to R5 receptors in the presence of drug have been described in viruses from some patients whose virus remained R5 at the time of virologic failure. A number of such mutations have been identified, and the phenotypic manifestation of this drug resistance is a reduction in the maximal percentage inhibition (MPI) rather than the increase in the 50% inhibitory concentration (IC_{50}; defined by fold increase) that is characteristic of resistance to other classes of antiretrovirals. The resistance profile for maraviroc is too complex to be depicted on the figures. The frequency and rate at which maraviroc resistance mutations emerge are not yet known.

26. Raltegravir failure was associated with integrase mutations in 2 distinct genetic pathways defined by 2 or more mutations including: (1) a signature (major) mutation at either Q148H/K/R or N155H; and (2) 1 or more minor mutations unique to each pathway. Minor mutations described in the Q148H/K/R pathway include L74M + E138A, E138K, or G140S. The most common mutation pattern in this pathway is Q148H + G140S; this Q148H + G140S pattern exhibits the greatest loss of drug susceptibility. Mutations described in the N155H pathway include this primary mutation plus either L74M, E92Q, T97A, E92Q + T97A, Y143H, G163K/R, V151I, or D232N (Hazuda et al, *Antivir Ther,* 2007).

(continued from page 62)

Please send your evidence-based comments, including relevant reference citations, to the IAS-USA at **resistance2008"at"iasusa.org** or by fax at 415-544-9401. Please include your name and institution.

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Financial Disclosures: The authors disclose the following affiliations with commercial organizations that may have interests related to the content of this article: Dr Brun-Vézinet has received grants and research support from GlaxoSmithKline, Tibotec Therapeutics, and Gilead Sciences, Inc, has served as a consultant to Merck & Co, Inc, Gilead Sciences, Inc, Virco Lab, Inc, Abbott Laboratories, Siemens Medical Solutions Diagnostics, and Tibotec Therapeutics, and has served as a paid lecturer for Bristol-Myers Squibb, Roche Pharmaceuticals, GlaxoSmithKline, Gilead Sciences, Inc, and Tibotec Therapeutics; Dr Clotet has served on scientific and marketing advisory boards and has received honoraria for lectures from Abbott Laboratories, Boehringer Ingelheim Pharmaceuticals, Inc, Merck Sharp & Dohme, Bristol-Myers Squibb, Gilead Sciences, Inc, GlaxoSmithKline, Panacos Pharmaceuticals, Inc, Pfizer, Inc, Roche Pharmaceuticals, and Tibotec Therapeutics; Dr Günthard has served as a scientific and medical advisor for Abbott Laboratories, Bristol-Myers Squibb, Boehringer Ingelheim Pharmaceuticals, Inc, GlaxoSmithKline, Novartis Pharmaceuticals Corp, and Tibotec Therapeutics. Furthermore, he has received unrestricted research and travel grants from Abbott Laboratories, Boehringer Ingelheim Pharmaceuticals, Inc, Bristol-Myers Squibb, Gilead Sciences, Merck Sharp & Dohme, and Roche Pharmaceuticals; Dr Johnson has received grant and research support from Agouron Pharmaceuticals, Bristol-Myers Squibb, GlaxoSmithKline, Monogram Biosciences, Inc, and Visible Genetics, Inc (later Bayer, now Siemens); has served on the speaker's bureaus or received honoraria from Abbott Laboratories, GlaxoSmithKline, and Monogram Biosciences, Inc; and has served on medical or clinical advisory boards of Bristol-Myers Squibb, GlaxoSmithKline, Monogram Biosciences, Inc, and Virco Lab, Inc. Dr Ku-

ritzkes has served as a consultant to, and has received honoraria from Abbott Laboratories, Avexa Ltd, Boehringer Ingelheim Pharmaceuticals, Inc, Bristol-Myers Squibb, Gilead Sciences, Inc, GlaxoSmithKline, Human Genome Sciences, Inc, Idenix Pharmaceuticals, Inc, Merck & Co, Inc, Monogram Biosciences, Inc, Pfizer, Inc, Roche Pharmaceuticals, Schering-Plough Corp, Siemens, and Trimeris, Inc. He has received research grant support from GlaxoSmithKline, Human Genome Sciences, Inc, Merck & Co, Inc, and Schering-Plough Corp. Dr Pillay has served as a consultant to Boehringer Ingelheim Pharmaceuticals, Inc, Bristol-Myers Squibb, Gilead Sciences, Inc, and Roche Pharmaceuticals. Dr Schapiro has served as a consultant to, advisor to, or speaker for Abbott Laboratories, Ambrilia Biopharma, Inc, Bristol-Myers Squibb, Boehringer Ingelheim Pharmaceuticals, Inc, Gilead Sciences, Inc, GlaxoSmithKline, Merck & Co, Inc, Monogram Biosciences, Inc, Pfizer, Inc, Roche Pharmaceuticals, Siemens, Tibotec Therapeutics, Virco Lab, Inc, and Virology Education; and has received research support from GlaxoSmithKline, Monogram Biosciences, Inc, Roche Pharmaceuticals, and Tibotec Therapeutics. Dr Richman has served as a consultant to Anadys Pharmaceuticals, Inc, Biota, Bristol-Myers Squibb, Boehringer Ingelheim Pharmaceuticals, Inc, Gilead Sciences, Inc, Idenix Pharmaceuticals, Inc, Koronis Pharmaceuticals, Merck & Co, Inc, Monogram Biosciences, Inc, Pfizer, Inc, Roche Pharmaceuticals, and Tobira Therapeutics, Inc. The International AIDS Society–USA has received grants in the past 3 years for selected continuing medical education activities that are pooled (ie, no single company supports any single effort) from Abbott Laboratories, Boehringer Ingelheim Pharmaceuticals, Inc, Bristol-Myers Squibb, Gilead Sciences, Inc, GlaxoSmithKline, Merck & Co, Inc, Roche Pharmaceuticals, Tibotec Therapeutics, and Pfizer, Inc.

Funding/Support: This work was funded by the IAS–USA. No private sector or government funding was used to support the effort. Panel members are not compensated.

Reference

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Top HIV Med. 2008;16(1): 62-68

Appendix II. Rega Version 7.1. Algorithm for the use of genotypic HIV-1 resistance data: 8 May 2007

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http://www.kuleuven.be/rega/cev/links/rega_algorithm/Rega_HIV1_Rules_v7.1.pdf.

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Algorithm for the use of genotypic HIV-1 resistance data
Rega v7.1 ©, Leuven, 8 May 2007

The rules include and score mutations for which *in vitro* phenotypic drug resistance or *in vivo* therapy response data have been reported, or for which retrospective associations with drug experience have been described. Some of the mutations do not necessarily result in high-level phenotypic resistance. Instead, they have been identified as key mutations along a pathway leading to high-level resistance (e.g., T215 revertants) or have proved to be predictive of therapy failure. Where supported by reliable evidence, the antagonistic or synergistic interaction effects of combinations of mutations are also incorporated (e.g., M184IV and TAMs). Three levels of interpretation criteria are applied: for HIV-1, criteria to consider an isolate as resistant, intermediately resistant or susceptible.

Genotypic susceptibility scores (GSS) are assigned to the three susceptibility levels for each drug class or individual drug. Although the same rules apply to each PI and its respective PI/r, different GSS are assigned to the boosted and un-boosted drug.

GSS	resistant	intermediate resistant	susceptible
NRTI - NtRTI	0	0.5	1
NNRTI	0	0.25	1
Etravirine	0	0.5	1
PI	0	0.5	1
PI/r	0	0.75	1.5
EI	0	0.25	1

Target GSS for the entire treatment combination regimen are proposed. Resistance development is expected when therapy changes with GSS below the target are installed.

Clinical situation	Target GSS
Therapy-naive persons with indications of transmitted drug resistance	≥3.5
Therapy-naive and therapy-experienced persons	≥3
Therapy-experienced persons with limited treatment options	≥2

	Criteria to consider an isolate resistant [a,b,c]	Criteria to consider an isolate intermediately resistant [a,b,c]
NRTI		
zidovudine	[at least 1 mutation of (T69X-XX,Q151M)] *or* [at least 4 mutations of (M41L,D67GN,T69AN,K70R,L210W,T215ACDEGHILNSVFY,K219EHNQR)] *or* [3 mutations of (M41L,L210W,T215Y) and not M184IV and not L74V] *or* [at least 3 mutations of (D67GN,K70R,T215F, K219EQ)]	[2 or 3 mutations of (M41L,D67GN,T69AN,K70R,L210W,T215ACDEGHILNSVFY,K219EHNQR)] *or* [T215FY and not M184IV]
didanosine	at least 1 mutation of (T69DGN,T69X-XX,Q151M) *or* [M184IV and at least 1 mutation of (K65RN,L74IV)] *or* [at least 5 mutations of (M41L,D67N,K70R,L210W,T215ACDEGHILNSVFY,K219EHNQR)]	at least 1 mutation of (K65NR,L74IV,V75T) *or* [3 or 4 mutations of (M41L,D67N,K70R,L210W,T215ACDEGHILNSVFY,K219EHNQR)]
stavudine	[at least 1 mutation of (Δ67,T69X-XX,V75AMST,Q151M)] *or* [at least 4 mutations of (M41L,D67N,T69ADGN,K70R,L210W,T215ACDEGHILNSVFY,K219EHNQR)] *or* [3 mutations of (M41L,L210W,T215Y)]	[2 or 3 mutations of (M41L,D67N,T69ADGN,K70R,L210W,T215ACDEGHILNSVFY,K219EHNQR)] *or* [T215FY and not M184IV]

lamivudine	M184IV *or* (K65NR and Q151M)	[at least 1 mutation of (Δ67,T69X-XX,K65NR,Q151M)] *or* {[at least 1 mutation of (E44AD,V118I)] and [at least 3 mutations of (M41L,D67N,T69AN,K70R,L210W,T215FY,K219EHNQR)]}
abacavir	[at least 1 mutation of (Δ67,T69G)] *or* [at least 2 mutations of (K65NR,T69X-XX,L74IV,Y115F,Q151M,M184IV)] *or* {(T69X-XX or Q151M) and [at least 3 mutations of (M41L,D67N,K70R,L210W,T215ACDEGHILNSVFY, K219EHNQR)]} *or* {[at least 1 mutation of (K65NR,L74IV,Y115F,M184IV)] and [at least 4 mutations of (M41L,D67N,K70R,L210W,T215ACDEGHILNSVFY,K219EHNQR]}	[1 mutation of (T69X-XX,Q151M)] *or* [1 mutation of (K65NR,L74IV,Y115F,M184IV)] and [2 or 3 mutations of (M41L,D67N,K70R,L210W,T215ACDEGHILNSVFY,K219EHNQR]} *or* [at least 3 mutations of (M41L,D67N,K70R,L210W,T215ACDEGHILNSVFY,K219EHNQR)]
emtricitabine	M184IV *or* (K65NR and Q151M)	[at least 1 mutation of (Δ67,T69X-XX,K65NR,Q151M)] *or* {[at least 1 mutation of (E44AD,V118I)] and [at least 3 mutations of (M41L,D67N,T69AN,K70R,L210W,T215FY,K219EHNQR)]}
NtRTI		
tenofovir	[at least 1 mutation of (K65NR,T69X-XX)] *or* [at least 5 mutations of (M41L,D67N,K70R,L210W,T215ACDEGHILNSVYF,K219EHNQR)]	[at least 1 mutation of (70EG,Q151M)] *or* [K65NR and M184IV] *or* [3 mutations of (M41L,L210W,T215Y)] or [at least 4 mutations of (M41L,D67N,K70R,L210W,T215ACDEGHILNSVYF,K219EHNQR)]
NNRTI		
nevirapine	[at least 1 mutation of (L100I,K101P,K103HNST,V106AM,Y181CIV,Y188CHL,G190ACEQSTV,M230L)] *or* [at least 4 mutations of (A98G,K101EQ,K103R,V106I,V108I,V179D,H221Y,F227L,K238T,Y318F)]	[2 or 3 mutations of (A98G,K101EQ,V106I,V108I,V179D,H221Y,F227L,K238T,Y318F)] *or* (K103R and V179D)
delavirdine	[at least 1 mutation of (L100I,K103HNT,V106AM,Y181CIV,Y188L,G190E,M230L,P236L)] *or* [at least 2 mutations of (K101P,K103S,Y188CH,G190Q,Y318F)] *or* [1 mutation of (K101P,K103S,Y188CH,G190Q,Y318F) and at least 2 mutations of (A98G,K101EQ,K103R,V106I,V108I,V179D,F227L)] *or* [at least 4 mutations of (A98G,K101EQ,K103R,V106I,V108I,V179D,F227L)]	[1 mutation of (K101P,K103S,Y188CH,G190Q,Y318F)] *or* [2 or 3 mutations of (A98G,K101EQ,V106I,V108I,V179D,F227L)] *or* (K103R and V179D)
efavirenz	[at least 1 mutation of (K103HN,V106M,Y181C,Y188L,G190CEQSTV,M230L)] *or* [at least 2 mutations of (L100I,K101P,K103ST,V106A,Y181IV,Y188CHF,G190A,P225H)] *or* [1 mutation of (L100I,K101P,K103ST,V106A,Y181IV,Y188CHF,G190A,P225H) and at least 2 mutations of (A98G,K101EQ,K103R,V106I,V108I,V179D,H221Y,F227L,K238T,Y318F)] *or* [at least 4 mutations of (A98G,K101EQ,K103R,V106I,V108I,V179D,H221Y,F227L,K238T,Y318F)]	[1 mutation of (L100I,K101P,K103ST,V106A,Y181IV,Y188CHF,G190A,P225H)] *or* [2 or 3 mutations of (A98G,K101EQ,V106I,V108I,V179D,H221Y,F227L,K238T,Y318F)] *or* (K103R and V179D)
etravirine	(V179DEFI and Y181CV) *or* [at least 1 mutation of (Y181I,F227C,M230L) and at least 1 mutation of (L100I,K101ENP,E138K,V179DEFI,Y181CV, Y188L,G190ES)] *or* [at least 1 mutation of (Y181I,F227C,M230L) and at least 2 mutations of (A98G,K101QR, K103N,V106AM,V108I,Y188CFH,G190ACQTV,H221Y,P225H,F227L,L234I,P236L,K238T,Y318T)] *or* [at least 3 mutations of (L100I,K101ENP,E138K,V179DEFI,Y181CV, Y188L,G190ES)] *or* [at least 2 mutations of (L100I,K101ENP,E138K,V179DEFI,Y181CV,Y188L,G190ES) and at least 2 mutations of (A98G,K101QR,K103N,V106AM,V108I,Y188CFH,G190ACQTV,H221Y,P225H,F227L,L234I,P236L,K238T, Y318T)] *or* [at least 1 mutation of (L100I,K101ENP,E138K,V179DEFI,Y181CV,Y188L,G190ES) and at least 4 mutations of (A98G,K101QR,K103N,V106AM,V108I,Y188CFH,G190ACQSTV,H221Y,P225H,F227L,L234I,P236L,K238T, Y318T)] *or* [at least 6 mutations of (A98G,K101QR,K103N,V106AM,V108I,Y188CFH,G190ACQTV, H221Y,P225H,F227L,L234I,P236L,K238T,Y318T)]	[1 mutation of (Y181I,F227C,M230L)] *or* [2 mutations of (L100I,K101ENP,E138K,V179DEFI,Y181CV, Y188L,G190ES)] *or* [1 mutation of (L100I,K101ENP,E138K,V179DEFI,Y181CV,Y188L,G190ES) and at least 2 mutations of (A98G,K101QR,K103N,V106AM,V108I,Y188CFH,G190ACQTV,H221Y,P225H,F227L,L234I,P236L,K238T,Y318T)] *or* [4 or 5 mutations of (A98G,K101QR,K103N,V106AM,V108I,Y188CFH,G190ACQTV,H221Y,P225H, F227L,L234I,P236L,K238T,Y318T)]

PI[d]		
saquinavir/r	score is at least 3.5 [add 1.5 to the score for every mutation in the following list: 48MV,54AST,84AC,90M; add 1 to the score for every mutation in the following list: 24I,53L,54V,71V,84V,88DS,89V; add 0.5 to the score for every mutation in the following list: 10F,20T,46IL,48A,50V,54LM,58E,71I,73STC, 74SP,89T; add 0.25 to the score for every mutation in the following list: score 0.25: 10IV,11I,20IMRV,62V,71T,74A,82AIFLMST,89I; distract 0.5 from the score for every mutation in the following list: 50L]	score is at least 2 [add 1.5 to the score for every mutation in the following list: 48MV,54AST,84AC,90M; add 1 to the score for every mutation in the following list: 24I,53L,54V,71V,84V,88DS,89V; add 0.5 to the score for every mutation in the following list: 10F,20T,46IL,48A,50V,54LM,58E,71I,73STC, 74SP,89T; add 0.25 to the score for every mutation in the following list: score 0.25: 10IV,11I,20IMRV,62V,71T,74A,82AIFLMST,89I; distract 0.5 from the score for every mutation in the following list: 50L]
indinavir/r	score is at least 3 [add 1.5 to the score for every mutation in the following list: 46IL,54AST,82AT,84A; add 1 to the score for every mutation in the following list: 10F,24I,32I,48MV,54V,76V,82FMS,88S,90M; add 0.5 to the score for every mutation in the following list: 20T,32X-X,43T,48A,54LM,66F,71I, 73STC,74P,84CV,88D, 89TV,95F; add 0.25 to the score for every mutation in the following list: score 0.25: 10IV,20IMRV,35DGN,43R,62V,71TV,74AS, 82I,89I; distract 0.5 from the score for every mutation in the following list: 50L]	score is at least 2 [add 1.5 to the score for every mutation in the following list: 46IL,54AST,82AT,84A; add 1 to the score for every mutation in the following list: 10F,24I,32I,48MV,54V,76V,82FMS,88S,90M; add 0.5 to the score for every mutation in the following list: 20T,32X-X,43T,48A,54LM,66F,71I, 73STC,74P,84CV,88D, 89TV,95F; add 0.25 to the score for every mutation in the following list: score 0.25: 10IV,20IMRV,35DGN,43R,62V,71TV,74AS, 82I,89I; distract 0.5 from the score for every mutation in the following list: 50L]
nelfinavir	score is at least 2.5 [add 1.5 to the score for every mutation in the following list: 30N,54AST,84AC,88S,90M; add 1 to the score for every mutation in the following list: 54V,82ATF,88D; add 0.5 to the score for every mutation in the following list: 10F,20T,23I,24I,32I,43T,46IL,48AV, 54LM,58E,66F,71I,73STC,74P,82LMS,84V,89TV,93M; add 0.25 to the score for every mutation in the following list: 10IV,20IMRV,33FI,35DGN,43R,62V,64V,71TV,74AS,82I,89I; distract 0.5 from the score for every mutation in the following list: 50L; distract 0.25 from the score for every mutation in the following list: 70R]	score is at least 1.5 [add 1.5 to the score for every mutation in the following list: 30N,54AST,84AC,88S,90M; add 1 to the score for every mutation in the following list: 54V,82ATF,88D; add 0.5 to the score for every mutation in the following list: 10F,20T,23I,24I,32I,43T,46IL,48AV, 54LM,58E,66F,71I,73STC,74P,82LMS,84V,89TV,93M; add 0.25 to the score for every mutation in the following list: 10IV,20IMRV,33FI,35DGN,43R,62V,64V,71TV,74AS,82I,89I; distract 0.5 from the score for every mutation in the following list: 50L; distract 0.25 from the score for every mutation in the following list: 70R]
fosamprenavir (/r)	score is at least 3.5 [add 1.5 to the score for every mutation in the following list: 32X-X,50V,84AC; add 1 to the score for every mutation in the following list: 47AV,48M,54MTV,76V,82F,84V; add 0.5 to the score for every mutation in the following list: 10F,20T,24I,32I,33F,43T,46IL,48A,54AL,58E,82AMST,89TV; add 0.25 to the score for every mutation in the following list: 10IV,20IRMV,33I,43R,48V,82I,89I,90M; distract 0.5 from the score for every mutation in the following list: 50L]	score is at least 2 [add 1.5 to the score for every mutation in the following list: 32X-X,50V,84AC; add 1 to the score for every mutation in the following list: 47AV,48M,54MTV,76V,82F,84V; add 0.5 to the score for every mutation in the following list: 10F,20T,24I,32I,33F,43T,46IL,48A,54AL,58E,82AMST,89TV; add 0.25 to the score for every mutation in the following list: 10IV,20IRMV,33I,43R,48V,82I,89I,90M; distract 0.5 from the score for every mutation in the following list: 50L]
lopinavir/r	score is at least 4 [add 1.5 to the score for every mutation in the following list: 32X-X,47A,54AT; add 1 to the score for every mutation in the following list: 47V,48M,50V,54SV,76V,82FS,84A; add 0.5 to the score for every mutation in the following list: 10F,20T,24FI,32I,33F,43T,46IL,48AV,53L,54LM, 71I,73STC, 82ALMT,84V,88D,90M; add 0.25 to the score for every mutation in the following list: 10IV,20IMRV,33I,43R,64MV,71TV,77ATV,82I; distract 0.5 from the score for every mutation in the following list: 50L]	score is at least 2.5 [add 1.5 to the score for every mutation in the following list: 32X-X,47A,54AT; add 1 to the score for every mutation in the following list: 47V,48M,50V,54SV,76V,82FS,84A; add 0.5 to the score for every mutation in the following list: 10F,20T,24FI,32I,33F,43T,46IL,48AV,53L,54LM, 71I,73STC, 82ALMT,84V,88D,90M; add 0.25 to the score for every mutation in the following list: 10IV,20IMRV,33I,43R,64MV,71TV,77ATV,82I; distract 0.5 from the score for every mutation in the following list: 50L]
atazanavir(/r)	score is at least 3.5 [add 1.5 to the score for every mutation in the following list: 48V,50L,54AT; add 1 to the score for every mutation in the following list: 20T,47V,54V,74P,82T,84V,88DS,90M; add 0.5 to the score for every mutation in the following list: 10F,24I,32I,46IL,54LM,58E,71IL,73ACFST,82ALMSF,85V; add 0.25 to the score for every mutation in the following list: 10IV,20IMRV,33FI,71TV,74AS,82I]	score is at least 2 [add 1.5 to the score for every mutation in the following list: 48V,50L,54AT; add 1 to the score for every mutation in the following list: 20T,47V,54V,74P,82T,84V,88DS,90M; add 0.5 to the score for every mutation in the following list: 10F,24I,32I,46IL,54LM,58E,71IL,73ACFST,82ALMSF,85V; add 0.25 to the score for every mutation in the following list: 10IV,20IMRV,33FI,71TV,74AS,82I]

tipranavir/r	score is at least 5 [add 1.5 to the score for every mutation in the following list: 47V,54A,82T,84V; add 1 to the score for every mutation in the following list: 11L,32I,33FM,38W,41T,54S,73T,82L; add 0.5 to the score for every mutation in the following list: 10F,20T,43T,45I,46IL,54MVT,58E, 71ILF,73SC,82ACSFM, 88DS,89VT,90M; add 0.25 to the score for every mutation in the following list: 10IV,13V,20IMRV,33IV,35DGN,36I,41K,43R,69K,71TV,82I,89I; distract 0.5 from the score for every mutation in the following list: 50L; distract 0.25 from the score for every mutation in the following list: 57R]	score is at least 3 [add 1.5 to the score for every mutation in the following list: 47V,54A,82T,84V; add 1 to the score for every mutation in the following list: 11L,32I,33FM,38W,41T,54S,73T,82L; add 0.5 to the score for every mutation in the following list: 10F,20T,43T,45I,46IL,54MVT,58E, 71ILF,73SC,82ACSFM, 88DS,89VT,90M; add 0.25 to the score for every mutation in the following list: 10IV,13V,20IMRV,33IV,35DGN,36I,41K,43R,69K,71TV,82I,89I; distract 0.5 from the score for every mutation in the following list: 50L; distract 0.25 from the score for every mutation in the following list: 57R]
darunavir/r	score is at least 5 [add 1.5 to the score for every mutation in the following list: 50V; add 1 to the score for every mutation in the following list: 76V,84ACV; add 0.5 to the score for every mutation in the following list: 32I,33F,47AV; add 0.25 to the score for every mutation in the following list: 11I,32L,33IMV,34V,35GN,41IT,46IL,54LM,70E,73ACFSTV, 74E,82L,85V,89ITV]	score is at least 3 [add 1.5 to the score for every mutation in the following list: 50V; add 1 to the score for every mutation in the following list: 76V,84ACV; add 0.5 to the score for every mutation in the following list: 32I,33F,47AV; add 0.25 to the score for every mutation in the following list: 11I,32L,33IMV,34V,35GN,41IT,46IL,54LM,70E,73ACFSTV, 74E,82L,85V,89ITV]
EI		
enfuvirtide	at least 2 mutations of (G36DESV,V38AEM,Q40H,Q41R,N42DEHKQT,N43DKQS,L44MV,L45MPQ)	1 mutation of (G36DESV,V38AEM,Q40H,Q41R,N42DEHKQT,N43DKQS,L44MV,L45MPQ)

[a] T69X-XX, X whatever amino acid. Δ67, deletion of amino acid 67. A, alanine; R, arginine; N, asparagine; D, aspartic acid; C, cysteine; Q, glutamine; E, glutamic acid; G, glycine; H, histidine; I, isoleucine; L, leucine; K, lysine; M, methionine; F, phenylalanine; P, proline; S, serine; T, threonine; W, tryptophan; Y, tyrosine and V, valine. [b] If more mutations are present at a certain position (e.g. T215Y and T215F), they are only counted as one mutation in the rules. [c] If none of the criteria to consider an isolate resistant towards a particular drug are full-filled, proceed to the criteria to consider an isolate intermediately resistant. If none of the latter criteria are full-filled, the isolate can be scored susceptible to that particular drug. [d] r, PI boosted with baby dose of ritonavir. NRTI, nucleoside reverse transcriptase inhibitor; NtRTI, nucleotide reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; EI, entry inhibitor.

Appendix III. The EuroSIDA study group

(National co-ordinators in parenthesis).

Argentina: (M Losso), A Duran, Hospital JM Ramos Mejia, Buenos Aires.

Austria: (N Vetter), Pulmologisches Zentrum der Stadt Wien, Vienna.

Belarus: (I Karpov), A Vassilenko, Belarus State Medical University, Minsk.

Belgium: (N Clumeck), S De Wit, B Poll, Saint-Pierre Hospital, Brussels; R Colebunders, Institute of Tropical Medicine, Antwerp.

Bulgaria: (K Kostov), Infectious Diseases Hospital, Sofia.

Croatia: (J Begovac), University Hospital of Infectious Diseases, Zagreb.

Finland: (M Ristola), Helsinki University Central Hospital, Helsinki.

Czech Republic: (L Machala), H Rozsypal, Faculty Hospital Bulovka, Prague; D Sedlacek, Charles University Hospital, Plzen.

Denmark: (J Nielsen), J Lundgren, T Benfield, O Kirk, Panum Institute, Copenhagen; J Gerstoft, T Katzenstein, A-B E Hansen, P Skinhøj, Rigshospitalet, Copenhagen; C Pedersen, Odense University Hospital, Odense; L Oestergaard, Skejby Hospital, Aarhus.

Estonia: (K Zilmer), West-Tallinn Central Hospital, Tallinn; J Smidt, Nakkusosakond Siseklinik, Kohtla-Järve.

France: (C Katlama), Hôpital de la Pitié-Salpétière, Paris; J-P Viard, Hôpital Necker-Enfants Malades, Paris; P-M Girard, Hospital Saint-Antoine, Paris; JM Livrozet, Hôpital Edouard Herriot, Lyon; P Vanhems, University Claude Bernard, Lyon; C Pradier, Hôpital de l'Archet, Nice; F Dabis, Unité INSERM, Bordeaux.

Germany: (J Rockstroh), Universitäts Klinik Bonn; R Schmidt, Medizinische Hochschule Hannover; J van Lunzen, O Degen, University Medical Center Hamburg-Eppendorf, Infectious Diseases Unit, Hamburg; HJ Stellbrink, IPM Study Center, Hamburg; S Staszewski, JW Goethe University Hospital, Frankfurt; J Bogner, Medizinische Poliklinik, Munich; G. Fätkenheuer, Universität Köln, Cologne.

Greece: (J Kosmidis), P Gargalianos, G Xylomenos, J Perdios, Athens General Hospital; G Panos, A Filandras, E Karabatsaki, 1st IKA Hospital; H Sambattakou, Ippokration Genereal Hospital, Athens.

Hungary: (D Banhegyi), Szent Lásló Hospital, Budapest.

Ireland: (F Mulcahy), St. James's Hospital, Dublin.

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Italy: (A Chiesi), Istituto Superiore di Sanità, Rome; R Esposito, I Mazeu, C Mussini, Università Modena, Modena; C Arici, Ospedale Riuniti, Bergamo; R Pristera, Ospedale Generale Regionale, Bolzano; F Mazzotta, A Gabbuti, Ospedale S Maria Annunziata, Firenze; V Vullo, M Lichtner, University di Roma la Sapienza, Rome; A Chirianni, E Montesarchio, M Gargiulo, Presidio Ospedaliero AD Cotugno, Monaldi Hospital, Napoli; G Antonucci, F Iacomi, P Narciso, C Vlassi, M Zaccarelli, Istituto Nazionale Malattie Infettive Lazzaro Spallanzani, Rome; A Lazzarin, R Finazzi, Ospedale San Raffaele, Milan; M Galli, A Ridolfo, Osp. L. Sacco, Milan; A d'Arminio Monforte, Istituto Di Clinica Malattie Infettive e Tropicale, Milan.

Latvia: (B Rozentale), P Aldins, Infectology Centre of Latvia, Riga.

Lithuania: (S Chaplinskas), Lithuanian AIDS Centre, Vilnius.

Luxembourg: (R Hemmer), T Staub, Centre Hospitalier, Luxembourg.

Netherlands: (P Reiss), Academisch Medisch Centrum bij de Universiteit van Amsterdam, Amsterdam.

Norway: (J Bruun) A Maeland, V Ormaasen, Ullevål Hospital, Oslo.

Poland: (B Knysz), J Gasiorowski, Medical University, Wroclaw; A Horban, Centrum Diagnostyki i Terapii AIDS, Warsaw; D Prokopowicz, A Wiercinska-Drapalo, Medical University, Bialystok; A Boron-Kaczmarska, M Pynka, Medical Univesity, Szczecin; M Beniowski, E Mularska, Osrodek Diagnostyki i Terapii AIDS, Chorzow; H Trocha, Medical University, Gdansk.

Portugal: (F Antunes), E Valadas, Hospital Santa Maria, Lisbon; K Mansinho, Hospital de Egas Moniz, Lisbon; F Maltez, Hospital Curry Cabral, Lisbon.

Romania: (D Duiculescu), Spitalul de Boli Infectioase si Tropicale: Dr. Victor Babes, Bucarest.

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Serbia: (D Jevtovic), The Institute for Infectious and Tropical Diseases, Belgrade.

Slovakia: (M Mokráš), D Staneková, Dérer Hospital, Bratislava.

Spain: (J González-Lahoz), V Soriano, L Martin-Carbonero, P Labarga, Hospital Carlos III, Madrid; B Clotet, A Jou, J Conejero, C Tural, Hospital Germans Trias i Pujol, Badalona; JM Gatell, JM Miró, Hospital Clinic i Provincial, Barcelona; P Domingo, M Gutierrez, G Mateo, MA Sambeat, Hospital Sant Pau, Barcelona.

Sweden: (A Karlsson), Karolinska University Hospital, Stockholm; PO Persson, Karolinska University Hospital, Huddinge; L Flamholc, Malmö University Hospital, Malmö.

Switzerland: (B Ledergerber), R Weber, University Hospital, Zürich; P Francioli, M Cavassini, Centre Hospitalier Universitaire Vaudois, Lausanne; B Hirschel, E Boffi, Hospital Cantonal Universitaire de Geneve, Geneve; H Furrer, Inselspital Bern, Bern; M Battegay, L Elzi, University Hospital Basel.

Ukraine: (E Kravchenko), N Chentsova, Kiev Centre for AIDS, Kiev.

United Kingdom: (S Barton), St. Stephen's Clinic, Chelsea and Westminster Hospital, London; AM Johnson, D Mercey, Royal Free and University College London Medical School, London (University College Campus); A Phillips, MA Johnson, A Mocroft, Royal Free and University College Medical School, London (Royal Free Campus); M Murphy, Medical College of Saint Bartholomew's Hospital, London; J Weber, G Scullard, Imperial College School of Medicine at St. Mary's, London; M Fisher, Royal Sussex County Hospital, Brighton; R Brettle, Western General Hospital, Edinburgh.

Virology group: B Clotet (Central Coordinators) plus ad hoc virologists from participating sites in the EuroSIDA Study.

Steering Committee: F Antunes, B Clotet, D Duiculescu, J Gatell, B Gazzard, A Horban, A Karlsson, C Katlama, B Ledergerber (Chair), A D'Arminio Montforte, A Phillips, A Rakhmanova, P Reiss (Vice-Chair), J Rockstroh.

Coordinating centre staff: J Lundgren (project leader), O Kirk, A Mocroft, N Friis-Møller, A Cozzi-Lepri, W Bannister, M Ellefson, A Borch, D Podlevkareva, C Holkmann Olsen, J Kjær, L Peters, J Reekie.

Appendix IV. The EuroSIDA December 2006 follow-up form

EuroSIDA dataset 25 form

December 2006 / 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: ☐ ☐ ☐

For women: Pregnancy in 2006? ☐ ☐ ☐

If Pregnancy, outcome:

☐ Spontaneous abortion ☐ Birth of HIV+ ☐ Birth of child with unknown HIV stat

☐ 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:_____):	☐	☐	☐	- -
Hepatic encephalopathy (stage III or IV) :	☐	☐	☐	- -
Pancreatitis (symtoms + objective evidence):	☐	☐	☐	- -
Renal disease, end stage (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:					
Peak 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:	- -		
S-lactate (not LDH) value:	- -		
Peak value since last follow-up for:			
S-lactate value (not LDH):	- -		
S-amylase value:	- -		

	Date of measurement (dd-mm-yyyy)	Value	Date of measurement (dd-mm-yyyy)	Value
Two most recently reported CD4 cell counts:	- -		- -	
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	- -				
	- -				
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:_____

	Date (dd-mm-yyyy)	Type	Not done
Last HIV-subtyping performed:	- -		
Last HCV-subtyping performed:	- -		
Last HIV resistance testing, if performed since last follow-up:	- -	Method used:	

If a test was performed - please attach a copy of the resistance test report when returning the form

Hepatitis virology/serology	Last reported		Most recent test (if any later than the last recorded)					
	Date	Result	Date	Positive	Negative	Unknown	Value	Unit
HBV antibody test	-		- -					
HBVsAg	-		- -					
HBV-DNA	-		- -					
HCV antibody test	-		- -					
HCV-RNA	-		- -					

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)

Fusion inhibitors:

ENF:	Enfurvirtide (Fuzeon/T20)
MAR:	Maraviroc

Integrase inhibitors:

Please specify full name

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			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 antiretroviral therapy since last follow-up
Please use the list of codes below or write full drug name for any drugs not listed

_____	- -			- -
_____	- -			- -

Fungal drugs
- AMPH: Amphotericin B, i.v.
- CASP: Caspofungin
- FLUC: Fluconazole
- ITRA: Itraconazole
- KETO: Ketoconazole

HBV and CMV/HSV drugs
- ADEF: Adefovir dipivoxil
- CONA: Continous Acyclovir
- CIDO: Cidofovir
- FOSC: Foscarnet
- GANC: Ganciclovir

HCV drugs
- INTF: Interferon
- RIBA: Ribavirin
- VORI: Voriconazole

Immunomodulating therapy
- IL2: Interleukin 2
- GCSF: G-CSF
- INTF: Interferon
- PINT: Peg-Interferon

Mycobacterium drugs
- CLAR: Clarithromycin/azithromycin
- ETHA: Ethambutole
- ISON: Isoniazide

Mycobacterium drugs
- PYRA: Pyrazinamide
- RIFA: Rifabutine
- RIFM: Rifampicine

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:	-	-	-	- -			- -
Lipid lowering agents:	-	-	-	- -			- -
Oral antidiabetic agents:	-	-	-	- -			- -

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

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: **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	Non-Hodgkin lymphoma:	
CRVC:	Cervical cancer	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**				
_____	- -	☐	☐	☐
_____	- -	☐	☐	☐

ANUS:	Anus cancer	Leukemia:		Metastasis:	
BLAD:	Bladder cancer		ALL: Acute lymphoid	MESC:	of squamuos cell carcinoma
BRCA:	Breast cancer		AML: Acute myeloid	MEAC:	of adenocarcinoma
CERV:	Cervical dysplasia/carcinoma in situ		CLL: Chronic lymphoid	MEOC:	of other cancertype
COLO:	Colon cancer		CML: Chronic myeloid	PENC:	Penile cancer
COTC:	Connective tissue cancer	LIPC:	Lip cancer	PROS:	Prostate cancer
HDL:	Hodgkin lymphoma	LIVR:	Liver cancer	RECT:	Rectum cancer
KIDN:	Kidney cancer	LUNG:	Lung cancer	STOM:	Stomach cancer
		MALM:	Malignant melanoma	TESE:	Testicular seminoma
		MULM:	Multiple myeloma	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 V. Statistical methods

This appendix gives a formal description of the statistical ideas presented throughout this thesis. For more details, see *An Introduction to Statistical Modelling* by W.J. Krzanowski, *Modelling Binary Data* by D. Collett and *Modelling Survival Data in Medical Research* by D. Collett [371,372,535]. Statistical models can be described in terms of a systematic component and a random component. Choice of an appropriate model depends upon the prior beliefs of the underlying probability distribution of the response variable and how the response can be related to other explanatory variables. The systematic component is made up of explanatory variables and *parameters*, i.e. constant values that are the coefficients in the equation, which define the relationship with the response. The random component is a random variable that takes values from a defined probability distribution to take account of individual deviation from the systematic component. Most commonly, these two components are combined together additively to form a model. For n observed values of a response variable Y, the ith observation in the model can be written as:

$$y_i = \eta_i + \varepsilon_i, \quad i = 1, 2, \ldots, n$$

where η_i is the systematic component and ε_i is the random component.

Logistic regression

The logistic regression model is one of a family of models called *generalised linear models* (GLMs). These are linear in terms of the parameters in the model, so that for every unit change in any parameter, the response variable experiences the same change in value. A GLM assumes that the response variable has a probability distribution from the *exponential family* (including a wide range of distributions) and relates the expected value of the response for the ith observation, μ_i, to the systematic component, η_i, via a *link function*.

Logistic regression models are used for binary data where data can be *grouped* or *ungrouped*. All the data in this thesis were ungrouped, i.e. each observation was for one individual and so this is the model that is described below. The response variable Y_i for the ith observation has a binomial probability distribution, $Y_i \sim B(1, p_i)$ with p_i = the probability of a 'success'. It is this probability that is predicted in the model using a *logistic* link function:

$$\text{logit}(p_i) = \log_e(p_i/(1-p_i)) = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \ldots + \beta_n x_{ni}$$

where $\{x_{1i}, x_{2i}, \ldots, x_{ni}\}$ are the ith observations on n explanatory variables $\{X_{1i}, X_{2i}, \ldots, X_{ni}\}$,

$\{\beta_0, \beta_1, \beta_2, \ldots, \beta_n\}$ are the parameters measuring the effects of the explanatory variables,

$\exp(\beta_0)$ is the odds of the outcome in patients with zero values for each of the explanatory variables,

$\exp(\beta_j)$ is the odds ratio of the outcome in patients with $x_j = 1$ compared to $x_j = 0$.

This link function transforms the probability p from taking values in the interval (0,1) to $(-\infty,\infty)$. An unconstrained model is better to avoid the possibility of predicting impossible values outside of the range and so ensures that the predicted probability is between zero and one.

Cox proportional hazards regression model

The basic model for time to event (survival) data is the Cox proportional hazards regression model, which relates the survival time to an event of interest to a set of explanatory variables. It does not assume a particular distribution for the survival times and so is a semi-parametric model. If the actual survival time of an individual is t, which is a value of the random variable T that can take any non-negative value, then the *survivor* function can be defined as:

$$S(t) = \mathrm{P}(T > t) = 1 - F(t)$$

where $F(t) = \mathrm{P}(T \leq t)$ is the probability *distribution* function. The probability *density* function, $f(t)$, is the derivative of $F(t)$ and gives the probability of the event of interest per unit of time t. However, it is more convenient to think in terms of the probability that an individual who has survived until time t will experience an event in the next small interval of time between t and $t + \delta t$. This is conditional on survival up to time t and is the instantaneous risk of developing the event defined by the *hazard* function:

$$h(t) = \lim_{\delta t \to 0} \{\mathrm{P}(t \leq T < t + \delta t \mid T \geq t) / \delta t\}$$

Assuming proportional hazards at all time points, the predicted hazard for a particular individual i is:

$$h_i(t) = h_0(t)\Psi_i$$

where $h_0(t)$ is called the baseline hazard and Ψ_i is a linear predictor consisting of explanatory variables. To ensure that this is a positive value, the exponential is taken:

$$\Psi_i = \exp(\beta_1 x_{1i} + \beta_2 x_{2i} + \ldots + \beta_n x_{ni})$$

where $\{x_{1i}, x_{2i}, \ldots, x_{ni}\}$ are the ith observations on n explanatory variables $\{X_{1i}, X_{2i}, \ldots, X_{ni}\}$,

$\{\beta_0, \beta_1, \beta_2, \ldots, \beta_n\}$ are the parameters measuring the effects of the explanatory variables.

This is a *log-linear* model from which hazard ratios (or relative hazards) can be estimated. For individuals i and k with covariate values $\boldsymbol{x_i} = \{x_{1i}, x_{2i}, \ldots, x_{ni}\}$ and $\boldsymbol{x_k} = \{x_{1k}, x_{2k}, \ldots, x_{nk}\}$, the relative hazards is given by:

$$h_i(t)/\ h_k(t) = \exp(\boldsymbol{\beta}(\boldsymbol{x_i} - \boldsymbol{x_k}))$$

As it is independent of t, the value is constant over time and the hazards for the two individuals are therefore proportional.

Poisson regression

The Poisson regression model is also a generalised linear model used for count data. The response variable Y_i for the ith observation has a Poisson probability distribution, $Y_i \sim P(\mu_i)$ with μ_i = the expectation of Y_i (the mean average), $E(Y_i)$. A log-linear link function is used:

$$\log(\mu_i) = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \ldots + \beta_n x_{ni}$$

where $\{x_{1i}, x_{2i}, \ldots, x_{ni}\}$ are the ith observations on n explanatory variables $\{X_{1i}, X_{2i}, \ldots, X_{ni}\}$,

$\{\beta_0, \beta_1, \beta_2, \ldots, \beta_n\}$ are the parameters measuring the effects of the explanatory variables.

Linear regression

The linear regression model is used when the data are normally distributed with $Y_i \sim N(\mu_i, \sigma^2)$ with μ_i = the expectation of Y_i, $E(Y_i)$ and σ^2, the variance of Y_i. A simple linear link function is used:

$$\mu_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \ldots + \beta_n x_{ni}$$

where $\{x_{1i}, x_{2i}, \ldots, x_{ni}\}$ are the ith observations on n explanatory variables $\{X_{1i}, X_{2i}, \ldots, X_{ni}\}$,

$\{\beta_0, \beta_1, \beta_2, \ldots, \beta_n\}$ are the parameters measuring the effects of the explanatory variables.

Estimating the parameters

For all the above models, the parameters $\boldsymbol{\beta} = \{\beta_0, \beta_1, \beta_2, \ldots, \beta_n\}$ are estimated via the method of maximum likelihood estimation, which selects values that make the data the most likely to have occurred. For a sample $x_1, x_2, \ldots, x_n$ of n values from a particular probability distribution with unknown parameter θ, the multivariate probability density function associated with the observed data is the likelihood function:

$$L(\theta) = f_\theta(x_1, x_2, \ldots, x_n | \theta)$$

The method of maximum likelihood estimation finds the value of θ that maximises $L(\theta)$. This requires iterative computational procedures such as the Newton-Raphson method.

Confidence intervals and testing for significance

In large samples the distribution of the parameters $\boldsymbol{\beta}$ can be approximated to a normal distribution. Therefore, confidence intervals and hypothesis tests for $\boldsymbol{\beta}$ can be performed in the usual way. Given a parameter estimate β_j and a standard error for this estimate (the square root of the estimated variance), a 95% confidence interval (CI) can be calculated as:

$$\beta_j \pm (1.96 \times \text{standard error of } \beta_j)$$

A Wald test is used to compare the value of β_j divided by its standard error to a standard normal distribution in order to obtain a p value. If the p value is less than 0.05, it is conventionally accepted that there is enough evidence to reject the null hypothesis of no significant difference from zero.

Appendix VI. Regional changes over time in initial virologic response rates to combination antiretroviral therapy across Europe

Removed due to copyright restrictions. See Bannister WP, Kirk O, Gatell JM, Knysz B, Viard J-P, Mens H, D'Arminio Monforte A, Phillips AN, Mocroft A and Lundgren J for the EuroSIDA Study. Regional changes over time in initial virologic response rates to combination antiretroviral therapy across Europe. J Acquir Immune Defic Syndr 2006; ***42:****229-237.*

Appendix VII. HIV-1 subtypes and response to combination antiretroviral therapy in Europe

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HIV-1 subtypes and response to combination antiretroviral therapy in Europe

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Background: Combination antiretroviral therapy (cART) may vary in ability to suppress viral load and increase CD4⁺ T-cell count in people infected with different HIV-1 subtypes, possibly due to differences in resistance development. Antiretroviral drugs have predominantly been developed in Western Europe/North America on the basis of the most prevalent subtype, B. However, non-B subtypes are increasingly spreading worldwide.

Objective: To compare virological and immunological response to cART between patients infected with B and non-B subtypes across Europe.

Design: EuroSIDA prospective, observational cohort with 11,928 HIV-1-infected patients.

Methods: Response to cART was analysed in patients with subtypes determined pre-cART, via multivariable logistic regression on the first measurements 6–12 months after starting cART. A virological response was defined as a viral load <500 copies/ml and immunological response as a CD4⁺ T-cell count increase of ≥100 cells/mm^3.

Results: Forty-five percent of patients were antiretroviral naive at initiation of cART. Virological suppression was achieved by 58% of 689 subtype-B-infected patients and 66% of 102 non-B-infected patients (P=0.159). After adjustment for potential confounders, there was no significant difference in odds of achieving virological suppression (non-B compared with B; odds ratio [OR]: 1.05, 95% confidence interval [CI]: 0.58–1.93, P=0.866). An immunological response was achieved by 43% of 753 B-infected patients and 48% of 114 non-B-infected patients (P=0.334). After adjustment, there was no significant difference in odds of an immunological response (OR: 1.17, 95% CI: 0.73–1.87, P=0.524).

Conclusions: There was no evidence of significant differences in virological or immunological response to cART between patients infected with HIV-1 B and non-B subtypes.

Introduction

HIVs are a genetically diverse group of retroviruses [1–6]. HIV-1 is the predominant form of the virus worldwide [7], classified into three groups according to its genetic structure [2,3,8,9]. The major group, 'M', represents the vast majority of all infections [4,7] and has nine distinct subtypes: A–D, F–H, J and K [9–11], as well as 19 circulating recombinant forms (CRFs) [12–16]: CRF01–CRF19.

It has been suggested that certain HIV-1 subtypes may be more susceptible to evolving mutations associated with antiretroviral resistance than others [10,11,17–22] and there is evidence that resistance is associated with a poorer virological response to antiretroviral therapy [23–25]. Historically, antiretroviral drugs have mostly been designed in North America and Western Europe [11], where subtype B is the most prevalent strain [26]. Consequently, they have been developed based on the biological and clinical findings of their effects on patients largely infected with this strain [10,20], even though, in 2000, subtype B was estimated to contribute to just 12.3% of the worldwide epidemic [26]. Non-B subtypes, which are widespread in Africa, Asia and much of Eastern Europe [26], are increasingly spreading worldwide through travel and migration [4]. This highlights the importance of researching response to antiretroviral

therapy in patients infected with different subtypes. So far, the limited research in this area has generally found no striking differences [27–35].

The aims of these analyses were to compare both virological and immunological response with combination antiretroviral therapy (cART) between patients infected with HIV-1 B and non-B subtypes across Europe.

Methods

Patients

The EuroSIDA study is a prospective, observational cohort of 11,928 HIV-1-infected patients in 83 centres across 28 European countries, Israel and Argentina [36]. It comprises six cohorts of consecutive HIV-1-infected patients with pre-booked clinic appointments, aged 16 or over, from May 1994 onwards. For Cohort I–III, eligible patients were those with a $CD4^+$ T-lymphocyte count of <500 cells/mm^3 in the previous 4 months. This restriction was removed for Cohorts IV–VI.

Information was provided on a standardized data collection form at baseline and every 6 months thereafter. Follow-up is to May 2005. Data collected includes all $CD4^+$ T-cell counts and viral loads measured since the last follow-up and starting and stopping dates of all antiretroviral drugs and prophylactic drugs used against opportunistic infections. All dates of diagnosis of AIDS-defining illnesses are also recorded, using the 1993 clinical definition of AIDS from the Centers for Disease Control [37]. Members of the coordinating office visit all centres to facilitate correct patient selection and to verify the information provided against case notes for a proportion of patients.

Patients were eligible for analyses if they started combination antiretroviral therapy (cART), defined as at least three antiretroviral drugs including a protease inhibitor (PI), a non-nucleoside reverse transcriptase inhibitor (NNRTI), or abacavir (ABC), with no previous PI/NNRTI/ABC experience, before May 2004 allowing for the potential of at least 1 year's follow-up, and had pre-cART test results available determining the subtype they were infected with.

Subtype determination

HIV-1 subtypes in EuroSIDA were primarily ascertained using plasma samples analysed in central virology laboratories. Phylogenetic analysis on genetic sequences of reverse transcriptase and protease was used to determine subtype in all but a few cases where sequences were incomplete and serology methods had been used. Phylogenetic trees were constructed as neighbour-joining trees based on Kimura 2-parameter distances. In the absence of plasma samples, clinics could provide paper copies of resistance test results including subtype determination, conducted in local laboratories. If these were also not available, any subtype assignment in the patients' case notes reported on the EuroSIDA follow-up forms was taken.

Statistical methods

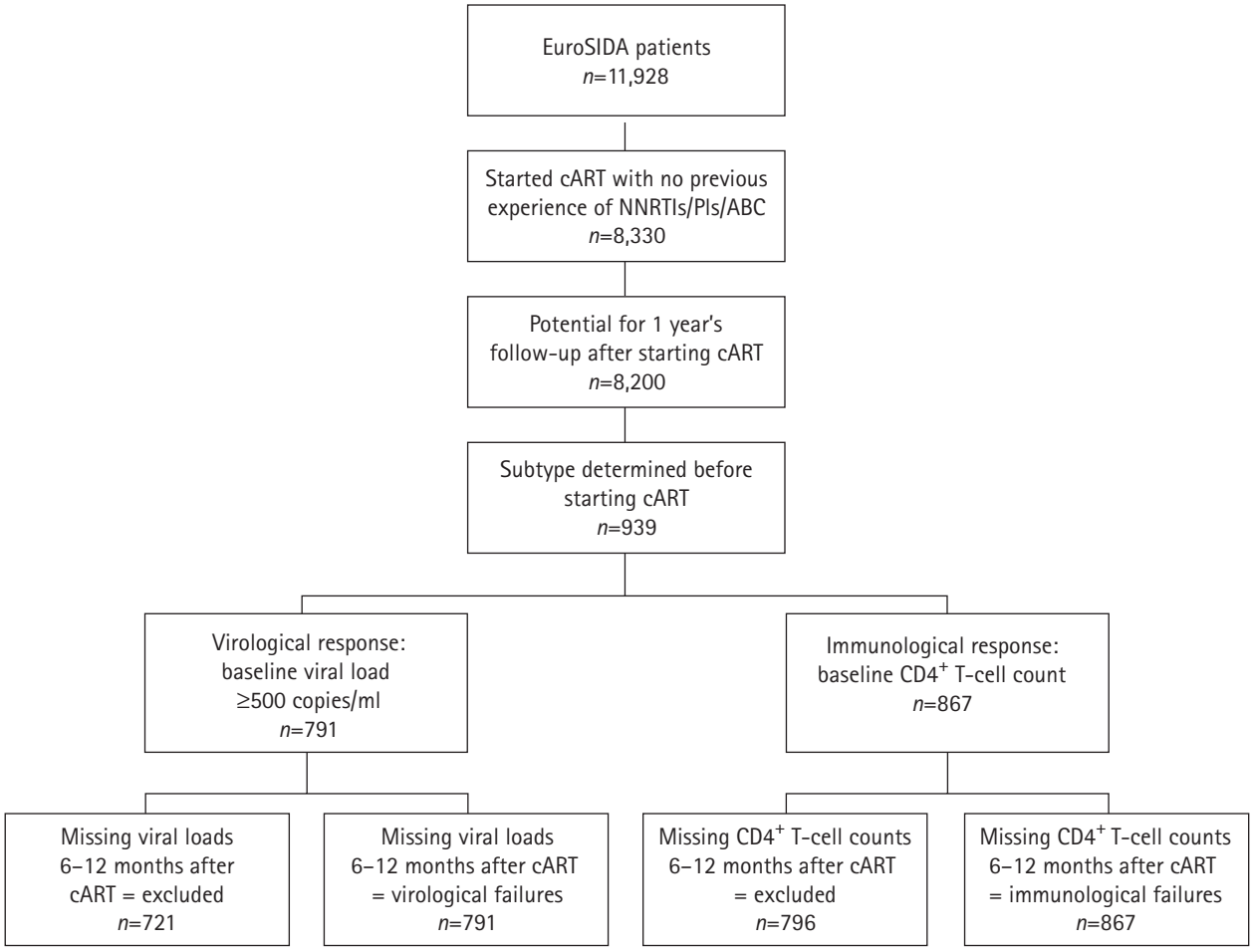
χ^2 and Kruskal–Wallis tests were used to test the significance of differences in demographics between patients infected with B versus non-B subtypes. All tests were two-sided and a $P<0.05$ was taken to be statistically significant. Resistance mutations were defined according to IAS-USA October/November 2005 [38]. SAS software version 8.2 (SAS Institute, Cary, NC, USA, 1999–2001) was used for all analyses.

Response to cART was analysed via logistic regression on the first measurements 6–12 months after initiation of cART. A virological response was defined as a viral load <500 copies/ml and immunological response as a $CD4^+$ T-cell count increase of ≥100 cells/mm^3 from the start of cART (baseline) to be consistent with previous EuroSIDA analyses [39,40]. For analyses of virological response, patients were required to have an unsuppressed baseline viral load of ≥500 copies/ml and for immunological response, patients needed a baseline $CD4^+$ T-cell count, measured within 6 months prior to starting cART. In the main analysis, patients with missing values in the period 6–12 months after cART were defined as virological/immunological failures to maximize the number of patients included. A sensitivity analysis was conducted excluding these patients instead. All analyses were intent-to-treat in the sense that no adjustments were made for stopping or changing any component of the regimen.

Multivariable models were developed to investigate the effects of subtype on virological/immunological response and were adjusted for potentially confounding variables found to be significant ($P<0.10$) in univariable analyses. A stepwise selection method was used to confirm final model selection. Variables investigated included baseline viral loads and $CD4^+$ T-cell counts, hepatitis B/C coinfection status, prior AIDS diagnosis, previous antiretroviral experience, current regimen, demographics, how subtype was determined and whether or not tested for resistance.

Sensitivity analyses with stricter inclusion criteria were conducted on subsets of antiretroviral-naive patients, patients with subtypes determined by phylogenetic analysis, patients who had their subtypes identified post-1999 due to subtype-specific problems with viral load assays before then [41–43] (for virological response only) and patients who started cART after enrollment into EuroSIDA. Alternative definitions of virological and immunological response were also investigated (a viral load <50 copies/ml and a $CD4^+$ T-cell count increase of 25% from baseline).

Figure 1. Patient numbers in analyses according to inclusion criteria



ABC, abacavir; cART, combination antiretroviral therapy; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PIs, protease inhbitors.

Results

Demographics

Of the 11,928 EuroSIDA patients, 8,200 (69%) started cART with no previous NNRTI/PI/ABC experience and the potential for at least 1 year's follow-up. Figure 1 displays a description of the patients included. Pre-cART subtype results were available for 939 (11%) of these. Compared with the 7,261 (89%) excluded patients, those with known subtypes had higher median baseline CD4+ T-cell counts (228 cells/mm^3 compared with 185 cells/mm^3, P<0.001), higher viral loads (4.7 compared with 4.5 $\log_{10}$ copies/ml, P<0.001), less previous AIDS diagnoses (22% compared with 30%, P<0.001) and less previous antiretroviral experience (55% compared with 63%, P<0.001).

Overall the analyses included 812 (86%) patients infected with subtype B, 23 (2%) with A, 42 (4%) with C and 62 (7%) with any other subtype, including 16 with CRF01_AE and 14 with CRF02_AG. Table 1 compares the demographics between the 812 infected with B and the 127 (14%) infected with non-B virus at the time of starting cART. There were similar proportions of antiretroviral-naive patients: 45% of B-infected and 42% of non-B-infected (P=0.481), and similar median baseline CD4+ T-cell counts: 230 cells/mm^3 for B-infected and 210 cells/mm^3 for non-B-infected (P=0.881). Viral loads were slightly higher for B-infected: 4.7 $\log_{10}$ copies/ml compared with 4.5 $\log_{10}$ copies/ml (P=0.045).

Resistance test results were available within 1 year before starting cART for 533 (57%) patients of whom 466 (87%) were infected with subtype B. Overall 218 (41%) patients had at least one IAS-USA NRTI resistance mutation, 35 (7%) had a major PI resistance

Table 1. Demographics at date of starting cART according to HIV-1 subtype B versus non-B

	Total		B		Non-B		
	n	%	*n*	%	*n*	%	*P*-value
All	939	100.0	812	86.5	127	13.5	–
Source of subtype ID							–
Phylogenetic analysis	729	77.6	656	80.8	73	57.5	<0.001
Other	210	22.4	156	19.2	54	42.5	–
Serology methods	6	0.6	6	0.7	0	0.0	–
Local lab results	30	3.2	21	2.6	9	7.1	–
EuroSIDA follow-up	174	18.5	129	15.9	45	35.4	–
Male	725	77.2	666	82.0	59	46.5	<0.001
HIV transmission mode							<0.001
Homosexual/bisexual	467	49.7	457	56.3	10	7.9	–
IDU	179	19.1	170	20.9	9	7.1	–
Heterosexual	223	23.7	128	15.8	95	74.8	–
Other	70	7.5	57	7.0	13	10.2	–
Ethnicity							<0.001
White	783	83.4	725	89.3	58	45.7	–
Other	156	16.6	87	10.7	69	54.3	–
Region							0.045
South	110	11.7	98	12.1	12	9.4	–
Central West	229	24.4	189	23.3	40	31.5	–
North	514	54.7	444	54.7	70	55.1	–
East	86	9.2	81	10.0	5	3.9	–
Previous AIDS	206	21.9	184	22.7	22	17.3	0.177
Hepatitis B status							0.582
Negative	662	70.5	574	70.7	88	69.3	–
Positive	68	7.2	56	6.9	12	9.4	–
Unknown	209	22.3	182	22.4	27	21.3	–
Hepatitis C status							0.013
Negative	418	44.5	351	43.2	67	52.8	–
Positive	153	16.3	143	17.6	10	7.9	–
Unknown	368	39.2	318	39.2	50	39.4	–
cART regimen							0.786
Single PI	532	56.7	461	56.8	71	55.9	–
Boosted PI	78	8.3	67	8.3	11	8.7	–
Single NNRTI	185	19.7	157	19.3	28	22.0	–
Other	144	15.3	127	15.6	17	13.4	–
Antiretroviral naive	419	44.6	366	45.1	53	41.7	0.481
Successful resistance test							
within 1 year pre-cART	533	56.8	466	57.4	67	52.8	0.327
NRTI resistance*	218	40.9	192	41.2	26	38.8	0.709
PI resistance*	35	6.6	27	5.8	8	11.9	–
NNRTI resistance*	24	4.5	17	3.6	7	10.4	-
Median (interquartile range)							–
GSS score according to	3.0	3.0–3.0	3.0	3.0–3.0	3.0	2.5–3.0	0.155
Rega algorithm (v6.4)*							
Date started cART regimen	Jan '98	Apr '97 to Dec '99	Feb '98	Apr '97 to Dec '99	Nov '97	Mar '97 to Mar '00	0.607
Date pre-cART subtype test	May '97	Jul '96 to Dec '98	May '97	Jul '96 to Dec '98	Apr '97	Jul '96 to Sep '98	0.752
Age, years	37	32–45	37	32–45	35	32–43	0.124
$CD4^+$ T-cell count, cells/mm^3							
Baseline	228	113–333	230	113–333	210	116–330	0.881
Nadir	172	87–260	171	87–260	176	84–257	0.843
Viral load, $\log_{10}$ copies/ml							
Baseline	4.7	4.0–5.2	4.7	4.0–5.2	4.5	3.8–5.0	0.045
Maximum ever	5.0	4.5–5.4	5.0	4.5–5.4	4.9	4.4–5.2	0.017

*In a subset of patients with a successful resistance test within 1 year pre-combination antiretroviral therapy (cART). GSS, genotypic sensitivity score; IDU, intravenous drug user; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Table 2. ORs and 95% CIs of virological and immunological response for HIV-1 subtype B versus non-B

	B			Non-B					
	n	% responders	Reference OR	*n*	% responders	Univariable OR (% CI)	*P*-value	Multivariable OR (% CI)	*P*-value
Virological response*									
Main analysis, missing=failure	689	58	1.00	102	66	1.37 (0.88–2.11)	0.160	1.05 (0.58–1.93)	0.866
Sensitivity analyses, missing=failure									
AR-naive	326	71	1.00	46	72	1.04 (0.53–2.07)	0.903	1.09 (0.44–2.70)	0.849
Subtypes by phylogenetic analysis	573	59	1.00	58	62	1.15 (0.66-2.01)	0.613	1.22 (0.58-2.56)	0.597
Subtypes post-1999	139	63	1.00	35	83	2.80 (1.09–7.20)	0.033	3.51 (0.80–15.47)	0.098
Excluding retrospective data	565	62	1.00	72	67	1.23 (0.73-2.06)	0.437	1.13 (0.56-2.29)	0.730
Endpoint viral load <50 copies/ml	689	29	1.00	102	42	1.76 (1.15–2.69)	0.010	1.39 (0.72–2.67)	0.330
Excluding missing	629	64	1.00	92	73	1.51 (0.93–2.46)	0.096	1.28 (0.65–2.54)	0.476
Immunological response†									
Main analysis, missing=failure	753	43	1.00	114	48	1.21 (0.82–1.80)	0.335	1.17 (0.73–1.87)	0.524
Sensitivity analyses, missing=failure									
AR-naive	353	48	1.00	49	55	1.31 (0.72–2.38)	0.383	1.35 (0.67–2.75)	0.401
Subtypes by phylogenetic analysis	623	43	1.00	65	48	1.21 (0.72–2.02)	0.470	1.27 (0.70–2.31)	0.425
Excluding retrospective data	614	44	1.00	85	49	1.26 (0.80–1.99)	0.317	1.34 (0.78–2.31)	0.285
Endpoint 25% $CD4^+$ T-cell increase	753	57	1.00	114	61	1.20 (0.80–1.79)	0.387	1.28 (0.78–2.09)	0.333
Excluding missing	691	47	1.00	105	52	1.21 (0.82–1.80)	0.334	1.17 (0.72–1.90)	0.537

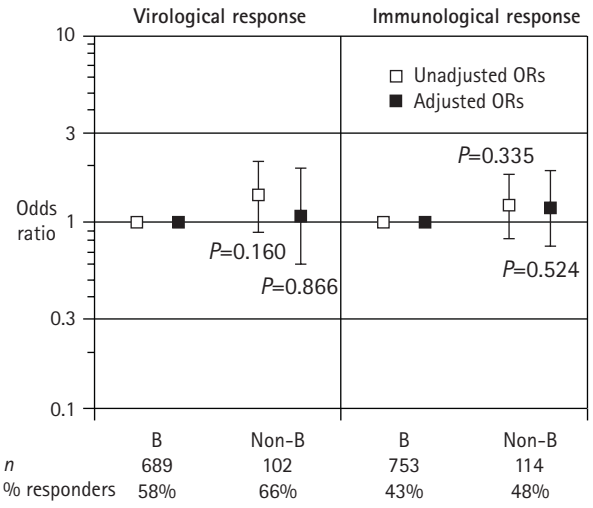
*Multivariable analyses adjusted for baseline maximum ever viral load (log_{10} copies/ml), baseline $CD4^+$ T-cell nadir (log_2 cells/mm^3), time from $CD4^+$ T-cell nadir, hepatitis B and C coinfection status, calendar year of starting combination antiretroviral therapy (cART) regimen, type of regimen, number of new drugs in regimen, whether or not antiretroviral (AR) naive at baseline, age, transmission risk group, region of clinical centre visited and ethnicity. †Multivariable analyses adjusted for baseline viral load (log_{10} copies/ml), baseline $CD4^+$ T-cell nadir (log_2 cells/mm^3), time from $CD4^+$ T-cell nadir, calendar year of starting cART regimen, type of regimen, number of new drugs in regimen, whether or not AR naive and transmission risk group. CI, confidence interval; OR, odds ratio.

mutation and 24 (5%) had an NNRTI resistance mutation. Genotypic sensitivity score (GSS), as defined by the Rega algorithm version 6.4, was also compared in this subset. The median scores in both groups were 3.0, *P*=0.155.

Virological response to cART

Table 2 displays the results of analysing virological response to cART in patients infected with B versus non-B subtypes. Patients with baseline viral loads missing or <500 copies/ml were excluded leaving 791 patients. Of these, 721 had a viral load measurement in the period 6–12 months after starting cart (median time to first measurement: 7 months, interquartile range [IQR]: 6–8 months, for both B-infected and non-B-infected patients, *P*=0.779) and a further 47 patients had a subsequent measurement recorded after 12 months. Counting patients with missing values during this period as virological failures gave a total of 469 (59%) patients out of 791 who achieved virological suppression (<500 copies/ml): 402 (58%) of 689 B-infected compared with 67 (66%) of 102 non-B-infected patients, *P*=0.159. Within the non-B subtypes, 8 (47%) of 17 patients

Figure 2. ORs of virological response (<500 copies/ml) and immunological response (100 CD4⁺ T-cell increase) after starting cART according to HIV-1 subtype B versus non-B



cART, combination antiretroviral therapy; ORs, odds ratio.

infected with subtype A, 25 (66%) of 38 patients with C and 34 (72%) of 47 patients with 'Other' achieved virological suppression, *P*=0.152.

Before adjustment for potentially confounding variables, the data showed no evidence of a significant difference between the odds of achieving virological suppression in non-B-infected compared with B-infected patients (odds ratio [OR]: 1.37, 95% confidence interval [CI]: 0.88–2.11, *P*=0.160). A multivariable model adjusting for baseline maximum ever viral load, CD4⁺ T-cell nadir, time from CD4⁺ T-cell nadir, hepatitis B and C coinfection, calendar year of starting cART, type of regimen, number of new drugs in regimen, whether or not antiretroviral-naive at baseline, age, transmission risk group, region of clinical centre visited and ethnicity also showed no significant difference (OR: 1.05, 95% CI: 0.58–1.93, *P*=0.866). Unadjusted and adjusted ORs are displayed in Figure 2. Further investigation into A, C and 'Other' subtypes showed adjusted ORs (95% CIs) of 0.32 (0.10–1.00; *P*=0.051), 1.07 (0.45–2.54; *P*=0.879) and 1.64 (0.73–3.67; *P*=0.233), respectively. Interactions between type of cART regimen and subtype were also considered. The power for this was limited; however, the interaction between PI-containing versus other regimens and subtype was not found to be significant.

All sensitivity analyses gave results consistent with those from the main analyses (results shown in Table 2).

Immunological response to cART

The results of analysing immunological response to cART in patients infected with B versus non-B are also displayed in Table 2. Patients with missing baseline CD4⁺ T-cell counts were excluded, leaving 867 patients. Of these, 796 had a CD4⁺ T-cell count 6–12 months after starting cART (median time to first measurement: 7 months, IQR: 6–8 months, for both B-infected and non-B-infected patients, *P*=0.973) and a further 47 patients had a subsequent measurement recorded after 12 months. Counting patients with missing values during this period as immunological failures gave a total of 382 (44%) patients out of 867 who experienced a successful immunological response: 327 (43%) of 753 B patients and 55 (48%) of 114 non-B patients (*P*=0.334). Of the non-B subtypes, 7 (35%) of 20 patients infected with subtype A, 18 (46%) of 39 patients with C and 30 (55%) of 55 patients with 'Other' experienced an immunological response (*P*=0.346).

Before adjustment for potentially confounding variables, there was no evidence of a significant difference in the odds of experiencing an immunological response between non-B-infected and B-infected patients (OR: 1.21, 95% CI: 0.82–1.80, *P*=0.335). A multivariable model adjusting for baseline viral load, baseline CD4⁺ T-cell nadir, time from CD4⁺ T-cell nadir, hepatitis B and C coinfection, calendar year of starting cART, type of regimen, number of new drugs in regimen, whether or not antiretroviral-naive at baseline, age and transmission risk group again found no significant difference (OR: 1.17, 95% CI: 0.73–1.87, *P*=0.524). Unadjusted and adjusted ORs are again displayed in Figure 2. ORs (95% CIs) for A, C and 'Other' subtypes compared to B were 0.64 (0.24–1.72; *P*=0.375), 1.06 (0.52–2.18; *P*=0.871) and 1.54 (0.83–2.86; *P*=0.168), respectively. Interactions between type of cART regimen (PI-containing versus other) and subtype were again considered; however, they were not found to be significant.

Sensitivity analyses also gave consistent results as those from the main analyses (results shown in Table 2).

Discussion

In this cohort of HIV-1-infected patients from across Europe, a comparison of patients infected with B and non-B subtypes showed that after adjustment for potentially confounding variables, there was no evidence of significant differences in virological or immunological response to cART. Relatively few patients were infected with non-B subtypes, but further investigation suggested that there were generally no significant differences between patients infected with A, C or 'Other' subtypes, compared with those with B.

However, due to the small numbers, the possibility that some differences do exist cannot be excluded.

The findings are consistent with previous research [27–35]. For example, Bocket *et al.* 2005 [28] compared time to virological suppression between 317 B-infected and 99 non-B-infected antiretroviral-naive patients in a French cohort and found no significant difference. A retrospective case–control study of 50 B-infected and 50 non-B infected patients starting cART looked at both initial and long-term virological response, finding no significant differences [33]. Analyses such as this have implications for increasing the availability of drugs in developing countries (where non-B subtypes dominate) [22], for future drug development and for potential vaccine development [3,7,44].

There is evidence that the frequency and pattern of drug-resistance mutations differ between subtypes, which could suggest that differences in virological, immunological and clinical outcomes would be expected [10,11,18,21,22]. The results from these analyses imply that any differences in drug resistance development between subtypes have not impacted greatly on patients' response to therapy.

The limitations of this study should be recognized when interpreting these results. The patients in this analysis were from an observational study and hence were not randomized to groups, so there may be unmeasured or unknown confounding variables. However, all measured potentially confounding variables were investigated or adjusted for in analyses to limit their influence on the results. The reason for patients having their subtypes determined may also introduce a bias. It is likely that subtypes were mostly determined at the same time as a resistance test and some patients may have been selected for testing if they were virologically or immunologically failing on a regimen. To limit the impact of this, patients were only included if their subtypes were determined prior to starting cART so that any who were tested for resistance (and also had their subtype determined) due to failing on cART were excluded.

The results from sensitivity analyses were found to be consistent with those of the main analyses. One analysis investigated another conventional way of dealing with missing values (exclusion) [45]. The reasons for these missing values are unknown, but some could be due to the varying frequency of measurements. This was the motivation for using a binary endpoint, as unlike a time to event endpoint, it was relatively robust to the number of measurements available. Another analysis took patients who had their subtypes identified after 1999 only, as earlier assays were identified as having problems measuring accurately in non-B subtypes [41–43]. A subset excluding patients with retrospective data was also analysed; that is, those who started cART before being enrolled into EuroSIDA. Patients from this subset were excluded, as they had already survived a period of time up to enrollment; therefore, they may have been more likely to have a successful response to therapy than those starting cART after enrollment.

In summary, the findings from these analyses showed no evidence of significant differences in virological or immunological response to cART between B-infected and non-B-infected patients. Those infected with non-B subtypes did not show a detrimental outcome compared with patients infected with B, as suggested by differences in resistance development found in other research. The continued expansion of the EuroSIDA resistance database and the exclusive use of phylogenetic analysis to determine subtypes will allow more sensitive analyses in the future with increased power to detect any true differences. Increasingly we will be able to focus on individual non-B subtypes and on responses to particular regimens.

Acknowledgements

Sponsorship: The EuroSIDA study was supported by grants from the BIOMED 1 (CT94-1637) and BIOMED 2 (CT97-2713) programs and the fifth framework program (QLK2-2000-00773) of the European Commission. Unrestricted grants were also provided by Bristol-Myers Squibb, GlaxoSmithKline, Boehringer Ingelheim and Roche. The participation of Swiss centres was funded by a grant from the Swiss Federal Office for Education and Science.

Additional files

The additional file 'The EuroSIDA Study Group' can be accessed via the Volume 11 Issue 6 contents page for Antiviral Therapy, which can be found at www.intmedpress.com (by clicking on 'Antiviral Therapy' then 'Journal PDFs').

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Accepted for publication 06 May 2006

Appendix VIII. Transmitted drug resistant HIV-1 and association with virologic and CD4 cell count response to combination antiretroviral therapy in the EuroSIDA Study

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Appendix IX. Comparison of genotypic resistance profiles and virological response between patients starting nevirapine and efavirenz in EuroSIDA

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Appendix X. Incidence of abacavir hypersensitivity reactions in EuroSIDA

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Original article

Incidence of abacavir hypersensitivity reactions in EuroSIDA

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Background: The aim of the study was to investigate the incidence of abacavir-related hypersensitivity reaction (HSR) and associated deaths in EuroSIDA HIV-1-infected patients.
Methods: Poisson regression models were developed to compare incidence of abacavir discontinuation according to the line of therapy within which abacavir was received, geographical regions, calendar time and drug formulation (abacavir/lamivudine combination tablet versus abacavir as a single drug or abacavir/zidovudine/lamivudine combination).
Results: Of 3,278 patients that started abacavir, 2,101 (64.1%) discontinued. Of these, 167 (5.1%) discontinued abacavir within 3 months due to HSR with an incidence of 22.1 (95% confidence interval [CI] 18.7–25.4) per 100 person-years of follow-up. After adjustment for gender, prior AIDS, hepatitis C serostatus, baseline CD4+ T-cell count, region and calendar time, HSR incidence was significantly higher in those starting abacavir in a first-line regimen compared with second-line (incidence rate ratio [IRR] 2.04 [95% CI 1.24–3.38]; *P*=0.005). There was no significant difference between regions. HSR incidence from 2005 onwards was significantly lower compared with 1999–2000 (IRR 0.54 [95% CI 0.32–0.92]; *P*=0.024). There was a lower observed incidence in patients starting abacavir/lamivudine compared with other formulations (IRR 0.33 [95% CI 0.13–0.88]; *P*=0.027), however, available data were limited.
Conclusions: Incidence of abacavir-related HSR is higher in patients starting abacavir in first-line therapy, which could indicate increased over-diagnosis. HSR incidence has decreased in recent years, which might reflect the wider availability of genetic screening and improved awareness of symptoms. There were no reported deaths due to abacavir HSR.

Introduction

Hypersensitivity reaction (HSR) occurs in approximately 4–8% of patients starting abacavir generally within 6 weeks of initiation [1–5]. It is characterized by fever, rash, neurological, gastrointestinal, musculoskeletal and respiratory symptoms [1,4–6] and if the drug is not discontinued immediately there is a risk of death [3,6,7]. Potentially fatal symptoms may reoccur within hours if the drug is restarted [8,9]. However, as patients are advised to stop immediately if symptoms arise, reported incidence of fatal HSR is fortunately rare. A recent study reported no HSR-related deaths in over 11,500 patients exposed to either Ziagen® (abacavir as a single tablet) or Trizivir® (abacavir, lamivudine and zidovudine) [10]. This study also found no differences in the frequency of abacavir HSR between patients starting either formulation.

The genetic allele HLA-B*5701 has been found to be strongly associated with abacavir-related HSR and is more prevalent in Caucasian individuals, which could account for differences in observed rates of HSR between ethnic groups [11–15]. In particular, the presence of HLA-B*5701, HLA-DR7 and HLA-DQ3 was found to have a positive predictive value of 100% and absence of

this combination had a negative predictive value of 97% [16]. Prospective genetic screening for HLA-B*5701 prior to prescribing abacavir has been shown to dramatically reduce the incidence of abacavir-related HSR, but is not widely available to all patients [11,14,15,17–21]. In clinical practice, diagnosis of HSR can be difficult as it includes a combination of non-specific symptoms. Patients who exhibit symptoms consistent with HSR may be given a skin patch test to immunologically confirm whether or not they are a true HSR case [19,21]. Recent findings from the PREDICT-1 study provided evidence that the presence of HLA-B*5701 was a necessary condition for abacavir HSR and that withholding the drug from these patients would eliminate the reaction [15]. The findings also highlighted the problem of clinical over-diagnosis of HSR that results from physicians not being able to risk missing any potential true cases of HSR. Further factors that have been linked to an increased risk of HSR include female gender, higher $CD8^+$ T-cell counts at initiation of abacavir and abacavir use during primary HIV infection [22–24]. Factors linked to a decreased risk include previous treatment experience and more advanced disease [22,25].

EuroSIDA collects prospective longitudinal data from a large, heterogeneous population in centres across Europe, which provides the opportunity to compare incidence of abacavir-related HSR across different patient subsets. The objective of these analyses was to investigate the incidence of abacavir discontinuation, particularly as a result of HSR, according to the line of therapy within which abacavir was received (first, second, third or subsequent regimens), geographical region, calendar time and coformulation of abacavir (as part of the combination tablet, Kivexa® [abacavir/lamivudine] or as part of either the combination tablet Trizivir® or as Ziagen®). The incidence of HSR within these patient subsets has not previously been evaluated in a large cohort study and is important to identify patients most at risk and to monitor trends. The rate of death associated with abacavir HSR was also determined.

Methods

Patients

The EuroSIDA study is a prospective, observational cohort of 14,310 HIV-1-infected patients in 93 centres across Europe, Israel and Argentina. The study has been described in detail previously [26]. In brief, patients were enrolled into seven cohorts from May 1994 onwards and median follow-up time was to January 2007. Information is collected on a standardized data collection form every 6 months, including all $CD4^+$ T-cell counts and viral loads measured since the last follow-up and starting and stopping dates of all antiretroviral drugs. The forms (available at www.cphiv.dk) provide a list of reasons for discontinuation of antiretroviral drugs from which sites are asked to indicate the most appropriate reason (with one reason chosen per drug stopped) on the basis of the physician's assessment. EuroSIDA also requests that all deaths are recorded on the forms with the cause of death selected from a list provided. To ensure correct patient selection and to verify that accurate data is supplied, members of the coordinating office visit all centres to check the information provided against case notes for all patients with clinical events and 10% of randomly selected patients per year.

Patients were included in the analyses if they initiated abacavir as Ziagen®, Trizivir® or Kivexa® for the first time during prospective follow-up after 1 January 1999 (the date at which EuroSIDA started collecting data on reasons for discontinuation of therapy).

Statistical methods

Patient characteristics were compared using χ^2 tests and Fisher's exact tests for categorical data and Kruskal–Wallis tests for continuous data. Only the first abacavir discontinuation with a corresponding reason was considered and baseline was defined as the date that the drug was first started. Incidence of abacavir discontinuation was assessed using a person-year analysis with person-years of follow-up (PYFU) measured from the date of starting abacavir to the earliest of the last follow-up visit, death or until abacavir was discontinued for any reason. The incidence of abacavir HSR was assessed in the 3 months after initiating abacavir treatment with follow-up time to the earliest of 3 months, death or abacavir discontinuation. No standardized guidelines were used to define HSR and cases of HSR were not reviewed centrally. Diagnosis was based on physician assessment.

Univariable Poisson regression models were used to identify factors that could potentially affect incidence of HSR-related discontinuation within 3 months of starting abacavir. Factors investigated were gender, HIV exposure group, ethnicity, prior AIDS diagnosis, hepatitis B/C coinfection serostatus, $CD4^+$ T-cell count, nadir $CD4^+$ T-cell count and viral load at date of starting abacavir (baseline), whether or not patients were previously antiretroviral-naive, age and concurrent use of other drugs that may cause HSR. The potential interactions between hepatitis C status and both HIV exposure groups and nevirapine use were also investigated. A multivariable Poisson regression model was then developed adjusting for the factors found to be significant (P-value <0.1) in the univariable models. The model also included factors such as whether patients started abacavir as part of a first-line regimen (containing at least one protease inhibitor [PI], non-nucleoside reverse transcriptase inhibitor [NNRTI] or abacavir), second regimen (containing a new PI, a new NNRTI or abacavir

and started at least 1 month after initiation of the first), third or subsequent regimen; other factors included were the geographical region of EuroSIDA the patients were seen in (categorized into South, Central West, North and East, as described in a previous analysis [27]) and calendar time of starting abacavir, which was divided into two-year periods (1999–2000, 2001–2002, 2003–2004 and 2005 onwards). A further factor considered in a separate model was whether a patient started abacavir for the first time as part of the coformulation Kivexa® or whether they started abacavir as the single drug Ziagen® or as part of Trizivir®. As Kivexa® was first introduced in January 2004, only patients who started abacavir after this time were included in this particular analysis.

All tests were two-sided and a *P*-value of <0.05 was taken to be statistically significant. SAS software version 9.1 (SAS Institute, Cary, NC, USA) was used for all analyses.

Results

Patient numbers

A total of 3,278 (22.9%) patients fulfilled the inclusion criteria with a median follow-up time of 1.4 years (range 0.0–8.5) and a total follow-up time of 6,803 person-years. Among the patients who started abacavir, there were 2,101 (64.1%) discontinuations, of which 193 (9.2%) were associated with HSR. As expected from the literature, HSR was experienced very soon after starting abacavir with a median time to HSR-related discontinuation of 1 month (interquartile range [IQR] 0.3–1.2). A further 344 (16.4%) patients discontinued due to treatment failure with a median time on abacavir treatment of 16 months (IQR 8.4–29.0). Other reasons for discontinuation reported by 317 (15.1%) patients included clinical fat abnormalities, dyslipidaemia, toxicities and structured treatment interruption. A further 285 (13.5%) patients had other causes, not specified in the list. Finally, 607 (28.9%) had the reason for discontinuation as patient or physician's choice and 357 (17.0%) had an unknown reason. As the median times to discontinuation for these groups were over a year (13.0 months [IQR 4.2–32.5] and 18.0 months [IQR 5.3–40.6], respectively), it is unlikely that these patients experienced HSR.

Patient characteristics at date of abacavir initiation

Table 1 shows a comparison of the patient characteristics at date of abacavir initiation between 1,177 patients who continued on abacavir therapy in the available follow-up time, 193 patients who discontinued abacavir due to HSR and 1,908 patients who discontinued due to a reason not reported as HSR. Patients who continued abacavir throughout their follow-up time started treatment most recently, followed by those who discontinued due to HSR and then those who discontinued for another reason (medians June 2004, June 2001 and January 2001, respectively, *P*<0.001). Patients who discontinued abacavir were more likely to be intravenous drug users (*P*<0.001) than those who continued on the drug, had lower baseline $CD4^+$ T-cell counts (*P*<0.001) and higher baseline viral loads (*P*<0.001). A higher percentage of patients who discontinued abacavir due to HSR were Caucasian (95.3%, *P*=0.011), hepatitis C positive (28.0%, *P*<0.001), antiretroviral-naive (5.7%, *P*=0.007) and were less likely to start abacavir in a triple nucleoside reverse transcriptase inhibitor (NRTI) regimen (11.9%, *P*<0.001). They were also more likely to have started nevirapine at the same time as abacavir (7.8%, *P*=0.042). There were similar percentages of patients across the three groups starting other drugs that have been linked to HSR, that is, efavirenz, amprenavir and cotrimoxazole.

Incidence of abacavir discontinuation and abacavir-related HSR

The incidence of abacavir discontinuation for any reason (including HSR) was 30.9 (95% confidence interval [CI] 29.6–32.2) per 100 PYFU and for HSR specifically the incidence was 2.8 (95% CI 2.4–3.2) per 100 PYFU. To avoid including patients who may have been misclassified as experiencing HSR, 167 patients (86.5% of the 193 reported to have discontinued abacavir due to HSR) who discontinued abacavir due to HSR within the first 3 months of abacavir treatment were investigated further. This accounted for 5.1% of all patients starting the drug. The incidence of abacavir discontinuation due to HSR within 3 months was 22.1 (95% CI 18.7–25.4) per 100 PYFU during 757 PYFU.

Incidence according to the line of therapy within which abacavir was received

A total of 252 (7.7%) patients started abacavir as part of a first-line regimen, 952 (29.0%) started it as part of a second regimen, 1,081 (33.0%) as part of a third and 993 (30.3%) as part of a fourth or subsequent regimen (Table 2). The highest incidence of abacavir discontinuation for any reason was in those who started abacavir as part of a fourth/subsequent regimen (*P*=0.002). Incidence of HSR-related discontinuation within the first 3 months was highest in those who started abacavir as part of a first-line regimen (*P*=0.002).

Incidence according to EuroSIDA region

A total of 929 (28.3%) patients were from the South region, 938 (28.6%) were from the Central West, 1,164 (35.5%) were from the North and 247 (7.5%) were from the East (Figure 1). Overall there was no significant difference in incidence of discontinuation due to any reason and incidence of discontinuation due to HSR between regions (*P*=0.797 and *P*=0.114,

respectively), however, the North region appeared to have a lower incidence of HSR-related discontinuation than the other regions.

Incidence according to date of initiation of abacavir

A total of 1,245 (38.0%) patients started abacavir between 1999 and 2000, 863 (26.3%) between 2001 and 2002, 450 (13.7%) between 2003 and 2004 and 720 (22.0%) from 2005 onwards (Figure 2). Incidence of abacavir discontinuation for any reason was similar over 1999–2000 and 2001–2002 followed by a slight increase during 2003–2004 and a drop from 2005 onwards, giving an overall significant difference (P=0.014). Incidence of abacavir discontinuation due to HSR within the first 3 months of abacavir treatment remained similar over the years until 2005 onwards when a sharp decrease to 11.4 cases per 100 PYFU during 166 PYFU was observed, giving an overall significant difference (P=0.004).

Table 1. Patient characteristics at start of abacavir therapy according to continuation of abacavir therapy, discontinuation of abacavir due to hypersensitivity reaction or discontinuation of abacavir due to another reason

Characteristic	Total	Continuation of abacavir	Discontinuation due to HSR	Discontinuation due to other reason	*P*-value
All patients, *n* (%)	3,278 (100.0)	1,177 (35.9)	193 (5.9)	1,908 (58.2)	–
Male gender, *n* (%)	2,539 (77.5)	937 (79.6)	141 (73.1)	1,461 (76.6)	0.047
HIV exposure					
Male homosexual intercourse, *n* (%)	1,507 (46.0)	591 (50.2)	80 (41.5)	836 (43.8)	<0.001
Heterosexual intercourse, *n* (%)	861 (26.3)	306 (26.0)	48 (24.9)	507 (26.6)	–
Intravenous drug use, *n* (%)	672 (20.5)	192 (16.3)	51 (26.4)	429 (22.5)	–
Other, *n* (%)	238 (7.3)	88 (7.5)	14 (7.3)	136 (7.1)	–
White ethnicity, *n* (%)	3,026 (92.3)	1,066 (90.5)	184 (95.3)	1,776 (93.1)	0.011
Previous AIDS	1,197 (36.5)	412 (35.0)	59 (30.6)	726 (38.1)	0.049
Hepatitis B status					
Negative, *n* (%)	2,561 (78.1)	976 (82.9)	143 (74.1)	1,442 (75.6)	<0.001
Positive, *n* (%)	206 (6.3)	58 (4.9)	14 (7.0)	134 (7.0)	–
Unknown, *n* (%)	511 (15.6)	143 (12.2)	36 (18.7)	332 (17.4)	–
Hepatitis C status					
Negative, *n* (%)	1,950 (59.5)	785 (66.7)	103 (53.4)	1,062 (59.5)	<0.001
Positive, *n* (%)	717 (21.9)	228 (19.4)	54 (28.0)	435 (22.8)	–
Unknown, *n* (%)	611 (18.6)	164 (13.9)	36 (18.7)	411 (21.5)	–
Type of regimen					
Single PI plus ≥2 NRTIs, *n* (%)	281 (8.6)	92 (7.8)	21 (10.9)	168 (8.8)	<0.001
Ritonavir-boosted PI plus ≥2 NRTIs, *n* (%)	687 (21.0)	326 (27.7)	39 (20.2)	322 (16.9)	–
Single NNRTI plus ≥2 NRTIs, *n* (%)	917 (28.0)	392 (33.3)	51 (26.4)	474 (24.8)	–
Triple NRTI, *n* (%)	722 (22.0)	182 (15.5)	23 (11.9)	517 (27.1)	–
Other, *n* (%)	671 (20.5)	185 (15.7)	59 (30.6)	427 (22.4)	–
Concurrent use of drugs that may cause HSR					
Nevirapine, *n* (%)	183 (5.6)	51 (4.3)	15 (7.8)	117 (6.1)	0.042
Efavirenz, *n* (%)	511 (15.6)	165 (14.0)	33 (17.1)	313 (16.4)	0.173
Amprenavir, *n* (%)	108 (3.3)	32 (2.7)	3 (1.6)	73 (3.8)	0.093
Cotrimoxazole, *n* (%)	42 (1.3)	14 (1.2)	3 (1.6)	25 (1.3)	0.903
Antiretroviral-naive at start of abacavir, *n* (%)	96 (2.9)	42 (3.6)	11 (5.7)	43 (2.3)	0.007
Median date of abacavir start (IQR)	Oct 01 (Apr 00–Jun 04)	Jun 04 (Jul 01–Feb 06)	Jun 01 (Jan 00–Feb 06)	Jan 01 (Jan 00–Sep 02)	<0.001
Median age, years (IQR)	41 (37–48)	42 (37–49)	41 (37–48)	41 (36–48)	0.005
Median CD4$^+$ T-cell count (IQR)					
At baseline, cells/mm^3*	357 (211–544)	394 (239–586)	327 (226–480)	336 (190–520)	<0.001
At nadir, cells/mm^3†	127 (50–215)	134 (51–218)	136 (63–200)	121 (46–214)	0.114
Median viral load (IQR)					
At baseline, $\log_{10}$ copies/ml‡	2.8 (1.7–4.4)	2.1 (1.7–4.0)	3.1 (1.7–4.4)	3.3 (1.7–4.6)	<0.001

Median CD4$^+$ T-cell counts and viral loads were based on measurements from *3,138 patients, †3,276 patients or ‡3,090 patients. *P*-values were obtained from χ^2, Fisher's exact and Kruskal–Wallis tests. HSR, hypersensitivity reaction; IQR, interquartile range; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Incidence according to use of Kivexa®

A total of 928 (28.3%) previously abacavir-naive patients started abacavir in January 2004 onwards, the date when the coformulation drug Kivexa® was first introduced into clinical practice. Of these 928 patients, 342 (36.9%) started Kivexa®. Overall, 305 (32.9%) patients discontinued abacavir, of which 27 (8.9%) discontinuations occurred within 3 months and were associated with HSR. Incidence of discontinuation due to any reason was significantly higher ($P<0.001$) in the non-Kivexa® group (38.6 [95% CI 33.9–43.3] cases per 100 PYFU during 676 PYFU) compared with the Kivexa® group (13.1 [95% CI 9.3–17.0] cases per 100 PYFU during 335 PYFU). There was also a significant difference ($P=0.036$) in incidence of discontinuation due to HSR within 3 months (16.2 [95% CI 9.4–23.0] cases per 100 PYFU during 136 PYFU) in the non-Kivexa® group compared with the Kivexa® group (6.3 [95% CI 2.0–14.6] cases per 100 PYFU during 80 PYFU).

Multivariable incidence rate ratios

In univariable models, female gender, positive hepatitis C coinfection serostatus, lack of prior AIDS diagnosis and a lower baseline CD4+ T-cell count were significantly associated with incidence of abacavir discontinuation due to HSR within 3 months of treatment. The concurrent use of drugs such as nevirapine, efavirenz, amprenavir and cotrimoxazole that can cause HSR, started at the same time as abacavir, were investigated to check that symptoms arising from one of these drugs had not been misdiagnosed as abacavir HSR. However, none were found to be significantly associated with HSR-related abacavir discontinuation. Ethnicity was also found to be unassociated with HSR-related abacavir discontinuation. However, the HSR incidence rate was lower in those with non-white ethnicity compared with those with white ethnicity, as expected from the literature, with an incidence rate ratio (IRR) of 0.66 (95% CI 0.34–1.30; $P=0.230$). A multivariable model was then developed that contained the above factors significantly associated with HSR in univariable analyses, as well as whether abacavir was a component of a first, second, third or subsequent regimen, geographical region and calendar time when abacavir was started. Figure 3 displays the adjusted IRRs and 95% CIs from this model.

The multivariable model provided evidence that the incidence of abacavir discontinuation due to HSR among patients starting abacavir as part of a first-line regimen was twice that observed among those starting abacavir in a second-line regimen (IRR 2.04 [95% CI 1.24–3.38]; $P=0.005$). Incidence of HSR was similar across regions, although it was lowest in the North. There was also evidence that among patients starting abacavir from 2005 onwards, the incidence of HSR-related discontinuation was almost half that observed among patients starting abacavir between 1999 and 2000 (IRR 0.54 [95% CI 0.32–0.92]; $P=0.024$).

A multivariable model was developed containing the same variables as before, but adjusting for the abacavir start date instead of the time period and with the addition of whether patients received Kivexa® or Ziagen®/Trizivir®. After adjustment, there was evidence of a significantly lower incidence of abacavir discontinuation due to HSR within 3 months in those starting Kivexa® compared with those starting Ziagen®/Trizivir® (IRR 0.33 [95% CI 0.13–0.88]; $P=0.027$).

Rate of death associated with abacavir HSR

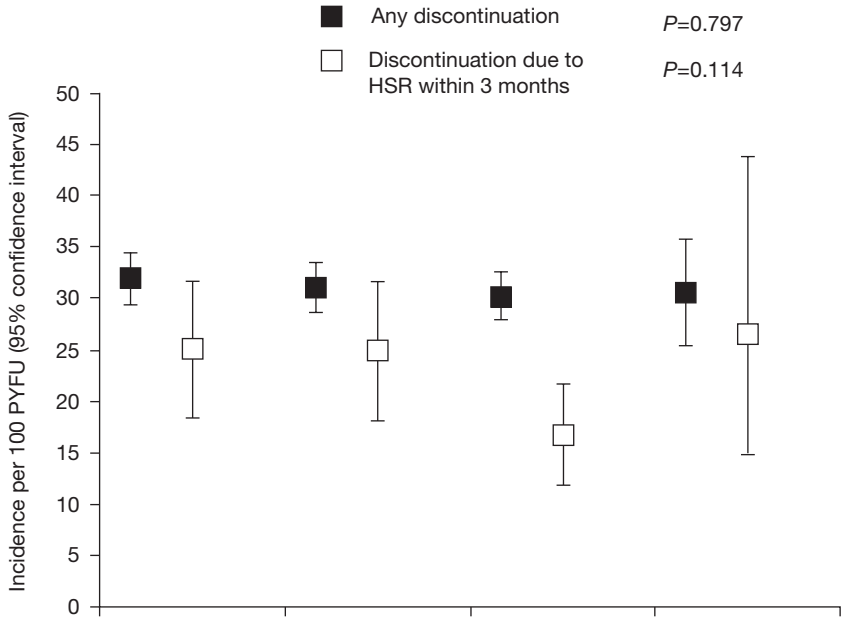
There were no fatal cases of HSR registered (defined as patients who discontinued abacavir therapy due to HSR and died within 1 month of discontinuation). A

Table 2. Abacavir discontinuation due to any reason or due to hypersensitivity reaction within 3 months according to the line of therapy within which abacavir was received

Number of patients who started abacavir	Discontinued abacavir due to any reason, *n* (%)	Discontinued abacavir due to HSR in 3 months: Of all those who discontinued, *n* (%)	Discontinued abacavir due to HSR in 3 months: Of all those started abacavir, %	Any discontinuation incidence per 100 PYFU (95% CI)	Total PYFU	Discontinuation due to HSR in 3 months incidence per 100 PYFU (95% CI)	PYFU up to 3 months
Overall *n*=3,278	2,101 (64.1)	167 (7.9)	5.1	30.9 (29.6–32.2)	6,803	22.1 (18.7–25.4)	757
As first-line therapy *n*=252	171 (67.9)	27 (15.8)	10.7	32.3 (27.5–37.2)	529	48.8 (30.4–67.2)	55
As second-line therapy *n*=952	585 (61.5)	44 (7.5)	4.6	27.4 (25.1–29.6)	2,138	19.9 (14.0–25.7)	222
As third-line therapy *n*=1,081	714 (66.1)	52 (7.3)	4.8	31.5 (29.2–33.8)	2,266	21.0 (15.3–26.7)	248
As fourth-line therapy or later *n*=993	631 (63.5)	44 (7.0)	4.4	33.7 (31.1–36.4)	1,870	19.0 (13.4–24.6)	232
P-value	0.095*	0.001*	<0.001*	0.002†	–	0.002†	–

P-values obtained from *χ^2 tests and †univariable Poisson regression models. CI, confidence interval; HSR, hypersensitivity reaction; PYFU, person-years of follow-up.

Figure 1. Abacavir discontinuation due to any reason or due to hypersensitivity reaction within 3 months according to EuroSIDA region



	South	Central West	North	East	*P*-value
n	929	938	1,164	247	–
Discontinued abacavir due to any reason, *n* (%)	593 (63.8)	659 (70.3)	715 (61.4)	134 (54.3)	<0.001
Discontinued due to HSR: of all discontinued, *n* (%)	54 (9.1)	53 (8.0)	45 (6.3)	15 (11.2)	0.127
Discontinued due to HSR: of all started, %	5.8	5.7	3.9	6.1	0.126
Total PYFU	1,861	2,130	2,372	440	–
PYFU up to 3 months	216	214	271	57	–

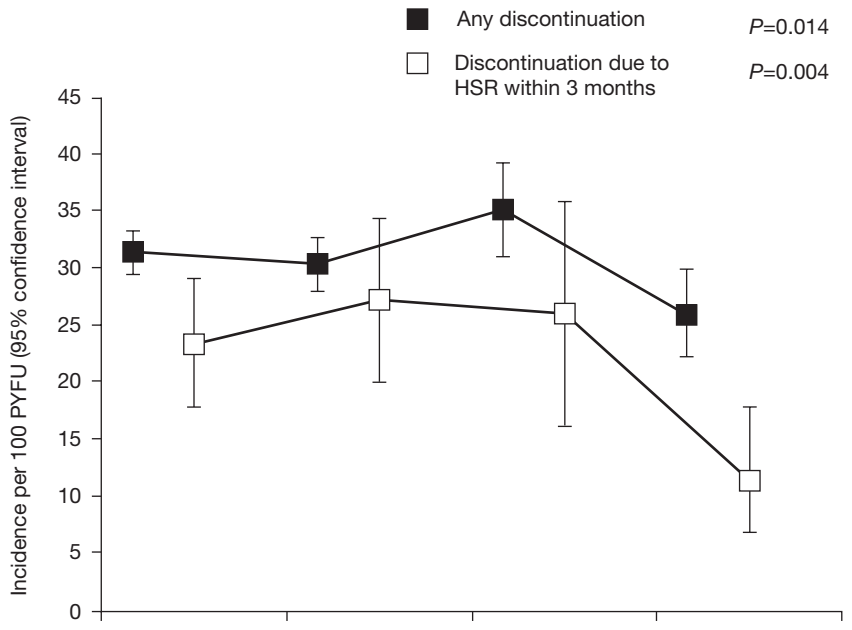
HSR, hypersensitivity reaction; PYFU, person-years of follow-up.

total of 111 (3.4%) patients died on abacavir therapy or within 1 month of a non-HSR related discontinuation. Of these, 55 had specific non-HSR-related causes of death recorded and of the remaining 56 patients, 33 had a previous AIDS diagnosis and 36 had received at least two or three PI/NNRTI-containing regimens prior to the abacavir regimen. Among these 111 patients, 15 died within 3 months of starting abacavir. The investigators classified 13 of these 15 deaths as due to specific and mainly AIDS-related causes rather than as a result of HSR. Non-AIDS-related causes of death included myocardial infarction and complications in hepatitis infection. The two remaining patients had unknown causes of death, but both died with low $CD4^+$ T-cell counts of 106 cells/mm^3 and 120 cells/mm^3.

Discussion

In this cohort of 3,278 HIV-1-infected patients who started abacavir across Europe, the incidence rate of abacavir discontinuation for any reason (including HSR) was 30.9 (95% CI 29.6–32.2) per 100 PYFU. In line with the 4–8% incidence found in other studies [1–5], the incidence of abacavir discontinuation due to HSR within 3 months was 22.1 (18.7–25.4) per 100 PYFU and this occurred in 5.1% of patients who

Figure 2. Abacavir discontinuation due to any reason or due to hypersensitivity reaction within 3 months according to date of initiation of abacavir



	1999–2000	2001–2002	2003–2004	2005 onwards	*P*-value
n	1,245	863	450	720	–
Discontinued abacavir due to any reason, *n* (%)	1,012 (81.3)	631 (73.1)	282 (62.7)	176 (24.4)	<0.001
Discontinued due to HSR: of all discontinued, *n* (%)	67 (6.6)	54 (8.6)	27 (9.6)	19 (10.8)	0.126
Discontinued due to HSR: of all started, %	5.4	6.3	6.0	2.6	0.006
Total PYFU	3,169	2,010	736	424	–
PYFU up to 3 months	287	200	104	135	–

HSR, hypersensitivity reaction; PYFU, person-years of follow-up.

started the abacavir treatment. This analysis assessed the incidence of HSR across different patient subsets in a heterogeneous population from centres all across Europe with a standardized data collection and checking system, as well as investigating the factors associated with abacavir-related HSR, which is important to identify patients with the highest risk.

One of the main findings was that the adjusted HSR incidence rate was found to be twice as high when abacavir was started as part of a first-line regimen compared with a second-line regimen. This supports research that showed a 42% decreased risk in patients with prior treatment experience [25]. Patients starting therapy for the first time might be more likely to be clinically diagnosed with suspected HSR, however, this might not reflect the incidence of true HSR cases. New patients could be more likely to report all symptoms experienced due to anxiety about starting treatment, whereas more experienced patients might not mention the more minor symptoms. This could result in physicians misdiagnosing HSR more often in patients starting first-line therapy to ensure that no true HSR cases are missed.

It is well-established that the HLA-B*5701 allele is associated with long-term non-progression of HIV

[28–30] and is linked to abacavir-related HSR in many studies [11–15]. Therefore, patients with this allele may be under-represented in the groups starting abacavir in second-, third- and fourth-line therapy, which could explain the increased incidence in the group starting a first-line regimen. It is more often found in Caucasian patients than in non-Caucasians [12–14], however, no significant association between white ethnicity and risk of HSR was found in this analysis and so ethnicity was ruled out as a potential confounder. This could be due to limited data as there were few non-Caucasians who discontinued due to HSR in this study (9 patients, 4.7%).

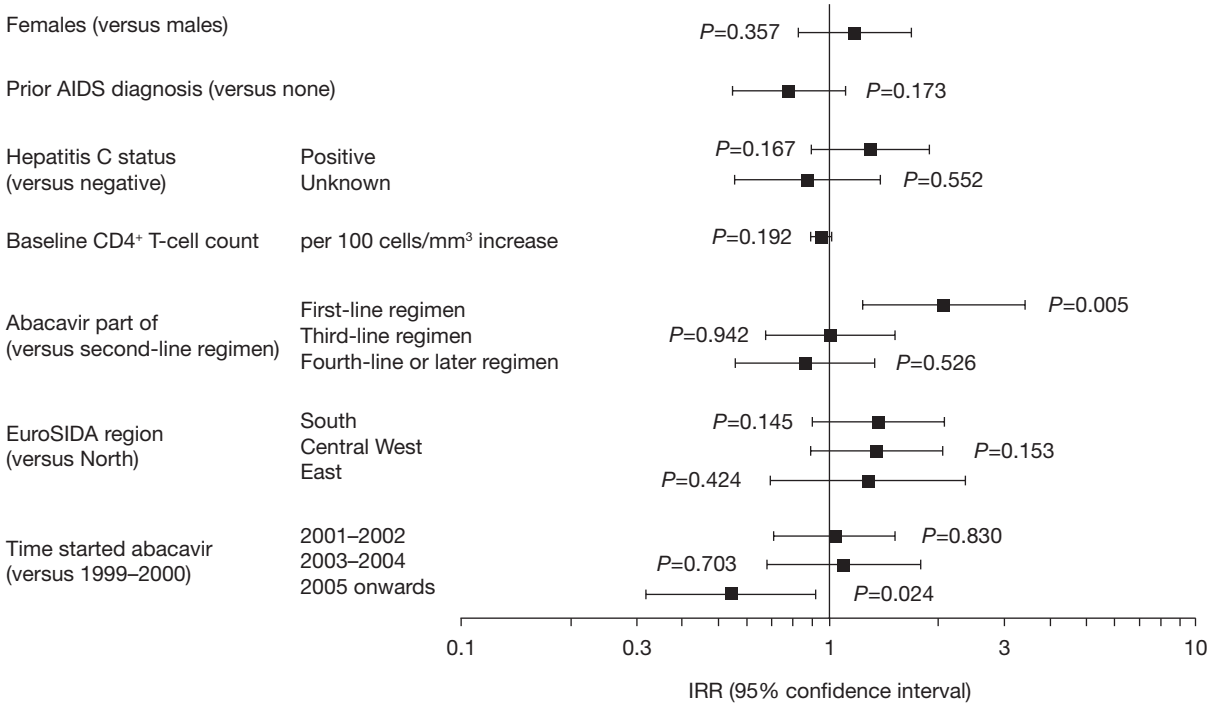
There was no evidence of a significant regional difference in abacavir discontinuation due to HSR within the first 3 months in this analysis. The frequency of HLA-B*5701 is known to vary across different populations and differences in frequency have been found across Europe [31]. Therefore, a possible explanation for this finding is that in areas of low prevalence of the allele, there could be more cases of clinical over-diagnosis of suspected HSR resulting in an overall similar incidence of abacavir HSR between regions.

A significantly higher incidence rate of HSR was found in earlier years compared with 2005 onwards. A reason for this could be that more clinics are using prospective genetic screening, which has been shown to effectively reduce the incidence of HSR by preventing the prescription of abacavir to those at high risk [11,14,15,17–21]. Also, as HSR symptoms are now better-documented, it could be more recognizable and therefore correctly identified more frequently, reducing the chance of over-diagnosis. If symptoms were misclassified as HSR in earlier years, this could explain the decrease in recent years.

A decreased incidence of abacavir discontinuation due to HSR within 3 months was observed in patients starting Kivexa® compared with those starting Ziagen® or Trizivir®. All known, measured variables that could potentially confound the results were investigated but a difference remained after adjustment. This could be due to the relatively small number of person-years available for the Kivexa® group and the small number of events during this time. Therefore, these results should be treated with caution.

Severe abacavir-related HSRs, which can lead to death, are rare but have been reported in a few case studies [7–9]. In this analysis, there were no reports of patients who discontinued abacavir due to HSR and then died within 1 month. Patients who died on abacavir therapy

Figure 3. Multivariable incidence rate ratios and 95% confidence intervals of abacavir discontinuation due to hypersensitivity reaction within 3 months



HSR, hypersensitivity reaction; IRR, incidence rate ratios; PYFU, person-years of follow-up.

or within 1 month after stopping mostly had advanced disease with known non-HSR causes of death. This suggests that any patients with severe reactions to abacavir were taken off the drug promptly to prevent fatality.

The main potential limitation of these analyses is that there could be bias in the reporting of reasons of discontinuation. The definition of HSR is unclear as it includes a combination of clinical symptoms. The incidences reported in this analysis are therefore made on the basis of a practical 'real life' approach to the diagnosis of HSR and could possibly reflect clinical diagnosis rather than true HSR. Examination of the data did not show any major differences in reported HSR between centres, suggesting that reporting bias across centres was minimal (results not shown). Furthermore, the EuroSIDA follow-up forms only collect one reason for discontinuation per drug and some patients may experience more than one toxicity, which could mean that HSR is not always reported. The forms also allow 'patient's wish' and 'physician's decision' as reasons for discontinuation, which could result in misclassification of HSR.

Another limitation is that EuroSIDA does not collect information on genetic screening. Thus, it is unknown if patients were tested for HLA-B*5701 and prescribed abacavir according to results. There is also no information on $CD8^+$ T-cell count so it was not possible to look at any association between this and HSR. Although it is assumed in this study that all deaths are recorded on the follow-up forms, it is possible that some were missed. Causes of death could also be misclassified or coded differently in different centres. The introduction of the CoDe ('Coding of Death in HIV') project in cohort studies (details at www.cphiv.dk) has helped to standardize the approach to collecting and reviewing causes of death.

In summary, incidence of abacavir discontinuation due to HSR appears to be higher in patients starting abacavir as part of first-line therapy, which might be explained by increased clinical over-diagnosis. Incidence of abacavir HSR has decreased in recent years, suggesting that prospective genetic screening, improved patient care and awareness of the symptoms of HSR may have prevented the use of this drug for high-risk patients. There appears to be a similar rate of abacavir discontinuation due to HSR across Europe. Patients starting the coformulation drug Kivexa® which was introduced in January 2004, appear to have a decreased risk to those starting Ziagen® or Trizivir®; however, limited data are available for the use of Kivexa® and so results remain preliminary. There were no reported deaths due to abacavir HSR in this analysis.

Acknowledgements

Primary funding 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) programs. Current funding support also includes unrestricted grants by Bristol–Myers Squibb, GlaxoSmithKline, Roche, Gilead, Pfizer, Merck and Co., Tibotec and Boehringer–Ingelheim. The participation of centres from Switzerland was supported by a grant from the Swiss Federal Office for Education and Science.

Disclosure statement

The authors declare no conflicts of interest.

Additional file

The additional file 'The EuroSIDA Study Group', which lists the members of the EuroSIDA study group, can be accessed via the Volume 13 Issue 5 contents page for *Antiviral Therapy*, which can be found at www.intmedpress.com (by clicking on 'Antiviral Therapy' then 'Journal PDFs').

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Accepted for publication 29 April 2008

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