Treatment strategies in HIV

THESIS

presented for the

DEGREE

of

DOCTOR OF PHILOSOPHY

in the Faculty of Medicine

(Field of study - Epidemiology)

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Declaration

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

Abstract

The introduction of highly active antiretroviral therapy (HAART) has led to significant improvements in survival and morbidity for HIV-positive patients. Though HIV can now be well managed with treatment, interlinked barriers to successful treatment are still prevalent. In particular, low adherence to therapy, resistance to drugs and drug toxicity can have a considerable impact on the success of HAART.

The potential of HAART is limited from the outset if patients are infected with a drug-resistant virus. Evidence suggests that a small minority of patients are starting HAART with drugs that the virus is resistant to, and consequently, are less likely to achieve virological suppression. A large proportion of resistance tests performed after patients' start HAART are not followed by a switch within 4 months of the result being received. This raises questions as to why the test was performed and whether limited future drug options are of concern.

A single abnormal laboratory value may be the result of random fluctuations and may not necessarily be a reason for concern over drug toxicity. I derive a more stringent definition of an ALT flare and compare this definition with that commonly found in the literature. Treatment interruptions, perhaps due to drug toxicity, should not take place when the viral load is detectable. Patients who have achieved viral suppression after interrupting treatment and who have failed a higher number of HAART regimens are at a greater risk of viral rebound, though this risk is reduced substantially as duration of suppression increases.

A score to characterise laboratory abnormalities is derived and used to predict mortality. Several methods were used; I felt the most appropriate was that based on the estimates from a regression model in which the current laboratory measurements were fitted; only three routinely measured laboratory measures were needed to calculate the score.

Acknowledgements

I would like to thank my supervisors, Caroline Sabin and Andrew Phillips, for the support, guidance and encouragement they have given me over the last 5 years. Thank-you for reading all the Chapters in such detail, and for the very helpful comments and feedback I received. I would also like to thank Alessandro Cozzi-Lepri, Colette Smith and Zoe Fox for taking the time to read a Chapter or two, and for the useful comments and advice they gave.

This thesis would not have been possible without the extensive work of all those involved in the UK CHIC Study and the UK HIV Drug Resistance Database. Thanks to those in the steering committees and co-ordinating teams of both studies, and in particular, to Teresa Hill, the UK CHIC Study co-ordinator. I know how much work is involved in getting a dataset ready, it's much appreciated.

Thank you to everyone else at the HIV Epidemiology and Biostatistics Group at the Royal Free and University College Medical School, London, for reminding me it will all be worthwhile, and for the encouragement and support they gave, especially in this last year; Jani Puredirejda, Elaine Harris, Jo Reekie, Rebecca Lodwick, Valentina Cambiano, Fiona Lampe, Daniel Grint, Fumiyo Nakagawa, David Kamara, Jeff McDonnell, Amanda Mocroft, Andrew Speakman and Jemma O'Connor.

Finally, I would like to thank my parents, especially my mum, without whom I would never have got this far. Thanks for wanting me to do this and for believing that I could, and for all the time, support and encouragement you have given me throughout the years – it's because of that, that I am able to finally hand this in.

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Frequently Used Abbreviations

AIDS:	Acquired immunodeficiency syndrome
ALB:	Albumin
ALK:	Alkaline phosphatase
ALT:	Alanine aminotransferase
AMY:	Amylase
ART:	Antiretroviral treatment
AST:	Aspartate transaminase
BHIVA:	British HIV Associaiton
BIL:	Bilirubin
BUN:	Blood urea nitrogen
CHL:	Cholesterol
CI:	Confidence interval
CKD:	Chronic kidney disease
CPK:	Creatinine phosphokinase
CRE:	Creatinine
eGFR:	Estimated glomerular filtration rate
ESRF:	End stage renal failure
GEE:	Generalised estimating equations
GGT:	Gamma-glutamyl transpeptidase
GLU:	Glucose
GSS:	Genotypic sensitivity score
HAART:	Highly active antiretroviral treatment
HAE:	Haemoglobin
HBV:	Hepatitis B virus
HCV:	Hepatitis C virus
HDL:	High density lipoprotein

HIV:	Human immunodeficiency virus
HIVAN:	HIV associated nephropathy
HR:	Hazard ratio
IAS:	International AIDS Society
IDU:	Intravenous drug users
IQR:	Interquartile range
LDL:	Low density lipoprotein
MDR:	Multi-drug resistance
MSM:	Men having sex with men
nNAMS:	Non-nucleoside analogue mutations
NNRTI:	Non-nucleoside reverse transcriptase inhibitor
NRTI:	Nucleoside reverse transcriptase inhibitor
OR:	Odds ratio
PI:	Protease inhibitor
PR:	Protease gene
PRAMS:	Protease inhibitor resistance associated mutations
PYS	Person-years
RR:	Rate ratio
RT:	Reverse transcriptase
SD:	Standard deviation
STI:	Structured treatment interruptions
TAM:	Thymidine analog mutation
TDR:	Transmitted drug resistance
TI:	Treatment interruption
TRI:	Triglycerides
ULN:	Upper limit of normal
URE:	Urea
VL:	Viral load

Chapter 1: Introduction

1.1. The HIV epidemic

The number of people living with human immunodeficiency virus (HIV) in 2007 worldwide was estimated to be around 33 million, with the number of new infections in that year standing at 2.7 million (1). Since the first described case of what was later recognised to be HIV in 1981, 25 million people have died of Acquired Immunodeficiency Syndrome (AIDS) (1). Although the issues surrounding the management of HIV have changed over the last 25 years, with better understanding of the mechanisms behind the virus, together with advances in therapy, the challenges faced by clinicians now reflect the difficulties of dealing with a viral infection for which a cure is still not available. Toxicities associated with therapy, the accumulation of resistance to antiretroviral (ART) drugs and the financial cost of a lifetime of therapy are just some of these issues.

1.2. History of HIV

Although blood samples taken from a man who died in Africa in 1959 have shown that he died from HIV-complications (2), HIV-associated illnesses are generally dated back to 1981. The emergence of Kaposi's Sarcoma (KS) and *Pneumocystis carinii* Pneumonia (PCP) amongst gay men in New York and California were the first signs of the epidemic (3). In 1982, the United States Centers for Disease Control (CDC) defined a case of AIDS as 'a disease, at least moderately predictive of a defect in cell-mediated immunity, occurring in a person with no known cause for diminished resistance to that disease. Such diseases include KS, PCP, and serious other opportunistic infections (OOI)' (4). Research by both French and American scientists (5;6) isolated the virus which was believed to cause AIDS, later named as HIV by an international committee of scientists (7).

1.3. Mechanisms of HIV

HIV belongs to a class of viruses called retroviruses, which have genes composed of ribonucleic acid (RNA) molecules. HIV attacks the immune system, the body's mechanism for protecting itself against bacteria and viruses that try to invade it. The virus infects particular types of white blood cells called CD4 lymphocytes (also known as T-cells or T-lymphocytes) which are needed to fight against infection. By copying its own genetic code into the DNA of the CD4 cells, the virus is able to

create new copies of HIV which are then released from the CD4 cell into the blood to infect and destroy other CD4 cells (and some other cells). This leads to a reduction in the number of CD4 cells, leaving the immune system with a decreased ability to fight certain infections and cancers (8-11). A healthy uninfected person usually has 800-1,200 CD4 cells per cubic millimetre (mm^3) of blood. During untreated HIV infection, this count falls towards zero – once the CD4 count cell has fallen below 200/mm^3, the body is particularly vulnerable to opportunistic infections (OIs). The CDC's most current definition of AIDS (12) includes all HIV-positive persons who have a CD4 cell count of less than 200 cells/mm^3, a CD4 cell percentage of total lymphocytes of less than 14, or one of 26 clinical indicator conditions. The European definition of AIDS differs somewhat from the CDC's definition, in that it does not include the CD4 lymphocyte count criteria.

1.4. Strains, subtypes and transmission of HIV

There are two types of HIV: HIV-1 and HIV-2. The predominant virus worldwide is HIV-1 and is the virus which was initially discovered by researchers at the Pasteur Institute in France (5). HIV-2 is largely confined to West Africa and is thought to be less easily transmitted than HIV-1 (13-15). HIV-1 is classified into three groups: M (major group), O (outlier group) and N (new group). The vast majority of infections worldwide fall into group M (16) and the rest of this thesis relates to this group only. Within Group M, there are known to be at least 9 different subtypes (clades) of HIV-1: A, B, C, D, F, G, H, J and K. Due to evolution of the virus over time (particularly in those exposed to greater than one strain), some cases are of a circulating recombinant form (CRF). CRF A/C, for example, is a combination of subtypes A and C. It is possible that further subtypes will be found in the future and hence this is not a complete list. Subtype B is the most common subtype in Europe, America, Japan and Australia, although other subtypes are becoming more frequent and now account for at least 25% of new infections in Europe (16).

There are 3 main transmission routes for HIV; unprotected penetrative sex with someone who is infected, sharing unsterilized injection equipment that has been used by someone who is infected and from mother to child during pregnancy, at birth and through breastfeeding. Other less common routes include transfusion of contaminated blood/blood products and oral sex (17;18). Historically, in Western countries, the virus has been spread mainly by anal intercourse between men and sharing of injection equipment, whilst in African countries heterosexual intercourse

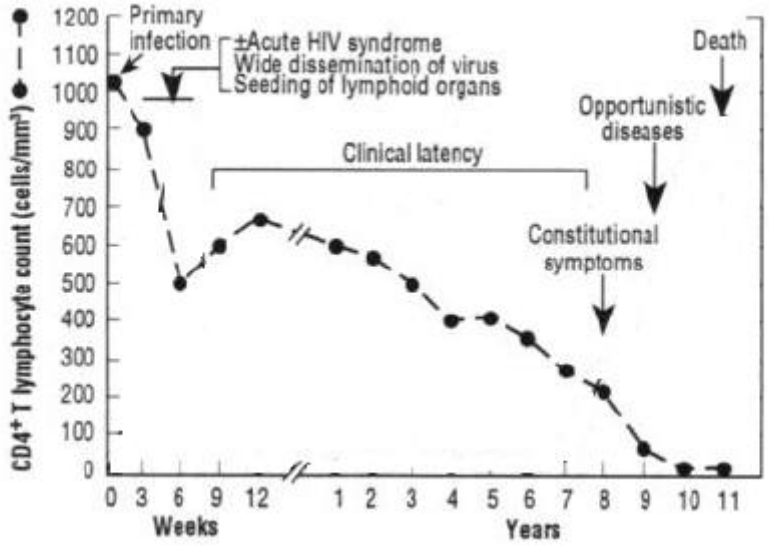
has been the main mode of infection (19). Trends over time show that although the number of infections acquired through heterosexual contact in the UK have been increasing since 2000, the vast majority of these infections were amongst those who were infected abroad (20). Further, the number of newly identified heterosexual infections in more recent years appears to be relatively stable, whilst infections acquired through homosexual contact have been increasing (21).

1.5. Stages of infection and markers of disease progression

After an individual is infected with HIV, it takes around 7-10 days for RNA from the virus to be detectable in plasma (this period is known as primary HIV infection); thereafter antibodies to the virus can be detected in serum using several different tests within a few weeks. The first of these to be introduced into clinical use was the ELISA test, which was licensed in 1985 (22). The development of antibodies (such that an individual goes from being HIV-negative to HIV-positive) may take up to 6 months and is known as 'seroconversion' (23;24). An HIV-specific cellular immune response is also generated (25). During this period, people may have symptoms such as a mild glandular fever, headache, skin rash and enlarged lymph nodes, lasting for around 2 weeks (26;27). The CD4 count declines rapidly and there is a high rate of viral replication, leading to a rise in HIV viral load (the number of viral particles found in each millilitre of plasma) (28-30) (Figure 1.5.1). This period is followed by an asymptomatic or 'silent' phase, during which time the person infected is likely to remain clinically healthy. The body continues to produce antibodies and though the virus is active in the lymph nodes (31), the antibody and cellular immune response reduces the rate of viral replication, i.e. the viral load falls to low levels amongst the majority of infected individuals. The fall in viral load is typically accompanied by a rise in CD4 cell count to sub-optimal levels (30;32). This asymptomatic stage lasts for an average of 10 years and other than swollen glands and some CDC 'B' events (12), the individual is generally free from major symptoms (33-36). As the disease progresses, the viral load increases and the number of CD4 cells declines (the rate of the CD4 cell count decline varies from individual to individual). This allows opportunistic infections, normally controlled by CD4 cell-mediated immunity, to occur. If the immune system is severely weakened, it is vulnerable to serious opportunistic infections and cancers and it is at this stage that a person who has acquired HIV is said to have AIDS (12).

Although there are several markers of HIV disease progression (37-43), the CD4 cell count and plasma viral load have long been established as the two most predictive markers (44-51) and hence are widely used in clinical care. The risk of AIDS is significantly increased with lower CD4 counts and higher viral loads (48;50;51), throughout the course of infection. Whilst the CD4 count captures the number of CD4 cells in the blood to give an indication of the state of the individual's immune system, the viral load provides a direct measure of the amount of virus in the blood (and is usually given in RNA copies per millilitre of blood plasma). These tests are continuously being improved; the first viral load test had a lower limit of quantification of 10,000 copies/mL whilst some current tests now have a lower limit of quantification of 10 copies/mL or lower, although most tests in routine practice have a lower limit of 40-50 copies/mL.

Figure 1.5.1: The natural history of the CD4 cell count during HIV infection (29)



Other co-factors which impact on the rate of disease progression include older age (52-54), co-infection with cytomegalovirus (52), low concentrations of albumin (38;41), low concentrations of haemoglobin (38;55-57), female gender (58-60) and black ethnicity (58;61).

1.6. Treatment of HIV

There is currently no vaccine to prevent HIV infection. ART drugs are used to treat HIV infection by inhibiting the replication of HIV. Table 1.6.1 shows the ART drugs currently licensed in the UK and the date of approval from the Food and Drug

Administration (FDA). Historically, there have been 3 classes of ART drugs, though drugs from new classes are now part of clinical practice. The first drugs approved by the FDA to fight HIV infection were from the nucleoside reverse transcriptase inhibitor (NRTI) class and worked by preventing the HIV RNA from converting into double-stranded DNA, thus preventing the virus from reproducing. NRTIs had been used previously to treat malignancies and herpes virus and were first introduced to treat HIV infection in 1987.

In 1995, the first protease inhibitor (PI) to treat HIV was approved by the FDA. Protease is one of HIV's enzymes. As the infected cell starts to produce viral proteins in order to produce new virions, it makes long chains of proteins and enzymes that will form new copies of HIV. However, in order to do this, the long chains have to be cut into smaller pieces by the HIV protease enzyme. PIs prevent this from happening. Although new copies of the virus are still made, these are not completely formed and so cannot infect other cells, slowing down the course of infection (62).

Shortly after, a third class, the non-nucleoside reverse transcriptase inhibitors (NNRTI), was approved by the FDA in 1996. Like NRTIs, NNRTIs prevent the virus from being able to reproduce by targeting the structure of reverse transcriptase to inhibit enzyme activity. Enzymes are prevented from converting RNA to DNA, stopping the virus from making DNA to be inserted in the cell's genetic material, and hence preventing it from producing new virus.
More recently, other classes of ARTs have been licensed by the FDA to treat HIV. Nucleotide reverse transcriptase inhibitors (NtRTIs) are similar to NRTIs but possess a phosphate molecule which NRTIs do not. This enables the NtRTI to skip the initial phosphorylation step needed by NRTIs for activation.

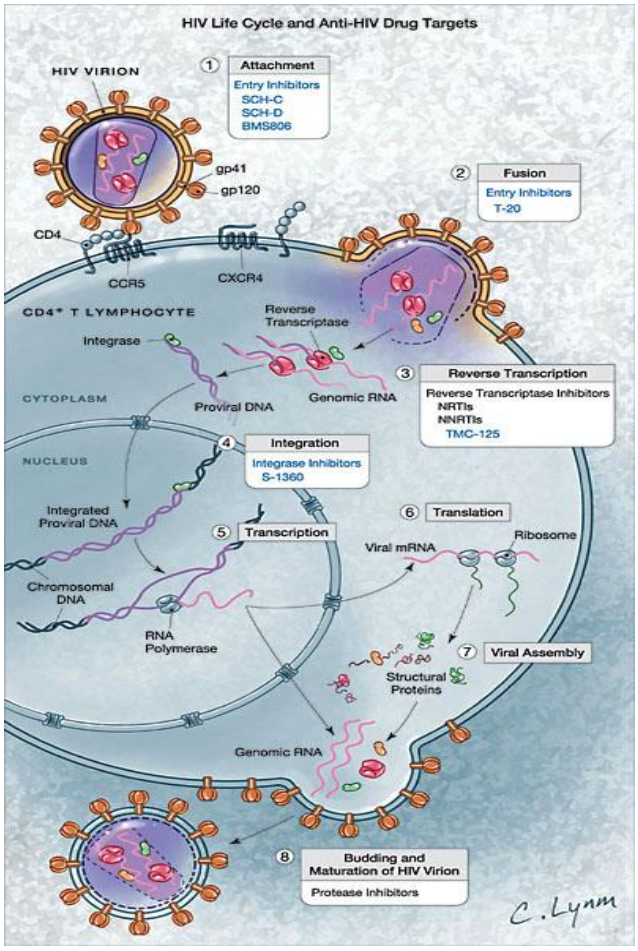
Fusion or entry inhibitors work at an earlier stage in the HIV cycle, by preventing the virus from entering CD4 cells. By attaching themselves to the protein on the surface of the CD4 cells (or on the surface of HIV), entry inhibitors stop HIV from binding to the CD4 cells. The first integrase inhibitor was licensed in 2007. If HIV RNA is able to convert to DNA (i.e. the NRTIs/NtRTIs are unable to block this process), in order for HIV to reproduce, the HIV DNA must be incorporated into the CD4 cells DNA. Integrase inhibitors work by blocking this integration. Figure 1.6.1 illustrates the targets of the various HIV drug classes.

Table 1.6.1: Antiretrovirals licensed in the United Kingdom

Antiretroviral agent	Brand name	Abbreviation	Date UK license	Common side effects
Nucleoside reverse transcriptase inhibitors (NRTIs)				
Zidovudine	Retrovir©	AZT or ZDV	1986	Headache, insomnia, nausea, stomach discomfort
Didanosine	Videx EC©	ddI	1991	Numbeness, tingling, diarrhoea, vomiting, rash
Zalcitabine	Hivid©	ddC	1992	Burning in hands/feet, numbeness, pain, tingling
Stavudine	Zerit©	d4T	1994	Numbeness, tingling, diarrhoea, vomiting, rash
Lamivudine	Epivir©	3TC	1995	Insomnia, nausea, vomiting, abdominal pain, rash, fever
Abacavir	ZiagenTM	ABC	1998	Insomnia, nausea, vomiting, abdominal pain, rash, fever
Emtricitabine	Emtriva	FTC	2003	Dizziness, insomnia, nausea, diarrhoea, hyperglycaemia
Protease Inhibitors (PIs)				
Saquinavir hard gel	Invirase©	SQV (HGC)	1995	Poor absorption, so rarely used
Indinavir	Crixivan©	IDV	1996	Kidney stones in 6-8%, occasional nausea, GI upset
Ritonavir	Norvir©	RTV	1996	Nausea, diarrhoea, numb lips, occasional hepatitis, hyperlipidaemia
Saquinavir soft gel	Fortovase©	SQV (SGC)	1997	
Nelfinavir	Viracept©	NFV	1997	Diarrhoea, nausea
Amprenavir	AgeneraseTM	APV	1999	Rash, diarrhoea, nausea
Lopinavir+ritonavir	Kaletra©	LPV	2000	GI side effects common but mild, hyperlipidaemia

Tipranavir	Aptivus TM	TPV	2005	Raised lipids, liver disease
Atazanavir	Reyatz ®	ATV	2003	High levels of bilirubin,
Fos-amprenavir	Telzir/LexivaTM	FPV	2003	Raised triglycerides,
Darunavir	Prezista	DRV	2007	Diarrhoea, nausea, headache, skin rashes
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)				
Nevirapine	Viramune©	NVP	1996	Transient rash, hepatitis
Efavirenz	SustivaTM	EFV	1998	Initial dizziness, insomnia, transient rash
Delavirdine (Not licensed in UK)			1997	
Nucleotide reverse transcriptase inhibitors (NtRTIs)				
Tenofovir disoproxil fumarate	VireadTM	TDF	2001	Generally well tolerated
Fusion (Entry)/CCR5 inhibitors				
Enfuvirtide	Fuzeon ®	T-20	2003	Skin reactions at injection site
Maraviroc	Selzentry	MRV	2007	Fever, upper respiratory infections, rash, diarrhoea
Integrase inhibitors				
Raltegravir	Isentress	RTG	2007	Diarrhea, nausea, headache, and fever, rash, depression

Figure 1.6.1: HIV life cycle and anti-HIV drug targets



Entry inhibitors work by stopping HIV getting into the CD4 cell

Reverse transcriptase inhibitors (NRTIs/NNRTIs) stop HIV changing from a single strand of RNA into a double strand of DNA

Integrase inhibitors block HIV from being 'integrated' into the cell's DNA

Protease inhibitors block new HIV from being cut into the right size proteins and this prevents new virus from being infectious

1.6.1. History of ART and treatment strategies

Although the first cases of AIDS were documented in 1981, it wasn't until 1987 that the first drug to treat HIV was approved by the FDA. Zidovudine (AZT), an NRTI, was initially administered as monotherapy. A randomised controlled trial comparing 145 patients receiving AZT with 137 receiving placebo was terminated early due to the significantly higher number of deaths in the placebo arm (64). However, further research showed that this effect wasn't sustained beyond around 6 months. The Concorde trial compared immediate with deferred AZT monotherapy in asymptomatic HIV-positive patients and found no difference in clinical outcome between the two groups (65), showing only a limited beneficial effect on overall survival amongst patients with HIV.

In the following years, three more NRTIs were approved by the FDA: didanosine (ddI) in 1991, zalcitabine (ddC) in 1992 and stavudine (d4T) in 1994. Although each of these drugs used alone were somewhat effective in slowing down the progression of HIV, when used in combination with AZT, outcomes were further improved (64;66-69). However, it soon became clear that these improvements were also short lived. Strategies that involved either sequencing (i.e. discontinuing one NRTI and replacing it with another) or concomitant use of two NRTIs were not potent enough to suppress viral replication for long, allowing the virus to develop drug resistance mutations (Section 1.8.3)

In 1995, the first PI, saquinavir invirase (hard gel capsules, SQV-HGC) was approved by the FDA. This essentially marked the beginning of the highly active antiretroviral (HAART) era. Used in conjunction with NRTIs (usually 2), SQV-HGC was associated with significantly better immunological and virological outcomes than dual NRTI therapy alone (70;71). Further, a statistically significant prolongation of time to first AIDS defining illness or death was observed in those receiving triple therapy compared with those receiving dual NRTIs. However, later studies showed that SQV-H was less potent than other PIs that subsequently became available, as it only had approximately 4% bioavailability (72), prompting the licensing of a soft-gel version of the drug in 1997 (saquinavir fortovase, SQV-SGC) (73).

Two other PIs, indinavir (IDV) and ritonavir (RTV) were approved by the FDA shortly after SQV-HGC and were associated with a dramatic improvement in HIV disease outcomes in both early and late disease (74-76). Different combinations of PIs and NRTIs were examined and evidence in favour of triple therapy again emerged. Due

to the high toxicity associated with full dose ritonavir (77-80), and the high interaction levels with other drugs (81;82), full dose ritonavir was not recommended. However, low doses of ritonavir were used to pharmacologically 'boost' the levels of other PIs when taken together, because it inhibits the breakdown of the drug in the liver. Regimens consisting of boosted PIs (in conjunction with 2 NRTIs) had fewer adverse events than when using full dose ritonavir and better clinical outcomes than single PIs (83-85).

In 1996 a third class of ART was introduced. Nevirapine was the first NNRTI approved by the FDA. Regimens containing nevirapine and two NRTIs not only showed superior efficacy than dual NRTI therapy alone (86;87) but also compared well against PI-containing regimens. The COMBINE Study compared 70 patients receiving two NRTIs and nelfinavir (a PI approved by the FDA in 1997) with 72 patients receiving two NRTIs and nevirapine; the study investigators concluded that the regimen containing the NNRTI was at least as effective as that containing the PI (88;89). Other studies have also reached similar conclusions (89;90).

However, as with earlier drugs, nevirapine also had disadvantages. Although the most common adverse event was mild or moderate rash, particularly amongst women (91;92), it soon became evident that the emergence of resistance was a major concern. Amongst patients who did not achieve viral suppression, resistance developed rapidly – the virus mutated and was able to multiply, reducing the ability of particular drugs to block HIV replication (Chapter 2.1) (86;93;94). The FDA approved two other NNRTIs in the following two years; delavirdine in 1997 and efavirenz in 1998. Although triple therapy including delavirdine (in combination with 2 NRTIs) was shown to be have somewhat better clinical efficacy than dual NRTI therapy (95), this difference was only modest (96). Further, delavirdine did not appear to provide any significant improvements over nevirapine (97), although its use amongst patients who had failed previous regimens, especially those containing PIs, was recommended (98;99). Consequently, delaviradine has not been licensed in Europe.

Efavirenz, on the other hand, compared well against nevirapine. Results from several cohort studies showed efavirenz to be either superior or equivalent to nevirapine, both in terms of CD4 increase and virological suppression (100-103). Further, adverse events (i.e. hepatotoxicity and rash amongst patients taking both drugs) were generally comparable (104-106), though in a large randomised trial, the

2NN study published in 2004, patients receiving NVP were at a greater risk of hepatotoxicity. Although it is well documented that patients using efavirenz are likely to experience short-term side effects associated with the central nervous system (107-109), data showing similar effects amongst patients using nevirapine also exist (110). The 2NN study found no significant differences in week-48 virological outcomes amongst those starting HAART with two NRTIs and either efavirenz or nevirapine (104). These results verified the clinical similarities between the two NNRTIs reported by authors of a smaller trial a few years earlier (111).

1.6.2. PI vs. NNRTI containing HAART

Given the relative success of both boosted PI- and NNRTI-containing HAART regimens, together with the number of NRTI drugs available (Lamivudine (3TC) was licensed in 1995 and Abacavir (ABC) in 1998) at the time when efavirenz was approved by the FDA, there was a choice of not only which drugs to start treatment with but also which class of drugs. Patients starting HAART with PI-containing regimens were likely to experience severe long-term toxicities, including an increased risk of lipodystrophy (this has since mainly been ascribed to the NRTIs in the regimen) and hyperlipidemia (112;113). However, these regimens had been associated with improved clinical outcomes and had the added advantage of requiring several mutations to confer resistance. Resistance to NNRTIs, on the other hand, is likely to develop quickly if viral suppression is not achieved or maintained. Only one resistance mutation is needed to confer resistance to either of the two original NNRTIs and there is the added complication of patients often being resistant to all drugs from the NNRTI class if they develop resistance to any NNRTI drug (114-116). However, NNRTIs have the advantages of fewer diet restrictions, less frequent dosing and greater tolerability, outweighing the risk of resistance for many clinicians and/or patients.

Many studies have compared the efficacy of PIs (boosted or single) with NNRTIs. A meta-analysis published in 2006 by Chou et al (117) found 12 trials of at least 24 weeks duration which directly compared NNRTI-based versus PI-based therapy in patients with limited or no previous exposure to ART. Virological results favoured NNRTI-based regimens, though the difference was reduced when a subset of higher quality trials was considered. No difference was found in death rates, disease progression or adverse events between the two groups. A previous meta-analysis published in 2004 had also shown NNRTI- to be superior to PI-based regimens (118). Other meta-analyses have found PI-regimens to be superior, but these are

often indirect comparisons of smaller and older trials, so newer drugs such as efavirenz were not included (119). The choice in the use of PI or NNRTI regimens as first-line therapy is also dependent on clinical site. Data from the UK Collaborative HIV Cohort (CHIC) Study published in 2008 showed that although the use of PIs in first-line regimens declined from 1997 to 2000 in most centres, the rate of decline varied between centres. An increase in the proportion of patients being prescribed NNRTIs was also observed over the same time period, and though this increase appeared to stabilise from 2000 to 2005 at around 80% for most centres, only 40% of patients were being prescribed NNRTI-regimens at one particular centre in 2005. Results from multivariable regression showed clinical site and calendar year to be independent predictors in the choice of first-line therapy (120). On the whole, in recent years, NNRTI-therapy is the preferred choice as a first-line-regimen, leaving PI-based regimens as options for those who do not achieve viral suppression.

1.6.3. Drugs approved in the last 10 years

Since 1998, several more ART drugs have been approved to fight HIV infection. Newer drugs from existing classes tend to have fewer side effects and require less frequent dosing. They offer options to patients who have virologically failed existing drugs and have resistance to those failed drugs and others with a similar resistance profile. Further, these new drugs are also used as part of first-line regimens. In the NRTI/NtRTI classes, emtricitabine (FTC) was approved in 2003 and tenofovir (TDF) in 2001. Six PIs have been licensed since 1998; amprenavir (APV) in 1999, Kaletra (consisting of lopinavir co-formulated with low dose-ritonavir, LPV) in 2000, atazanavir (ATV) in 2003, fos-amprenavir (FPV) in 2003, tipranavir (TPV) in 2005 and darunavir (DRV) in 2006. These PIs are now rarely used without low-dose ritonavir. Etravirine (ETR) is the newest NNRTI to be licensed (2008) and unlike efavirenz/nevirapine, resistance to other NNRTIs does not seem to always confer high level resistance to etravirine (121), though this is likely to depend on which mutations are present.

The first, and thus far only, fusion inhibitor to be licensed was enfuvirtide (T-20) in 2003. It was thought that this drug may open treatment options for patients who had advanced HIV infection and were unable to achieve viral suppression using existing drugs. However, T-20 has to be injected twice daily, can cause local painful skin reactions and some patients using T-20 may develop bacterial pneumonia. Further, T-20 is the most expensive ART drug used to treat HIV. These issues have led to T-

20 being rarely used for any patients, other than those with documented resistance to the three main drug classes. Maraviroc is an entry inhibitor which was approved for treatment experienced patients in 2007. Also known as a CCR5 antagonist, clinical trials showed that patients taking maraviroc (with other ART drugs) were more likely to achieve viral suppression than those taking placebo (with the same other drugs) and reported no clinically relevant differences in safety between the two groups (122). Raltegravir is the only integrase inhibitor to be currently approved. Licensed in late 2007, raltegravir is recommended for use amongst patients who are resistant to other HIV drugs/classes. Patients taking raltegravir in clinical trials achieved viral suppression sooner than those taking NNRTIs and PIs. Research on how raltegravir alters HIV viral dynamics and decay is ongoing (123).

1.6.4. Treatment guidelines – what to start therapy with

The British HIV Association (BHIVA) was founded in 1995 and is a well established organisation that produces guidelines for the management of HIV infection for the UK. The International AIDS Society (IAS) is a worldwide organization of professionals and was established in 1985. The IAS also produces a wide range of guidelines for both patients and clinicians for managing HIV infection. Other advisory boards include the European AIDS Clinical Society (EACS) and the United States Department of Health and Human Services (DHHS).

First-line regimens recommended by these groups have changed to reflect the steady emergence of new drugs since 1985. Table 1.6.4.1 shows both BHIVA and IAS simplified guidelines for the first-line treatment of HIV infection in adults since 1996. Factors such as initial CD4 count, pregnancy and transmitted drug resistance may influence treatment choice.

Table 1.6.4.1: Simplified IAS and BHIVA guidelines for first-line treatment of HIV infection

	IAS-USA		BHIVA	
Year of publication	**Nucleoside backbone**	**'third' drug**	**Nucleoside backbone**	**'third' drug**
2008 (124;125)	TDF+FTC or ABC+3TC	EFV or PI/r[1]	TDF+FTC or ABC+3TC	EFV
2006 (126;127)	TDF+FTC or AZT+3TC or ABC+3TC	EFV or PI/r[2]	TDF+FTC or AZT+3TC or ABC+3TC	EFV or LPV/r or FVP/r
2005 (128)	-	-	TDF+FTC or AZT+3TC or ABC+3TC	EFV or LPV/r
2004 (129)	AZT+3TC/FTC or TDF+3TC/FTC	EFV or PI/r[3]	-	-
2003 (130)	-	-	2 NRTIs	NNRTI or LPV/r or SQV/r
2002[4] (131)	2 NRTIs	NNRTI or PI/r or ABC	-	-
2001 (132)	-	-	2 NRTIs	NNRTI
2000 (133;134)	2 NRTIs	NNRTI or PI/r	2 NRTIs	PI or PI/r or NNRTI
1998 (135;136)	2 NRTIs	PI (unboosted)	2 NRTIs	PI or PI/r or NNRTI
1997 (137;138)	2 NRTIs	PI (unboosted)	2 NRTIs	Na
1996 (139)	2 NRTIs	-	-	Na

[1]LPV, AZV, FPV, DRV or SQV [2]LPV, AZV, FPV or SQV [3]LPV, AZV, SQV or IDV [4]Specific drugs not specified before and including 2002

1.7. Treatment of HIV in children and pregnant women

Up to 30% of babies born to HIV-positive women are likely to become infected with HIV if the mother does not receive antiretroviral treatment during pregnancy (140). It has been reported that up to a further 20% are likely to be infected through breast-feeding. However, the risk of HIV infection in babies can be reduced to less than 2% if the mother is receiving antiretroviral treatment during pregnancy and if the baby is bottle-fed. Elective caesarean section and prophylactic treatment for the newborn baby also contribute to reducing this risk (141;142). Although the rate of disease progression varies greatly in children, children are likely to progress to AIDS more quickly than adults (143;144). The antiretroviral drugs used to treat HIV-positive children are the same as those licensed to treat adults, though doses are dependent on age. Although children have been shown to achieve virological suppression and CD4 increases whilst taking HAART, the degree of response is somewhat less than that seen in adults (145).

1.8. Limitations of ART

HIV replication occurs at an extremely high rate in untreated individuals. The number of new virions created each day is estimated to be in the region of 1 to 10 billion copies (146;147). ART drugs are unable to completely stop all HIV replication (new replication may be stopped but if ART is discontinued, infected cells are activated and start producing virus) and so the aim of therapy is to suppress viral replication for as long as possible. However, there are several interlinked barriers to successful treatment. Adherence to therapy, toxicities associated with ART drugs and resistance to ART drugs/classes are major issues which may prevent the optimal treatment of HIV.

1.8.1. Adherence

Maintaining a high level of adherence to an ART regimen is vital for sustaining viral suppression (148-151). It has been reported that in order for HIV medications to effectively reduce viral load, adherence rates must be around 95% (152) if receiving unboosted PIs, though this percentage is lower amongst patients receiving NNRTI-based regimens (153). However, toxicities associated with ART may make this a difficult target to achieve. Busy lifestyle choices and the need to take certain drugs with particular foods may reduce adherence rates further. Studies have shown certain groups of patients are less likely to be adherent to medication than others. In particular, patients who contracted HIV via intravenous drug use, those of younger

age, females and patients of black African ethnicity (154-159) are likely to have lower adherence, leading to poorer virological response.

1.8.2. Drug toxicity

Drug toxicity is a major barrier to successful HIV treatment. Regimens containing drugs with high toxicities are likely to result in lower levels of adherence (160) and thus patients are less likely to achieve virological suppression. HIV drug toxicity was first described with the introduction of AZT and has since been associated in some form with almost all HIV antiretroviral drugs. Toxicities may be acute or long-term and either class specific or drug-specific.

One of the most serious side effects of NRTIs/NtRTIs is mitochondrial toxicity. Associated in particular with the 'd-drugs', d4T, ddI and ddC, mitochondrial toxicity can lead to pancreatitis and peripheral neuropathy (161;162). The newer NRTI, ABC, has been shown to cause hypersensitivity reactions in around 8% of patients (163;164) and ABC has also been associated with a raised risk of myocardial infarction (165). Dyslipidemia, (e.g. high total and LDL cholesterols, elevated triglycerides and reduced HDL-C) and gastrointestinal side effects are generally associated with PIs (166). As mentioned earlier, efavirenz has been associated with a risk of dysphoria (107). Short-term milder toxicities, such as nausea, vomiting, diarrhoea, anaemia and headaches have been associated with most available drugs (Table 1.6.4.1). Hepatotoxicity (can also be acute), renal disease and ALT elevations are examples of longer-term toxicities. Definitions of such toxicities vary considerably and are discussed in detail in Chapter 2.2.1.

1.8.3. Resistance

At the time of HIV infection, usually a single virus replicates (i.e. individuals are infected with a single strain), producing new virions as mentioned above. As the RNA genome is transcribed to viral DNA, replication of the virus is highly error-prone. There is a high frequency of alterations to the genetic code, resulting in genetic mutations in the replicated copies of the virus (167). Mutations cause different strains of the virus to be produced. Some of these mutations are harmful to the virus – they cause changes in the viral proteins that are needed for viral replication.

There are some mutations, however, which are advantageous to the virus. In the presence of therapy, genetic mutations may exist that allow the virus to replicate,

despite the presence of antiretrovirals. This strain, with mutations conferring resistance to antiretroviral drugs is more likely to survive than one which is drug-sensitive (i.e. wild type) and hence is known to have a higher level of *fitness* in the presence of therapy.

Resistance mutations are often drug specific but can also be class specific. If a mutation occurs in the reverse transcriptase or protease region of the virus, antiretroviral drugs designed to inhibit viral replication in these areas may have a reduced effect or no effect at all. Such mutations are often referred to as primary resistance mutations. In the presence of ART, strains with resistance mutations will tend to out-compete other strains because they are able to replicate in the presence of ART. If therapy is subsequently stopped, the wild type virus is likely to have higher fitness than the resistant strain and hence will become the dominant strain again (168). For example, if a patient receiving 3TC has developed a strain of virus resistant to 3TC and subsequently stops therapy, the HIV variants with the mutation associated with 3TC (M184V) rapidly disappear (169). The resistant virus however does remain a minority sub-species, and so if the patient was reintroduced to 3TC monotherapy, the resistant virus is likely to again become the dominant virus, making virological suppression unlikely.

In the absence of ART, the strain of virus which is able to replicate the most quickly is known as the 'wild type' virus. Since some or most resistance mutations tend to result in some reduced replicative capacity of the virus, when the associated drug is no longer used they tend to get outcompeted and replaced by virus without such mutations.

A strain of HIV which is resistant to antiretroviral drugs is more likely to dominate amongst patients in whom viral suppression is not achieved. For example, patients who were exposed to sub-optimal therapy such as AZT monotherapy were unlikely to achieve low viral loads – the ongoing replication of virus results in a higher number of mutations and therefore a higher chance of these mutations being resistance mutations. When such resistance mutations arise, they will be selected for as they have the advantage of the presence of a particular drug (if this particular drug was not present, the resistance mutations would not be selected). Patients with low levels of adherence to therapy are also at increased risk of developing resistance since their viral loads are unlikely to be suppressed.

Drug resistance may also be present amongst patients who receive optimal HAART therapy as their first-line of treatment. These patients are likely to have been infected with a strain of virus which was already resistant to specific drugs/classes. This, together with the impact of resistance amongst patients who start second-line HAART, is discussed in detail in Chapter 2.2.1.

These limitations of ART are all interlinked - for example, a patient may be infected with a drug resistant virus which will limit his/her treatment options from the outset. After starting treatment, toxicity to drugs may result in poor adherence and even discontinuation of treatment altogether. This is then likely to result in accumulation of resistance, prompting a switch in regimen and potential for further toxicities.

1.9. Focus of thesis

My thesis will be focused on the barriers to successful treatment which are of key importance in the developed world. A more detailed literature review forms Chapter 2 of the thesis and the methodology of the thesis is discussed in Chapter 3 – this includes a description of the processes involved when establishing and linking the datasets, as well as a summary of trends over time in key markers of clinical success within the UK CHIC Study. Chapter 4 describes the frequency of transmitted resistance and the impact of such resistance on the selection of first-line therapy. In Chapter 5, I investigate how well the selection of second-line therapy relates to identified resistance mutations of first-line therapy. I discuss a particular toxicity to treatment in Chapter 6 (ALT flares). In this Chapter, I also justify the need for a new definition of an ALT flare and derive such a definition. The impact of treatment interruptions on long-term virological outcomes is discussed in Chapter 7. In Chapter 8, I develop a combined score for a range of toxicities to predict mortality. Finally, an overall summary and discussion of the above chapters is provided in Chapter 9.

Chapter 2: Literature review

2.1. HIV drug resistance

2.1.1. HIV drug resistance testing

Resistance testing is now recommended in all newly diagnosed patients and those who are looking to change treatment because of virological failure (124). Currently there are two types of resistance tests: genotypic and phenotypic.

2.1.1.1. Genotypic resistance testing

Genotypic resistance testing generates a list of mutations which are known to confer resistance to antiretrovirals; such tests are generally recommended for those who have recently been infected or have used only a limited number of HIV antiretrovirals. There are two main types of genotypic tests: sequencing assays and point-mutation assays. Sequencing assays are used to detect mutations in genes that are involved in the replication of HIV, i.e. the reverse transcriptase and protease genes. The assays give a complete nucleotide sequence by means of cycle sequencing (dideoxy chain termination) or gene chip sequencing. Point mutation assays detect mutations in the specific genes that are known to cause antiretroviral drug resistance and are cheaper and easier to perform and interpret. As a result, these are performed more often than sequencing assays.

A blood plasma sample is used to examine whether the virus in the blood contains any mutations. This is done by comparing the genetic code in the virus in the plasma sample to that of wild type virus. This code is a chain of molecules called nucleotides; each group of three nucleotides (defined as a 'codon') defines a specific amino acid which forms part of any new virus produced. Mutations are described using a combination of letters and numbers. The K103N mutation for example, correlates with high-level resistance to efavirenz and confers cross resistance to nevirapine and delavirdine (114;116). The first letter 'K' is the code for the amino acid in the wild type virus. The position of the codon is identified by the number '103', and the last letter 'N' is the code for the altered amino acid in the mutated virus. Interpretation of mutations is not straight-forward. A single mutation could result in resistance to one drug but make the virus more sensitive to a different drug. Further, in some cases, several mutations are required to exist in order for the

virus to be resistant to a single antiretroviral drug, whilst in other cases, only one particular mutation may be required for HIV to be resistant to an entire class of antiretrovirals.

Several genotypic resistance interpretation systems have been developed, most of which are rule based (http://hivdb.stanford.edu/, http://www.hivfrenchresistance.org/, http://www.kuleuven.ac.be/rega/cev/links/rega_algorithm/index.htm). Each algorithm outputs a genotypic sensitivity score (GSS), which predicts how resistant the virus is to each drug. However, substantial differences in the interpretation of genotypic results using these different systems have been reported (170-172). Genotypic tests do not provide a direct measure of resistance and tests may not be useful if a patient's viral load is below 1000 copies/mL as it may not be possible to amplify the virus. Further, patients may have many different strains of virus and genotypic tests are unable to detect minority mutations, which may occur in less than 20% of the virus population (173). Genotypic tests are, however, relatively cheap, have a quick turn-around time and are now commonly used in clinical practice to test for resistance in HIV-positive patients.

2.1.1.2. Phenotypic resistance testing

Phenotypic tests measure the ability of HIV to reproduce in the presence of antiretrovirals. Blood plasma samples are divided into many test tubes and mixed with different antiretroviral drugs. The concentration of each drug is gradually increased until it is high enough to stop viral replication. The results are compared to a wild type virus that is known to be 100% susceptible to all antiretrovirals and a resistance 'profile' is created (174). Clinical cut-offs are used to determine whether the virus is resistant to each drug and, if resistance does exist, the level of resistance (high, intermediate, low) is defined; results are, however, somewhat open to interpretation (175;176). A lower cut-off is used to determine the value at which a particular drug has partial susceptibility and an upper cut off defines the point at which no clinical response is expected.

Since phenotypic tests measure the combined effect of all mutations, they may be more useful in patients who are likely to have multiple mutations. Phenotypic tests also provide a quantitative measure of resistance, which is particularly useful for patients who have limited treatment options. A disadvantage of phenotypic testing is that it is unable to measure how combinations of drugs work together (although this

is a challenge also when using genotypic testing). Phenotypic testing is also considerably more expensive and time-consuming than genotypic testing.

2.1.1.3. Virtual resistance testing

This method uses genotypic tests to predict phenotypic results. A database containing matched genotypic and phenotypic data of over 10,000 patients is used to match the results of a genotypic assay to that of other patients in the database. The corresponding phenotypes are extracted and a predicted result, i.e. the virtual phenotype, is synthesized (177).

2.1.2. Primary and secondary drug mutations

Resistance mutations are generally defined as either primary or secondary mutations. Each antiretroviral drug is known to be associated with at least one primary mutation and it is these mutations that are associated with some level of resistance. However, the identification of a primary mutation does not guarantee high-level resistance as further mutations may reverse the effect. Secondary mutations cannot cause drug resistance unless a primary mutation is present. Combinations of secondary mutations are complex to interpret and confer varying levels of resistance.

2.1.3. Transmitted drug resistance (TDR)

There has been evidence of the transmission of drug-resistant virus strains since the AZT monotherapy era (178-180). Newly infected patients were found to be resistant to AZT despite never having taken the antiretroviral before. Since then, the number of antiretroviral drugs used to treat HIV infection has increased considerably and hence the number of patients infected with a virus resistant to specific antiretrovirals also initially increased (though is now decreasing), limiting future treatment options for patients infected with resistance virus from the outset.

2.1.3.1. Prevalence of TDR

The prevalence of TDR globally has been reported to range from 1 to 25% (181). This difference is likely to be explained by the variation in study patients/design, the access and availability of antiretroviral drugs, the sensitivity of the resistance assays and the differences in interpretation of the results from the resistance tests. The IAS-USA drug resistance mutation list maintains a current list of mutations associated with resistance (182). This list has been edited by Shafer et al, who have compiled a

list of drug-resistance mutations for epidemiologic estimates of transmitted resistance (183) (most recently updated in 2009 by Bennett et al (184)). Recently published studies found little difference between the two lists and other interpretation algorithms when estimating overall rates of resistance in a cohort study (185;186).

Transmitted resistance mutations can exist for several years since there is no archived dominant wild type virus present in the infected individual (187-189). A recent study, based on expected infection duration, has suggested that transmitted resistance mutations may exist for up to 8 years (190). TDR, though reported worldwide, is most prevalent in the United States of America (US) and Europe. A study published by Shet et al on a New York City cohort of newly infected HIV individuals found that the prevalence of TDR had increased from 13% in 1995-8 to 24% in 2003-4 (191). This is the current highest reported prevalence of TDR worldwide. Of note, rates of TDR are often conservative. Even amongst patients who have been infected with a virus resistant to ART, the majority virus is likely to revert back to wild type in the absence of therapy. In this case, the person has clinically significant resistance (which is likely to re-emerge if the relevant drug is taken), but this is not detectable using standard resistance testing. Hence the longer the duration between infection and resistance testing, the less accurate the test result.

Since the vast majority of patients currently living with HIV in developed countries have access to HAART, the literature review on TDR will focus on those papers published from 1996 onwards in Europe, North America, South America and Australia. A summary of these papers is presented in Table 2.1.3.1.1.

Table 2.1.3.1.1: Studies investigating the prevalence of TDR

Author, region and year of publication	Year diagnosed	N	Main risk	Age	Interpretation algorithm	Prevalence (%) of TDR					Risk factors for TDR/other main results
						Overall	MDR	NRTI	NNRTI	PI	
Hattori, Japan 2010[192]	2003-2008	2573	MSM	35	Stanford	7.7	na	na	na	na	Subtype B
Stanczak, Poland 2010[193]	2008	95	MSM	32	Stanford	5.3	0.0	na	1.1	4.2	na
Murillo, Hondruas 2010[194]	2004-2007	200	Het	31	ANRS	7.0	2.5	3.0	5.0	0.5	na
Wheeler, US 2010[195]	2006	1997	MSM	na	Stanford	14.6	2.6	5.6	7.6	4.5	na
Haidara, Mali 2010[196]	2007-2008	101	na	35	Virco	9.9	1.0	5.0	6.0	0.0	na
Liao, China 2010[197]	2004-2005	676	Blood doners	38	Stanford	3.8	na	1.6	2.1	0.4	na
Riva, Italy 2010[198]	1992-2003	112 CAS[1] 271 SPR	MSM Het	31 35	IAS-USA	15.1 12.2	0.0 2.6	8.0 7.7	0.9 5.5	0.0 2.9	na
Vercauteren (SPREAD), Europe 2009[199]	2002-2005	2793	MSM	37	SDRM-list	8.4	1.0	4.7	2.3	2.9	Subtype B
Bracciale, Italy 2009(200)	1996-2007	1690	Het	36	SDRM-list	15.1	3.7	11.0	6.0	4.0	Low VL, high CD4, subtype B
Green, UK 2008[185]	1997-2008	8272 sample	na	na	1)IAS-USA 2)SDRM-list 3)Stanford	1) 10.0 2) 9.2 3) 10.4	na	na	na	na	na
Madsen, Greenland 2008[201]	1999-2007	60	Het	na	Stanford	25.0	10.0	na	na	na	na
Payne, UK 2008[202]	2005-2007	406	70.6% Het	35	IAS-USA & Stanford	3.3	0.0	0.5	1.8	1.0	None
Bannister, UK 2008	1996-2004	525	na	na	SDRM-list &	11.4	1.7	9.3	1.0	3.0	HCV+, region.

(203)					Stanford						TDR did not impact on CD4/VL response
Huang, US 2008(204)	2005-2006	228	55.7% MSM	37	Stanford	12.1	2.2	4.5	9.8	1.8	na
Vercauteren, Belgium 2008(205)	2003-2006	285	55.0% MSM	36	SDRM-list & REGA	9.5	na	7.0	3.5	1.8	Subtype B, Belgian nationality
SPREAD Program, Europe 2008(206)	2002-2003	1050	44.0% MSM/Bi	37	IAS-USA & REGA	9.1	0.1	5.4	2.6	3.0	na
Brenner, Canada 2008(207)	1997-Jul 07	848	MSM/IDU	na	SDRM-list	14.9	2.1	6.8	7.6	5.0	na
Cachay, US 2007(208)	2004-2006	115	89% MSM	32	Genseq^{Tm} & IAS-USA	23.0	1.7	10	11	2.6	Methamphetamine use
Booth, UK 2007(209)	2004-2006	239	52.7% MSM	35	IAS-USA & Stanford	7.1	0.4	4.2	1.7	1.7	Born in the UK, high baseline CD4
Yerly, Switzerland 2007(210)	1996-2005	822	42% MSM	na	1)Stanford 2)ANRS	1) 7.9 2) 7.7	na	1) 6.0 2) 5.5	1) 2.0 2) 1.9	1) 2.5 2) 2.7	Subtype B
Poggensee, German 2007(211)	1988-2004 (majority >2001)	504	88.0% MSM	32	IAS-USA & Stanford	16.0	2.0	9.1	2.0	2.8	TDR did not impact on VL response
Parker, US 2007(212)	2000-2004	151	53.0% Het	39	IAS-USA & Stanford	11.3		6.6	5.3	0.7	na
Peuchant, France 2007(213)	2004-2005	55	76.4% MSM	35	IAS-USA & ANRS	20.0 res to T-20	na	na	na	na	na
UK Collab/UK CHIC /UK Seroconv 2007(214)	1996-2004	4454 sample	na	na	IAS-USA	9.2 in 2004	na	4.6	4.4	2.1	na
Ross, US 2007(215)	2007	317	74% MSM	na	1)Phenosense 2)IAS-USA	1) 8.0 2) 0.0	na	1) 1.0 2) 0.0	1) 7.0 2) 0.0	1) <1 2) 0.0	White ethnicity
Fox,UK 2007(216)	2005-2006	140	61% MSM	34	IAS-USA	9.4	na	2.7	4.0	0.7	na
Sagir, Germany 2007(217)	2001-2005	831	51.0% MSM	na	IAS-USA & geno2pheno	9	1.3	na	na	na	Non-white ethnicity, non-B subtype, non-MSM
Rodrigues, Brazil 2006(218)	2004	108	~80% Het	31	Stanford	3.0	na	na	na	na	na

Petroni, Argentina 2006[219]	2004-2005	52	52.0% Het	36	IAS-USA	7.7 RT 0.0 PI	na	na	na	na	na
Oette, Germany 2006[220]	2001-2003	269	48.3 %MSM	39	IAS-USA	11.2	1.5	8.6	3.7	1.5	TDR did not impact on CD4/VL response
Fox, UK 2006[189]	2000-2004	140	~90% MSM	32	Stanford	6.0	1.4	na	na	na	TDR did not impact on VL response
Truong, US 2006[221]	2004	129	78% MSM	41% >35	IAS-USA & Stanford	13.2	3.1	na	8.5	na	na
Jayaraman, Canada 2006[222]	2000-2001	715	na	na	IAS-USA	8.1	1.0	4.1	1.4	1.5	na
Shet, US 2006[191]	2003-2004	116	97.0% MSM	35	IAS-USA	24.1	9.8	16.1	13.4	7.1	TDR did not impact on CD4/VL response
Bezemer 2006 UK[223]	1984-2005	440	na	na	IAS-USA & Stanford	14.8	na	na	na	na	na
Babic 2006 Slovenia[224]	2000-2004	77	62.3% MSM	36	IAS-USA & Stanford	3.9	na	3.9	0.0	0.0	na
Cane, UK 2005[225]	1996-2003	2357	76.4% MSM	na	Stanford	14.2	2.0	9.9	4.5	4.6	na
Masquelier, UK 2005[226]	1987-2003	438	74.9% MSM	32	IAS-USA & Stanford4	10.3	0.5	5.7 >1 NRTI	3.4	3.0	No sig factors associated with TDR
Paraskevis, Greece 2005[227]	2002-2003	101	55.0% MSM	37	IAS-USA	9.0	0.0	5.0	4.0	0.0	na
De Mendoza, Spain 2004[228]	1997-2003	89	75.3% MSM	na	IAS-USA	16.8	4.5	14.6	3.4	3.4	na
Weinstock, US 2004[229]	1997-2001	1082	45.3% Het 44.5% MSM	na	Automated sequencing	8.3	1.3	6.4	1.7	1.9	na
Ammaranond, Australia 2003[230]	1992-2000	130	na	na	na	na	na	30.8	na	0.8	na
Little, US 2002[231]	1995-2000	301	77.0% MSM	35	IAS-USA	12.0	na	na	na	na	TDR associated with longer time to VS
Simon, US 2002[232]	1995-2001	154	>90% MSM	na	IAS-USA	13.2 - 19.7	2.6 – 4.0	13.2 - 18.4	na	1.3 - 5.3	na
Violin, Italy 2002[233]	1995-1998	68	na		na	na	1.5	14.7	0.0	5.9	na
Grant, US 2002[234]	1996-2001	225	86.2% MSM	35	IAS-USA	23.1	13.2 in 00/01	20.9 in 00/01	13.2 in 00/01	7.7 in 00/01	Higher CD4 counts /TDR associated with longer time to VS
Goudsmit, Holland	1990-1998	43	na	na	IAS-USA	7.0	na	na	na	na	na

2001[235]											
Briones, Madrid 2001[236]	1997-1999	30	70.0% MSM	31	IAS-USA	26.7	6.7	23.3	3.3	6.7	na
Duwe, Germany 2001[237]	1996-1999	64	89.1% MSM	na	US Los Alamos database	Pheno: 13%	na	9.4		4.7	na
Garcia, US 2001[238]	1997-1999	603	na	na	Phenotypic	3.3 ZDV	na	na	na	na	na
UK Collaborative Group, 2001[239]	1994-2000	69	84.1% MSM	na	IAS-USA	14.0	2.9	na	na	na	Later calendar year
Descamps, France 2001[240]	1998	404	48.5% MSM	35	IAS-USA & ANRS	3.4	na	3.3	0.8	1.9	Low baseline viral load
Weinstock, US 2000[241]	1993-1998	99	57.0% Het	29	IAS-USA	7.0	1.0	4.0		1.0	na
Balotta, Italy 2000[242]	1994-1997	38	44.7% MSM	na	Phenotypic	21% ZDV	na	na	na	na	TDR did not impact on CD4/VL response
Tamalet, France 2000[243]	1995-1998	48	na	na	IAS-USA	na	na	17.0		2.0	TDR did not impact on CD4/VL response
Salomon, Canada 2000[244]	1997-1999	81	64.3% MSM	na	Phenotypic	na	na	18.5		12	na
Boden, US 1999[245]	1995-1999	80	93.8% MSM	na	US Los Alamos database	na	3.8	12.5	7.5	2.5	na
Yerly, Switzerland 1999[246]	1996-1998	82	40% MSM, 40% Het	34	Schinazi 1997	na	1.2	10		4.9	na

Age is summarised as 'median' age.

MDR: Multi-drug resistance (resistance to >1 class of ART drugs), MSM: Men having sex with men, Het: Heterosexual, Bi: Bisexual, na: Not applicable

1: CAS:CASCADE, SPR:SPREAD Study

2.1.3.2. Early reports of TDR (pre-2000)

Though reported rates of transmitted resistance (also referred to as 'primary resistance') to antiretroviral therapy vary considerably from study to study, few studies have published rates of less than 10% in the years following the introduction of HAART. A large number of patients infected prior to the year 2000 were likely to have been exposed to some degree of AZT monotherapy/nucleoside dual therapy and thus were unable to maintain viral suppression. These patients were more likely to transmit a virus resistant to specific nucleosides and hence primary resistance to nucleosides peaked before the introduction of HAART in 1996. A study by Balotta et al published in 2000 reported that 21% of patients newly infected in Italy in 1994-1997 were resistant to AZT (242). Of those infected in 1994, 1995, 1996 and 1997, 12.5%, 28.6%, 22.2% and 14.3% had mutations conferring resistance to AZT, illustrating the peak in resistance rates prior to the widespread use of HAART. Other European and American studies have reported similar rates. Tamalet et al found 17% of patients newly infected in France in 1995-1998 had a mutation in the reverse transcriptase gene and 2% had a resistance mutation associated with PIs (243). In Switzerland, Yerly et al reported a TDR prevalence rate of 10% amongst those infected in 1996-1998 (246). A German cohort study published in 2001 found that 13% of newly infected HIV-positive patients carried at least one primary resistance mutation (237). The proportion of patients with primary resistance mutations in 1995 had increased to 16% from 7% in 1994 but a decrease was seen thereafter, with 11% of patients reported to have TDR in 1998. Rates of TDR in Italy and Madrid amongst those infected before 1999 were 14.7% and 26.7% respectively (233;236). A study published in 2001 by the UK Collaborative Group on Monitoring the transmission of HIV Drug Resistance found that 14% of individuals infected in 1994-2000 had at least one primary mutation (239). However, rather than a decreasing trend after the introduction of HAART, authors reported a significant increase over time, with 27% of those infected in the year 2000 having resistance mutations.

US studies have reported a wide range of prevalence rates, differing with area, sample size and interpretation algorithms used as described above. A study of 80 predominately men having sex with men (MSM) in New York and Los Angeles found that 16% were likely to have been infected with a resistant virus before the year 2000 (245). A considerably larger study was conducted by Little et al in North America (231). Of 301 patients seen across 10 clinics in 1995-2000, 12% were found to have at least one resistant mutation. Like the UK based study published in 2001 (239), Little et al also found that an increasing proportion of patients had TDR

after the introduction of HAART, with prevalence figures increasing from 8% in 1995-1998 to 22.7% in 1999-2000. Simon et al reported similar figures in their study of 154 individuals in New York City. TDR rates increased from 13.2% in 1995-1998 to 19.7% in 1999-2001 (232). An even higher rate was reported by Grant et al (234). Amongst 225 patients first seen for care in 1996-2001, 23.1% were found to have at least one primary resistance mutation.

However, other studies have reported considerably lower rates of TDR. In a large nationwide study in France, 404 patients were tested for primary resistance mutations in 1998 and only 3.4% were identified as having a resistant virus (240). Garcia et al reported a similar rate of AZT TDR amongst 603 patients seen across 10 American cities in 1997-1999 (3.3%) (238). Although seen for care a little earlier, Weinstock et al published a study in 2001 which assessed the prevalence of mutations in 6 United States cities (241). Of the 99 patients first attending for care in 1993-1998, only 5% were found to have a primary resistance mutation. A smaller study in Holland analysed 43 antiretroviral naive patients, first seen for care in 1990-1998, and reported a TDR prevalence rate of 7% (235).

2.1.3.3. TDR trends after 2000

There has also been considerable variability in the rates of TDR reported since the year 2000. Whilst some studies have reported relatively high rates, both across Europe and America (191;220;221;223;225-228), other studies have reported rates of less than 10% (189;218;219;222;224). The SPREAD study is the largest study analysing rates of primary drug resistance across Europe. By analysing genotypic data of 1245 newly infected patients in 2002-2003 from 17 countries, organisers reported a TDR prevalence rate of 9.1% (206). Several studies were able to assess trends over time. Shet et al reported an increasing trend of TDR from 1995 to 2004; rates of TDR in 1995-1998, 1999-2000, 2001-2002, 2003-2004 were 13.2%, 19.7%, 16.7% and 24.1% respectively. This study was completed in New York City and consisted of predominately MSM (191). A UK study by Cane et al investigated the prevalence rates of TDR by using different interpretation systems and also concluded that TDR had increased markedly from 1996 to 2003 (225). De Mendoza et al in Spain reported rates of TDR dropping considerably between 1997-1999 and 2000-2001 from 27.6% to 4.1% but rising again in 2002-2003 to 16.0% (228). However, a UK Study by Fox et al reported stable incidence rates of TDR between 2000 and 2004 of 6% (189). Rates of TDR in Switzerland were also stable at around 7% between 1996 and 2005 (210).

More recent studies generally have reported a TDR prevalence rate of around 10%. Green et al used the IAS mutation list, the list of mutations suggested by Shafer et al and the Stanford algorithm to calculate rates of resistance amongst newly infected patients in 1997-2008 and found all 3 lists provided similar estimates of 10.0%, 9.2% and 10.4% respectively (185). A study using data from the EuroSIDA cohort reported a TDR rate of 11.4% amongst 525 patients seen across Europe in 1996-2004 (203). Similar rates have been reported in Germany (211;217), Belgium (205) and Canada (207). Rates of recent TDR in the US are not as varied as those in earlier calendar years and are also generally around 10% (204;212;215). A lower prevalence of TDR has been reported in Switzerland and in parts of the UK (202;209;210). There are also reports of considerably higher prevalence rates than the 10% reported above. Madsen et al used the Stanford algorithm to identify resistance mutations amongst newly infected patients in 1999-2007 (201). Of the 60 predominately heterosexual patients tested in Greenland, over a quarter were found to carry at least one primary mutation. Trends over time in recent studies also vary. Bannister et al used data from 1994 to 2004 to describe trends of TDR across Europe (203); a decrease in the prevalence of primary resistance was seen between 1994 and 2001 (from 20.3% to 6.5%) but this was followed by an upward trend in 2002-2004. The rate of TDR in the latter period was reported to be 9.1%, suggesting that the proportion of patients with primary resistance is increasing. Brenner et al also reported an increase of TDR in Canada (207), from 15.7% in 1997-2001 to 21.2% in 2004-2005. The UK Collaborative Group on HIV Resistance analysed data on over 4000 samples from treatment naive patients (214) and reported prevalence rates from 1996-1998 to 2004. Rates were generally around 10% though after an initial increase, appeared to be declining towards the end of the period: rates in 1996-1998, 1999, 2000, 2001, 2002, 2003 and 2004 were 8.4%, 10.0, 11.2%, 14.2%, 13.0%, 15.6%, 12.5% and 9.2% respectively .

In the recently published SPREAD study, TDR prevalence rates across Europe stood at 8.4% between 2002 and 2005. Though no statistical significant decrease was seen in overall prevalence rates, authors did find that the proportion of patients with PI and NNRTI resistance were decreasing and concluded that the overall rates of TDR were stabilising (199).

2.1.3.4. Resistance patterns

Most reported resistance mutations occur in the reverse transcriptase (RT) gene, though actual frequencies vary considerably from study to study. A relatively low

prevalence of mutations conferring resistance to NRTIs (3.3%) was reported by Descamps et al in 2001, in a nationwide study in France (240). Other studies published before 2002 have reported similar rates (241;245). However, a study in Madrid of 30 newly infected patients reported 23.3% had NRTI mutations (236). This estimate is markedly higher than other estimates published both earlier and later. Earlier trends in time of the prevalence of RT resistance also vary. Duwe et al reported an overall decline in TDR in Germany from 1996 to 1999 but saw an increase in the proportion of patients with RT resistance (237). In France, however, a decline in RT resistance was reported between 1995 and 1998 (243). Simon et al also reported that the proportion of patients conferring mutations resistant to NRTIs had declined between 1995 and 2001, though in the same period the proportion of patients with mutations conferring resistance to NNRTIs had increased from 5% to 8.1% (232). In a larger study in the US, the proportion of NRTI resistance mutations decreased from 25% to 7% between 1996/7 and 1998/9 but then increased to 20.9% in 2000/1. Authors also reported the proportion of NNRTI resistance mutations steadily increasing from 0.0% in 1996/7 to 13% in 2000/1 (234).

In recent years, the proportion of patients with mutations conferring resistance to NRTIs has been reported to be fewer than 10%. In the EuroSIDA study, 9.3% of patients were resistant to NRTIs and 1.8% were resistant to NNRTIs (203). Other large studies have reported lower rates of NRTI resistance but higher rates of NNRTI resistance. For example, the SPREAD study reported an NRTI mutation prevalence rate of 5.4% and an NNRTI mutation prevalence rate of 2.6% (206). A study in Switzerland reported similar rates of 5.5% and 1.9% respectively (210). A large study in the UK reported similar rates of mutations conferring resistance to NRTIs and NNRTIs of 4.6% and 4.4% respectively (214), whilst data published in America and Canada suggest that the proportion of patients with NNRTI resistance mutations is now higher than that with NRTI resistance mutations (204;207).

Mutations conferring resistance to the protease gene (PR) have generally been reported to occur in fewer than 5% of newly infected patients (202-205). Although an Australian study found no difference in the proportion of patients with either primary or secondary PR mutations before and after the widespread use of PIs (230), other studies have reported an increasing trend in the proportion of patients with primary muations. Simon et al reported PR mutation prevalence rates of 1.7% in 1995-1998 increasing to 5.4% in 1999-2001 (232). Another US study reported PR mutation rates increasing from 2.5% to 7.7% between 1996 and 2001 (234). A third US study

conducted by Shet et al reported a markedly higher rate of PR mutations. Of those newly infected in 2003-2004, 13% harboured a mutation conferring resistance to a PI (191)

2.1.3.5. Multi-drug resistance (MDR)

Patients who are infected with a virus which is resistant to more than one class of antiretrovirals are defined as having MDR. The management of these patients is difficult since many drugs which are used as part of first-line treatment are ineffective amongst patients who have MDR. Further, there is a risk that patients with MDR will transmit their resistant virus to others, resulting in a potential increase of prevalence of MDR.

As early as 1999, Boden et al identified 3 (3.8%) patients who were resistant to two or more classes of antiretrovirals (245). A significantly higher rate of MDR was reported in a Spanish study conducted by Briones et al (236). Albeit with a relatively small sample size of 30, Briones et al reported that 6.7% of patients who were seen in 1997-1999 had MDR. Estimates of MDR from the CASCADE study over a similar time period were lower; between 1987 and 2003, Masquelier et al reported a MDR prevalence rate of 0.5% (226). A UK study conducted by Cane et al reported 2% of newly infected patients had MDR (225). Canadian studies also reported similar estimates (207;222). The rate of MDR in more recent studies appears to be stable or even declining (231). A stochastic model (a method of modelling in which one or more variables within the model are randomly distributed, e.g. mode of infection) developed by Phillips also predicted a decline in the proportion of cases with MDR, from 4.7% in 2006 to 1.5% in 2010 (247). A suggested reason for this decline is that most infectious people are actually not receiving antiretroviral therapy and those that are have successfully suppressed their viral load.

2.1.3.6. Predictors and outcomes of TDR

There is conflicting evidence on the predictors of TDR. A study by Sagir et al published in 2007 found patients of ‘other’ ethnicity (compared to white), those who were infected with non-B subtypes of HIV and those who weren’t MSM were more likely to have a resistant strain of HIV (217). However, a US study published in the same year found that patients of white ethnicity were actually more likely to have TDR (215) and a Belgium study of newly infected patients in 2003-2006 reported that patients infected with subtype-B HIV were more prone to having primary resistance (205).

Another study conducted in the US in 2004-2006 focused on recreational substance use amongst HIV-positive patients (208). The prevalence of alcohol, marijuana and methamphetamine use were 98%, 71% and 64% respectively. Authors reported a TDR prevalence rate of 23% and found methamphetamine use to be significantly associated with TDR. Positive hepatitis status was associated with TDR in the EuroSIDA study (163) and low viral load was reported to be significantly linked to primary resistance mutations in the study by Poggensee in Germany (211). Amongst 239 predominately MSM patients in the UK, Booth et al identified an association between high CD4 counts, being born in the UK and TDR (209).

Though being infected with a resistant virus limits treatment options, it does not necessarily result in worse long-term immunological and virological outcomes than those infected with wild type virus. In the period where monotherapy/dual therapy was the most common form of treatment for HIV, Balotta et al concluded that that there was no difference in 6 month outcomes between patients infected with an AZT resistant virus and those infected with wild type virus (242). Tamalet et al analyzed outcomes between those with TDR and those without over a similar calendar period and also concluded that there was no significant difference in outcomes between the two groups (243). Although in these particular studies, sub-optimal therapy is likely to explain the similarities between the two groups, later studies have reached similar conclusions. Oetta et al analyzed outcomes of 269 patients infected with TDR in 2001-2003 in Germany and also found no difference between this group and those not infected with TDR (220). Shet et al also reached similar conclusions in their cohort of US patients seen for care in 2003-2004 (191).

2.1.3.7. Summary of TDR

The introduction of routine resistance testing amongst newly infected HIV-positive patients has contributed immensely to patients with TDR being able to achieve similar immunological and virological outcomes as those without TDR. Though these patients often do not have the option of using recommended first-line HAART, clinicians are able to select effective antiretroviral drugs individually suited to patient needs.

Estimates of TDR are conservative yet still show that the prevalence of primary resistance in developed countries stands at around 10%. There is conflicting evidence on trends of TDR, though with effective HAART and higher rates of viral suppression, it is anticipated that the prevalence of TDR is likely to decrease.

Though patients infected with a MDR virus have significantly fewer treatment options than those infected with a single drug resistant virus, the prevalence rate of MDR is relatively low at below 5%. Mutations conferring resistance to NRTIs are most frequently detected, though those conferring resistance to NNRTIs appear to be on the rise. The proportion of newly infected patients conferring resistance to PIs is relatively low. With baseline resistance testing now recommended in all newly infected patients, virological and immunological responses amongst patients with TDR are comparable to those without primary resistance.

2.1.4. Resistance testing post-ART

2.1.4.1. BHIVA guidelines

Current BHIVA guidelines recommend that all patients who experience virological failure, i.e. a detectable viral load (>50 copies/mL) on their current treatment regimen after 24-36 weeks, should consider changing therapy (124). This decision should be guided by the availability of a treatment option that is likely to suppress viral loads to undetectable levels. Resistance testing whilst the patient is still on the failing regimen is recommended as resistant viruses may cease to dominate when therapy is stopped, and hence may not be as detectable using current resistance tests which are unable to detect all mutations in a non-dominant virus. Patients who remain on failing regimens for prolonged periods of time are likely to continue developing resistance mutations, particularly to NRTIs (248), and hence resistance testing needs to be performed rapidly and results need to be analysed quickly to avoid the potential of cross resistance when choosing a new regimen.

2.1.4.2. Prevalence of resistance mutations in pre-treated patients

A recent study published in 2007 assessed the prevalence of resistance mutations amongst patients in Italy who had at least 6 months of antiretroviral experience and were experiencing virological failure (2 consecutive viral loads >1000 copies/mL) (249). Most patients (over 50% in years 1999-2004) had started treatment with suboptimal NRTI mono/dual therapy and had failed a median of 5 ART regimens. Of the total number of patients experiencing virological failure in 1999-2005, 36.1% had had a genotypic resistance test in the same calendar year. Authors reported that the prevalence (based on the results of the last genotypic test result available) of any drug resistance had declined from 95% in 1999 to 53% in 2005. The proportions of patients with resistance mutations to NRTIs, NNRTIs and PIs had changed from 89.3%, 33.6% and 63.9% in 1999 to 47.4%, 10.3% and 23.1% in 2005, respectively.

Independent predictors of the detection of resistance mutations were earlier calendar year, male sex, higher CD4 counts and suboptimal NRTI therapy.

The UK Collaborative Group on HIV Drug Resistance also published a study that reported the prevalence of drug mutations amongst treated patients, though did not restrict analyses to those experiencing virological failure (250) after a defined period of time. A total of 4218 patients were identified as having a resistance test whilst receiving therapy in 1998-2002. When using only the last resistance test result available, between 75% and 82% of patients with resistant tests were found to have resistant mutations to at least one class during follow-up. Mutations conferring resistance to PIs decreased over time, whilst mutations conferring resistance to NNRTIs increased. However, if the results of all prior resistance tests were also taken into account, together with the number of patients receiving treatment used as the denominator (rather than the number of patients with resistance tests), these proportions were considerably lower. An increase in the proportion of treated patients with a resistance mutation, however was still evident (4.5% by the end of 1998 to 15.3% by the end of 2000), though this did stabilise by the end of 2002 (17.0%). As noted by the authors, these proportions are likely to be underestimates since only around 30% of patients experiencing virological failure had a resistance test performed.

Other studies have used various numerators/denominators to calculate the proportion of treated patients with detectable resistance mutations. Tamalet et al reported the prevalence of resistance mutations in a French cohort, using the total number of patients with resistance tests as a denominator (251). The proportion of patients with mutations indicating resistance to at least one NRTI was reported to be stable at around 80% during 1999-2002, whilst a marked increase was noted in the proportion of patients carrying resistance mutations to at least one NNRTI (40.4% in 2002). For PIs, the proportion of patients with resistant viruses reached a high of 50.9% in 1999 but a downward trend was observed in the following years (41.9% in 2002). The proportion of patients with resistance to 3 classes increased from 3.4% in 1998 to 25.9% in 2000, after which time it remained stable. Authors of an earlier US study had used the number of patients with viral loads >500 copies/mL as the denominator and reported that 76.3% of patients tested had at least one drug resistance mutation during the study follow-up period (1996 to 1998) (252). The proportion of patients who were resistant to at least one NRTI, NNRTI and PI was reported to be 71.4%, 40.5% and 25.2% respectively. MDR was detected in 47.7%

of the viremic population, with 13.1% of patients having mutations conferring resistance to all 3 classes. A higher prevalence of resistance was detected amongst patients with lower current viral load and lowest self-reported CD4 count. Scott et al published similar estimates in 2004 (253) relating to 91, 92 and 92 patients on treatment who were under follow-up with viral loads >2000 copies/mL in 1998,1999 and 2000 respectively. The proportion of patients with any resistance mutations to at least one drug increased from 69% to 88% between 1998 and 1999, and then dropped to 55% in 2000. As in other studies, a significant rise was seen in the proportion of patients with NNRTI resistance and MDR.

Hertogs et al focussed specifically on patients with mutations conferring resistance to PIs (254). Of those with a resistance test performed whilst having a detectable viral load of >1000 copies/mL, 17% to 25% of patients were found to harbour a mutation conferring resistance to a PI. The prevalence of drug resistance amongst patients experiencing viral rebound was analysed in the Frankfurt HIV clinic cohort (255). In total, 45.1% and 88.9% of patients were identified as having mutations conferring resistance to the PI or NNRTI in their initial HAART regimen. These proportions appear to be markedly higher than others – one possible reason for this is that phenotypic resistance testing was performed (rather than genotypic as in most studies). A cross sectional study performed in 2000 in Spain identified that 79% of pre-treated patients carried resistance mutations (256); 77% carried NRTI mutations, 53% carried PI mutations and 42% carried NNRTI mutations. These results are similar to those published earlier in Spain, despite varying denominators and definitions of resistance (257;258).

2.1.4.3. Summary of the prevalence of resistance mutations in pre-treated patients

The proportion of pre-treated patients with a resistant virus peaked in around 1999-2000. Although comparability between estimates is difficult due to differing denominators/definitions of resistance, around 70% of patients in the above mentioned studies appeared to harbour mutations resistant to at least one drug. The proportion of patients with mutations conferring resistance to NRTIs was high, at above 75% in most studies, whilst the proportion of patients conferring resistance to PIs was somewhat lower at below 50%. Mutations conferring resistance to NNRTIs appeared to be on the increase in most studies. One possible explanation for this is that prescribing patterns have changed over the last 10 years, moving towards an increase in NNRTI prescribing. Further, the genetic barrier to resistance differs

considerably between the two classes. NNRTI resistance is significantly more likely to occur than PI resistance in patients taking HAART, explaining the lower rates of PI resistance seen across studies (259;260). Another explanation for these differences is that denominators between studies often vary; in some studies only those currently receiving drugs from the class of interest are included, whilst in other studies, this restriction is not enforced.

Improved management of patients infected with HIV, together with improvements in therapy has led to the prevalence of resistance amongst treated patients declining in recent years. Around 50% of those who have had resistance tests performed after 2000 were identified as carrying resistant mutations (214;261). This downward trend was noted particularly in the proportion of patients with NRTI resistance and PI resistance, again likely to be explained by changing prescribing patterns.

An important issue that has not been well addressed is the impact of number of prior regimens on treatment failure and hence prevalence of resistant mutations. Patients on their first line of HAART are less likely to experience virological failure than those on subsequent regimens, and thus this should be taken into account when calculating resistant mutation prevalence rates.

2.1.4.4. Genetic barriers of drugs

Together with drug susceptibility, viral fitness, drug concentration and drug interactions, the number of mutations required to confer resistance defines the genetic barriers of drugs to the emergence of resistance. The genetic barrier is intermediate for most NRTIs and low for 3TC, FTC, EFV and raltegravir.
For example, for 3TC, only one mutation is needed to produce a resistant virus which can replicate better than the current virus. However, if 3TC is used in conjunction with AZT, the genetic barrier to resistance is higher.

Ritonavir boosted PIs have the highest genetic barrier and hence are least likely to result in development of a resistance virus. At least two mutations are needed for resistance to PIs; a single mutation is a step towards resistance but that intermediate virus can't replicate as well as the current virus, so the second mutation is needed before the barrier is overcome. The virus with just the one mutation is likely to be outcompeted and die out before the second mutation has a chance to appear. The current list of mutations associated with resistance to each antiretroviral is provided by Johnson et al (182).

2.2. HIV drug toxicities

Although the introduction of HAART in 1996 saw a rapid decline in the number of new AIDS illnesses and deaths (262), the association between HAART and toxicities was soon well recognised. Toxicities associated with antiretrovirals can be mild to severe in intensity, either short-term or long-term and either drug-specific or class-specific. Short-term toxicities were discussed in Chapter 1.6 (Table 1.6.1). Occurring usually in the first few weeks/months of starting treatment, early adverse effects tend to be mild/moderate and can include headaches, nausea, diarrhoea and vomiting (128). As patients remain on treatment for longer periods of time, the emergence of more severe and life-threatening drug related toxicities has become apparent. These include lactic acidosis, fat redistribution, hepatotoxicity, renal disease and peripheral neuropathy (263-267).

Drug-related toxicity is a major factor leading to the interruption of treatment, which in turn is likely to lead to virological failure (152;268). In this Section, toxicities associated with HAART, i.e. hepatotoxicity (in particular rises in alanine transaminase [ALT]) will be described. Further, renal-related toxicities (both due to HIV itself and anti-HIV drugs) and the relationship between HIV, HAART and dyslipidaemia (demonstrated by abnormal total cholesterol, triglycerides, low density lipoprotein (LDL)- and high density lipoprotein (HDL)-cholesterol levels) will be discussed. Finally, I will briefly summarise other abnormal measures associated with HAART, including abnormalities in haemoglobin, neutrophil, urate, and creatinine levels.

2.2.1. Hepatotoxicity

Hepatotoxicity has been reported to develop in patients taking all of the original three antiretroviral classes. An increase in liver enzymes in the blood, in particular ALT, aspartate aminotransferase (AST) and gamma–glutamyltransferase (GGT) are early signs of liver disease. Numerous studies have investigated the association between HAART and liver disease, often concluding that HAART may cause a rise in ALT levels (269-278). These studies are summarised in Table 2.2.1.1. However, the prevalence of hepatotoxicity and the magnitude of the association between hepatotoxicity and HAART vary considerably from study to study. For example, Livry et al reported the incidence of ALT elevations to be 20.9% (269), whilst a study by Saves et al found only 2.2% of patients in their cohort experienced ALT elevations (279). Other studies have reported incidence figures in the region of 10%

(270;277;280;281), whilst a study by Butt et al in 2007 concluded that up to 58% of patients had signs of ALT elevation (282).

These differences are largely due to the choice of definition used to assess ALT elevations. Some studies include new ALT elevations which persist, while others refer only to transient elevations which return to baseline, or to within the normal range. Many studies use the AIDS Clinical Trials Group (ACTG) classification of hepatotoxicity (283), with severe hepatotoxicity being classed as either grade 3 or grade 4, referring to ALT levels >5-times and >10-times the upper limit of normal (ULN), respectively. However, even the ULN is subject to variability, ranging from 31 to 40 (284;285) IU in different laboratories. Furthermore, not all studies use the ACTG classification (282) whilst others use an amended version (277).

Chronic infection with hepatitis C virus (HCV) or hepatitis B virus (HBV) is an independent risk factor for liver damage in HIV-positive patients (269;279;286-288). The prevalence rates of both HCV and HBV vary greatly between studies, partially explained by differing definitions of HCV and HBV. Authors may use a positive HCV antibody result or alternatively use the detection of HCV RNA to define HCV-positive status. Positive HBV status may be defined by a single positive surface antigen result or either 2 consecutive results measured a defined period of time apart (272;285). Further, hepatitis status is associated with means of HIV transmission and so reported prevalence rates will vary greatly according to the population being studied. Intravenous drug users (IDUs) and those infected through blood products are highly likely to be co-infected with HCV and, in some cases, HBV, whilst those with other risk contacts, i.e. heterosexual and homosexual, are likely to have a significantly lower rate of co-infection (289). Hence, even amongst studies with similar definitions of HCV, the prevalence of HCV may vary considerably, impacting directly on the prevalence of hepatotoxicity. For example, studies by Palmon and Sulkowski used similar definitions of HCV but reported prevalences of HCV of 10% and 52% respectively (277;290). This difference is most likely to be attributable to the proportion of IDUs in each study (4% in Palmon's study and 42% in Sulkowski's study).

Specific antiretrovirals have also been associated with ALT rises and so the prevalence of ALT elevations in a study may also be dependent on the frequency of prescribing these drugs. Several studies have found NVP and RTV, in particular, to be associated with hepatotoxicity. Wit et al found that patients who were currently

taking NVP or RTV were 9.6 times and 4.9 times, respectively, more likely to experience grade 4 liver enzyme elevations, independent from hepatitis status (272). In a case control study published by Requena et al, higher NVP levels in blood plasma were found to be independently associated with ALT rises. Further, amongst patients with HCV, NVP concentrations above 6 microg/ml were associated with a 92% risk of liver toxicity (276). Other studies have reported similar findings. Amongst patients starting HAART with a NVP based regimen, Martinez et al found that hepatotoxicity developed in 12.5% of patients. Each additional year of NVP use was associated with a 10% higher risk of hepatotoxicity (275). RTV use was found to be associated with an 8.6 times higher risk of severe hepatotoxicity by Sulkowski et al (277) and similarly, Bonfanti et al reported that patients taking RTV were twice as likely to experience liver toxicity than those who had not been exposed to RTV (271). It should be noted that the majority of studies which have found an association between RTV and liver toxicity tended to include patients on high dose RTV (i.e. 1000mg, or single RTV). Since RTV is now only prescribed as a booster for other PIs, this association has weakened considerably, though has not disappeared completely (274). Several studies have shown that mitochondrial toxicity is associated with the use of certain NRTIs and may lead to liver enlargement/fatty liver (291;292). In particular, the “d” drugs, ddI, ddC and d4T, are more likely to have an effect on mitochondria than the other NRTIs (293). These drugs have been shown to increase the risk of hepatic steatosis, especially amongst those infected with HCV (294;295).

Table 2.2.1.1: Summary of studies examining risk factors for hepatotoxicity

Author, region and year published	Study type	N	IDU %	HCV %	HBV %	ART inclusion criteria	Definition of hepatotoxicity	Incidence/ prevalence rate of hepatotoxicity	Independent risk factors
Aaron, America 2010[296]	Retrospective cohort	612	12.4	15	na	Initiating new ART regimen	ALT/AST >2.6 times baseline	6%	HCV+
Piazza, America 2010[297]	Cross-sectional	432	na	0	0	None	Liver fibrosis	8%	Diabetes, high VL
Kovari, Switzerland 2010[298]	Prospective cohort	2365	1	0	0	None	ALT>ULN on 2 occasions	16%: 3.9/100 p-yrs	White ethnicity, high CD4, VL >100,000
Chaudhry, America 2009[299]	Prospective cohort	1358	45	49	8	None	Liver fibrosis (APRI)	11.6% with APRI>1.5	Male gender, HBV+, HCV+, IDU, hazardous alcohol consumption
Yunihastuti, Indonesia 2009[300]	Retrospective cohort	284	78	100	0	ART naive	ALT >5 times ULN	21%	High baseline ALT
Vergara, Spain 2007[301]	Retrospective cohort	116	94	100	na	12 months on HAART	Liver fibrosis	36%	ALT >5 times ULN, >3.5 times baseline if abnormal at baseline
Chihrin, Canada 2007[274]	Retrospective cohort	102	45	100	9	Receiving HAART	ALT >5 times ULN	22%	None
Butt, America 2007[282]	Prospective cohort HCV mono HIV/HCV co-infected	390 315 75	 60 68	 100 100	na	None	ALT>50	 58% 41%	HCV+, prior treatment for HCV and HCV mono-infection
Maggiolo, Italy 2007[302]	Prospective cohort	582	56	55	na	Receiving NVP HAART	Grade 3 toxicity	5.3/100 p-yrs	Not tested
Maida, Spain 2006[303]	Cross Sectional	3200	na	na	na	None	Persistently elevated LFTs in absence of other causes	0.5%	ddI

Cooper, Canada 2006[304]	Retrospective cohort	186	75	100	5	Receiving first HAART regimen	ALT >5 times ULN	7%	Not tested
Uberti Foppa, Italy 2006[305]	Prospective cohort	326	87	100	0	None	Liver fibrosis	66%	Older age, CD4<500 cells/mm3 and ALT>55 for men, >50 for women
Manfredi, Italy 2006[306]	Retrospective cohort	742	na	14	3	Receiving NVP/ EFV ≥12 mths	>2 fold increase in AST/ALT	NVP: (27%) EFV: (14%)	NVP
Torti, Italy 2005[273]	Prospective cohort of Naïve (N) & Experienced (E)	1038 N:155 E:833	N:68 E:77	N:100 E:100	N:10 E:6	Receiving HAART	AST/ALT >5 times ULN	N:18/100 p-yrs E: 8/100 p-yrs	N: univariable only, higher baseline ALT E:previous hepatotoxicity, higher baseline ALT, and NNRTI regimen
Olalla, Spain 2005[307]	Retrospective cohort	145	82	73	na	None	AST/ALT >5 times ULN, >3.5 times baseline if abnormal at baseline	28%	HCV+ and higher baseline ALT and
Servin-Abad, America 2005[308]	Retrospective case control	85	60	100	77	Receiving HAART	ALT >5 times ULN, plus an increase of >100 IU/L	11%	Univariable only: high baseline AST, low abumin levels and high INR
Bonfanti, Italy 2005[309]	Prospective cohort	551	40	40	7	Either naive or experienced starting LPV/r	ALT >5 times ULN, >3.5 times baseline if abnormal at baseline	0.59/100 p-yrs	Not tested
Mocroft, Europe 2005[310]	Prospective cohort	10937	24	17	5	(1) None (2) Those who had received HAART	Death from liver related disease	(1) Overall: 3.5/1000 p-yrs (2) 12% increase in risk per year longer	(1) IDU, HBV+, HCV+, lower current CD4 count, 3TC and later calendar year (2) Taking HAART < 2 yrs
Sanne, South Africa 2005[311]	RCT	468	na	na	NVP: 4	Naïve receiving NVP or EFV and randomized to d4T/3TC or d4T/FTC	>5 times ULN	NVP: 66 (17%) EFV: 0 (0%)	NVP arm only: female sex, low BMI, low VL, low albumin, low AST, low LDH, low MCV, at baseline

Macias, Spain 2004[312]	Cross sectional study	152	95	100	na	41% naive, 59% received HAART (of whom 87% had received a PI)	Fibrosis stage ≥F3* ALT 2.5* baseline	39% Not tested	Older age, no prior PI, prior NVP, lower CD4 count
Sulkowski, America 2004 [313]	Prospective cohort	1161	43	45	10	PI-naïve, starting PIs	AST/ALT>5 times ULN, >3.5 times baseline if abnormal at baseline.	9.9/100 p-yrs	HCV+, low CD4 count, high VL, IDR, IDV/r, SQV/r
Meraviglia, Italy 2004[314]	Prospective cohort	782	42	39	47	Treatment experienced starting LPV/r	>100 IU/L higher than baseline if abnormal at baseline	9.1%	Higher baseline ALT,GGT, younger age, EFV
Livry, France 2003[269]	Retrospective cohort	239		14	5	None	ALT 2.5 times baseline	9.9/100 p-yrs	HBV+, HCV+, ART, CDC Stage 3, high alkaline phosphatise
Puoti, Italy 2003[315]	Prospective cohort	755	65	70	7	Previously ART-naïve	AST/ALT >5 times ULN, >2.5 times baseline if abnormal at baseline	17/100 p-yrs	Univariable only: male, IDU, younger age, HBV+, HCV+, HDV+, higher baseline ALT, higher bilirubin, <75% prothrombin time, prior alcohol abuse
Aceti, Italy 2002[316]	Retrospective cohort	1325	50	47	4	Receiving at least 1 PI for 6 months	ALT>5 times ULN, or if high baseline then relative to baseline value	3.2%	Univariabe only: HBV+, HCV+
Wit, Netherlands 2002[272]	Retrospective cohort	560	7	9	11	Previously naïve starting HAART	AST/ALT >5 times ULN, >100 U/L compared to baseline value	17%	HBV+, HCV+, recent discontinuation of 3TC, use of NVP, use of RTV

Sulkowski, America 2002[317]	Prospective cohort	568	42	43	8	Starting NNRTIs (NVP (N) or EFV (E))	AST/ALT>5 times ULN, >3.5 times baseline if abnormal at baseline	N: 16% E: 8%	NVP, HCV+, HBV+, concurrent PI use
Palmon, America 2002[290]	Retrospective cohort	272	4	12	9	Receiving NNRTIs	>5 times ULN, >3.5 times baseline if abnormal at baseline.	1.1%	None
Martin-Carbonero, Spain 2002[318]	Retrospective cohort	42	88	100	0	Previously naive starting HAART	>5 times ULN, >3.5 times baseline if abnormal at baseline	14%	HCV+
Bonfanti, Italy 2002[271]	Prospective cohort	1477	48	47	7	Starting PI containing ART	AST/ALT>5 times ULN, changes from baseline if abnormal baseline	2.7/100 p-yrs	IDUs, hepatitis, RTV, HBV+, HCV+
Nunez, Spain 2002[111]	Retrospective cohort	222	48	38	5	Naïve starting HAART.	AST/ALT>5 times ULN or >3.5 times baseline if abnormal at baseline	9%	Alcohol abuse, HCV+ and older age
D'Arminio Monforte, Italy 2001[278]	Prospective cohort	1255	38	47	7	Starting HAART, 214 ART-experienced	ALT>200 U/L or 5 times ULN or stopping regimen due to hepatotoxicity	4.5%	Pre-treated, higher baseline ALT, HBV+, d4T containing regimens
Bica, America 2001[319]	Retrospective cohort	84	70	na	na	None	Death (causes of)	na	End-stage liver disease leading cause of death
Martinez, Spain 2001[275]	Prospective cohort	610	35	46	9	Starting NVP-containing regimen	AST/ALT >3 times increase from baseline	12.5%	Longer duration of ART, HCV+, higher baseline ALT

Sulkowski, America 2000[277]	Prospective cohort	298	54	52	3	Starting ART	AST/ALT>5 times ULN or >3.5 times baseline if abnormal at baseline	10.4%	RTV, CD4 increase >50
Den Brinker, Netherlands 2000[285]	Retrospective cohort	394	9	14	7	PI-naïve starting PIs	AST/ALT>5 times ULN, at least >100 from baseline	18%	HBV+, HCV+, abnormal baseline ALT levels
Saves, France 2000[279]	Prospective cohort	1047	17	26	4	All starting PIs	ALT>5 times ULN	5/100 p-yrs	HBV+, HCV+
Melvin, America 2000[281]	Prospective case-control	95	na	Case:100 Ctrl: 0	na	Receiving ART	ALT>5 times ULN	Case:17.0% Ctrl: 6.8%	HCV+
Gisolf, Belgium & Netherlands 2000[270]	RCT	208	5%	8% HCVAb	12% HBsAg	Previously naive starting RTV/SQV or RTV/SQV/d4T	ALT/AST>5 times ULN plus >100 U/L compared to baseline value	9%	HBV, d4T
Saves, France 1999[280]	Prospective cohort	1) 1253 2) 748	1) 27 2) 21	1) 27 2) 35	1)9 2)13	1) 2 NRTI 2) PI-containing HAART	ALT>200 U/L or 5 times the ULN	1) 5.7/100 p-yrs 2) 7.3/100 p-yrs	1) history of cytolysis, HBV+, HCV+ 2) history of cytolysis, higher baseline ALT, HBV+, HCV+
Rodriguiz, Spain 1998[286]	Prospective cohort	187	48	na	na	Starting HAART	ALT >2 times baseline	na	HCV+

IDU: Injecting drug users Na: not applicable

2.2.2. Renal failure

Several factors can confer an increased risk of renal disease amongst HIV-positive patients; HIV-associated nephropathy (HIVAN) has been reported to be one of the leading diagnoses, followed by noncollapsing focal segmental glomeruosclerosis and acute interstitial nephritis (320-323). Patients who experience HIVAN are likely to have high levels of protein present in urine, high levels of waste products in the blood and scarring of blood vessels in the kidneys. Kidney disease is tested for with urinalysis, a creatinine test and/or a blood urea nitrogen test (BUN). Healthy individuals usually have 0.8-1.4mg/dl and 7-20mg/dl of creatinine and BUN, respectively. Elevated levels of either laboratory marker may indicate kidney disease. Creatinine measurements are used to estimate an individual's glomerular filtration rate (eGFR). The most common formula to calculate the eGFR takes into account age, weight, gender and ethnicity. The eGFR is used to determine how well kidneys are able to filter blood over a period of time; measurements below 60 units normally indicate kidney disease.

Although HIV itself can infect the cells inside the kidneys causing HIVAN, antiretrovirals used to treat HIV have also been linked to kidney damage. In particular, NRTIs such as TDF and the PI IDV have been shown to cause kidney damage amongst HIV-positive patients (324;325); it is recommended that patients taking these drugs undergo annual blood and urine tests to evaluate kidney function. If diagnosed early, HAART can be used to either treat HIVAN (i.e. if the cause of HIVAN is HIV infection itself) or can be modified so drugs which are causing HIVAN are switched.

Kidney complications amongst HIV-positive patients were first noted in 1984, a few years after cases of HIV/AIDS were initially identified. A variety of renal lesions were found amongst a small group of patients diagnosed with AIDS (326) and mortality rates amongst these patients were found to be significantly worse than those in AIDS patients without renal problems. Before the HAART era, the prevalence of HIVAN was reported to be between 1-10% (322) with mortality rates approaching 50% at one year after diagnosis and 70% at three years after diagnosis (327). Patients with acute renal failure had poorer outcomes than those not infected with HIV (328) and in many cases were only diagnosed with HIVAN at the time of AIDS diagnosis (329;330). HIVAN was found to be closely associated with ethnicity – over 90% of HIVAN cases were seen in black HIV-positive patients (322;331-333). Hence

prevalence rates of HIVAN appear to be closely associated with geographical area - this is likely to be explained by the ethnicity distribution in certain areas.

After the introduction of HAART, initial reports suggested a rise in the incidence of acute renal failure amongst hospitalised HIV-positive patients in New York. Compared to the 2.9% with HIVAN in 1995, 6.0% of hospitalised patients had HIVAN in 2003 (334). However, there were reports of a beneficial effect of ART even before the year 2000 (335) so the rise in acute renal failure may be explained by other factors. For example, in the same time period there was also a rise in HIV negative patients diagnosed with acute renal failure and hence renal failure may have been more recognised in the population as a whole.

Chronic kidney disease (CKD) is of increasing importance in the HAART era. As HIV-positive patients are living longer, the prevalence of CKD is increasing, though not necessarily due to HIV-infection itself. Major risk factors of CKD are older age, hypertension and diabetes (336-338). Amongst patients with HIV infection, HCV, lower nadir CD4 count and AIDS diagnoses have also been shown to be associated with CKD (339). Although early HIVAN is not associated with impaired renal function in patients on HAART (340), patients with HIVAN do progress more rapidly to end stage renal failure (ESRF) (320;321). As with HIVAN, prevalence rates of CKD vary greatly depending on geographical area and the proportion of black ethnicity patients in the study population. There have been several recent studies investigating the prevalence of CKD (stages 3-5, GFR<60mL/min/1.73^2), in Europe, Asia and America. A study using the EuroSIDA dataset published in 2007 found only 3.5% of patients experienced CKD (339). Patients included in this analysis were of predominately white ethnicity (85.1%) and risk factors for CKD were much the same as those found in the general population, i.e. older age, diabetes and hypertension. Lower nadir CD4 count and AIDS diagnosis were also found to be independently associated with CKD. Data from Spanish HIV-positive patients showed a slightly higher prevalence of CKD of 7.6% (341). Again, this study consisted of predominately non-black patients and older age was found to be a risk factor for CKD. Female gender was also found to be associated with CKD, though this has not been reported by other studies. Similar prevalence rates were reported by Cheung et al in a Chinese cohort study and Wyatt et al in an urban American study (342;343). Interestingly, when a less restrictive definition of CKD was used by Wyatt et al (all stages of CKD, without the restriction of GFR <60mL/min/1.73^2), the prevalence increased to 15.5%. CKD was most prevalent in patients of black and

Hispanic ethnicities, those with HCV and lower CD4 cell counts. Another American study reported a CKD prevalence rate of 9.7%, observed in patients seen in 2004. This was notably higher than the prevalence of CKD in the general American population and amongst other factors, black African Americans were found to be at a significantly higher risk of CKD than whites (344).

2.2.2.1. HAART and renal disease

Before the introduction of HAART, the number of new cases of HIVAN rose significantly (345). ZDV, the only antiretroviral available at the time was reported to slow down the progression of HIVAN considerably (335;346;347). Since 1996, the incidence of ESRF has declined (345) but the impact of HAART on overall renal function is unclear. A beneficial effect of antiretroviral therapy has been reported in some small studies (348;349). For example, Wali et al reported the beneficial effect of NFV-based HAART on renal function in 1998 and Szczech et al reported similar findings for PIs in 2002. Whether these benefits are related directly to the use of specific antiretrovirals or the effect of the antiretrovirals on viral suppression is not well understood. However, of concern, many other studies have reported a detrimental effect of HIV drugs, in particular IDV and TDF, on renal function (350-355). In the EuroSIDA study, Mocroft et al also found that patients who were taking either TDF or IDV were at an increased risk of experiencing chronic renal failure (339). Though the benefits of HAART are undoubtedly immense, it is important to recognise that the management of HIV should take into account not only the specific patient risk factors, i.e. ethnicity, that are related to toxicities, but also the HIV drugs which are most prone to contribute to these toxicities.

2.2.3. Relationship between HIV, HAART and other laboratory markers

HIV and HAART have been linked to an adverse change in a number of laboratory markers. Although CD4 count and HIV RNA are the two most recognised markers of disease progression, other laboratory markers may provide useful information on outcome that cannot fully be captured using CD4 count and HIV RNA alone. The association between HAART and adverse changes in routinely collected laboratory markers has been investigated in several studies, though it is difficult to attribute these changes to a single drug used in a HAART regimen. The known effects of HIV, HAART and adverse changes in routinely collected laboratory markers are briefly discussed below.

2.2.3.1. Haemoglobin

Haemoglobin is a protein in red blood cells. Normal values are generally defined as >13.5 g/dL for men and >11.5 g/dL for women. Individuals with haemoglobin values below these points are defined as being anaemic. HIV positive patients frequently experience anaemia, both due to HIV infection itself and the drugs used to treat HIV. A relationship between anaemia and shorter survival has been frequently reported. As early as 1988, a study consisting of almost 5000 patients showed that only 56% of AIDS patients with baseline haemoglobin values of <100 g/L survived to the end of the follow-up period, compared to 83% of patients with baseline haemoglobin values of >120 g/L (356). A much smaller study published a year later also found a highly significant relationship between low haemoglobin levels and death (357). The results of these two studies were confirmed by several other subsequent studies (57;358-362).

The association between ZDV and anaemia has been well documented (363-365). Even when used as part of combination therapy, ZDV use has been shown to be a risk factor for anaemia. A study published in 2007 reported that patients who received ZDV-containing HAART had a 3-fold higher incidence of anaemia in the first 6 months of receiving therapy compared to those who received non-ZDV-containing regimens (366). ddI and d4T have also been associated with anaemia, though not as frequently or as strongly as ZDV (367;368). Combination therapy consisting of 3TC and ZDV has further been linked to anaemia, as has 3TC alone (369-371), although larger studies have not found this association (67;69).

The introduction of HAART has been associated with a decrease in the incidence of anaemia. Semba et al investigated the impact of HAART in 361 HIV-positive women and found that 26% of women who were receiving HAART developed anaemia, compared to 37% of those receiving non-HAART regimens and 45% of those receiving no antiretrovirals. Further, after 1 year of treatment, HAART was associated with a 32% reduction in anaemia (372). In a predominately male population, Moore et al reported a significantly higher proportion of patients with haemoglobin levels >140g/L receiving HAART than those who did not receive HAART, irrespective of ZDV use (373). Receipt of ZDV-containing HAART was found to be an independent predictor of not being anaemic during 1 year of follow-up. Similar findings were reported amongst IDUs in a sub-study of the ALIVE study (372).

2.2.3.2. Neutrophil count

Neutrophils are white blood cells that fight bacterial infections. The normal range of neutrophils is between 45% and 70% of total white blood cells (or >500 absolute neutrophil count). A low neutrophil count is associated with an increased risk of bacterial infections and occurs mostly in HIV-positive patients who are severely immnosuppressed. Low neutrophil count has been reported to be an independent risk factor for bacterial infection in patients with advanced HIV disease (374) and occurs frequently amongst patients infected with HIV (375;376). Episodes of low neutrophil count usually last around 2 weeks and are more common amongst patients with late stage disease and amongst those receiving ZDV (377).

2.2.3.3. Platelet count

Platelets are cells found in the blood that help it to clot. The normal range for platelets is 150,000 – 400,000/ml^3. Very low platelet counts (thrombocytopenia) increase the risk of bleeding and can be life-threatening. The prevalence of thrombocytopenia in HIV-positive patients has been reported to range from 10% to 50% and occurs in around 10% of newly infected patients (378;379). HIV-infection itself can cause thrombocytopenia, though malignancies and medication may also play a part (380;381). A case-control study by Migues-Burbano et al showed that patients with low platelet counts were more likely to experience CD4 counts <200 cells/mm^3, independent of anaemia and neutropenia (382). Thrombocytopenia is an 'uncommon' side effect of AZT (as stated on the drug label provided with AZT) but the drug has, in fact, been shown to increase platelet counts. Jackson et al reported an increase within several weeks of initiating treatment with AZT, which was not sustained after treatment cessation (383). HAART has also been shown to improve platelet count. A study by Servais et al reported a mean platelet count increase of 70,000 after 6 months of treatment in patients receiving HAART (384) and a significant increase after 3 months of receiving HAART has been reported by Carbonara et al (385). However, Miguez et al have reported conflicting results (386). After 2 years of follow-up, 70% of patients with persistent thrombocytopenia, most of whom were on HAART, still had a low platelet count. Over 50% of patients in this cohort were IDUs and hence the authors argue that thrombocytopenia may be associated with more rapid HIV disease progression in IDUs, despite antiretroviral therapy.

2.2.3.4. Urate

The normal reference range for uric acid is between 3.6mg/dL and 8.3mg/dL. Uric acid above this range (hyperuricemia) can lead to a type of arthritis known as gout and has been associated with cardiovascular disease and diabetes. Hypouricemia refers to uric acid below the normal range. Though not a medical condition in itself, hypouricemia has been associated with renal failure. Both hyperuricemia and hypouricemia are frequent amongst HIV-positive patients (387-389). Together with HIV infection itself, antiretroviral drugs have also been reported to be associated with adverse changes in uric acid. In particular, ddI has been associated with an increased risk of hyperuricemia (390;391). Patients receiving 3TC and PIs have also been reported to be at an increased risk of hyperuricemia, whilst patients receiving TDF may be at an increased risk of hypouricemia (392;393).

2.2.3.5. Bilirubin

Bilirubin is produced through the normal breakdown of haemoglobin and is usually in the range of 0.3 to 1.9 mg/dL. Increased levels of bilirubin are linked to jaundice associated with hepatitis, cirrhosis and anaemia. High bilirubin may be caused by liver disease, blood disease and drug toxicity. AZV and IDV, in particular, have been associated with increased bilirubin levels. Some patients may be genetically prone to bilirubin elevations when taking AZV. In a large study of treatment-naive individuals comparing EFV with AZV, one third of patients receiving AZV had elevated bilirubin levels and clinical jaundice was reported in 7% of patients (394). However, discontinuation of AZV due to these adverse events was rare and this was confirmed in a later study by the same authors (395). Of note, raised bilirubin levels amongst patients receiving AZV are unlikely to directly cause liver disease and are generally associated with direct inhibition of glucorinidation. IDV is associated with up to a 25% incidence of elevated bilirubin levels and has prompted drug discontinuation amongst patients (75;396-399).

2.2.3.6. Amylase

Amylase is an enzyme that breaks starch down into sugar and is mainly produced by the pancreas. A normal serum amylase level is 50-160 IU/L and a level above this range is often a sign of pancreatitis. Although pancreatitis is a relatively rare condition amongst HIV-positive patients, the cells that produce insulin may be damaged in patients with this condition, effectively causing diabetes and adverse changes in glucose levels in the blood. The most common cause of pancreatitis is alcohol, though other infections that affect the gall bladder, high levels of

triglycerides and certain HIV drugs have also been associated with raised amylase levels. HIV-positive patients with certain genetic markers may also be more prone to pancreatitis (400). The most common HIV drug reported to cause raised amylase levels is ddI. Although ddI causes only mild elevations of amylase in most patients, there have been reports of deaths caused directly by ddI-related pancreatitis. Patients exposed to a higher dose of ddI appear to be at a greater risk of pancreatitis and this is further increased amongst patients with low CD4 counts (401). Drugs such as TDF increase the concentration of ddI in the blood and so also may increase the risk of pancreatitis (402;403). A large study consisting of over 2500 patients reported the incidence rate of pancreatitis amongst patients receiving ddI with Hydroxyurea as 6.25/100 person years, significantly higher than those receiving NRTIs other than ddI (404)

2.2.3.7. Lactate

Lactic acid is produced by cells during chemical processes in the body that do not require oxygen. It is normally removed from the blood by the liver but when this process is not fully functional, lactate acid may accumulate causing hyperlactatemia which can lead to lactic acidosis. Lactate levels above 5mmol/dL are regarded as abnormal and can be life threatening. Mortality associated with lactic acidosis has been linked to NRTIs (161;405-407). d4T, in particular, has been reported to be associated with high levels of lactic acid (408-410). A study published by Lo et al reported that duration of treatment with NRTIs was also an independent risk factor for elevated lactate levels (411).

2.2.4. Summary of toxicities associated with HAART

Abnormalities in laboratory markers can result in mortality and hence it is important to identify the underlying cause of such elevations. Given the large number of mechanisms involved in HIV and in HAART, it is difficult to attribute laboratory abnormalities to single specific causes. Further, one abnormality often results in another and hence it may also be difficult to attribute cause of death to a single laboratory marker.

2.3. Viral rebound

The ultimate goal of HAART is to fully suppress HIV replication as this minimizes the risk of resistance evolution and results in the greatest potential for immune recovery. However patients who do manage to suppress their viral load to below detectable levels may experience viral rebound. This is often associated with the emergence of

resistance, though can also be caused by other factors such as an inability to maintain long term adherence and a lack of treatment options. Rates of viral rebound have been reported to be as high as 40% amongst patients who have achieved viral suppression (412-416). Several factors have been shown to be associated with viral rebound and the most frequently reported factors are discussed below.

2.3.1. Sub-optimal therapy before HAART

Many studies have shown that the use of NRTI single or dual therapy before HAART is associated with a raised risk of viral rebound. In the Swiss HIV Cohort Study of 2232 patients who achieved an undetectable viral load after the initiation of HAART, those who had been treated with prior mono/dual therapy were reported to have a viral rebound rate of 35-40% after 2 years of achieving an undetectable viral load, compared to 20.1% of treatment-naïve patients (417). Similar results were reported in the EuroSIDA cohort. Amongst 925 patients who had achieved an undetectable viral load, 35% of those who were pre-treated experienced viral rebound by 6 months, compared to 18% of those who were naïve at HAART initiation (418). Sub-optimal therapy before HAART was found to be an independent risk factor for viral rebound. Another large study in France showed patients starting HAART with a PI-containing regimen had a 72% increased risk of viral rebound if they had been pre-treated with NRTI mono/dual therapy (412). An even greater risk was reported by Phillips et al; patients with prior nucleoside experience were 2.86 times more likely to experience viral rebound than naïve patients (413). A later study by the EuroSIDA group confirmed earlier published findings. Taking into account the improved efficacy of HAART by 2003, Mocroft et al concluded that the rate of viral rebound among treatment-naïve patients remained approximately half that of treatment-experienced patients (414). Although the efficacy of HAART has continued to improve since 2003, recent findings still show that treatment experienced patients have a raised risk of viral rebound compared to those starting HAART from naïve (415;416).

Patients who received NRTI mono/dual therapy before initiating HAART are likely to experience higher rates of viral rebound as these patients are more likely to harbour resistance mutations (419). Several studies have demonstrated a significant correlation between drug resistance and virological response to new treatment regimens amongst patients who have failed previous regimens (116;420).

2.3.2. Age

Several studies have found that patients of older age are less likely to experience viral rebound. Mocroft et al reported that patients in the EuroSIDA study were 14% less likely to experience viral rebound for every 10 year increase in age (414). A greater reduction in risk was found by authors of an American study published in 2008 (421). In this study, older age at seroconversion was associated with a 32% reduction (per 5 year increase) in the risk of viral rebound. Older age was also found to be significantly associated with a lower risk of viral rebound by Le Moing et al in France (412). Results from the UK CHIC Study showed a similar reduction in risk of viral rebound in older patients amongst both those who were treatment-naive and experienced (422). Estimates from other studies have also shown that older patients are at a lower risk of experiencing viral rebound, albeit not always reaching statistical significance (416;423).

The largest study to date looking at the response to ART in different age groups is the COHERE study (145). Authors of this study evaluated virological and immunological responses to HAART in 49,921 patients. Though the authors did not analyse factors associated with viral rebound, they did find that patients of older age were more likely to experience a virological response after HAART initiation than those of younger age.

One possible reason for the association seen between older age and a lower risk of viral rebound may be that patients of older age have better adherence to HIV medications and therefore better virological outcomes. Although many studies have found a positive correlation between older age and adherence (155;424;425), other studies have been able to control for adherence and have still found age to be an independent predictor of virological response (412). Hence other factors such as pharmacokinetics of ART drugs in older patients may also explain the association seen between age and viral rebound, though there is little evidence in the literature to support this argument.

2.3.3. Viral load at baseline

Studies have suggested that patients with higher baseline viral loads at start of ART are at an increased risk of experiencing viral rebound or long term virological failure. A 39% increase per 1 log copies/mL higher in the risk of viral rebound was observed by Le Moing et al (412), whilst results from the EuroSIDA study found that patients with viral loads >400 copies/mL at the start of HAART were at an increased risk of

viral rebound (414;418). Similar results were reported by Staszewski et al and Grabar et al when analysing virological response to PI therapy (426;427). A 9% increase per 1 log copy/ml higher was reported in a recent study by Smith et al (416), although this association was not seen amongst a subset of highly treatment experienced patients. It has been well established that high levels of plasma RNA are correlated with HIV disease progression (51;428) and there is general consensus on the explanation for the association seen between baseline viral load and viral rebound.

2.3.4. Ethnicity and duration of suppression

Patients of black ethnicity have also been found to be at an increased risk of viral rebound. Results from the UK CHIC study showed that patients of black ethnicity had a 75% higher risk of viral rebound if they started HAART from naive and over a 2-fold increase of viral rebound if they had received prior mono/dual therapy (422). A study directly comparing virological outcomes between African patients and British/European patients also found evidence of a significantly higher increase in viral load after initial suppression amongst African patients compared to non-African patients (158). This may in part be related to socio-economic factors. Patients with increasing duration of virological suppression have also been shown to be less likely to experience viral rebound (413;414;429). This association has been reported for both treatment naive and experienced patients. The finding of an association between duration of suppression and viral rebound may be explained by a selection effect. Patients who manage to suppress their viral loads for long periods of time are likely to be those who are most adherent and have the fewest pre-existing drug mutations. Hence patients who are likely to experience virological failure will have been selected out as the time from initial response increased.

2.3.5. Specific drugs

The role of specific HIV antiretrovirals on viral rebound has been investigated in many studies. In a EuroSIDA study of 2120 patients, regimens were classified according to nucleoside pairs and the ‘third’ drug. Of the nucleoside pairs, only ZDV/ddI (compared to ZDV/3TC) was significantly associated with an increased risk of viral rebound. However, with regards to the ‘third’ drug in the regimen, compared to EFV, NVP, NFV and, in particular, ABC was associated with an increased risk of viral rebound (430). Other observational studies have also shown patients receiving NFV are at an increased risk of viral rebound (412;422) and randomised controlled trials comparing EFV to NFV have shown similar results (431;432). Analyses from

the Swiss HIV Cohort Study have suggested that treatment experienced patients receiving ABC are at an increased risk of viral rebound (433), whilst results from the UK CHIC Study also reached similar conclusions (422). Interestingly, ABC was not associated with an increased risk of viral rebound amongst patients who started HAART from naive in any of the above studies. In line with findings from the EuroSIDA study, naive patients in the UK starting HAART with either ZDV/ddI or d4T/ddI compared to ZDV/3TC have been shown to be at a higher risk of experiencing viral rebound (422). This study also found that naive patients receiving IDV/r compared to EFV were at an increased risk of viral rebound, whilst in treatment experienced patients, the reverse was true. Countless studies have directly compared antiretroviral drugs in relation to virological response – these studies have been briefly discussed in Chapter 1.6.

It is important for patients to maintain virological suppression as viral rebound is often associated with the emergence of resistance mutations. Even amongst patients who have experienced viral rebound, the aim of HAART is to reduce viral replication to undetectable levels. As the occurrence of viral rebound increases, viral suppression becomes less likely and hence the number of regimens patients have previously failed is an important factor to consider when analysing the risk of viral rebound.

2.3.6. Treatment interruption

Eradication of HIV appears to be unlikely with current antiretroviral regimens. Although HIV infection is manageable with lifelong therapy, the side effects, toxicities, high costs and inconvenience of taking HIV drugs are reasons why patients may consider short term treatment breaks. Further, in order for medication to work effectively, adherence is of key importance. Many patients find this lifelong commitment to HIV treatment an extremely difficult task and hence much research has been conducted into structured treatment interruptions (STI) for HIV-positive patients. It has also been suggested that STI may provide other benefits. For example, viral rebound after full suppression of viral replication may enhance the body's natural immune responses, known as 'auto-immunization'. Secondly, amongst treatment experienced patients with resistant virus, STI may allow the virus to revert back to wild-type which, it has been argued, will improve subsequent virological response to treatment. Early studies investigating the impact of STIs were small, often with sample sizes of fewer than 20 patients and hence conclusions were

difficult to reach. Table 2.3.6.1 summarises studies of larger sample size, published from 2000 onwards.

Until the publication of the Strategies in Management of Antiretroviral Therapies (SMART) trial in 2006, there was little consensus on the impact of treatment interruptions amongst those with low viral loads on ART. Whilst some studies had shown that those who interrupted treatment had significantly worse virological, immunological and/or disease progression outcomes than those on continuous therapy (434-440), other studies had shown little difference between the two groups or no additional risk for those who interrupted therapy (441-448). Of the cohort studies, those of larger size generally found treatment interruptions associated with negative outcomes. Results published from the Swiss HIV Cohort Study in 2002 showed that of the 4720 patients under follow-up, 1299 interrupted treatment at least once and these patients were less likely to experience a CD4 cell increase. In this study, however, no difference between those with prior treatment interruptions and those without was detected in terms of morbidity, mortality or disease progression (434). Authors of a nested cohort study of 687 patients in the US reported those who interrupted treatment had over a 2-fold risk of experiencing a 1 log increase in viral load (437) and results from the ICONA study in Italy showed that amongst the 3142 patients under follow-up, those who had interrupted treatment for over 2 weeks were almost 3 times as likely to experience clinical progression compared to those who had never interrupted treatment (438). Two relatively large randomised trials published before the SMART trial reached different conclusions. The Trivican Study in Africa consisted of 326 patients with CD4 cells counts of 150-300 cells/mm^3, randomised to either continued therapy or deferred therapy until CD4 counts dropped to below 250 cells/mm^3 (440). Although no difference was found between the two groups with regards to mortality, patients in the deferred group were found to be at a 2-fold increased risk of morbidity and the authors concluded that treatment interruption should not be recommended. The Staccato Study consisted of 430 patients in Thailand, Australia and Switzerland with CD4 counts >350 cells/mm^3 and VL <50 copies/mL, randomised to either continued therapy or deferred therapy until CD4 counts fell below 350 cells/mm^3. Low CD4 counts were more frequent in the deferred arm but no difference was found with regards to the primary outcome, VL <50 copies/mL (448). Authors of this study concluded that the cost of drugs were reduced substantially in the deferred arm and boosted PI regimens could be interrupted without the fear of resistance emerging.

The largest study to date investigating the impact of treatment interruptions on clinical endpoints is the SMART study, consisting of 5472 patients (449). Patients with CD4 counts of >350 cells/mm^3, irrespective of whether or not they had received or were currently receiving ART were assigned to either continued therapy (viral suppression group) or deferred therapy (drug conservation group) until CD4 counts fell below 250 cells/mm^3. Patients in the viral suppression group received uninterrupted treatment, whilst those in the drug conservation group received ART only when their CD4 counts fell below 250 cells/mm^3 or if symptoms of HIV disease infection were present. For these patients, ART was discontinued if/when their CD4 counts reached 350 cells/mm^3.

The primary outcome of the study was opportunistic disease or death from any cause. Patients were followed for an average of 16 months before the study protocol was modified for those in the deferred arm. Of the 167 patients who experienced the primary end point, 120 (72%) had been randomised to the drug conservation arm. The unadjusted hazard ratio for this group compared to those who were randomised to the viral suppression arm was 2.6 (95% CI: 1.9, 3.7) and though adjusting for latest CD4 count and viral load reduced the hazard ratio to 1.5 (1.0, 2.1), this was still statistically significant. This effect was partly explained by patients spending longer periods of time with reduced CD4 counts and authors concluded that treatment strategies incorporating episodic treatment interruptions were 'deleterious' and were not to be recommended.

Since the publication of the SMART Study, several other studies looking at the effect of treatment interruptions have been published. Results of two cohort studies in Europe showed little difference in outcomes between those who interrupted treatment and those who did not (450;451), whilst results from the DART trial in Africa (n=813) showed that patients who were randomised to the STI arm of the study were significantly more likely to experience AIDS/death (452). Results from the recent LOTTI Study consisting of 329 patients (both treatment naïve and experienced) with very high CD4 counts (>700 cells/mm^3) and low VLs, randomised to either continued treatment or deferred treatment until CD4 counts fell below 350 cells/mm^3, found no difference in the proportion of patients experiencing AIDS/death in each group (453). Although the differences between this trial and the SMART trial are likely to be explained by the inclusion criteria, (i.e. the former trial included only those with CD4 counts >700 cells/mm^3), patients in the STI arm of the LOTTI trial were less likely to experience cardiovascular events, in contrast to results from the

SMART Study (453). These results suggest that despite the large number of patients included in the SMART Study, the question of whether interrupting treatment is safe remains unanswered. Although newer drugs are less toxic and easier to administer, other reasons for interrupting therapy as described above remain and hence the long term risks of interrupting treatment need further investigation.

Table 2.3.6.1: Studies investigating the impact of treatment interruptions amongst HIV-positive patients

Author, region and year of publication	N	% Male	Inclusion criteria	Interruption strategy	Endpoint	Main results	Other results/conclusions
Randomised controlled trials							
Maggiolo, Italy 2009 (LOTTI)[453]	329	73	CD4>700, VL <50	Continuous (C) vs. STI until CD4<350	AIDS/death	12% C vs. 12% STI, p=0.85	Clinical events influencing the cardiovascular risk of patients were significantly (p < 0.0001) more frequent among controls.
DART, Uganda/ Zimbabwe 2008[452]	813	27	CD4>300 at week 48 or 72 after ART initiation	Continuous (C) vs. STI 12 weeks on, 12 weeks off	AIDS/death	C: 2.4/100 person-yrs STI:6.4/100 p-yrs, HR (STI vs. C)=2.73, p=0.007	ART change due to toxicity occurred more frequently in continuous arm
Walmsley, Canada 2007[454]	147	87	CD4>50,VL >1000 >3 months	Immediate (IS) vs. 12 week deferred switch (STI)	VL <50 >3 months	51% STI vs. 64% IS	No difference in CD4 count / VL at week 60
Ruiz, Spain & Italy 2007[455]	201	73	On continuous HAART >1 year, CD4>500,VL <400	Continuous (C) vs. STI until CD4<350	% of adverse events	HR (STI vs, C)=2.71, p<0.0001	Week 96 CD4 counts significantly lower in patients with TI. TI is not as safe as continuing therapy
SMART Study Group, Europe & Australia 2006[449]	5472	73	CD4>350	Continued (C) vs. deferred (D) until CD4<250	Opportunistic disease or death	HR (D vs. C)=2.6, p<0.0001	Continuous use superior to episodic use of ART
Staccato Study Group, Thailand, Switzerland, Australia 2006[448]	430	46	CD4>350, VL <50, no evidence of resistance	Continued vs. Deferred until CD4<350	VL <50 at trial end	Difference in patients with VL <50: 1.3%, p=0.90	Low CD4 counts more frequent in STI arm, 62% saving on cost
Trivacan Study Group, Africa 2006[440]	326	21	150<CD4<350	Continued (C) vs. Deferred until CD4<250 (STI)	Mortality and morbidity at trial end	Mortality RR (C vs STI) =0.48, p=0.57. Morbidity RR=0.38,p<0.001	CD4-guided structured treatment interruption strategy should not be recommended
Beatty, America 2006[456]	30	na	On ART, VL >500, 3 class resistance	Immediate (I) vs. deferred (D) enfuvirtide	VL <75 48 weeks after initiating enfuvirtide	53% I vs. 35% D, p=NS	Baseline phenotypic susceptibility score predicted virological response in multivariable analyses
Benson, America 2006(457)	41	na	VL >10,000, CD4>150, MDR	Immediate (I) vs. deferred (D) ART	VL <400 48 weeks after	19% I vs. 33% D, p=0.44	
Ananworanich,	74	na	CD4>350, VL <50	Continuous (C) vs	CD4>350, VL	CD4>350: 96% C vs.	1 week on, 1 week off stopped

Thailand 2005[447]			> 6 months	CD4 guided STI vs, 1 week on, 1 week off	<400 at week 108	100% STI VL:96% vs. 91%	early due to high rates of failure. STI well tolerated and cost-saving
Cardiello, Thailand 2005[446]	74	49	CD4>350, VL <50 for 6 months	Continuous (C) vs. CD4 guided (STI) vs. 1 week on, 1 week off (W)	AIDS/death and CD4>350 at week 48	No deaths, 1 AIDS event in W arm.CD4>350:100% (C) vs. 87% (STI) vs. 96% (W), p=0.03	Virological/ immunological failure in W and C arms: 31% vs. 0% respectively. STI is clinically comparable to C
Katlama, France 2004[458]	68	97	VL >50,000, CD4<200, on ART	Continuous (C) vs. 8-week deferred (STI)	1 log decrease in VL at 12 weeks	26% C vs. 62% STI, p=0.01.	STI led to an increase in the number of sensitive drugs (P=0.004)
Maggiolo, Italy 2004[444]	69	25	CD4>800, VL <50 on HAART	Continuous (C) vs. STI until VL <400	CD4>400 at every 8 weeks	No statistical difference at any time point	Nadir CD4 predicted CD4 decline (p<0.001).
Papasavvas, America 2004[443]	42	90	CD4>400, VL <500 > 6 months	Continuous (C) vs. 2, 4 and 6 week repeated interruptions (STI)	Time to viral rebound (VL >5000)	C : 4 (1, 8) weeks, STI: 5 (4, 8) weeks, p=0.36	No difference between groups in terms of safety, immune reconstitution, and clinical therapy failure
Lawrence, America 2003[436]	270	91	VL >500, MDR	Continuous vs. 4 month STI	Disease progression or death	HR for STI group: 2.7, p=0.004	STI did not confer immunologic or virologic benefits or improve the overall quality of life.
Ananworanich, Thailand,Switzerland, Australia 2003[435]	36	na	CD4>350, VL <50, no evidence of resistance	1 week on, 1 week off	VL >500	53%.	Unacceptably high failure rate and hence terminated.
Ruiz, Spain 2003[441]	46	na	Heavily experienced, VL >1000	Immediate (I) vs. 12 week deferred (D) 5-drug regimen	VL <50 at 48 weeks	45% I vs. 46% D, p=0.62	No difference in CD4 counts at week 48. Only no. of NRTI mutations were significantly associated with VL <50
Cohort studies							
Sanchez, Spain 2007[451]	20	75	TI >4 weeks, prior VL <50 for 6 months	Interrupted due to toxicity, choice or surgery	VL >50 after week 24 after restarting	0 patients experienced failure	45% developed resistance mutations. Interruption safe for those with viral suppression
Boschi, Italy 2006[450]	112	72	CD4>500, undetectable VL, HAART>1 year	Deferred until CD4<200/patient choice	Duration of TI, AIDS,virological failure	Median duration: 1 year. 1 case of AIDS, 1 case of failure.	The strategy of TI is safe if the criteria for restarting therapy are applied correctly
Mata, Spain 2005[459]	141	76	VL <50 for >6 months, baseline	Interrupted with clinicians approval	>300 cell decline in CD4 count	Risk factors: lower CD4 nadir, greater CD4 increase after starting HAART, higher CD4 count pre interruption, higher	

			CD4>500	until CD4<350		viral load rebound.	
Wolf, Germany 2005[439]	399	na			CD4 increase, 2 years AIDS free survival	Patient with TI had fewer cells increase than those on continuous ART, p<0.001. No difference in survival. TI did not lead to increased disease progression.	
d'Arminio Monforte, Italy 2005[438]	3142	72	Initiating HAART	Interruption of all drugs for >2 weeks	Clinical progression	HR for those with prior interruption: 2.75 (p=0.03)	23% had interrupted treatment at a median of 41 months
Li, America 2005[437]	687	na	Initiating HAART	(i)TI for >2 days within 6 months (ii) ART discontinuation	>1 log VL increase	OR=2.62 and 43.32 (p<0.005) for TI and discontinuation compared to continuous HAART	Long interruptions have negative virological and immunological consequences
Boschi, Italy 2004[445]	71	51	CD4>500, low VL, stable ART	Interrupt until CD4<200/patient choice	AIDS/ VL >50 at 6 months after restarting ART	No AIDS. 1 patient had VL >50 after restarting ART	Treatment interruptions in patients with high CD4 counts do not reduce the efficacy of therapy when re-started
Fagard, Switzerland 2003[442]	133	69	VL <50 6 months, baseline CD4>300	4* 2 weeks off, 8 weeks on ART	VL <5000	17%. Risk factors: low pre HAART VL, lack of rebound	STI did not provoke clinical complications. Results do not favour autovaccination
Yerly, Switzerland 2003[460]	133	69	VL <50 6 months, baseline CD4>300	4* 2 weeks off, 8 weeks on ART	VL >50 after retreatment	29%	The M184V/I mutation is frequently selected during repeated treatment interruptions
Metzner, Switzerland 2003[461]	28	29	1st line HAART >6 months, without failure	4* 2 weeks off, 8 weeks on ART	Detection of drug resistance	M184V and L90M detected in 56% and 12% respectively	Multiple STIs can result in the emergence of drug-resistance
Tarwater, America 2003[462]	105	na	Interrupted ART with intention to resume	na	Resuming therapy	43% at a median of 114 weeks.	Lower CD4 count at interruption significantly associated with resuming therapy
Taffe, Switzerland 2002[434]	4720	71	Receiving HAART	TI for at least 1 month	Mortality, CDC stage C, >50 cell CD4 increase	TI not associated with mortality/morbidity	TI significantly decreased the likelihood of CD4 increase. TI neither worsen nor improve disease outcome
Miller 2000 Germany[463]	48	92	ART experienced	>2 month interruption at detectable VL	Drug susceptibility	28/45 patients reverted to wild type	Shift to wild type was significantly associated with response to re-initiation

Na: Not applicable, STI: structured treatment interruption, TI: treatment interruption, VL: viral load, MDR: multi-drug resistance

Chapter 3: Data collection and methods

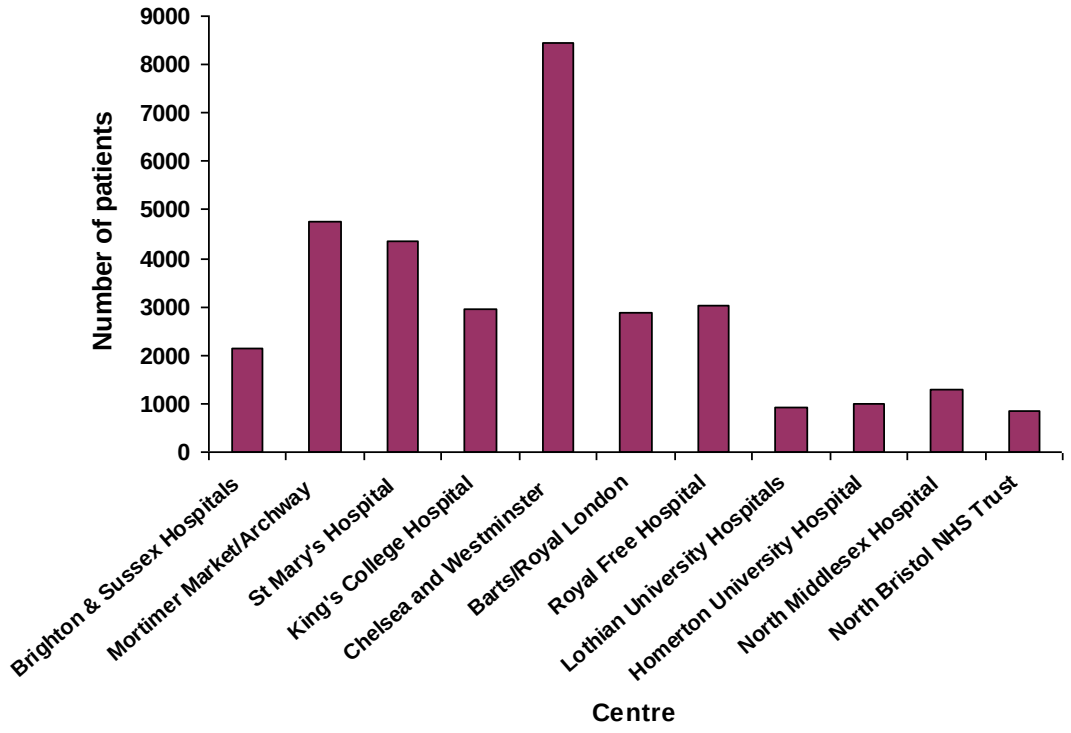
3.1. Introduction

All analyses presented in this thesis have been undertaken using data from the UK Collaborative HIV Cohort (CHIC) Study. Data from the UK HIV Drug Resistance Database have been linked to the UK CHIC dataset for analyses involving HIV drug resistance. This chapter describes the data collection methods for these datasets, the approach taken to link the datasets and a summary of the key clinical findings in the UK CHIC dataset used for analyses in this thesis.

3.2. The UK CHIC Study

The UK CHIC Study was initiated in 2001 with the aim of collating routinely collected data on HIV-positive individuals attending some of the largest clinical centres in the UK since 1st January 1996. Initial objectives of the UK CHIC Study included describing changes over time in the frequency of AIDS-defining illnesses, describing the uptake of and response to HAART and identifying factors associated with virological and immunological responses to HAART. The collaboration originally included data on 13,833 patients from 7 centres: Chelsea and Westminster NHS Trust; King's College Hospital; Mortimer Market Centre; St. Mary's Hospital; St. Thomas' Hospital; The Royal Free Hospital; and Brighton and Sussex University Hospital. Annual data downloads from the original centres and from new centres are provided to ensure that the database is up-to-date. The current dataset includes data from an additional 4 centres (Homerton University Hospital, Bristol NHS Trust, North Middlesex NHS Trust and Edinburgh Western General), and the 11 participating centres provide data on a total of 32,607 individuals (Figure 3.2.1).

Figure 3.2.1: Number of patients from each centre providing data to UK CHIC in 2008 (N=32,607)



3.2.1. Data collection

All HIV-positive patients aged 16 years or above, attending one of the centres for care at any time after 1st January 1996 are eligible for inclusion in the study. Electronically formatted data are provided by each centre in specified datasets. Information collected includes demographics (date of birth, gender, risk-group, ethnicity), AIDS diagnoses, CD4 and CD8 counts, viral loads and HAART history (start and stop date of each drug taken). Table 3.2.1.1 lists all variables currently included in the main CHIC dataset. In addition, data are also provided on hepatitis status (Table 3.2.1.2) and laboratory markers of HAART-related toxicities. All data provided are pseudo-anonymised in accordance with data protection policy. Patients' names are replaced with initials and soundex codes and all clinic numbers are replaced by a unique study identifier before the dataset is distributed for analysis.

Table 3.2.1.1: Data collected in The UK CHIC Study

Demographics	Gender
	Ethnicity
	Date of birth
	Primary risk factor for HIV transmission
Clinical data	Date of first recorded HIV positive test
	Date of last recorded HIV negative test
	Date of first clinic visit
	Date of last clinic visit
	Date of death
	Cause of death
	Date and description of each AIDS-defining event
Laboratory	Date and result of CD4 cell count for all available measurements
Markers	Date and result of CD8 cell count for all available measurements
	Date and result of CD4 percentage for all available measurements
	Date and result of CD8 percentage for all available measurements
	Date and result of HIV RNA viral load for all available measurements
Antiretroviral	Date of starting all antiretrovirals
Treatment	Date and up to 3 reasons for stopping all antiretrovirals
PCP	Date of starting PCP prophylaxis
Prophylaxis	Date of stopping PCP prophylaxis
	PCP prophylaxis taken
Hepatitis[1]	Date of hepatitis test
	Hepatitis test
	Hepatitis test result (negative/positive/unknown)

[1] See Table 3.2.1.2

Table 3.2.1.2: Hepatitis data collected

Hepatitis Test
Hep A antibody (total)
Hep B surface antigen (HbsAg)
Hep B surface antibody (anti-HBs)
Hep B core antibody (anti-HBc)
Hep B e antigen
Hep B e antibody
Hep C antibody
Hep C virus PCR/bDNA
Hep B core antibody (IgM)
Hep A antibody (IgM)
Hep B DNA (Type unknown)
Hep D antibody (total)
Hep B surface antigen (titre)
Hep D antibody (IgM)
Other
Not known

3.2.2. Data checks

Data from each centre are checked for accuracy and completeness by the study manager (Teresa Hill). A range of data queries is used to highlight errors in the datasets provided, for example HIV positive test results that predate HIV negative test results and dates of any laboratory tests that occur after the patient was last seen. All highlighted errors are reported back to individual centres. This gives centres the opportunity to improve their existing databases and reduces the number of errors identified in future downloads. If errors are resolved (by examining patient records for example) and more accurate information is established, the UK CHIC database is updated accordingly.

The datasets containing HAART history are checked extensively for completeness. Most centres provide start and stop dates for all individual drugs a patient has received in text format. I import these data into SAS and as an initial check, ensure that all stop dates occur after the start dates for each drug. If stop dates do occur before start dates, it is assumed that the dates were inputted under the wrong headings and hence are switched with each other. This assumption is based on a

sample of records which were sent back to the centres with inconsistent stop/start dates and confirmed by the clinician to have been inputted incorrectly.

I then highlight any instances in which one record is completely nested within a second record of the same drug. For example, a patient may appear to have started taking AZT on 01/01/2004 and stopped on 01/12/2004 but may also have a second record of starting AZT on 01/03/2004 and stopping on 01/06/2004. In situations involving such cases, the second record is removed from the dataset. Overlapping dates are edited in a similar manner. Gaps in treatment history of less than 2 weeks are also removed from the dataset. For example, if a patient appears to have started and stopped receiving AZT on 01/01/2004 and 01/06/2004 respectively, but has a second record of receiving AZT on 14/06/2004 and stopping on 01/12/2004, the stop date of the first record and the start date of the second record are removed, so the AZT history would then read from 01/01/2004 to 01/12/2004. HAART data from Kings College Hospital is provided in the form of prescription data and hence contains only start dates for drugs received. After importing the text file into SAS, I impute stop dates at 3 months after each start date for treatment data received from this centre. I then apply the above algorithm to remove any inconsistent records and to close any gaps which may have resulted from the imputation of stop dates. Data in the form of regimens is provided by Chelsea and Westminster NHS Trust. This text file is imported into SAS and regimens in the form of text characters are changed into individual records. Records may contain regimens which are theoretically identical but appear differently, e.g. 'AZT+3TC+NVP' and 'ZDV+TTC+NVP'. Each drug name is individually extracted and recoded to a standard CHIC code, i.e. '3TC' to '5' before the above algorithms are applied. Since not all drug names contain three letters (e.g. SQVH), this process involves a certain degree of manual editing in the text file before it can be imported in to SAS. After the treatment data have been cleaned, they are exported back to text files and passed to the data manager to link back to the other datasets provided by the centres.

3.2.3. Linkage to death registries and Health Protection Agency (HPA)

In addition to the data provided by the centres, the Office for National Statistics (ONS) and General Registrar Office for Scotland (GRO) death registers are used to ascertain whether patients lost to follow-up had subsequently died. UK CHIC records which matched a record in the ONS or GRO database based on first name initial, soundex, date of birth and soundex were identified and any missing date of deaths in UK CHIC were replaced by the ONS/GRO date of death for those patients

identified as having died. For those patients who were not recorded as having died in UK CHIC, matches were reported back to the appropriate centres for verification before being updated on the CHIC database.

The number of deaths in UK CHIC at each centre, stratified by year of death is also compared to those reported to the HPA through national surveillance schemes. Discrepancies are reported back to individual centres and information on date of death is then updated on both databases accordingly.

3.2.4. Duplicate records

Since the current UK CHIC Study dataset contains information from 11 centres, it is possible that the same individual has attended two or more of these centres, resulting in two or more separate records for a single individual. Such potential matches are initially identified using date of birth, initials and soundex codes. A computerised algorithm which utilises information on several demographic and clinical factors is then used to determine whether each potential match is a definite match, a definite non-match or remains indeterminate. The indeterminate matches are manually checked by two independent investigators who decide whether the records are likely to be matches or non-matches. A third investigator manually checks any records where consensus is not reached – if he/she is unable to make a decision on the two records, they are left as distinct individuals in the final dataset. All records which were determined to be related to the same individual are merged using a computer program which updates any missing information from one record using the matched record. The example below shows two separate records (in simplified form) which have been matched to give the merged record (Table 3.2.4.1). In this case, record B is identical to record A in all aspects, other than the HIV positive date (missing in record B). These records will have been identified as being from the same individual and the merged record will contain all information from records A and B.

Table 3.2.4.1: Example of a manual record check

Record A	Record B	Merged record
Male	Male	Male
MSM	MSM	MSM
White ethnicity	White ethnicity	White ethnicity
HIV POS: 01/05/2002	**HIV POS: No date**	HIV POS: 01/05/2002
DOB: 21/12/1976	DOB: 21/12/1976	DOB: 21/12/1976
ART HISTORY:	ART HISTORY:	ART HISTORY:
01/05/02 – 23/12/2004	01/05/02 – 23/12/2004	01/05/02 – 23/12/2004
AZT/3TC/NVP	AZT/3TC/NVP	AZT/3TC/NVP
Death: No date	Death: No date	Death: No date

3.2.5. Final dataset

After the datasets have been cleaned and duplicate records have been merged, a final version of the combined dataset is generated, with all patient identifiers removed and study numbers assigned. Using this final dataset, I run several queries, for example, removing duplicate laboratory data and ensuring last seen dates occur before date of deaths. Treatment history is also re-checked to highlight any inconsistencies which may have resulted from the de-duplication process. Finally, all datasets are merged together using the unique study identifier and a final SAS dataset is made available for distribution for analysis. A copy of this dataset is archived should it be required in the future.

3.2.6. Toxicity data and Hepatitis B/C status

Information on HAART-related toxicities is also provided by the majority of the centres. A full list of the variables collected is shown in Table 3.2.6.1. These data are provided in the form of text files and are checked for inconsistencies using SAS. Any duplicate records are removed and checks are carried out to ensure that the unit of measurement is consistent both within and between centres. All toxicity measurements are merged using the unique study identifier and are stored in a SAS dataset which can be linked to the main dataset using the CHIC study identifier.

Table 3.2.6.1: HAART-related toxicity data collected

Laboratory marker	Normal range and units
Total cholesterol	<5.2 mmol/l
HDL-cholesterol	>1 mmol/l
LDL-cholesterol	<4 mmol/l
Triglycerides	<2.3 mmol/l
Lactate	0.5-2.0 mmol/l
Glucose	Fasting <5 days: 0.7-4.2 mmol/l
	Fasting >5 days: 2.9-5.3 mmol/l
AST	5-40 IU/l
ALT	5-40 IU/l
Albumin	35-50 g/l
Bilirubin	5-17 Umol/l
Alkaline phosphate	42-128 IU/l
Gamma GT	Male: 9-54 IU/l
	Female: 8-35 IU/l
Urea	3.0-6.5 mmol/l
Creatinine	60-97 µmol/l
White blood count	3.7-9.5 x 10^9 cells/l
Haemoglobin	Male: 13.5-17.5 g/dl
	Female: 11.5-15.5 g/dl
Platelets	140-400 x 10^9 cells/l

3.3. Overview of trends over time in UK CHIC

Since the original UK CHIC dataset was made available for analysis in 2002, information on almost 20,000 new patients has been provided, with the latest dataset consisting of 32,607 records. This number represents around 40% of all HIV-positive patients in the UK. The London-based HIV-positive population is well represented in UK CHIC (8 out of 11 centres are London-based), as are HIV-positive individuals located in Brighton and Bristol. Currently, one centre from Scotland provides data and it is extremely likely that centres from the Midlands will also soon provide data to UK CHIC. I will be using the current dataset (n=32, 607) for all analyses in this thesis. Table 3.3.1 summarises the demographics of the cohort.

Table 3.3.1: Demographics of current (2008) UK CHIC dataset

		N	%
Total number of patients		32607	100.0
Sex	Female	7856	24.1
Risk group	MSM	16960	52.0
	IDU	1372	4.2
	Heterosexual	10353	31.8
	Other/not known	3922	12.0
Ethnicity	White	18989	58.2
	Black African	7728	23.7
	Not known	1618	5.0
	Other	4272	13.1
Median (IQR[1]) age at first entry into cohort (years)		30	24-36

[1] IQR: Interquartile range

3.3.1. Demographic changes over time (2000-2007)

In order to assess demographic changes over time, I first identified the number of patients under follow-up in UK CHIC at the mid-point of each year from 2000-2007. I chose the year 2000 as the reference year for these analyses since this is when HAART was widely available and recommended for all patients. Further, clinical practices may have been somewhat different in the early HAART-era (1996-1999) and hence these analyses were restricted to 2000 onwards. In order to be defined as under follow-up in a given year, patients had to have at least one viral load and/or CD4 count measured in the second half of the year (on or after July 1st) and the earliest date of each patient's first viral load and CD4 was required to be before July 1st of that year. Using this criterion, the number of patients under follow-up increased from 9041 in 2000 to 14,812 in 2007. Table 3.3.1.1 shows the changing characteristics of patients under follow-up in each year. Females now represent almost a quarter of the cohort compared to 14.5% in 1996. There has also been an increase in the proportion of heterosexual individuals under follow-up in later calendar years, whilst the proportion of homosexual individuals appears to be declining. In 1996, 75% of the cohort was of white ethnicity, compared to 60% in 2007. This is consistent with an increasing proportion of black-Africans under follow-up in later calendar years (23% in 2007 compared to 10% in 1996). Over 75% of patients under follow-up were receiving HAART (defined as 3 or more drugs) in 2007 and over 80% had received HAART at some point whilst under care.

Table 3.3.1.1: Characteristics of patients under follow-up in each year

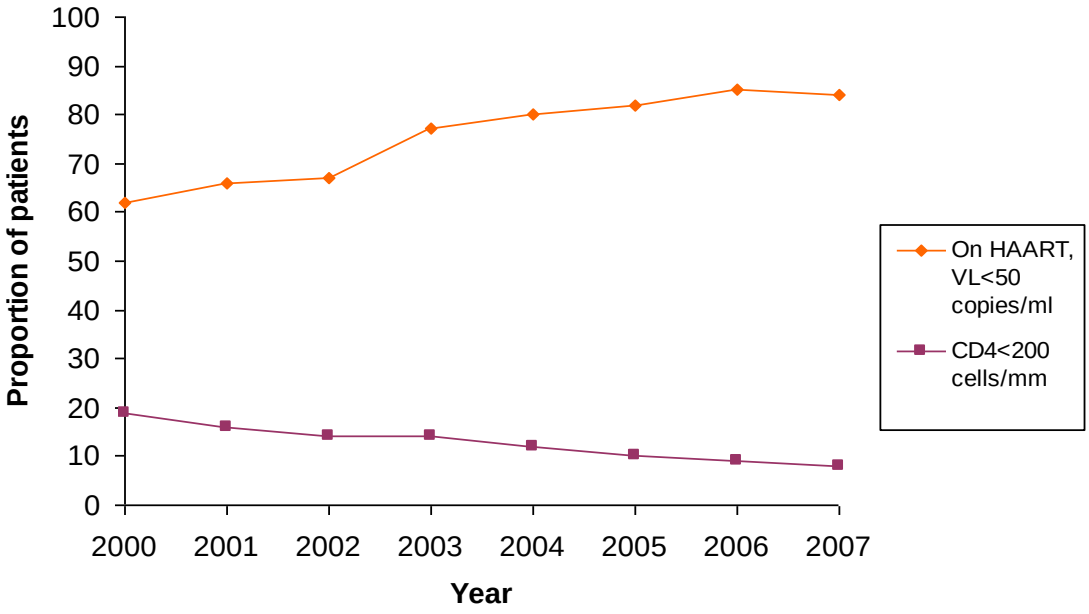
		2000	2001	2002	2003	2004	2005	2006	2007
No. under follow-up		9041	10230	11373	12648	13781	15003	16709	14812
Male N (%)		7462 (83)	8280 (81)	9067 (80)	9888 (78)	10654 (77)	11508 (77)	12831 (77)	11380 (77)
Risk group N (%)	MSM	5954 (66)	6543 (64)	7059 (62)	7702 (61)	8242 (60)	8915 (59)	9897 (59)	8640 (58)
	Heterosexual	2138 (24)	2721 (27)	3256 (29)	3868 (31)	4383 (32)	4893 (33)	5447 (33)	4666 (32)
	Other	949 (10)	966 (9)	1058 (9)	1078 (9)	1156 (8)	1195 (8)	1365 (8)	1506 (10)
Ethnicity N (%)	White	6507 (72)	7043 (69)	7578 (67)	8148 (64)	8649 (63)	9314 (62)	10246 (61)	8984 (61)
	Black African	1449 (16)	1864 (18)	2257 (20)	2777 (22)	3185 (23)	3516 (23)	3939 (24)	3454 (23)
	Other	1085 (12)	1323 (13)	1538 (14)	1723 (14)	1947 (14)	2173 (14)	2524 (15)	2374 (16)
On HAART		6249 (69)	7093 (69)	7862 (69)	8905 (70)	9933 (72)	11014 (73)	12451 (74)	11211 (76)
Started treatment with <3 drugs		2688 (30)	2634 (26)	2562 (23)	2505 (20)	2474 (18)	2428 (16)	2458 (15)	1981 (13)
Started treatment with >3 drugs		3561 (39)	4459 (44)	5300 (47)	6400 (51)	7459 (54)	8586 (57)	9993 (60)	9230 (62)
HAART-experienced		7017 (78)	7945 (78)	8819 (78)	9888 (78)	10927 (79)	11963 (80)	13366 (80)	11937 (81)
NNRTI experienced (%)		4532 (65)	5737 (72)	6679 (76)	7730 (78)	8836 (81)	9654 (81)	10663 (80)	9676 (81)
PI experienced (%)		4421 (63)	4704 (59)	5022 (57)	5390 (54)	5921 (54)	6648 (56)	7632 (57)	6716 (56)
Experienced 3 original classes[1] (%)		2450 (35)	2994 (38)	3385 (38)	3793 (38)	4286 (39)	4715 (39)	5250 (39)	4706 (39)

[1] NRTIS, NNRTIs and PIs

3.3.2. CD4 and viral load changes over time

For all patients under follow-up, the proportion of patients on HAART with viral load suppression below 50 copies/mL and the proportion of all patients with a low CD4 count (<200 cells/mm^3) were calculated and are shown in Figure 3.3.2.1. Of those receiving HAART, 84% had an undetectable viral load (<50 copies/mL) in 2007, compared to 62% in 2000. Amongst all patients under follow-up, the proportion of patients with a CD4 count below 200 cells/mm^3 decreased from 19% in 2000 to 8% in 2007.

Figure 3.3.2.1: Proportion of patients with current CD4 <200 cells/mm^3 and proportion of patients on HAART with viral load <50 copies/mL

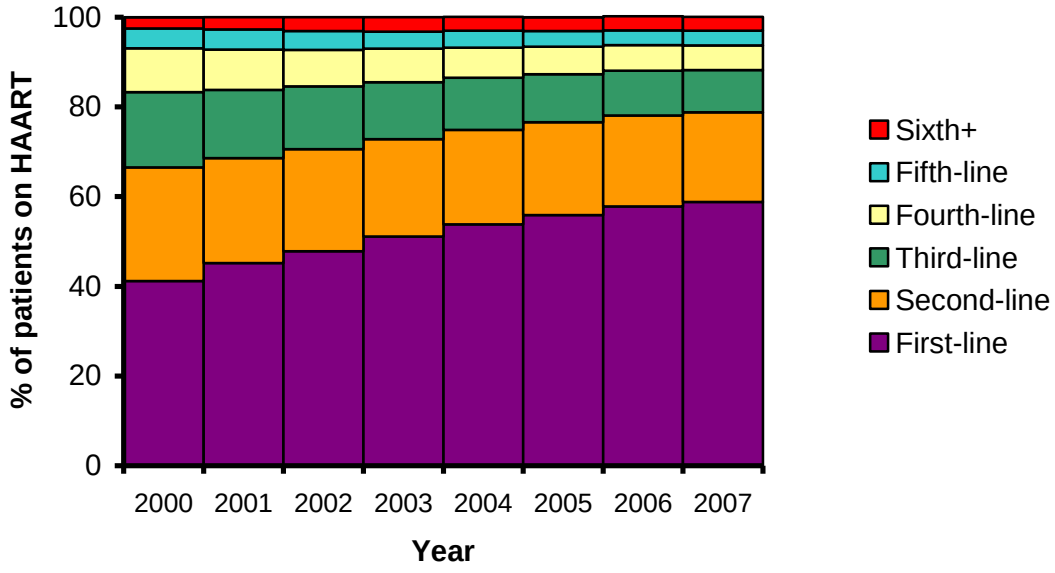


3.3.3. Number of regimens received

The number of regimens patients had received in each calendar year is shown in Figure 3.3.3.1. In order to be defined as starting a new regimen, patients had to virologically fail their current regimen. Virological failure of a drug was defined as having occurred if 2 consecutive viral loads >400 copies/mL were measured in an individual, despite at least 4 months of continuous use of the drug. All drugs received at the time of failure were also regarded as 'failed drugs'. Patients could only experience a new episode of virological failure if they were receiving at least one drug which they had not previously failed.

The proportion of patients on first-line HAART has steadily increased from 2000 onwards and was almost 60% in 2007. Consequently, the proportion of patients on subsequent regimens has been declining – only 9% of patients were on their third-line regimen in 2007.

Figure 3.3.3.1: Number of regimens received



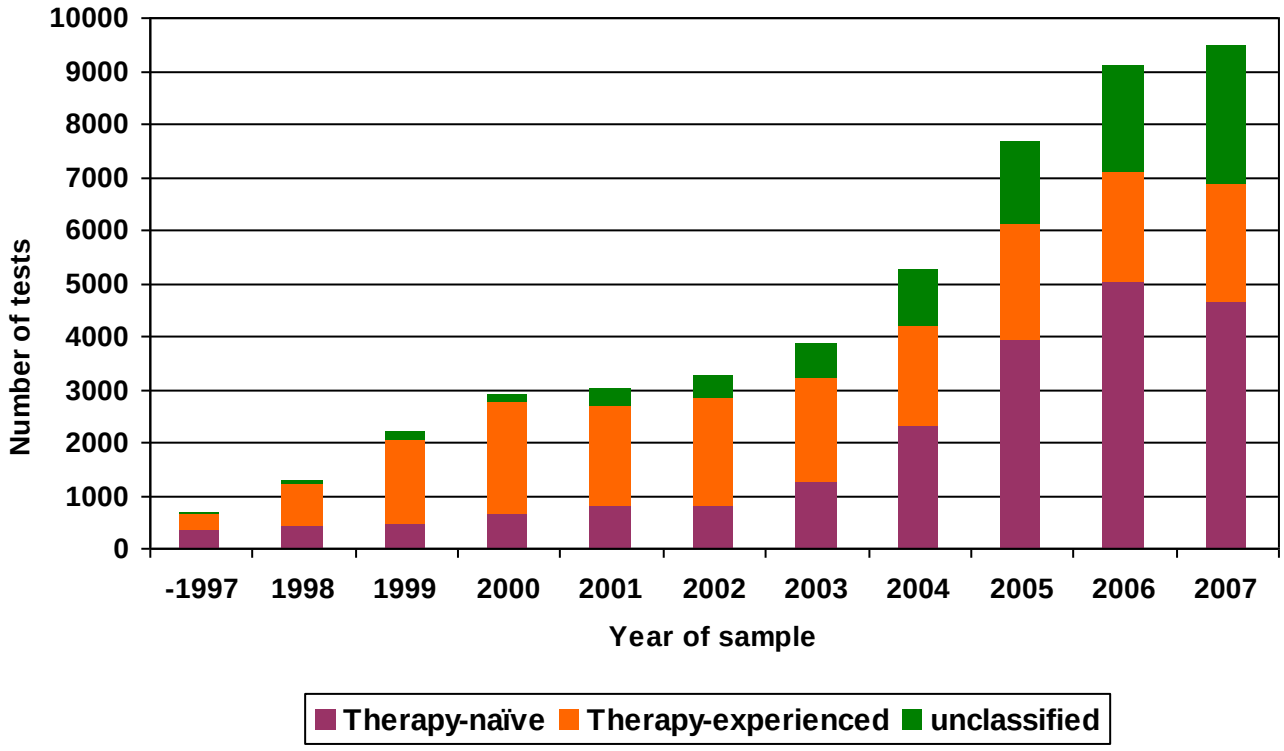
3.4. The UK HIV Drug Resistance Database

The UK HIV Drug Resistance Database was established in 2001 as a central repository of resistance tests performed as part of routine clinical care in the UK.

Resistance tests from clinical centres (in the form of blood samples) are sent to virology laboratories for analysis. Currently, 14 virology laboratories provide data to the study, usually in the form of viral gene sequences. The current database contains the results of over 51,000 test results from approximately 36,500 patients. Figure 3.4.1 shows the number of resistance tests submitted each year to the database, stratified by therapy status. In earlier years, the majority of tests were performed in HAART-experienced patients but with new guidelines now in place, there has been a steady increase in the number of resistance tests performed in HAART-naïve patients. The initial objectives of the study were to estimate the prevalence of drug resistance in untreated infections within the UK and describe changes over time; to describe the pattern of drug resistance in patients failing therapy; and to assess the effect of specific mutations on virological response.

The UK HIV Drug Resistance Database is co-ordinated by the MRC Clinical Trials Unit in London and is currently funded by the MRC.

Figure 3.4.1: Number of resistance tests submitted each year to the UK HIV Drug Resistance Database, by therapy status and year of sample



3.4.1. Data collection and linkage to UK CHIC

Laboratories provide the results of the resistance tests as full nucleotide sequences performed in the previous calendar year for all patients. Data are sent in a variety of formats including access databases, Excel spreadsheets and individual FASTA files (text-based format for representing either nucleotide sequences or peptide sequences, in which base pairs or amino acids are represented using single-letter codes). These data are imported into a central SQL database by the study coordinator (Esther Fearnhill). The nucleotide sequences are then processed through the Stanford University Genotypic Resistance Interpretation Algorithm (HIVdb) to obtain aligned sequences, amino acid mutations and drug susceptibility data. These are stored in the database along with subtype data from the Rega Institute. Data are also collected on patient demographics, clinical details, HAART history and laboratory markers at the time of the resistance test. Data checks, such as ensuring the reason for the resistance test matches the treatment status of the patient, are performed and samples are matched to patients in the UK CHIC

database using clinical centre and pseudo-anonymised patient identifiers. Further checks are performed, e.g. checking that the date of birth in the resistance database matches that in the CHIC database. Given the substantial overlap between the patients in the two databases, the clinical information is co-ordinated between the studies. There are a total of 15,550 (47.7%) patients in UK CHIC who have resistance data in the UK HIV Drug Resistance Database. Amongst patients who have had a resistance test, the median number (range) of tests is 1 (1, 16).

3.5. Statistical methods

The specific statistical methods used in each of the Results chapters are described in the appropriate chapter. I have, however, used some generic regression methods in all chapters; these are summarised below.

3.5.1. Linear regression

In situations where there is a single continuous outcome measure per individual *(dependant variable) y*, linear regression is used to predict the value of the outcome *y*, from a set of *explanatory* variables, $x_1, x_2, x_3, \ldots, x_n$. The magnitude of the effect of the explanatory variables on the dependant variable is estimated using a sample of observations from the population and this association is assumed to be of the following form:

Expected value of $y = \alpha + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + ... + \beta_n x_n + \varepsilon$.

The parameter estimate for β_i gives the impact of a unit increase in x_i on *y*, when all other independent variables are kept constant.

3.5.2. Logistic regression

Logistic regression, like linear regression, is used to investigate the impact of a set of explanatory variables on a dependant variable. However, the dependant variable *y*, is not continuously distributed. Instead, it is a binary variable and can only take the values 0 and 1. The estimates of β_i obtained from the model give the log odds ratio for the impact of a unit increase in x_i on the probability that y=1, and the exponential of β_i gives the odds ratio

3.5.3. Poisson regression

Poisson regression is similar to Logistic regression, in that the event of interest is a binary outcome. However, rather than modelling the probability that an event occurs, Poisson regression is used to model the *rate* at which an event occurs. Poisson

regression allows for differing follow-up time amongst patients and assumes that the rate of an event is constant over time over the whole study period (unless time on study is fitted in the model, for example as a categorical variable). The estimates of β_i obtained from the model give the impact of a unit increase in x_i on the log of the rate, and the exponential of β_i gives the rate ratio.

3.5.4. Cox proportional hazards regression models

Survival methods, such as the Cox regression model are used when the time to the occurrence of an event is of importance. Given that not all individuals will experience the event and are likely to have different lengths of follow-up time, data are censored, usually at the last known point at which an individual is known to experience the event or not. However, these individuals may still experience the event at some point in the future and hence we must account for the censored data.

A hazard is calculated for each individual, *h(t)*, and this is the instantaneous risk of having an event at any point in time (t), given that the event of interest has not already been experienced up until this time point. Generally, it is the hazard ratio which is of interest, rather than the hazard itself. The hazard ratio provides an estimate of the impact of a unit increase in a factor of interest on the hazard. Cox proportional hazard regression models take the following form:

$$h(t) = h_0(t)\exp(\beta_1 x_1 + \beta_1 x_1 + ...\beta_n x_n) \qquad t > 0$$

Here, $h_0(t)$ is the baseline or underlying hazard (usually not of interest in its own right, and not estimated in a Cox model) and the estimate of $exp(\beta_i)$ gives the hazard ratio for factor x_i.

It is assumed that the multiplicative impact of factor x_1 on the hazard remains constant, regardless of the current time point – the *proportional hazards assumption.*

In all regression analyses, all variables which were statistically significant in univariable analyses ($p<0.10$), or were of known clinical significance (e.g. CD4/viral load), were included in multivariable analyses.

Chapter 4: The impact of transmitted drug-resistance on treatment selection and outcome of first-line HAART

4.1. Introduction

As discussed in Chapter 2.1, transmitted drug resistance (TDR) can compromise an individual's response to HAART, especially in the context of NNRTI-based regimens (189;464-468). The British HIV Association (BHIVA) and the International AIDS Society (IAS) treatment guidelines recommend that all newly diagnosed HIV-positive individuals have a baseline resistance test performed to determine the presence of TDR and inform the selection of first-line HAART (126;130). However, resistance concerns are only one of a number of factors that may be considered when choosing an initial treatment regimen for a given patient. In particular, concerns about possible toxicities and likely adherence, together with patients' views on the ability of any particular regimen to accommodate lifestyle requirements, and hepatitis-B co-infection are major determinants of the selection of first-line regimens. In some cases, information about TDR may only become available after the patient has started treatment. In other cases, the significance of certain genotypic changes (e.g. the presence of T215 revertants) may be missed by the treating physician, especially if phenotypic tests are used (469). The overall resilience of the regimen to rapidly accumulating drug-resistance – commonly referred to as the "genetic barrier" - may also lead to a preference for regimens based on ritonavir-boosted protease inhibitors (PI/r) rather than NNRTIs as a compensation for the use of a partially active NRTI backbone. These considerations may lead to some patients starting treatment regimens that, according to their baseline resistance profile, contain fewer than 3 fully-active drugs. The extent to which virological responses to treatment are compromised by these choices is unclear.

In this chapter, I investigate TDR amongst patients who underwent genotypic resistance testing whilst antiretroviral drug-naïve and subsequently started first-line HAART. In this group, I determined the genotypic sensitivity score (GSS) of the initial regimen and assessed the impact of GSS on virological outcomes, including whether plasma viral load (VL) becomes suppressed to <50 copies/mL and, if so, the subsequent risk of virological rebound >400 copies/mL.

4.2. Methods

4.2.1. Study population

Linked data (as described in Section 3.4.1) from the UK HIV Drug Resistance Database and the UK CHIC Study were used for these analyses. All patients who were antiretroviral naive and subsequently started HAART (defined as a standard regimen of ≥3 drugs including NRTIs in combination with either one NNRTI, one or two (including ritonavir) PIs or another NRTI) between 1st January 1999 and 30th December 2007 were eligible for analyses (n=13073). January 1999 was chosen as the earliest inclusion date as resistance testing in treatment-naïve patients was not recommended in routine practice before this date and hence tests were only performed on selected individuals.

Patients receiving combinations without NRTIs, with 2 PIs excluding ritonavir or with both PIs and NNRTIs were excluded as these were non-standard treatment regimens. Patients were also excluded from analyses if their initial HAART regimen contained drugs with recorded start and stop dates but without drug identifiers, i.e. 'not known', 'other' and 'blinded treatment in clinical trial' and if they were receiving either fusion inhibitors (Maraviroc, T20) or integrase inhibitors (Raltegravir) as these were also non-standard regimens. These regimens are likely to have been taken by patients who are clinically dissimilar to those on standard regimens and hence results obtained from these individuals may not be generalisable.

In addition, those who had a VL <50 copies/mL before the start date of HAART were removed from the dataset as an undetectable pre-HAART VL may be an indication of either missing or inaccurate early treatment records. Patients were identified as having a routine resistance test performed before starting HAART if the test was performed after January 1999 and both the date of the test and the date when the result of the resistance test was conveyed to the clinician preceded the date of starting HAART. All resistance tests meeting these criteria were analysed (i.e. more than 1 test per patient was included if there was more than one test available). The results of multiple tests were combined, for example resistance mutations identified using the first test but not the second test (and vice-versa) were included in the analyses.

4.2.2. Interpreting resistance mutations

Amongst patients who did have a resistance test performed, mutations indicative of TDR were identified according to Shafer's 'Resistance surveillance lists' compiled in 2007 and updated in 2009 (183). This is a standardised list of drug resistance

mutations used to define transmitted resistance. It includes only those mutations which either cause or contribute to drug resistance amongst patients receiving HAART and excludes any mutations which occur as polymorphisms amongst patients not receiving HAART. The list is particularly useful in epidemiological studies where it is otherwise difficult to compare estimates of drug resistance due to the high number of (distinct) resistance mutation lists already in existence.

The Stanford interpretation algorithm (Version 5.1.2) was used to calculate drug susceptibility levels. All resistance mutations which were identified using the 'Resistance surveillance lists' and had a Stanford mutation penalty score >0 (each drug resistance mutation is assigned a drug penalty score; the total score for each drug is derived by adding the scores of each mutation associated with resistance to that drug) for each drug were used to calculate the proportion of patients with resistance mutations to any drug used as part of their initial HAART regimen, with separate analyses for resistance-associated mutations for the NRTIs (NAMs: M41L, D67N, K219Q, M184V, L210W, T69D, K219E, K70R, M184I, K65R, D67E, L74V, V75M, F77L, K219R, V75T, Y115F, D67G, K70E, L74I, V75A, V75S, F116Y, Q151M; TAMs: T215D, T215S, T215C, T215Y, T215E, T215I, T215V, T215F), the NNRTIs (nNAMs: K103N, Y181C, K101E, G190A, V106A, V106M, Y188L, K103S, G190S, P225H, L100I, K101P, M230L, V179F, Y181V, Y181I, Y188H, Y188C, G190E) and the PIs (PRAMs: L90M, M46I, M46L, V82L, F53L, I85V, I47V, I54V, G73S, V82A, V32I, N88D, N88S, L23I, D30N, I54L, I54S, L76V, V82T, L24I, I47A, G48M, G48V, I50L, I50V, F53Y, I54A, I54M, I54T, G73C, G73T, G73A, V82C, V82F, V82M, V82S, N83D, I84A, I84C, I84V).

Using the total drug score, the Stanford algorithm classifies the level of resistance into 5 groups: susceptible, potential low-level, low-level, intermediate and high-level. In these analyses, the genotypic sensitivity scores were determined by assigning a score of 1 to susceptible/potential low-level resistance, 0.5 to low-level/intermediate resistance and 0 to high-level resistance. Hence, a patient starting HAART with 3 drugs and no resistance mutations would have a GSS of 3.

These analyses were also stratified according to the number of drugs in the initial HAART regimens, where low-dose ritonavir was not counted as a separate drug.

4.2.3. Statistical analyses

Characteristics of patients who started HAART and who had a resistance test performed whilst HAART-naive in the study period were compared to those who did not have a resistance test performed using Chi-squared and Mann-Whitney tests.

Among patients with a resistance test, logistic regression was used to identify factors associated with starting a first-line HAART regimen with a GSS score <3. Models were adjusted for the following potential confounders: age, gender, ethnicity (white, black, 'other'), HIV transmission risk group (MSM, heterosexual, 'other'), HIV-1 subtype (B, non-B), calendar year of HAART initiation (1999-2001, 2002-2004, 2005-2007), interval between the last resistance test prior to HAART and the HAART start date, pre-HAART CD4 and VL, and type of HAART regimen started (PI-based, NNRTI-based or 'other' [triple NRTIs]). PI-based therapy included both non-boosted and ritonavir-boosted PIs (PI/r).

Further analyses using logistic regression were performed in order to investigate whether patients with nNAMs were less likely to start HAART on an NNRTI-based regimen, whether patients with PRAMs were less likely to start HAART on PI-based regimens and whether patients with TAMs were less likely to start HAART with the TAM-affected drugs zidovudine, stavudine, abacavir, didanosine and tenofovir.
Cox regression was used to identify factors associated with the rate of achieving an undetectable VL (<50 copies/mL) (patients without baseline and/or follow-up VL measurement excluded). Amongst patients who achieved an undetectable VL within a year of starting HAART, virological rebound was defined as 2 consecutive VL measurements >400 copies/mL or 1 VL >400 copies/mL followed by any treatment change. Hazard (rate) ratios of virological rebound were also calculated using Cox regression (patients without follow-up VL measurements excluded). In these models, GSS was fitted as a continuous variable if a linear trend between GSS and the outcome of interest was observed in univariate analyses.

The time to a CD4 increase of 50 cells from start of HAART was also analysed using Cox regression. In this analysis, viral load was incorporated as a time-updated variable. This again ensured that the effect of GSS on CD4 response was independent of VL (VL has been shown to be associated with CD4 response).
A summary of all outcomes considered and eligibility for each analysis is given in Table 4.2.3.1.

Table 4.2.3.1: Summary of outcomes considered and eligibility criteria for each analysis

Outcome considered	Inclusion criteria
Differences between those who had a resistance test whilst HAART-naive compared to those who did not have a resistance test	All patients starting HAART
Factors associated with starting a first-line HAART regimen with GSS <3	All patients with resistance tests performed whilst HAART-naive who subsequently started HAART
Were patients with nNAMS less likely to start NNRTI-based regimens?	Patients starting HAART with nNAMS
Were patients with PRAMS less likely to start PI-based regimens?	Patients starting HAART with PRAMS
Factors associated with achieving VL <50 copies/mL	Patients with baseline and follow-up viral load measures
Factors associated with virological rebound	Patients achieving VL <50 copies/mL within 1 year of starting HAART and with at least one follow-up VL
Factors associated with a CD4 cell increase of 50 cells from start of HAART	Patients with baseline and follow-up CD4 measures

4.2.4. Sensitivity analyses restricted to patients who had their resistance test performed within 6 months prior to starting HAART

As discussed in Chapter 1.8.3, in the absence of therapy, resistance mutations tend to fade out over time. Hence, sensitivity analyses were performed in which only patients who had their resistance test performed within 6 months prior to starting HAART were included. In this analysis, all current resistance mutations present in an individual are likely to be identified. The proportion of patients with mutations

conferring resistance to the first-line regimen was calculated and time to virological response, virological rebound and CD4 increase of >50 cells/mm^3 were investigated using Cox regression as detailed in Section 4.2.3.

4.3. Results

4.3.1. Patients starting HAART

Of the 32,607 patients in UK CHIC, 13,073 (40.1%) started a HAART regimen containing 3 or more drugs from naïve between January 1999 and December 2007. Three hundred and sixty-two (2.8%) of these patients were removed from the dataset for the following reasons; 245 patients received both PIs and NNRTIs, 73 patients received >1 PI excluding ritonavir, 30 patients received either unknown drugs or fusion/integrase inhibitors and 14 patients did not receive any NRTIs. A further 825 (6.3%) patients had a recorded viral load of <50 copies/mL before starting HAART and were also removed from the dataset, leaving 11,886 patients eligible for analysis.

4.3.1.1. Differences between patients with and without resistance tests before starting HAART

Amongst the 11,886 patients starting HAART from naïve, 2,931 (24.7%) underwent pre-HAART resistance testing. Ten of these patients had their first resistance test performed before 1999 (before resistance testing was recommended in naïve patients) and hence these tests were not considered to be part of routine clinical care. Two of these patients had a second resistance test performed after 1999 and still before starting HAART and were therefore categorised as having a resistance test performed whilst HAART-naive, leaving 2,923 patients with pre-HAART resistance tests performed as part of routine clinical care.

Compared to those who did not undergo resistance testing, patients with pre-HAART resistance tests were more likely to be male (28.2% of males tested vs. 16.0% of females, $p<0.0001$), of MSM exposure (33.3% of MSM tested vs. 15.6% of heterosexuals, $p<0.0001$) and of white ethnicity (30.9% of whites tested vs. 15.7% of blacks, $p<0.0001$) (Table 4.3.1.1.1). Patients with pre-HAART resistance tests were also more likely to start HAART with a PI/r-regimen (38.9% of those starting HAART with PI/r tested vs. 23.1% of those starting HAART with NNRTI-based regimens, $p<0.0001$) and had higher median pre-HAART CD4 counts (210 vs. 173 cells/mm^3, $p<0.0001$), though no clinical difference was seen in pre-HAART log viral loads (4.9 log copies/mL in those with and without pre-HAART resistance tests).

Table 4.3.1.1.1: Differences between patients starting HAART with a resistance test performed whilst naïve and those without a resistance test performed

		Resistance test performed whilst naive		P-value
		No	Yes	
N		8963	2923	
Sex N (%)	Male	6041 (71.9)	2366 (28.1)	<0.0001
	Female	2922 (84.0)	557 (16.0)	
Ethnicity N (%)	White	4189 (69.1)	1877 (30.9)	<0.0001
	Black	3785 (84.3)	705 (15.7)	
	Other	989 (74.4)	341 (25.6)	
HIV transmission risk group N (%)	MSM	3758 (66.7)	1877 (33.3)	<0.0001
	Heterosexual	4194 (84.4)	778 (15.6)	
	IDU	277 (82.7)	58 (17.3)	
	Other	734 (77.8)	210 (22.2)	
HAART regimen started	NNRTI	6485 (76.9)	1946 (23.1)	<0.0001
	PI/r	1392 (61.1)	885 (38.9)	
	Single PI	637 (95.5)	30 (4.5)	
	NRTI only	449 (87.9)	62 (12.1)	
CD4 count at HAART initiation (cells/mm^3)	Median (IQR)	173 (78, 269)	210 (140, 285)	<0.0001
Viral load at HAART initiation (log copies/mL)	Median (IQR)	4.9 (4.3, 5.4)	4.9 (4.3, 5.3)	0.005

4.3.2. Baseline characteristics of those with resistance tests before starting HAART

Table 4.3.2.1 shows the baseline characteristics of patients with resistance tests before starting HAART. The vast majority of patients (2,436, 83.3%) had only a single resistance test performed, though up to 5 tests were performed on some HAART-naive individuals. Patients with pre-HAART resistance tests were mostly male (80.9%), of white ethnicity (64.2%) and of MSM transmission group (64.2%). Testing was more frequent in later calendar years, reflecting the uptake of guidelines first introduced in 1999. The median (interquartile-range [IQR]) time

between the last resistance test before starting HAART and starting HAART was 1.9 (0.7, 8.3) months. The majority of patients (76.3%) who started HAART after 6 months of the date of the resistance test did so in later calendar years (2005-2007) and started HAART with an NNRTI-based regimen.

Two-thirds of all patients started an NNRTI-based regimen and almost one-third a PI/r-based regimen. The median CD4 count at start of HAART was lower than that at the time of the resistance test (210 vs. 251 cells/mm^3), though there was little difference between the median log viral loads at these time points (4.9 log copies in both groups).

Table 4.3.2.1: Baseline characteristics of 2931 patients who initiated first-line HAART following a baseline genotypic resistance test

Total N (%)		**2923 (100.0)**
Number of resistance tests before starting HAART N (%)	Median (range)	1 (1, 5)
Time between last resistance test pre-HAART and starting HAART (months)	Median (IQR)	1.9 (0.7, 8.3)
Sex N (%)	Male	2366 (80.9)
Ethnicity N (%)	White	1877 (64.2)
	Black	705 (24.1)
	Other	341 (11.7)
HIV transmission risk group N (%)	MSM	1877 (64.2)
	Heterosexual	778 (26.6
	IDU	58 (2.0)
	Other	210 (7.2)
Calendar year of resistance test N (%)	1999-2001	141 (4.8)
	2002-2004	950 (32.5)
	2005-2007	1832 (62.7)
Calendar year of starting HAART N (%)	1999-2001	80 (2.7)
	2002-2004	683 (23.4)
	2005-2007	2160 (73.9)
Subtype B virus N (%)		2012 (68.8)
Age at last resistance test before HAART (years)	Median (IQR)	37 (31, 42)
CD4 count at last resistance test before HAART (cells/mm^3)	Median (IQR)	251 (164, 354)
CD4 count at HAART initiation (cells/mm^3)	Median (IQR)	210 (140, 285)
Viral load at last resistance test before HAART (log copies/mL)	Median (IQR)	4.9 (4.4, 5.3)
Viral load at HAART initiation (log copies/mL)	Median (IQR)	4.9 (4.3, 5.3)
Initial HAART regimen N (%)	NNRTI	1946 (66.6)
	PI/r	885 (30.3)
	Single PI	30 (1.0)
	Nucleoside only	62 (2.1)

4.3.3. Prevalence of TDR

Overall, 272/2923 (9.3% (95% CI: 8.3%, 10.4%) patients had at least one detectable mutation indicative of TDR. Table 4.3.3.1 lists the frequency of individual mutations. Among NAMs, T215 revertants and M41L were the most frequently detected mutations and occurred in 85 (2.9%) and 48 (1.6%) patients respectively. The K103N and the L90M mutations were the most commonly detected nNAMs and PRAMs respectively, and occurred in 62 (2.1%) and 20 (0.7%) patients; 87/272 (32.0%) patients had more than 1 mutation. The median (range) number of mutations amongst those who had at least one mutation was 1 (1, 14); patients had a median of 1 (0, 5) NAM mutations, 0 (0, 4) nNAM mutations and 0 (0, 5) PRAM mutations.

The number of patients with at least one detectable mutation indicative of TDR increased substantially in later calendar years, from 5 in 2000 to 65 in 2005. However, this number appeared to plateau after 2005, with 61 patients identified with TDR in 2006 and 66 patients in 2007 (Figure 4.3.3.1). The number of patients with NAMs identified increased until 2006, after which a decline was observed (41 patients with NAMs were identified in 2006, compared to 36 in 2007). There was no evidence of a decrease in the number of patients with nNAMs in later calendar years, though after 2005, a clear decline was seen in the number of patients with PRAMs (15 patients in 2005 compared to 11 in 2006 and 9 in 2007).

Interestingly, if all patients with resistance tests performed whilst HAART-naive (including those who never started HAART) were included in the prevalence analyses, the number of patients with at least one detectable mutation indicative of TDR increased steadily from 1 in 1999 to 179 in 2007. These numbers did not appear to plateau as seen in the analysis in which only patients who started HAART were included. The number of patients with NAMs and nNAMs also increased from 1999 to 2007 though the number of patients with PRAMS decreased in 2007 to 34 (40 in 2006).

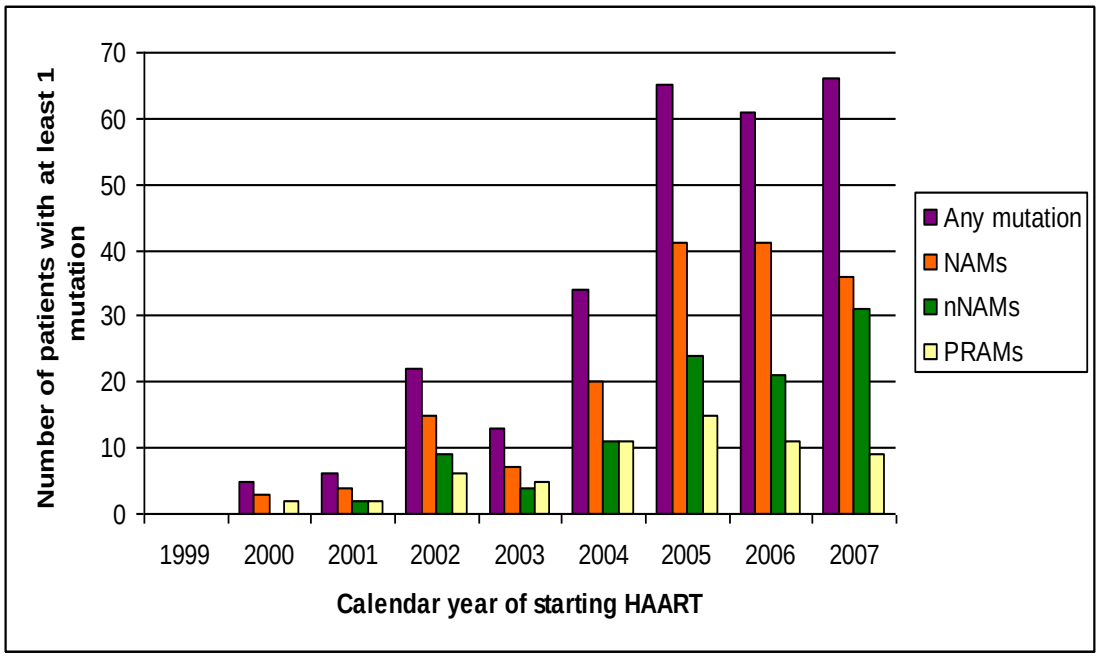
Table 4.3.3.1: Frequency of detection of mutations indicative of transmitted drug resistance among 2923 patients, of which 272 had at least one mutation[a]

NRTI N (%)		NNRTI %		Major PI %	
Any	**167 (5.7)**	**Any**	**102 (3.5)**	**Any**	**61 (2.1)**
T215rev[b]	85 (2.9)	K103N	62 (2.1)	L90M	20 (0.7)
M41L	48 (1.6)	Y181C	24 (0.8)	M46I	9 (0.3)
T215D	32 (1.1)	K101E	14 (0.5)	M46L	7 (0.2)
T215S	26 (0.9)	G190A	14 (0.5)	V82L	7 (0.2)
D67N	22 (0.8)	V106A	3 (0.1)	F53L	6 (0.2)
K219Q	21 (0.7)	V106M	3 (0.1)	I85V	6 (0.2)
M184V	20 (0.7)	Y188L	3 (0.1)	I47V	3 (0.1)
L210W	18 (0.6)	K103S	2 (0.1)	I54V	3 (0.1)
T215C	13 (0.4)	G190S	2 (0.1)	G73S	3 (0.1)
K219N	12 (0.4)	P225H	2 (0.1)	V82A	3 (0.1)
T215Y	10 (0.3)	L100I	1 (0.0)	V32I	2 (0.1)
T215E	9 (0.3)	K101P	1 (0.0)	N88D	2 (0.1)
T69D	7 (0.2)	M230L	1 (0.0)	N88S	2 (0.1)
T215I	7 (0.2)	V179F	0 (0.0)	L23I	1 (0.0)
T215V	7 (0.2)	Y181V	0 (0.0)	D30N	1 (0.0)
K219E	7 (0.2)	Y181I	0 (0.0)	I54L	1 (0.0)
K70R	6 (0.2)	Y188H	0 (0.0)	I54S	1 (0.0)
T215F	4 (0.1)	Y188C	0 (0.0)	L76V	1 (0.0)
M184I	3 (0.1)	G190E	0 (0.0)	V82T	1 (0.0)
K65R	2 (0.1)			L24I	0 (0.0)
D67E	2 (0.1)			I47A	0 (0.0)
L74V	2 (0.1)			G48M	0 (0.0)
V75M	2 (0.1)			G48V	0 (0.0)
F77L	2 (0.1)			I50L	0 (0.0)
K219R	2 (0.1)			I50V	0 (0.0)
V75T	1 (0.0)			F53Y	0 (0.0)
Y115F	1 (0.0)			I54A	0 (0.0)
D67G	0 (0.0)			I54M	0 (0.0)
K70E	0 (0.0)			I54T	0 (0.0)
L74I	0 (0.0)			G73C	0 (0.0)
V75A	0 (0.0)			G73T	0 (0.0)
V75S	0 (0.0)			G73A	0 (0.0)
F116Y	0 (0.0)			V82C	0 (0.0)
Q151M	0 (0.0)			V82F	0 (0.0)
				V82M	0 (0.0)
				V82S	0 (0.0)
				N83D	0 (0.0)
				I84A	0 (0.0)
				I84C	0 (0.0)
				I84V	0 (0.0)

[a] Patients may have more than one mutation within each drug class and for more than one class

[b] Sum of all patients with at least one T215 revertant

Figure 4.3.3.1: Number of patients with at least one mutation, stratified by calendar year of starting HAART



Of the total number of patients starting HAART, 161 (5.5%) patients had at least one detectable mutation associated with resistance to the drugs used in their initial HAART regimen (Table 4.3.3.2). This included 142 (4.9%) patients starting NRTIs, though this percentage varied by the NRTI received. In addition, 7/259 (2.7%) and 19/1688 (1.1%) patients who started nevirapine or efavirenz respectively had detectable resistance to these drugs. Overall, 15/915 (1.6%) of patients starting a PI (boosted or non-boosted) had detectable resistance mutations to the PI in their regimen, but this again varied by drug. Eleven patients had mutations associated with resistance to both NRTIs and PIs in their initial regimen and a further 11 patients had mutations associated with resistance to both NRTIs and NNRTIs in their initial regimen.

Table 4.3.3.2: Proportion of patients starting HAART with one or more resistance mutation to any drug used as part of their initial HAART regimen

	Number of patients starting drug as part of initial HAART regimen	N (%)[a] of patients with at least one mutation to specific drug
Total N (%)	**2923 (100.0)**	**161 (5.5)**
Any NRTI	**2923 (100.0)**	**142 (4.9)**
AZT	646 (22.1)	20 (3.2)
DDI	105 (3.6)	14 (13.3)
d4T	54 (1.8)	6 (11.1)
3TC	1662 (56.9)	50 (3.0)
ABC	880 (30.1)	52 (5.9)
TDF	1468 (50.2)	70 (4.8)
FTC	1170 (40.0)	20 (1.7)
Any NNRTI	**1946 (66.6)**	**26 (1.3)**
NVP	259 (8.9)	7 (2.7)
EFV	1688 (57.7)	19 (1.1)
Any PI	**915 (31.3)**	**15 (1.6)**
SQV	141 (4.8)	1 (0.7)
IDV	1 (0.0)	0 (0.0)
RTV	885 (30.3)	~
NFV	20 (0.7)	1 (0.1)
APV	1 (0.0)	0 (0.0)
LPV	474 (16.2)	12 (2.5)
ATV	224 (7.7)	1 (0.4)
TPV	2 (0.1)	0 (0.0)
DRV	12 (0.4)	0 (0.0)
FPV	40 (1.4)	0 (0.0)

DDI: didanosine, ABC: abacavir, d4T: stavudine, TDF: tenofovir, 3TC: lamivudine, AZT: zidovudine, FTC: emtricitabine, NVP: nevirapine, EFV: efavirenz, SQV: saquinavir, NFV: nelfinavir, LPV/r: lopinavir/ritonavir, ATV: atazanavir, TPV: tipranavir, FPV: fosamprenavir, IDV: indinavir, APV: amprenavir, DRV: darunavir

No patients received zalcitabine (ddC), delavirdine, etravirine,

[a] Denominators for percentages are the number of patients starting specific drugs

4.3.4. GSS of first-line regimen

In calculations involving GSS, RTV was not counted as a separate drug. Hence patients who were on exactly 3 drugs including RTV, were excluded from all further analyses (N=3), leaving 2920 patients in the dataset. Therefore, all patients remaining in the dataset were required to have a GSS of $\geq$3 to denote a fully active regimen.

The GSS of the first-line regimen was 3 in 2747 (94.1%) patients, >3 in 76 (2.6%) patients, and <3 in 97 (3.3%) patients, the latter comprising scores of 0.5, 1, 1.5, 2 and 2.5 in 3 (0.1%), 4 (0.1%), 6 (0.2%), 34 (1.2%) and 50 (1.7%) patients respectively. The 7 patients with a GSS of $\leq$1 were all receiving NNRTI-based regimens (4 were receiving NVP and 3 were receiving EFV). Table 4.3.4.1 shows the GSS of patients stratified by the number of drugs received. Of those receiving 3 drugs, 96.9% of patients had the maximum GSS of 3 and 0.3% of patients had a GSS <2. Of those receiving >3 drugs, the majority of patients had a GSS >3 (86.4%), though around 10% of patients had a GSS between 2 and 3. No patients receiving >3 drugs had a GSS below 1.

Table 4.3.4.1: GSS stratified by the number of drugs received as first-line HAART

	Number of drugs received N (%)		
GSS ↓	**3**	**>3**	**Any**
>3	-	76 (86.4)	76 (2.6)
3	2745 (96.9)	2 (2.3)	2747 (94.1)
2.5	47 (1.7)	3 (3.4)	50 (1.7)
2	29 (1.0)	5 (5.7)	34 (1.2)
1.5	4 (0.1)	2 (2.3)	6 (0.2)
1	4 (0.1)	0 (0.0)	4 (0.1)
0.5	3 (0.1)	0 (0.0)	3 (0.1)
Total	2832	88	2920

Characteristics of the 97 (3.3%) patients with GSS <3 were compared to the 2823 (96.7%) patients with GSS $\geq$3 (Table 4.3.4.2). A higher proportion of patients with GSS <3 were of white ethnicity (75.3% vs. 63.8%), of MSM risk group (72.3% vs. 63.8%) and had started HAART in earlier calendar years (10.3% vs. 4.6%)

compared to those with GSS $\geq$3. Patients with GSS <3 were also more likely to carry a subtype B virus (80.4% vs. 68.4%) and start HAART with PI-based regimens (PI/r: 36.1% vs. 30%, single PI: 3.1% vs. 1.0%) than those with GSS $\geq$3. No statistical differences were found between CD4 counts and viral loads amongst the two groups.

Table 4.3.4.2: Characteristics of patients stratified according to whether the Genotypic Sensitivity Score (GSS) of the first-line HAART regimen was < or ≥3

		GSS <3	GSS ≥3	P-value[1]
Total		97 (100.0)	2823 (100.0)	
Gender (%)	Male	85 (87.6)	2278 (80.7)	0.09
Ethnicity (%)	White	73 (75.3)	1802 (63.8)	0.02
	Black African	12 (12.4)	693 (24.5)	
	Other	12 (12.4)	328 (11.6)	
HIV transmission group (%)	MSM	73 (72.3)	1801 (63.8)	0.03
	Heterosexual	15 (15.5)	763 (27.0)	
	Other	9 (9.3)	259 (9.2)	
Calendar year of resistance test (%)	1999-2001	10 (10.3)	130 (4.6)	0.0001
	2002-2004	45 (46.4)	905 (32.1)	
	2005-2007	42 (43.3)	1788 (63.3)	
Months from resistance test to start of HAART	Median (IQR)	1.9 (0.6, 11.4)	1.9 (0.7, 8.3)	0.86
Subtype	B	78 (80.4)	1931 (68.4)	0.01
	Non B	19 (19.6)	892 (31.6)	
Age at time of test (years)	Median (IQR)	37 (31, 41)	37 (31, 43)	0.49
CD4 count at time of resistance test	Median (IQR)	261 (195, 386)	250 (163, 351)	0.20
CD4 count at HAART initiation	Median (IQR)	225 (153, 298)	210 (140, 284)	0.32
Viral load (VL) at time of resistance test	Median (IQR)	4.8 (4.3, 5.4)	4.9 (4.4, 5.3)	0.32
VL at HAART initiation	Median (IQR)	4.8 (4.4, 5.4)	4.9 (4.3, 5.3)	0.79
HAART regimen started N (%)	NNRTI	46 (47.4)	1900 (67.3)	<0.0001
	PI/r	35 (36.1)	847 (30.0)	
	PI	3 (3.1)	27 (1.0)	
	Triple NRTI	13 (13.4)	49 (1.7)	

[1] Calculated using Chi-squared or Mann Whitney tests

CD4 count measured in cell/mm^3 and VL measured in log_{10} copies

In adjusted analyses, factors significantly associated with a GSS <3 were starting HAART in earlier calendar years (Odds ratio (OR) 3.53; 95% Confidence Interval (CI): 1.49, 8.34, comparing 1999-2001 with 2005-2007 and 1.60 (0.99, 2.57) comparing 2002-2004 with 2005-2007), and starting HAART with a non-NNRTI regimen (5.08 (1.40, 18.37) comparing PI- to NNRTI-based regimens, 2.03 (1.27, 3.23) comparing PI/r- with NNRTI-based regimens and 10.18 (4.26, 24.35) comparing triple NRTI- with NNRTI-based regimens).

Given that patients starting HAART with PI-based regimens were more likely to have a GSS <3, these patients were investigated in greater detail. Of the 97 patients showing a GSS <3, 35 started PI/r-based HAART and 3 started single PI-based HAART. Analysing the resistance mutations of these 38 patients, 2 (5.3%) had mutations to only the PI in their initial regimen, 7 (18.4%) had mutations to both the PI and the NRTI in their initial regimen, and 28 (73.7%) patients had mutations to only the NRTIs in their initial regimen. Amongst the 9 patients with resistance mutations to PIs, 7 patients had started LPV/r (5 of whom had intermediate resistance to the drug), 1 patient had started NFV (with intermediate resistance to the drug) and 1 patient had started AZV/r (with intermediate resistance to the drug). Amongst the 35 patients with resistance mutations to the NRTIs in their initial regimen, the majority of patients (n=26) had mutations to 1 NRTI (most commonly ABC (10 patients) and TDF (10 patients), whilst the remaining 9 patients had resistance mutations to greater than one NRTI.

In both unadjusted and adjusted models, patients were less likely to start an NNRTI if they had any nNAMs detected (adjusted OR=0.16; 95% CI: 0.10, 0.25), whereas there was no evidence to suggest that patients with PRAMs were less likely to start boosted or non-boosted PI-based regimens (adjusted OR 1.32; 95% CI: 0.74, 2.33). Amongst patients with TAMs, in unadjusted analyses patients were less likely to start AZT (unadjusted OR: 0.47; 95% CI: 0.24, 0.97) but this effect was non-significant after adjusting for potential confounders (adjusted OR=0.52; 95% CI: 0.24, 1.16). No significant relationship was found between having TAMs and starting HAART with any of the other TAM-affected drugs, ddI, 3TC, ABC and TDF.

4.3.5. Virological suppression after starting first-line HAART

Of the 2923 patients, 2785 (95.4%) had follow-up viral-loads measured. There was no difference in the sex (p=0.34), ethnicity (p=0.75) or HAART regimen started (p=0.32) amongst patients who were included and excluded in the virological

suppression analysis. However patients included in this analysis were more likely to be of MSM risk group (96.2% of MSMs included compared to 91% of those from 'other' risk groups, p=0.01). No clinical difference was seen in the CD4 count and viral load at start of HAART, though p-values suggested borderline significance (p=0.07 and 0.10 respectively). Patients included in the analysis were also more likely to start HAART in earlier calendar years (99.1% in 2002-2004 compared to 94.2% in 2005-2007, p<0.0001).

Among the 2785 patients for whom follow-up VL measurements were available, 2511 (90.2%) patients achieved an undetectable VL (<50 copies/mL) at a (Kaplan-Meier) median of 3.6 (IQR: 3.5, 3.7) months after starting HAART. Follow-up was censored at the date of the last viral load measurement for patients who did not achieve an undetectable viral load. The majority of patients who achieved an undetectable viral load (1707/2511 (68.0%)) were receiving NNRTI-based regimens; 751/2511 (29.8%) were receiving PI-based regimens and 53/2511 (2.1%) were receiving triple NRTI regimens. Among the 2511 patients who achieved virological suppression, the GSS of the regimen started was ≥3 in 2426 (96.6%) patients, 2.5 in 43 (1.7%) patients, 2 in 32 (1.3%) patients, and ≤1.5 in 10 (0.4%) patients. Of the 274 (9.8%) patients who did not achieve virological suppression, the GSS was 4 in 7 (2.6%) patients, 3 in 259 (94.5%) patients, 2.5 in 4 (1.5%) patients, 2 in 2 (0.7%) patients and ≤1.5 in 2 (0.7%) patients. Some patients did switch therapy prior to virological suppression and hence the GSS at time of virological suppression was also calculated. Surprisingly, the GSS at time of virological suppression was generally lower than the GSS of the initial regimens – 2366 (94.2%) of those who achieved VL <50 copies/mL had a GSS of ≥3, whilst 123 (5.8%) patients had a GSS of <3. Patients may have switched drugs due to toxicity, and hence the GSS may not have been as important a factor as tolerability when these switches took place.

Amongst the 93 patients with GSS <3, 85 (91.4%) patients achieved virological suppression and 8 (8.6%) patients did not achieve virological suppression (Figure 4.3.5.1). These percentages appeared to be similar to those for patients with GSS ≥3 since they do not take into account censoring. The HAART regimens of the 85 patients who achieved virological suppression despite a GSS <3 were NNRTI-based in 41 patients (17 with NAMs, 15 with nNAMs, and 9 with both NAMs and nNAMs), PI-based in 2 patients (1 with PRAMs and 1 with NAMs), PI/r-based in 30 patients (2 with PRAMs, 22 with NAMs and 6 with NAMs and PRAMs), and triple NRTIs in 12 patients, 3 of which had NAMs.

Of the 8 patients with GSS <3 who did not achieve virological suppression, 4/8 started NNRTI-based regimens (2 with NAMs and 2 with NAMs and nNAMs). Three patients started PI-based regimens (lopinavir/r, nelfinavir and atazanavir/r); none had PRAMs whilst all 3 had NAMs. One patient with NAMs started a triple NRTI regimen.

As mentioned in Section 4.3.4, 9 patients with low GSS had resistance to the PIs in their initial regimens. Eight of these patients were included in this analysis and all 8 achieved virological suppression on the regimen they started with.

Figure 4.3.5.1: Flow chart outlining those included in the viral suppression analysis

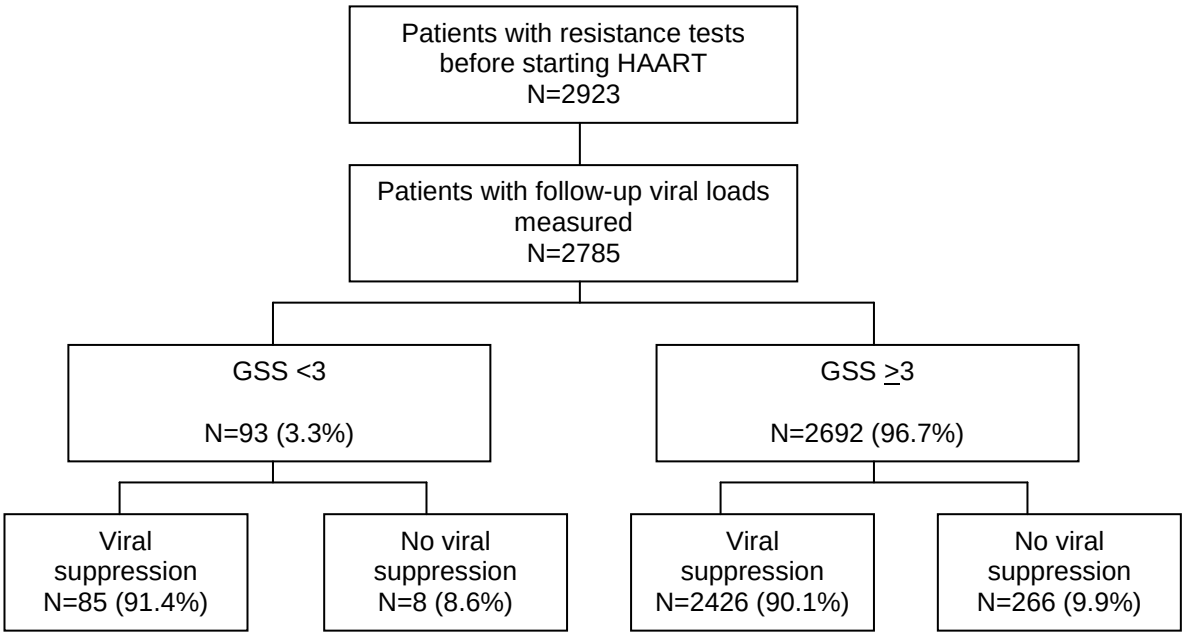


Table 4.3.5.1 shows the baseline characteristics of the 2785 patients eligible for the virological suppression analysis, stratified by whether or not they achieved an undetectable viral load after starting HAART. A higher proportion of patients who achieved viral suppression were male (81.6% vs. 76.3% of those who did not achieve viral suppression), of MSM risk group (65.2% vs. 59.9%) and had their resistance tests performed in 2002-2004 (35.6% vs. 13.5%). Patients who started HAART with NNRTI-based regimens were also more likely to achieve viral suppression (68.0% vs. 54.0% of those on other regimens).

In unadjusted analyses, patients with a GSS <3 were significantly less likely to achieve viral suppression than those with a GSS >3 (HR=0.72 (0.58, 0.89)). Patients were also less likely to achieve viral suppression if they had higher viral loads at start of HAART, were receiving a PI-based regimen (compared to NNRTI-based regimens), started HAART in earlier calendar years or made any switch in therapy before the end of follow-up (Table 4.3.5.2). Patients of black-African ethnicity (compared with those of white ethnicity) and of MSM risk group (compared with those of heterosexual risk group) were more likely to achieve viral suppression. Other than ethnicity and risk group, all of the above variables remained significantly associated with viral suppression after adjusting for potential confounders (Table 4.3.5.2).

In separate analyses where only those patients with GSS $\geq$3 were included, there was still evidence of a significant association between GSS and viral suppression (HR=1.32 (1.14, 1.53) per 1 unit higher). Further analyses in which GSS was incorporated as a categorical variable also showed that those with GSS <3 were less likely to achieve viral suppression compared to those with a GSS of 3 (0.68 (0.54, 0.85)) but there was no significant difference amongst patients with GSS >3 compared to those with GSS of 3 (1.01 (0.78, 1.32)).

An interaction test between calendar year (fitted as a categorical variable) and GSS (fitted as a continuous variable) showed no significant association between the two (p=0.88).

Table 4.3.5.1: Characteristics of patients included in virological suppression analysis, stratified by whether or not they achieved an undetectable (<50 copies/mL) VL after starting HAART (N=2785)

		VL <50 copies/mL	VL >50 copies/mL
Total N		2511 (100.0)	274 (100.0)
GSS	<2	10 (0.4)	2 (0.7)
	2 – 2.5	75 (3.0)	6 (2.2)
	3	2357 (93.8)	259 (94.5)
	> 3	69 (2.7)	7 (2.6)
Sex N (%)	Males	2049 (81.6)	209 (76.3)
Ethnicity N (%)	White	1616 (64.4)	176 (64.2)
	Black	502 (20.0)	56 (20.4)
	Other	393 (15.6)	42 (15.3)
Risk group N (%)	MSM	1638 (65.2)	164 (59.9)
	Heterosexual	664 (26.4)	73 (26.6)
	Other	209 (8.3)	37 (13.5)
Calendar year of test N (%)	1999-2001	124 (4.9)	12 (4.4)
	2002-2004	895 (35.6)	37 (13.5)
	2005-2007	1492 (59.4)	225 (89.1)
Subtype	B	1743 (69.4)	178 (65.0)
Calendar year of starting	1999-2001	68 (2.7)	8 (2.9)
HAART N (%)	2002-2004	656 (26.1)	21 (7.7)
	2005-2007	1787 (71.2)	245 (89.4)
Age at time of test	Median (IQR)	37 (32, 43)	36 (30, 41)
CD4 count at time of test	Median (IQR)	250 (163, 343)	287 (162, 410)
CD4 count at HAART initiation	Median (IQR)	210 (140, 280)	220 (141, 322)
VL at time of test	Median (IQR)	4.9 (4.4, 5.3)	5.0 (4.4, 5.3)
VL at HAART initiation	Median (IQR)	4.9 (4.3, 5.3)	5.0 (4.3, 5.4)
HAART regimen started N (%)	PI	27 (1.1)	2 (0.7)
	Boosted PI	724 (28.8)	115 (42.0)
	NNRTI	1707 (68.0)	148 (54.0)
	Other	53 (2.1)	9 (3.3)

CD4 measured in cells/mm^3, VL measured in $\log_{10}$ copies/mL

Table 4.3.5.2: Factors associated with achieving viral suppression (VL <50 copies/mL) after starting HAART

		Univariable		Multivariable	
		Hazard ratio (95% CI)	**P-value**	**Hazard ratio (95% CI)**	**P-value**
GSS	≥3	1	-	1	-
	<3	0.72 (0.58, 0.89)	0.003	0.68 (0.54, 0.85)	0.001
Gender	Male	0.92 (0.83, 1.01)	0.09	1.01 (0.88, 1.117	0.84
Ethnicity	White	1	-	1	-
	Black-African	1.18 (1.07, 1.30)	0.001	1.09 (0.93, 1.28)	0.29
	Other	1.22 (1.09, 1.36)	0.0004	1.14 (1.01, 1.28)	0.03
HIV transmission risk group	MSM	1	-	1	-
	Heterosexual	1.20 (1.09, 1.31)	0.0001	1.10 (0.94, 1.29)	0.22
	Other	0.91 (0.78, 1.07)	0.24	0.85 (0.70, 0.99)	0.04
Age at resistance test	Per 10 years higher	1.03 (0.99, 1.08)	0.12	1.02 (0.98, 1.07)	0.31
CD4 at start of HAART	Per 50 cell higher	1.00 (0.98, 1.01)	0.75	0.99 (0.97, 1.00)	0.07
Viral load at start of ART	Per 1 log higher	0.76 (0.73, 0.80)	<0.0001	0.73 (0.70, 0.77)	<0.0001
Regimen started	NNRTI	1	-	1	-
	PI	0.55 (0.38, 0.81)	0.003	0.53 (0.35, 0.79)	0.002
	PI/r	0.85 (0.78, 0.93)	0.0002	0.77 (0.70, 0.84)	<0.0001

	Other	0.78 (0.59, 1.03)	0.08	0.80 (0.60, 1.06)	0.12
Subtype	B	1	-	1	-
	Non B	1.09 (1.00, 1.18)	0.05	0.88 (0.60, 1.06)	0.12
Calendar year at start of HAART	1999-2001	0.43 (0.34, 0.56)	<0.0001	0.39 (0.30, 0.50)	<0.0001
	2002-2004	0.72 (0.66, 0.79)	<0.0001	0.73 (0.67, 0.81)	<0.0001
	2005-2007	1	-	1	-

4.3.6. Assigning 1.5 to active PIs (instead of 1)

It has been suggested that a fully active PI/r should be given a score of 1.5 rather than 1 (470). Assigning this score reduced the number of patients with GSS <3, from 97 to 75 and increased the number of patients with GSS ≥3 from 2823 to 2845. Using the same definition for viral suppression as outlined above (Section 4.3.5), in unadjusted analysis, the HR for patients with a GSS of <3 compared to ≥3 was 0.72 (0.56, 0.92). After adjusting for potential confounders, the HR for GSS <3 compared to ≥3 was very similar to that seen in the main analysis (0.66 (0.51, 0.85).

Since assigning 1.5 to fully active PIs instead of 1 made little difference to the adjusted HR (0.66 compared to 0.68 when a score of 1 was assigned), the rest of this chapter is based on assigning a score of 1 to fully active PIs.

4.3.7. Virological rebound after achieving viral load suppression

Among 2379 patients who had achieved virological suppression within a year of starting HAART, virological rebound (VL >400 copies/mL on two consecutive occasions or 1 VL >400 copies/mL followed by a treatment change) occurred in 228 (9.6%) patients, at a median 5.9 (2.8, 12.0) months after achieving an undetectable VL. Of patients with GSS <3, 80 were included in these analyses, 12 (15.0%) of whom experienced virological rebound at a median 3.0 (2.1, 5.0) months. Seven of these patients had started HAART with an NNRTI-based regimen; 4 patients had resistance mutations to the NNRTI in their initial regimen, 1 patient had mutations to the NRTI in the initial regimen and 2 patients had mutations to both the NRTIs and the NNRTIs in their initial regimen. Four of the 12 patients experiencing virological failure with GSS <3 started HAART on PI-based regimens; 3 of these patients had mutations to the NRTIs in their initial regimen, whilst one patient had mutations to the PI in their initial regimen. The remaining patient had started HAART on an NRTI-based regimen and had no resistance mutations.

In univariable analyses, patients with a GSS <3 were at an increased risk of experiencing virological rebound, though this effect was not significant (HR=1.49 (0.83, 2.67), p=0.18). After adjusting for potential confounders, this effect remained non-significant (1.64 (0.90, 2.99)). Though most other co-variates appeared to be associated with virological rebound in univariable analyses (Table 4.3.7.1), in multivariable analyses, only a higher CD4 count at start of HAART, starting HAART with a PI-based regimen (compared to an NNRTI-based regimen) and starting HAART in earlier calendar years were associated with an increased risk of

virological rebound. Older age was associated with a decreased risk of virological rebound.

Table 4.3.7.1: Factors associated with virological rebound after achieving an undetectable viral load

		Univariable		Multivariable	
		Hazard ratio (95% CI)	**P-value**	**Hazard ratio (95% CI)**	**P-value**
GSS	≥3	1	-	1	-
	<3	1.49 (0.83, 2.67)	0.18	1.64 (0.90, 2.99)	0.11
Gender	Male	0.36 (0.28, 0.48)	<0.0001	0.64 (0.41, 1.01)	0.06
Ethnicity	White	1	-	1	-
	Black-African	1.91 (1.41, 2.59)	<0.0001	1.05 (0.65, 1.70)	0.84
	Other	1.79 (1.27, 2.53)	0.001	1.55 (1.06, 2.26)	0.02
HIV transmission risk group	MSM	1	-	1	-
	Heterosexual	2.18 (1.66, 2.89)	<0.0001	1.63 (0.98, 2.71)	0.06
	Other	2.54 (1.61, 4.01)	<0.0001	1.65 (0.97, 2.85)	0.07
Age at resistance test	Per 10 years higher	0.63 (0.53, 0.74)	0.0002	0.77 (0.65, 0.91)	0.002
CD4 at start of HAART	Per 50 cell higher	1.15 (1.11, 1.19)	<0.0001	1.10 (1.06, 1.15)	<0.0001
Viral load at start of ART	Per 1 log higher	0.69 (0.60, 0.78)	<0.0001	0.92 (0.78, 1.08)	0.29
Regimen started	NNRTI	1	-	1	-
	PI	11.48 (6.15, 21.44)	<0.0001	4.97 (2.47, 9.99)	<0.0001
	PI/r	3.08 (2.34, 4.05)	<0.0001	2.40 (1.78, 3.22)	<0.0001

	Other	2.21 (1.11, 4.38)	0.02	1.62 (0.80, 3.28)	0.18
Subtype	B	1	-	1	-
	Non B	1.71 (1.31, 2.22)	<0.0001	0.85 (0.54, 1.34)	0.49
Calendar year at start of HAART	1999-2001	2.90 (1.73, 4.85)	<0.0001	3.29 (1.89, 5.75)	<0.0001
	2002-2004	1.07 (0.80, 1.44)	0.63	1.22 (0.89, 1.66)	0.22
	2005-2007	1	-	1	-

4.3.8. CD4 increase of >50 cells from start of HAART

Two hundred and forty-one patients did not have follow-up CD4 counts and hence were excluded from the analysis of CD4 increases. Patients included were more likely to be male (92.3% of males included compared to 89.6% of females, p=0.04), of MSM risk group (92.5% of MSMs included compared to 86.7% of others, p=0.02) and have started HAART in earlier calendar years (96.2% of those included started HAART in 1999-2001 compared to 90.6% in 2005-2007). There was no difference in ethnicity (p=0.43) or regimen started (p=0.26) amongst those included and excluded. Patients included in this analysis also had lower CD4 counts (210 vs. 243 cells/mm^3, p=0.002) and higher viral loads at HAART initiation (4.9 vs. 4.7 log copies/mL, p=0.04).

Of the 2679 patients with CD4 measurements available both at the time of starting HAART and after starting HAART, 2508 (93.6%) achieved a CD4 increase of at least 50 cells/mm^3 from baseline. Patients who did not experience a CD4 increase of >50 cells/mm^3 had shorter follow-up times (median (IQR) 0.6 (0.2, 1.3) vs. 1.8 (1.0, 3.0) years) and fewer CD4 measurements (3 (1, 5) vs. 8 (4, 12)). Amongst the 171 patients who didn't achieve a 50 cell increase, 6 (3.5%) had a GSS <3. Three of these patients had started HAART on NNRTI-based regimens, 2 of whom had resistance mutations to the NRTIs in the initial regimen. The remaining 3 patients had started HAART on PI/r regimens – all had resistance to the NRTIs in the initial regimen and 1 patient had further resistance to the PI in the initial regimen.

Factors associated with more rapidly achieving a CD4 increase of >50 cells/mm^3 are shown in Table 4.3.8.1. In univariable analyses, patients of male gender and with current undetectable viral loads were more likely to experience a CD4 increase. Patients of black-African ethnicity (compared to white ethnicity), heterosexual risk group (compared to MSM), higher CD4 counts at start of HAART, and those who started HAART in earlier calendar years were less likely to experience a CD4 increase of >50 cells/mm^3. Patients starting HAART with PI-based regimens and those with non-B subtypes were also less likely to experience a CD4 increase of >50 cells/mm^3. There was no association between GSS and achieving a CD4 increase of >50 cells/mm^3. After adjusting for potential confounders, only CD4 count at start of HAART (HR=0.93 (0.92, 0.94)), starting HAART with PI/r-based regimens (1.11 (1.01, 1.22) compared to NNRTI-based regimens), 'other' ethnicity (0.68 (0.57, 0.85)) compared to white ethnicity and starting HAART in earlier calendar years

(0.74 (0.59, 0.95) comparing 1999-2001 with 2005-2008) remained significantly associated with experiencing a CD4 increase of >50 cells/mm^3.

Since patients starting HAART with PI/r-based regimens were more likely to achieve a CD4 increase than those on NNRTI-based regimens, sensitivity analyses were performed to investigate whether this was a robust effect. In the first instance, the CD4 threshold was increased to >100 cells/mm^3 and >150 cells/mm^3 from baseline. The HRs for starting HAART with PI/r-based regimens compared to NNRTI-based regimens were 1.10 (1.00, 1.21) and 1.17 (1.05, 1.29) respectively. PI/r-based regimen was not significantly associated with a CD4 increase in univariable analysis, and only after adjusting for baseline CD4 count did the effect reach significance. The median baseline CD4 count amongst those receiving PI/r-based regimens was 227 (140, 328) cells/mm^3 and for those receiving NNRTI regimens was 205 (139, 268) cells/mm^3 ($p<0.0001$). However, a test for interaction between first HAART regimen and baseline CD4 count was not significant ($p>0.20$). Further, the median change in CD4 count for those receiving PI/r-based regimens was 108 (72, 170) cells/mm^3, whilst for those receiving NNRTI-based regimens was 110 (78, 159) cells/mm^3.

Finally, a sensitivity analysis that considered CD4 increases after a year of starting HAART was performed. Of the 1621 patients with CD4 counts available within a year and 18 months after starting HAART, 1396 (86.1%) patients had achieved a CD4 increase of >50 cells/mm^3 from baseline. After adjusting for potential confounders, PI/r boosted regimen was not found to be significantly associated with a CD4 increase from baseline (OR=0.88 (0.63, 1.24)).

Table 4.3.8.1: Factors associated with achieving a CD4 increase of >50 cells/mm^3 after starting HAART

		Univariable		Multivariable	
		Hazard ratio (95% CI)	**P-value**	**Hazard ratio (95% CI)**	**P-value**
GSS	≥3	1	-	1	-
	<3	1.14 (0.91, 1.41)	0.26	1.20 (0.96, 1.50)	0.11
Gender	Male	1.12 (1.01, 1.24)	0.03	0.89 (0.77, 1.03)	0.11
Ethnicity	White	1	-	1	-
	Black-African	0.87 (0.78, 0.96)	0.01	0.88 (0.75, 1.03)	0.10
	Other	1.01 (0.91, 1.13)	0.82	0.98 (0.87, 1.10)	0.69
HIV transmission risk group	MSM	1	-	1	-
	Heterosexual	0.87 (0.79, 0.95)	0.002	0.86 (0.74, 1.01)	0.06
	Other	0.75 (0.64, 0.88)	0.001	0.68 (0.57, 0.85)	<0.0001
Age at resistance test	Per 10 years higher	0.98 (0.94, 1.03)	0.45	0.96 (0.92, 1.00)	0.07
CD4 at start of HAART	Per 50 cell higher	0.94 (0.92, 0.95)	<0.0001	0.93 (0.92, 0.94)	<0.0001
Current VL	<50 copies/mL	1.77 (1.11, 2.84)	0.02	1.47 (0.91, 2.36)	0.11
Regimen started	NNRTI	1	-	1	-
	PI	0.63 (0.43, 0.92)	0.02	0.86 (0.58, 1.26)	0.43

	PI/r	1.06 (0.97, 1.15)	0.20	1.11 (1.01, 1.22)	0.02
	Other	0.93 (0.71, 1.21)	0.57	1.03 (0.78, 1.35)	0.85
Subtype	B	1	-	1	-
	Non B	0.84 (0.77, 0.91)	<0.0001	0.92 (0.80, 1.06)	0.24
Calendar year at start of HAART	1999-2001	0.70 (0.55, 0.88)	<0.0001	0.74 (0.59, 0.95)	0.02
	2002-2004	0.88 (0.80, 0.96)	0.63	0.87 (0.79, 0.96)	0.004
	2005-2008	1	-	1	-

4.3.9. Analyses restricted to patients whose resistance test is within 6 months prior to starting HAART

Amongst the 2923 patients who had resistance tests performed whist HAART-naïve, 2023 (69.2%) patients started HAART within 6 months of having had the resistance test performed. Of these patients, 8.8% had at least one resistance mutation indicative of TDR and 104 (5.1%) patients had resistance to a drug in their first-line regimen. Sixty-five patients (3.2%) had a GSS of <3 and though calendar year was not significantly associated with a GSS <3 in adjusted analyses (as it was in the main analysis), patients starting HAART with an NRTI-only regimen were at increased risk of having a low GSS (7.18 (2.48, 20.79)).

Of the 1947 patients included in the virological suppression analyses, 1766 (90.7%) achieved VL <50 copies/mL. In adjusted analyses, patients with a GSS <3 were less likely to achieve virological suppression (HR: 0.58 (0.44, 0.77)) – this effect was stronger than that seen in the main analyses (0.68 (0.54, 0.85)). One hundred and seventy-seven patients experienced virological rebound (10.6%). GSS was not associated with virological rebound in adjusted analyses (1.52 (0.70, 3.31)) and this effect was weaker than that seen in the main analyses (1.64 (0.90, 2.99)).

The vast majority (93.7%) of patients who had resistance tests performed within 6 months prior to starting HAART achieved a CD4 increase of >50 cells/mm^3. Again, GSS was not associated with this outcome (1.18 (0.89, 1.55)) and the effect was similar to that seen in the main analyses (1.20 (0.96, 1.50)).

4.4. Discussion

This chapter has focussed on the use of genotypic resistance testing among drug-naïve patients in routine care in the UK. Resistance testing was more commonly performed in white MSM compared with other demographic groups during the period 1999-2007. This finding reflects the overall higher risk of resistance traditionally observed in this group relative to heterosexuals, women and persons of black ethnicity (181;209). In addition, it is only in recent years that national treatment guidelines have started to clearly recommend testing in all newly diagnosed patients regardless of risk group and ethnicity (127).

The prevalence of at least one detectable mutation indicative of TDR was 9.3% in this cohort, and therefore consistent with previous estimates from the UK and elsewhere in Western Europe (206;209;210;214;221;226;464). In particular, the prevalence estimate of 9.3% is in line with the largest study analysing rates of primary drug resistance across Europe, in which organisers reported a TDR prevalence rate of 9.1%. It is also in line with the most recently published studies on TDR worldwide, which report rates around 10% (188;203;211;217;471). There was also evidence of an increasing trend of TDR prevalence in later calendar years, though of note, these analyses are restricted to patients who start HAART and so may not be directly comparable to other estimates. Though data on trends over time of TDR are conflicting, with some studies showing a declining prevalence, i.e. the UK Collaborative Group on HIV Resistance reports lower rates of TDR in later calendar years (12.5% in 2003 and 9.2% in 2004) (214), the findings reported in this chapter do consist of more recent data and are consistent with findings from the EuroSIDA study in which an upward trend in TDR prevalence was observed from 2001 to 2004 (6.5% to 9.1%) (163). Brenner et al also reported an increase of TDR in Canada (188), from 15.7% in 1997-2001 to 21.2% in 2004-2005.

The TAMs T215rev and M41L for the NRTIs, and the K103N mutation for the NNRTIs were the most prevalent mutations, in line with previously published findings (181;191;209). Importantly, the majority of patients started first-line HAART with a regimen comprising at least 3 fully active drugs. Those starting HAART in earlier calendar years and on PI-based regimens were at an increased risk of having low GSS. One explanation for the significant calendar year finding may be that in earlier years the likely impact of TDR was underappreciated. Patients may have been selectively tested, resulting in a higher proportion of patients appearing to have TDR. A second explanation is that the choice of HAART drugs was also limited in

earlier years. Availability of TDF, in particular, in later calendar years may have contributed to the higher GSS seen in this period. This may have reflected the evolution of resistance interpretation algorithms, combined with the range of available drug options. It should be noted that interpretation systems for genotypic drug resistance have evolved significantly over time. I have not attempted to adjust for this as I was interested in exploring the impact of GSS using a uniform interpretation approach. Hence in a small minority of cases, though the GSS was <3, it may have been equal to 3 according to the interpretation system used by the clinician at the time of the resistance test.

The lower GSS in patients receiving PI-based regimens compared to NNRTI-based regimens was only infrequently related to the presence of PRAMs. Rather, the majority of patients in this group had NAMs affecting the NRTIs they received. These findings suggest that in a cohort setting where NNRTI-based therapy is the generally preferred initial HAART regimen (127), PI/r-based regimens were selected specifically in order to compensate for the presence of resistance to the NRTI backbone.

A further consideration is that I used an interpretation algorithm which assigned a GSS of 1 to a fully active PI/r. It has been proposed that a fully active PI/r should be given a score of 1.5 (470). Using a higher GSS score for PI/r increases the overall GSS for each patient with TDR, but had little impact on the number of patients in the GSS <3 category (from 97 to 75).

Although some studies (163;188;206) have reported a higher proportion of patients who started a PI-based regimen despite having mutations associated with resistance to the selected drugs than I found (1.6%), my estimate is in line with those reported by most other recently published studies (202;204;208;209;214;220;471). This is also true of patients starting NNRTI based regimens despite having resistance mutations to the NNRTIs in their initial regimen (1.3%) (163;202;209), though again, other studies have reported higher estimates (188;204;208;471). The proportion of patients with mutations conferring resistance to the NRTIs in the initial regimen is variable across studies, even in recent years, ranging from 0.5% reported by Payne et al in 2008 (202) to 10.0% reported by Cachay et al in 2007 (208). I found 4.9% of patients had mutations conferring resistance to the NRTIs in their initial regimen. This is similar to other recently published studies (204;206;209;210;471), albeit utilising a different interpretation list to identify resistance mutations.

Interestingly, there was no significant association between having TAMs and receiving NRTIs that can be affected by these mutations. Although in unadjusted analyses, I found that persons with TAMs were less likely to receive zidovudine, this effect was not significant after adjusting for potential confounders. This is surprising, given the well recognised effect of these mutations on responses to particular drugs (469). I did however find that patients with nNAMS were less likely to start NNRTI-based regimens - this is consistent with the current understanding of the low genetic barrier of such regimens (472).

While only a small minority (3.3%) of patients started regimens with a GSS <3, this group of patients experienced reduced virological responses. This effect was still significant after adjusting for potential confounders, in particular, starting HAART with PI/r-based regimens. This suggests that starting HAART with PI/r (and in particular, lopinavir/r in this cohort) did not fully compensate for the presence of resistance to the NRTI backbone. This finding is consistent with the superior virological activity of lopinavir/r in combination with 2 NRTIs relative to lopinavir/r monotherapy in first-line HAART (473). Other studies have shown that TDR has not impacted on viral response (189;191;203;211;220), though again, different resistance interpretation systems will impact on the comparability of these results. It should also be noted that the number of patients in these studies is considerably smaller than that in the UK CHIC Study and hence whilst the proportion of patients with resistance mutations achieving viral suppression is generally smaller than those without mutations achieving viral suppression, there may not have been enough power to detect a significant difference. However, some studies have been able to detect such an association. Little et al analysed the resistance profiles of 202 patients in North America and reported a significant association between patients having resistance mutations and virological suppression (231). The time to virological suppression after starting HAART was longer, whilst the time to virological failure was shorter amongst patients with resistance mutations. Similar results were reported by Grant et al, also in 2002 (234). The time to virological suppression amongst patients with evidence of genotypic mutations was 12 weeks, compared to 5 weeks amongst patients without mutations.

In line with previous studies (418;474;475), patients with higher viral loads at start of HAART were less likely to achieve virological suppression. Patients starting HAART on PI-based regimens compared to NNRTI regimens were also less likely to achieve suppression – as discussed earlier, these patients are likely to be carrying NRTI

mutations and the use of a PI does not seem to have fully compensated for resistance to the NRTI backbone. Earlier calendar year and non-B subtype were also associated with a decreased probability of achieving viral suppression. Fewer drugs were available in earlier calendar years, some of which were associated with high levels of toxicities. Hence, patients receiving HAART in earlier calendar years may have received drugs which were not adhered to due to the toxicities associated with them. It has been well documented that adherence is a strong predictor of virological response to HAART (148-151). There are conflicting reports on the association between subtype and virological response. Whilst some studies have reported patients of non-B subtype are no less likely to achieve virological suppression than those harbouring a subtype B virus (476), other studies have reported a significant negative association between these variables (477). Patients harbouring non-B subtypes may be those of poorer socio-economic status and factors correlated with this status may also affect the levels of virological response. Also, the proportion of patients with non-B subtype virus is small in this dataset – this is also likely to play a part in the significant finding.

No relationship was seen between the GSS of the first-line regimen and risk of virological rebound, though this lack of association may be explained by the small number of patients who experienced rebound (<10%). Factors associated with virological rebound included younger age, higher pre-HAART CD4 counts and starting HAART with either a non-boosted or boosted PI-based regimen compared to an NNRTI-based regimen. These observations are mostly consistent with previous findings. It has been well documented that patients of older age are likely to have a better response to HAART, possibly due to better adherence to medication (418;423;475). The effect of CD4 count was surprising as though some studies have shown no association between CD4 count and viral rebound (474), most studies have shown a positive association between lower CD4 count and virological rebound (426;475;478). While the CD4 effect I found may represent a chance finding, the number of patients with pre-HAART CD4 counts >350 cells/mm^3 represented less than 15% of the total population and this may somewhat explain the association.

The association between starting HAART with PI-based regimens and viral rebound is as expected, given the findings discussed above on the resistance profiles of these patients. Other factors may also contribute to this finding (i.e. unmeasured

confounding), and in particular, it may simply be the case that NNRTI-regimens are more efficacious.

Finally, GSS was not significantly associated with a CD4 increase of >50 cells/mm^3 from pre-HAART levels. Patients with higher pre-HAART CD4 counts were less likely to achieve a CD4 increase of >50 cells/mm^3. This is likely to be explained by the greater scope for an increase amongst those with lower pre-HAART counts. This finding has been reported in other studies (479;480), though conflicting results have also been reported (481;482). It should be noted that though pre-HAART CD4 counts were found to be significantly associated with a CD4 increase of >50 cells/mm^3 in this chapter, the actual effect was small in magnitude, and hence may not have any significant clinical implications.

Patients starting HAART in earlier calendar years were also less likely to achieve a CD4 increase of >50 cells/mm^3. One possible explanation for this is that the importance of adherence may have been less well recognised in earlier years, and new methods to improve adherence may have made an impact on virological/immunological outcomes in later calendar years (483;484). Improvements in drug development, together with better management of drug toxicities may also contribute to the higher proportion of patients achieving increases in later calendar years. Interestingly, patients receiving PI/r-based regimens were more likely to achieve CD4 increases of >50 cells/mm^3 than those receiving NNRTI-based regimens, despite having higher CD4 counts at the start of HAART. It has been reported in some studies that immunological responses are better amongst those receiving PI-based regimens than those receiving NNRTI-based regimens (485-489), however, it is important to note that the significant effect found in my analysis may simply be a chance finding. In sensitivity analyses using the CD4 measure after a year of initiating HAART, there was no significant association seen between HAART regimen and CD4 increase of >50 cells/mm^3 from baseline.

In analyses in which only patients who had a resistance test within 6 months prior to starting HAART were included, the main results remained in line with those seen in the main analyses.

There are limitations to this analysis. Not all patients underwent resistance testing prior to starting HAART and selection in favour of white homosexual males was clearly evident. Given the data used in this study is derived from an observational

cohort study, there may be an effect of unmeasured confounders which may play a part in a patient having both a low GSS and a poorer virological outcome on first-line HAART. For example, patients with a GSS <3 were less likely to start HAART with NNRTI-based regimens. While this suggest adjustments in treatment decisions based upon the results of the resistance test, other factors influencing the choice of regimen, which may be related to the outcome of interest, may not have been captured in this study. Further, I have been unable to fully investigate the viral evolution of patients with GSS <3 who experienced virological failure.

4.5. Summary

In conclusion, I have shown that most but not all patients starting HAART do so on fully active regimens. This proportion is likely to increase with the availability of new drugs in recent calendar years. However, despite an attempt at compensating for the presence of NRTI TDR by using a PI/r-based regimen, patients with a GSS <3 showed poorer virological outcomes than those starting on fully active regimens. These findings indicate that selection of first-line HAART should take into account the presence of TDR, together with other recognised predictors of virological outcomes. The contribution of NRTI TDR to virological failure of PI/r-based regimens has potential clinical significance and warrants further investigation.

Chapter 5: Drug resistance testing post-ART

The main aim of work described in this Chapter is to investigate the impact of resistance testing on changes to treatment. I have also included a sub-section focussing on patients who commenced PI/r-based HAART after failing a first-line NNRTI-based regimen. In this latter Section, I investigate factors associated with failing second-line therapy and, in particular, assess the association between the number of new/fully active NRTIs in the second-line regimen and virological success rates.

5.1. The impact of resistance testing on changes to treatment

5.1.1. Introduction

Resistance testing is recommended for all HIV-positive patients experiencing virological failure of ART to guide changes in therapy (124;490;491). Guidelines also state that any changes in therapy amongst ART-experienced patients should be guided by the results of a resistance test.

Although there has been an attempt to assess the clinical utility of resistance testing using randomised control trials (RCTs), in most instances, randomisation has been performed after a decision to switch therapy has already been made (420;492-496). Some of these studies have shown little benefit of genotypic resistance testing (492;493), instead arguing that adherence and residual treatment options are more important predictors of virological response. However, the majority of these trials highlight the benefits of genotypic resistance testing in treatment experienced patients. For example, Tural et al found that genotypic testing was independently associated with achieving viral load (VL) <400 copies/mL at 24 weeks amongst those who had experienced virological failure (495). Similarly, results from the VIRADAPT trial showed that those who had had genotypic tests performed whilst on a failing HAART regimen in order to guide therapy switches had a sustained reduction in VL compared to those without resistance tests performed (420;497).

Although guidelines for resistance testing have been in place for some time, few studies have assessed the extent to which resistance tests are performed and

whether the results of the resistance tests have led to changes in treatment in routine clinical practice, or the factors associated with specific changes. Clinicians may decide to change only those drugs for which resistance mutations are present, rather than all of the ART drugs in the failing regimen. The level of resistance to the current regimen, the line of current ART regimen (first-line, second-line etc) and the time since starting ART may all impact on this decision.

In this Chapter, I will first investigate changes made to patients' regimens following a resistance test result and identify factors associated with switching regimen, with particular focus on the genotypic sensitivity score (GSS) of the current regimen and the number of regimens previously failed.

5.1.2. Methods

Linked data from the UK HIV Drug Resistance Database (HDRD) and the UK Collaborative HIV Cohort (CHIC) Study were used for these analyses.

5.1.2.1. Inclusion criteria

Patients who had a resistance test performed over the period 1998-2007 whilst on therapy were eligible for inclusion in the analysis if they had at least 4 months follow-up after the date of the result of the resistance test was made available, and if there was no change to their regimen between the date when the blood sample was drawn for the resistance test and the date when the result of the resistance test became available. Chi-squared and Mann-Whitney tests were used to assess differences between tests included and excluded from the analyses.

5.1.2.2. Genotypic sensitivity score (GSS)

The GSS of the current regimen was calculated using the Stanford interpretation algorithm (version 5.1.2). Patients with drug-susceptible virus were assigned a sensitivity score of one, and those with low-level, intermediate, high-level and very high-level resistance were assigned scores 0.75, 0.50, 0.25 and 0 respectively. In Chapter 4, these scores were condensed into just 3 groups (GSS of 1, 0.5 and 0), but with the higher number of patients in this analysis, I felt it was suitable to use 5 groups. If ritonavir was used in conjunction with another PI, it was assumed that ritonavir was used only as a pharmacologic booster, and hence was not considered when calculating the GSS.

5.1.2.3. Defining switches

For the main analysis, I focussed on whether a switch to the existing regimen occurred within 4 months of the date of the resistance test result. Since the definition of ‘switching’ is arbitrary, I used two different methods to calculate the number of patients switching. Using the first method, a patient was defined as having switched regimen subsequent to the resistance test if s/he started two or more drugs on the same date after the resistance test result. This ‘strict’ definition was used in order to identify patients who may have substituted only a single drug, perhaps for toxicity reasons. Using the second method, a switch was defined as starting or stopping at least one drug within 4 months of the resistance test result. This ‘relaxed’ definition was used to capture all switches made after the resistance test report and may have included some switches made for reasons other than drug resistance.

5.1.2.4. Statistical methods

The overall cumulative proportion of patients who switched regimen at any time (not restricted to within 4 months of the resistance test result) was estimated using Kaplan Meier analyses, stratified by whether or not mutations were present at the time of the resistance test. Patient follow-up was censored at the earliest of their last VL and the date of the report of any subsequent resistance test. Logistic regression (using generalised estimating equations to account for more than one test per individual) was used to identify factors independently associated with switching regimen within 4 months of the test result. In these analyses, the following covariates were included: GSS of current regimen, age at time of test, ethnicity, sex/exposure group, year of resistance test, regimen at resistance test (NRTI+NNRTI only, NRTI+PI only, NRTI+PI/r only, NRTI only or Other), CD4 count and viral load (VL) at resistance test, whether or not the VL had ever been <50 copies/mL at any point before the date of the resistance test and the number of regimens previously failed. Failure of a regimen was defined as having a VL >200 copies/mL despite at least one drug in the regimen (which had never been failed in the past) having been used continuously for at least 4 months.

If a patient had taken any drugs before January 1996 they were defined as having failed one line of ART by this time, and all drugs taken before January 1996 were counted as having been failed. I also adjusted for the number of potential drug options available to each patient at the time of the resistance test. This was determined by calculating the total GSS of the drugs that had not been used up to the point of the resistance test, taking into account drug availability at the date of the resistance test (drug availability dates are outlined in Table 1.6.1, Chapter 1.6). Formal tests for interaction between the effects of (i) regimen at resistance test and GSS, (ii) calendar year of resistance test and GSS and (iii) VL at resistance test and GSS were also undertaken.

Sensitivity analyses were performed in which only those patients for whom resistance mutations (GSS <1 for any drug) were present were included in the logistic regression model. Finally, amongst patients who did switch therapy within 4 months of the resistance test result, I compared the GSS of the old regimen to that of the regimen switched to.

5.1.3. Results

5.1.3.1. Differences between patients with tests included and excluded from the analyses

In total, 4,722 of the 22,353 UK CHIC patients who started ART before March 2008 had at least one resistance test performed after starting therapy (8,924 tests in total). Over three-quarters of tests (n=7,211, 80.8 %) were performed whilst patients were taking ART and, of these, 6,059 (84.0%) were amongst patients who had made no change to their regimen between the date of the resistance test and the date of the result of the resistance test. After excluding a further 936 tests without 4 months potential follow-up, 5,123 test results, from 2,567 patients, were included in the analyses. Statistically significant differences were detected amongst patients who had at least one resistance test included in these analyses and those with no resistance tests included, though often the absolute difference was marginal.

Patients with at least one test included in the analyses were more likely to be of MSM risk group (56.1% of MSMs had tests included compared to 46.6% of heterosexuals, p=<0.0001), of white ethnicity (56.1% of patients of white ethnicity had tests included compared to 50.4% of those of black ethnicity, p=0.002) and have started ART in earlier calendar years (of those who started ART before 1996, 65.9% of patients were included in the analyses, compared to 42.1% of patients who started ART after 2005, p<0.0001). Patients with tests included were also more likely to have failed $\geq$5 previous regimens before their first resistance test (81.6% of patients who had failed $\geq$5 regimens had at least one test included, compared to 30.5% of those who had failed no previous regimens, p<0.0001), had lower VLs (3.9 vs. 4.4 log copies/mL, p<0.0001) and higher CD4 counts (283 vs. 260 cells/mm^3, p<0.0001) at the time of their first resistance test.

5.1.3.2. Characteristics of patients with tests included in the analyses

Patients had a median (IQR) 42.1 (39.3, 45.6) months of follow-up after the date of the report of the resistance test and the results of the resistance test were available at a median (IQR) 3.0 (2.0, 4.4) weeks after the resistance test was performed. The characteristics of patients with resistance tests included in the analysis are shown in Table 5.1.3.2.1, with baseline defined as the date on which the resistance test was performed. The median (range) number of tests per patient was 1 (1, 12) and patients had achieved VL <50 copies/mL at some point before the resistance test in 3069 (59.9%) cases. A similar number of tests were performed on patients taking

an NRTI+NNRTI regimen (1524 (29.8%)) and on those taking a NRTI+PI/r regimen (1518 (29.6%)). Over half of tests were performed amongst patients who had experienced all 3 of the original drug classes (NRTI, NNRTI and PI) (52.0%) and amongst patients who had been taking ART for at least 3 years (70.9%). The median (IQR) CD4 and log VL at time of resistance test was 269 (157, 410) cells/mm^3 and 3.8 (3.1, 4.6) copies/mL respectively. In over 10% of cases, patients had failed at least 5 regimens before their resistance test.

Table 5.1.3.2.1: Characteristics and demographics of patients who had ≥1 resistance test after starting ART

Total number of resistance tests		**5123**
Number of resistance tests per patient	Median (range)	1 (1,12)
Year of starting ART n (%)	<1996	1504 (29.4)
	1996-1998	1939 (37.9)
	1999-2001	805 (15.7)
	2002-2004	618 (12.1)
	2005-2008	257 (5.0)
Year of resistance test n (%)	1998-2001	1699 (33.2)
	2002-2004	1761 (34.4)
	2005-2007	1663 (32.5)
Regimen at resistance test n (%)	NNRTI	1524 (29.8)
	Single PI	700 (13.7)
	PI/r	1518 (29.6)
	Nucleoside only	895 (17.5)
	Other	486 (9.5)
Number of classes (NRTI, NNRTI and PI)	1	337 (6.6)
experienced n (%)	2	2172 (42.4)
	3	2614 (51.0)
Drug options available at resistance test	0-3	795 (15.5)
	4-6	1183 (23.1)
	7-9	1237 (24.1)
	≥10	1908 (37.2)
Time since start of ART n (%)	≤3 months	203 (4.0)
	4 – 12 months	394 (7.7)
	1.1 - 3 years	893 (17.4)
	3.1 - 5 years	1037 (20.2)
	5.1 – 7 years	894 (17.5)
	7.1 – 9 years	760 (14.8)
	≥10 years	942 (18.4)
Ethnicity n (%)	White	3206 (62.6)
	Black	1396 (27.3)

	Other	521 (10.2)
Sex/Exposure n (%)	MSM	3062 (59.8)
	Heterosexual male	673 (13.1)
	Heterosexual female	909 (17.7)
	Other	479 (9.4)
CD4 count at resistance test (cells/mm^3)	Median (IQR)	269 (157, 410)
Viral load at resistance test (log copies/mL)	Median (IQR)	3.8 (3.1, 4.6)
Age at resistance test (years)	Median (IQR)	35 (31, 45)
Number of regimens failed before resistance test[1] n (%)	0	436 (8.5)
	1	1410 (27.5)
	2	1171 (22.9)
	3	890 (17.4)
	4	615 (12.0)
	5 or more	601 (11.7)
VL <50 copies/mL at any point before resistance test n (%)	Yes	3069 (59.9)

[1] Failure defined as VL >200 copies/mL despite 4 months of continuous use of any drug. Drugs taken before 1996 for >122 days are defined as a line of failure and will not be counted as subsequent failures after 1996.

5.1.3.3. GSS at the time of the resistance test

Table 5.1.3.3.1 shows the GSS at the time of the resistance test, stratified by the drug class basis of the current regimen. Amongst resistance tests which were performed in those taking an NRTI+NNRTI regimen, the GSS was <1 in 603 (39.6%) cases, compared to 357 (23.5%) cases in which tests were performed amongst those receiving an NRTI+PI/r regimen and 258 (36.9%) cases in which tests were performed in those receiving an NRTI+single-PI regimen. The GSS was more likely to be $\geq$3 amongst tests performed on patients receiving an NRTI-PI/r therapy (48.3%) than among those receiving NRTI+NNRTI (26.6%) or NRTI+single PI (21.4%) based therapy.

Table 5.1.3.3.1: Genotypic sensitivity score (GSS) stratified by regimen at resistance test

		Regimen at resistance test n (%)					
		NNRTI + NRTIs	**PI/r + NRTIs**	**PI + NRTIs**	**NRTIs only**	**Other**	**Total**
	N	1524	1518	700	895	486	5123
	Mean (SD) GSS	2 (1.3)	3 (1.4)	2 (1.2)	2 (1.1)	2 (1.4)	2 (1.4)
GSS	**<1**	603 (39.6)	357 (23.5)	258 (36.9)	525 (58.7)	203 (41.8)	1946 (38.0)
	1-1.75	342 (22.4)	199 (13.1)	179 (25.6)	188 (21.0)	112 (23.1)	1020 (19.9)
	2-2.75	173 (11.4)	229 (15.1)	113 (16.1)	76 (8.5)	59 (12.1)	650 (12.7)
	3-3.75	340 (22.3)	590 (38.9)	136 (19.4)	67 (7.5)	43 (8.9)	1176 (23.0)
	>4	66 (4.3)	143 (9.4)	14 (2.0)	39 (4.4)	69 (14.2)	331 (6.5)

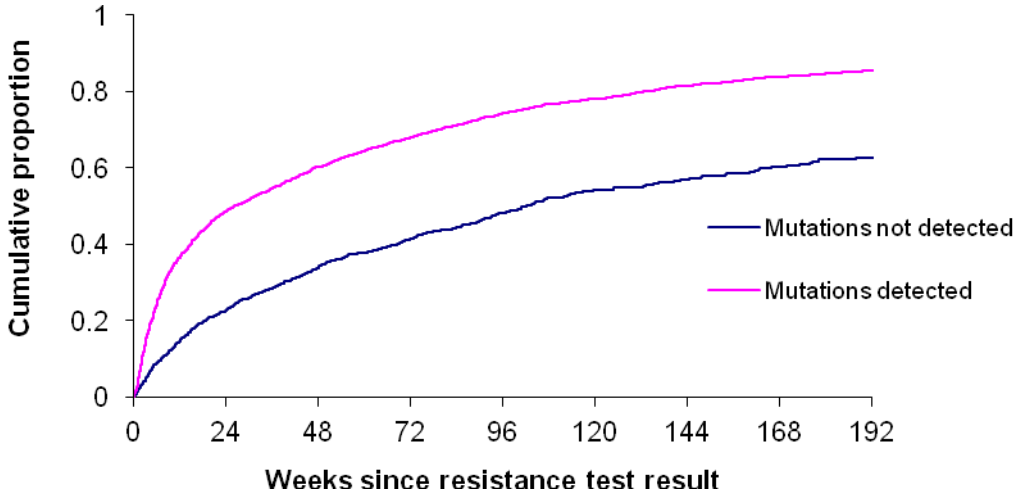
SD: Standard deviation

5.1.3.4. Switches to regimen at any time after the date of the result of the resistance test

Using the strict definition of a switch, 3471/5123 (67.8%) patients switched therapy at a Kaplan-Meier median (95% CI) time of 42 (39, 46) weeks after having received the result of the resistance test. Switches were made in 2775/3620 (76.7%) instances where mutations were detected and in 696/1503 (46.3%) cases where mutations were not detected. Figure 5.1.3.4.1 shows the Kaplan Meier time to switching stratified by whether or not mutations were detected. The median (95% CI) time to switching was significantly shorter amongst those who had mutations detected (26 (24, 30) weeks) compared to those who had no mutations detected (102 (93, 115) weeks) (p<0.0001).

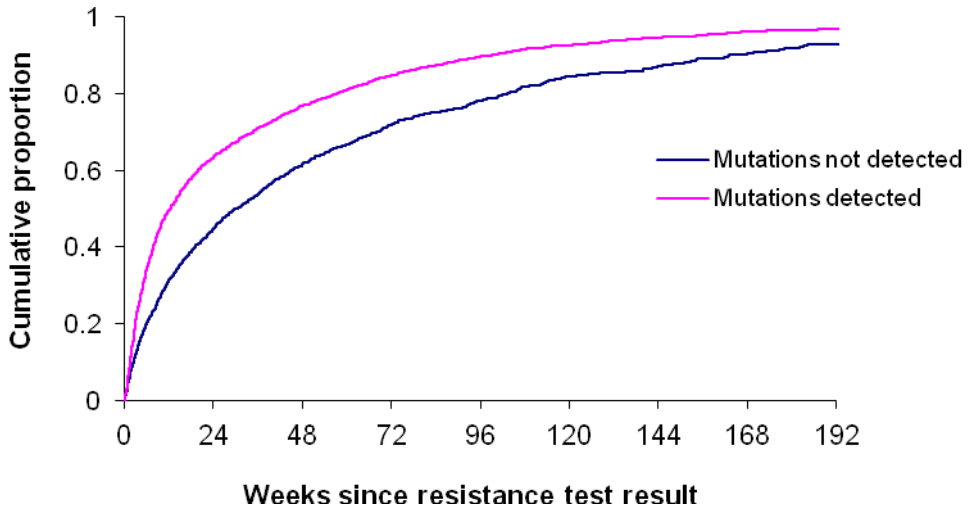
Using the relaxed definition of a switch, 4610 (90.0%) patients switched therapy at a median (95% CI) 16 (15, 17) weeks after the date of the result of the resistance test. Switches were made in 3413/3620 (94.3%) cases at a median (95% CI) 12 (11, 14) weeks where mutations were detected and in 1197/1503 (79.6%) cases at a median (95% CI) 31 (27, 34) weeks where mutations were not detected (p<0.0001) (Figure 5.1.3.4.2).

Figure 5.1.3.4.1: Kaplan Meier estimates of the proportion of patients switching regimen at any time after resistance test result: strict definition of switching



N	5123	2778	1922	1378	978	722	534	403	320

Figure 5.1.3.4.2: Kaplan Meier estimates of the proportion of patients switching regimen at any time after resistance test result: relaxed definition of switching



N	5123	2085	1250	783	513	331	223	142	102

5.1.3.5. Switches to therapy within 4 months of the resistance test result

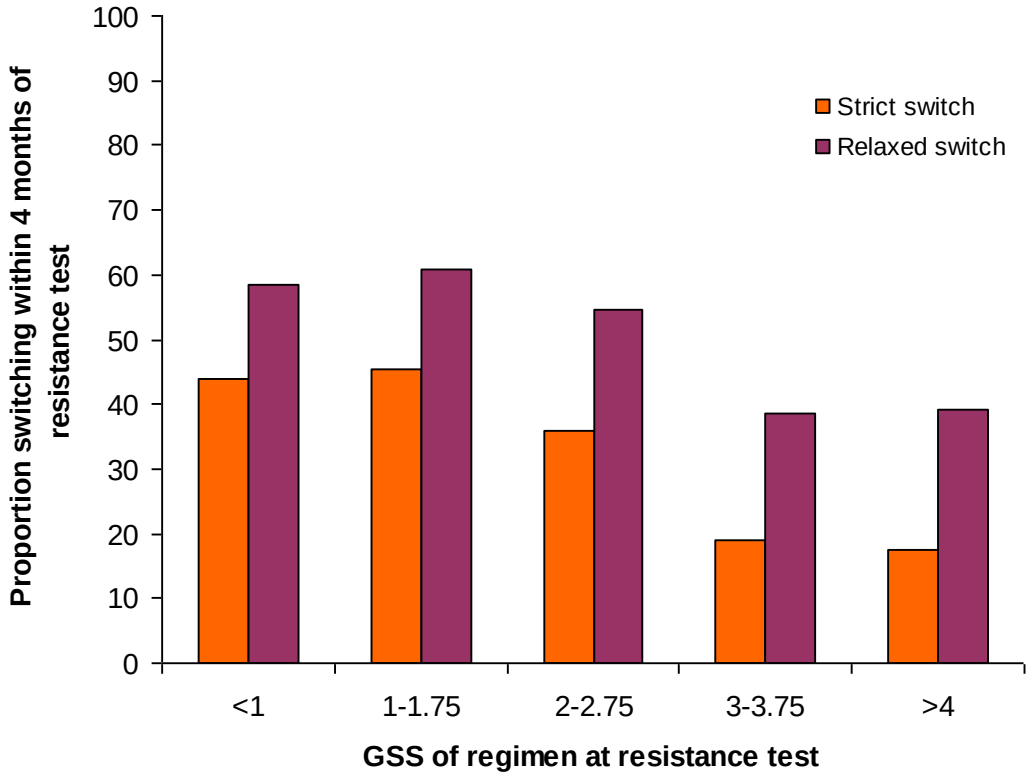
Using the strict definition, a switch to therapy occurred in 1,834 (35.8%) cases within 4 months of the resistance test result. 1550 (42.8%) of resistance tests for which resistance mutations were detected were followed by a switch in the next 4 months, compared to 284 (18.9%) resistance tests in which mutations were not detected ($p<0.0001$). The number of patients switching therapy within 4 months of the resistance test result when using the relaxed definition of a switch was considerably higher at 2693 (52.6%). Of those tests for which mutations were detected, 2118 (58.5%) were followed by a switch in the next 4 months, compared to 575 (38.3%) resistance tests in which mutations were not detected ($p<0.0001$). All subsequent analyses are restricted to 4 months follow-up after date of resistance test result.

5.1.3.6. GSS and switches to therapy

A decreasing trend was seen in the proportion of tests followed by a strict switch with increasing GSS of the current regimen; of those with GSS <1, 1-1.75, 2.-2.75, 3-3.75 and $\geq$4, 44.0%, 45.3%, 36.0%, 19.0% and 17.5% of resistance tests were followed by a switch within 4 months of the resistance test result (Figure 5.1.3.6.1). A similar trend was observed using the relaxed definition of a switch, albeit with higher absolute proportions in each category: of those with GSS <1, 1-1.75, 2.-2.75,

3-3.75 and ≥4, 58.4%, 60.7%, 54.5%, 38.5% and 39.3% of resistance tests were followed by a switch within 4 months of the resistance test result (p<0.0001).

Figure 5.1.3.6.1: Proportion of patients who switched regimen within 4 months of resistance test result, stratified by GSS



5.1.3.7. Future drug options

Patients had a median of 8 (5, 11) future drug options available. Table 5.1.3.7.1 shows the available future drug options stratified by the GSS at the time of the resistance test. A third of patients with a GSS <1 had 0-3 future drug options available, whilst half of those with GSS ≥4 had ≥10 future drug options available. Generally, the number of future drug options available increased as the GSS of the current regimen increased.

Table 5.1.3.7.1: Future drug options stratified by GSS at the time of the resistance test

		GSS of current regimen at time of resistance test				
		<1	**1-1.75**	**2-2.75**	**3-3.75**	**≥4**
Number of future drug options available	**0-3**	622 (32.0)	102 (10.0)	45 (6.9)	16 (1.4)	10 (3.0)
	4-6	599 (30.8)	253 (24.8)	145 (22.3)	114 (9.7)	72 (21.8)
	7-9	463 (23.8)	288 (28.2)	167 (25.7)	235 (20.0)	84 (25.4)
	≥10	262 (13.5)	377 (37.0)	293 (45.1)	811 (69.0)	165 (49.9)

5.1.3.8. Differences between those who switched regimen and those who did not

Table 5.1.3.8.1 shows the characteristics of all patients with resistance tests included in the analyses, stratified by definition of switch, and by whether or not a switch took place. Using the strict definition of switching, no associations were seen between year of starting HAART and switching, whilst using the relaxed definition, borderline significant associations were identified with in particular, patients being less likely to switch in later calendar years. Other than risk group and ethnicity, statistically significant associations were identified between all other variables and switching, regardless of the definition of switch. However, there were marked differences in proportions of patients switching according to the definition used. In particular, 20.7% of patients receiving PI/r-based regimens switched therapy under the strict definition, compared to 43.4% under the relaxed definition.

Table 5.1.3.8.1: Characteristics of patients with resistance tests post-ART according to whether a switch was made to the regimen within 4 months of resistance test result

		Strict[1] definition of switching			Relaxed[2] definition of switching		
		No switch	**Switch**	**P-value[3]**	**No switch**	**Switch**	**P-value[3]**
		3289	**1834**		**2430**	**2693**	
Mutations detected n (%)	No	1219 (81.1)	284 (18.9)	<0.0001	928 (61.7)	575 (38.3)	<0.0001
	Yes	2070 (57.2)	1550 (42.8)		1502 (41.5)	2118 (58.5)	
GSS	<1	1089 (56.0)	857 (44.0)	<0.0001	809 (41.6)	1137 (58.4)	<0.0001
	1-1.75	558 (54.7)	462 (45.3)		401 (39.3)	619 (60.7)	
	2-2.75	416 (64.0)	234 (36.0)		296 (45.5)	354 (54.5)	
	≥3	273 (82.5)	58 (17.5)		924 (61.3)	583 (38.7)	
Year of starting HAART	<1996	968 (64.4)	536 (35.6)	0.36	737 (49.0)	767 (51.0)	0.06
n (%)	1996-1998	1253 (64.6)	686 (35.4)		918 (47.3)	1021 (52.7)	
	1999-2001	500 (62.1)	305 (37.9)		356 (44.2)	449 (55.8)	
	2002-2004	391 (63.3)	227 (36.7)		282 (45.6)	336 (54.4)	
	2005-2008	177 (68.9)	80 (31.1)		137 (53.3)	120 (46.7)	
Regimen at resistance test	NNRTI + NRTIs	851 (55.8)	673 (44.2)	<0.0001	637 (41.8)	887 (58.2)	<0.0001
n (%)	Single PI +NRTIs	349 (49.9)	351 (50.1)		267 (38.1)	433 (61.9)	
	PI/r +NRTIs	1203 (79.3)	315 (20.7)		859 (56.6)	659 (43.4)	

	NRTIs only	552 (61.7)	343 (38.3)		434 (48.5)	461 (51.5)	
	Other	334 (68.7)	152 (31.3)		233 (47.9)	253 (52.1)	
Time between start of HAART	≤3 months	142 (70.0)	61 (30.0)	<0.0001	109 (53.7)	94 (46.3)	<0.0001
and resistance test (months)	3.1 – 12 months	216 (54.8)	178 (45.2)		145 (36.8)	249 (63.2)	
N (%)	1.1 - 3 years	521 (58.3)	372 (41.7)		373 (41.8)	520 (58.2)	
	3.1 - 5 years	658 (63.5)	379 (36.6)		470 (45.3)	567 (54.7)	
	5.1 – 7 years	592 (66.2)	302 (33.8)		427 (47.8)	467 (52.2)	
	7.1 – 9 years	525 (69.1)	235 (30.9)		409 (53.8)	351 (46.2)	
	≥10 years	635 (67.4)	307 (32.6)		497 (52.8)	445 (47.2)	
CD4 at resistance test (cells/mm^3)	Median (IQR)	290 (172, 430)	243 (137, 370)	<0.0001	290 (172, 430)	250 (140, 390)	<0.0001
Viral load at resistance test (log copies/mL)	Median (IQR)	3.7 (3.1, 4.5)	4.0 (3.4, 4.6)	<0.0001	3.8 (3.1, 4.5)	3.9 (3.3, 4.6)	<0.0001
Age at start of HAART (years)	Median (IQR)	40 (35, 45)	39 (35, 44)	0.07	40 (35, 45)	39 (35, 44)	0.004
Number of drugs	3	530 (58.4)	377 (41.6)	<0.0001	435 (48.0)	472 (52.0)	<0.0001
experienced	4-6	905 (59.0)	629 (41.0)		654 (42.6)	880 (57.4)	
	7-9	977 (66.1)	502 (33.9)		686 (46.4)	793 (53.6)	
	10-12	636 (72.0)	247 (28.0)		482 (54.6)	401 (45.4)	
	13-15	199 (75.4)	65 (24.6)		144 (54.6)	120 (45.5)	
	>15	42 (75.0)	14 (25.0)		29 (51.8)	27 (48.2)	

Number of classes	1	223 (66.2)	114 (33.8)	<0.0001	181 (53.7)	156 (46.3)	<0.0001
experienced	2	1262 (58.1)	910 (41.9)		938 (43.2)	1234 (56.8)	
	3	1804 (69.0)	810 (31.0)		1311 (50.2)	1303 (49.9)	
Ethnicity n (%)	White	2088 (65.1)	1118 (34.9)	0.20	1520 (47.4)	1686 (52.6)	0.97
	Black	873 (62.5)	523 (37.5)		665 (47.6)	731 (52.4)	
	Other	328 (63.0)	193 (37.0)		245 (47.0)	276 (53.0)	
Sex/Exposure n (%)	MSM	1981 (64.7)	1081 (35.3)	0.50	1429 (46.7)	1633 (53.3)	0.15
	Heterosexual (m)	417 (62.0)	256 (38.0)		312 (46.4)	361 (53.6)	
	Heterosexual (f)	577 (63.5)	332 (36.5)		440 (48.4)	469 (51.6)	
	Other	314 (65.6)	165 (34.5)		249 (52.0)	230 (48.0)	
Number of regimens failed	0	121 (72.9)	45 (27.1)	0.07	99 (59.6)	67 (40.4)	0.01
before resistance test n	1	606 (66.0)	312 (34.0)		464 (50.5)	454 (49.5)	
(%)	2	606 (61.5)	379 (38.5)		456 (46.3)	529 (53.7)	
	3	596 (63.4)	344 (36.6)		434 (46.2)	506 (53.8)	
	4	537 (63.9)	304 (36.1)		395 (47.0)	446 (53.0)	
	5	823 (64.7)	450 (35.3)		582 (45.7)	691 (54.3)	
VL <50 copies/mL at any	No	1284 (62.5)	770 (37.5)	0.04	944 (46.0)	1110 (54.0)	0.08
point before resistance test	Yes	2005 (65.3)	1064 (34.7)		1486 (48.4)	1583 (51.6)	

[1] Starting at least two drugs within 4 months of the resistance test result; [2] Starting/stopping at least one drug within 4 months of the resistance test result;

[3] Obtained by Chi-Squared/Mann-Whitney tests

5.1.3.9. Factors associated with switching regimen

With the exception of mutations detected, number of drugs experienced and number of classes experienced, all variables in Table 5.1.3.8.1 were included in logistic regression analyses. I chose to include GSS in the models, as opposed to whether mutations were, or were not detected, since GSS better captures clinically relevant resistance carried by patients. The number of drugs/classes experienced is somewhat captured by the number of regimens previously failed. I felt the latter variable was also more clinically relevant and hence did not include the former variables due to co-linearity with the number of regimens failed. In univariable analyses, using the strict definition of switching, the GSS of the current regimen was a significant predictor of switching regimen within 4 months of the resistance test result (OR (95% CI) for GSS <1 compared to $\geq$3: 3.43 (2.93, 4.02)). Patients receiving PI/r-based regimens were less likely to switch than those receiving NNRTI-based regimens (0.33 (0.28, 0.39)), as were patients who had failed a greater number of previous regimens (0.92 (0.88, 0.95) per additional regimen). A reduced, albeit still significant, effect of GSS was seen when using the relaxed definition of switching (2.23 (1.94, 2.56) for GSS <1 compared to $\geq$3). Patients receiving PI/r-based regimens were again less likely to switch than those receiving NNRTI-based regimens (0.55 (0.48, 0.64)), as were patients who had failed a greater number of previous regimens (0.94 (0.91, 0.98) per additional regimen).

After adjusting for other possible confounders, the GSS of the current regimen was a significant predictor of switching regimen within 4 months of the resistance test result, regardless of the definition used for switching (4.82 (3.92, 5.93) and 2.91 (2.42, 3.51) for GSS <1 compared to GSS $\geq$3 using the strict definition and relaxed definition, respectively, Table 5.1.3.9.1). Using the strict definition, patients with fewer drug options were less likely to switch (0.46 (0.34, 0.61) comparing 0-3 drug options with $\geq$10 drug options), whilst no association was seen between the number of regimens previously failed and switching regimen (0.98 (0.92, 1.05) per additional failed regimen). Tests performed amongst patients receiving PI/r-based regimens were less likely to result in a switch than those performed on patients receiving NNRTI-based regimens (0.47 (0.39, 0.56)), whilst tests performed on those receiving single PI-based regimens were more likely to result in a switch than those performed on patients receiving NNRTI-based regimens (1.41 (1.16, 1.72)). Lower CD4 count at time of resistance test (2.06 (1.74, 2.44) for those with CD4<200 cells/mm^3 compared to CD4>350 cells/mm^3 and higher VL (0.36 (0.27, 0.49) comparing VL <400 with VL >100,000 copies/mL) at the time of the resistance test

were also independently associated with switching regimen. Patients who had achieved viral suppression (at any time point, not necessarily on the regimen they were failing) before having the resistance test performed were more likely to switch regimens than those who had not (1.27 (1.10, 1.47)).

Estimates using the relaxed definition of switching were generally in the same direction as those obtained using the strict definition of switching, though were less pronounced and hence closer to one in most cases.

Table 5.1.3.9.1: Independent factors associated with switching regimen, stratified by definition of switching

		Strict definition[1]		Relaxed definition[2]	
		OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**
GSS at time of resistance test	<1	4.82 (3.92, 5.93)	<0.0001	2.91 (2.42, 3.51)	<0.0001
	1-1.75	4.09 (2.02, 3.14)		2.73 (1.57, 2.33)	
	2-2.75	2.52 (2.02, 3.14)		1.24 (1.09, 1.42)	
	≥3	1		1	
Drug options remaining	0-3	0.46 (0.34, 0.61)	<0.0001	0.59 (0.45, 0.77)	<0.0001
	4-6	0.85 (0.68, 1.07)		0.94 (0.76, 1.16)	
	7-9	0.91 (0.75, 1.11)		0.90 (0.75, 1.08)	
	≥10	1		1	
Age	Per 10 years older	0.99 (0.91, 1.07)	0.81	0.94 (0.88, 1.02)	0.13
Year of resistance test	≤1999	1	0.68	1	0.81
	1999-2001	0.94 (0.76, 1.17)		0.96 (0.78, 1.19)	
	2002-2003	0.97 (0.77, 1.23)		1.01 (0.81, 1.26)	
	2004-2005	1.06 (0.82, 1.37)		0.93 (0.73, 1.19)	
	2006-2007	0.93 (0.69, 1.23)		0.90 (0.69, 1.17)	
Regimen at resistance test	NNRTI + NRTIs	1	<0.0001	1	<0.0001
	PI/r + NRTIs	0.47 (0.39, 0.56)		0.77 (0.66, 0.91)	

	Single PI + NRTIs	1.41 (1.16, 1.72)		1.22 (1.00, 1.49)	
	NRTIs only	0.68 (0.56, 0.81)		0.69 (0.58, 0.83)	
	Other	0.72 (0.56, 0.92)		0.95 (0.76, 1.20)	
Time between start of ART and resistance test (months)	≤3	1.04 (0.69, 1.57)	0.01	1.23 (0.85, 1.78)	<0.0001
	4 – 24	1.44 (1.13, 1.83)		1.77 (1.41, 2.23)	
	25 – 36	1.30 (1.01, 1.69)		1.51 (1.18, 1.93)	
	37 – 60	1.08 (0.88, 1.31)		1.30 (1.08, 1.56)	
	61 – 84	0.97 (0.80, 1.17)		1.17 (0.98, 1.40)	
	>84	1		1	
Ethnicity	White	1	0.86	1	0.84
	Black	1.06 (0.86, 1.29)		0.95 (0.79, 1.14)	
	Other/unknown	1.03 (0.83, 1.28)		0.99 (0.81, 1.21)	
Sex/Exposure	MSM	1	0.51	1	0.10
	Heterosexual male	1.13 (0.90, 1.41)		1.02 (0.82, 1.25)	
	Heterosexual female	1.02 (0.82, 1.28)		0.93 (0.76, 1.14)	
	Other/unknown	0.91 (0.73, 1.14)		0.78 (0.64, 0.96)	
Number of regimens failed before resistance test	Per 1 regimen greater	0.98 (0.92, 1.05)	0.55	1.00 (0.94, 1.06)	0.97
CD4 at resistance test cells/mm^3	≤200	2.06 (1.74, 2.44)	<0.0001	1.69 (1.44, 1.97)	<0.0001
	200-350	1.35 (1.15, 1.59)		1.13 (0.97, 1.30)	

	>350	1		1	
	Missing	1.20 (0.66, 2.15)		1.25 (0.73, 2.14)	
VL <50 copies/mL before resistance test	Yes	1.27 (1.10, 1.47)	0.001	1.24 (1.09, 1.42)	0.001
VL at resistance test	<400 copies/mL	0.36 (0.27, 0.49)	<0.0001	0.47 (0.37, 0.62)	<0.0001
	401-1000 copies/mL	0.76 (0.56, 1.02)		0.81 (0.61, 1.07)	
	1001-10,000 copies/mL	0.68 (0.55, 0.84)		0.78 (0.63, 0.96)	
	10,000-30,000 copies/mL	0.82 (0.65, 1.04)		0.79 (0.63, 0.98)	
	30,000-100,000 copies/mL	0.78 (0.61, 0.99)		0.81 (0.64, 1.02)	
	>100,000 copies/mL	1		1	
	Missing	0.56 (0.42, 0.76)		0.62 (0.47, 0.81)	

[1] Starting at least two drugs within 4 months of the resistance test result

[2] Starting/stopping at least one drug within 4 months of the resistance test result

5.1.3.10. Interactions with GSS

A significant interaction was identified between regimen at the time of the resistance test and the GSS (fitted as a continuous variable, likelihood ratio test p=0.04 and p=0.01 using strict and relaxed definitions respectively), after adjusting for other variables. This was mainly driven by the 'other' regimen category – amongst those with a GSS of 0, those who were receiving other regimens were less likely to switch their regimen than those receiving NNRTI-containing regimens (OR=0.54, p<0.0001), but this effect attenuated as the GSS increased (OR for interaction=1.32, p=0.001). The ORs (95% CIs) for 'other' regimen when the GSS was <1, 1-1.75, 2-2.75 and $\geq$3 were 0.74 (0.50, 1.09), 0.59 (0.35, 0.98), 0.37 (0.17, 0.80) and 1.25 (0.70, 2.21) respectively.

There was also a significant interaction between calendar year and GSS (p=0.03 and 0.001 using the strict and relaxed definitions respectively). Patients with resistance tests performed in later calendar years were more likely to switch regimen when they had a GSS of 0 (OR=1.55 comparing resistance tests performed between 2004 and 2005 to before 1999, p=0.01) but this effect was reduced as the GSS increased (OR for interaction=0.75, p=0.003). The ORs (95% CIs) comparing resistance tests performed between 2004 and 2005 to before1999 when the GSS was <1, 1-1.75, 2-2.75 and $\geq$3 were 0.95 (0.65, 1.39), 1.52 (0.85, 2.70), 0.91 (0.41, 2.01) and 0.98 (0.47, 2.05) respectively

There was no significant interaction between VL at resistance test and GSS (p=0.93).

5.1.3.11. Restricting analyses to only those with mutations detected

I then considered analyses in which patients without detectable resistance mutations were removed from the dataset. Of the 3,620 patients remaining in the dataset, 1,550 (42.8%) made a switch (strict definition) to their regimen within 4 months of the resistance test result. The proportion of patients with GSS <1, 1-1.75, 2-2.75 and $\geq$3 who switched regimen were 44.1%, 45.6%, 37.4% and 43.9%, respectively.

Factors associated with switching regimen in univariable and multivariable analyses are shown in Table 5.1.3.11.1. The independent association between GSS and probability of switching regimen was weakened compared to the main analysis, but was still significant (2.08 (1.41, 3.08)) for GSS <1 compared to GSS $\geq$3). The number of future drug options available (0.42 (0.31, 0.58) for 0-3 drugs compared to $\geq$10 drugs) was also still independently associated with switching regimen after

adjusting for potential confounders. Patients who had resistance tests performed whilst receiving a PI/r regimen were less likely to switch regimen than those receiving an NNRTI-based regimen (0.43 (0.34, 0.54)), whilst those who were receiving a single PI-based regimen were more likely to switch (1.35 (1.08, 1.68)). Patients who had failed a greater number of regimens before having a resistance test performed were less likely to switch in univariable analyses (0.81 (0.78, 0.85) per additional failed regimen) but this association was not significant after adjusting for potential confounders (0.96 (0.89, 1.03)).

Similar results were seen when using the relaxed definition of switching. Of the 3,620 patients with mutations detected, 2118 (58.5%) switched regimen within 4 months of the resistance test result. Patients with a GSS <1 were more likely to switch (1.45 (1.01, 2.07) compared to GSS $\geq$3), though again, this estimate was weaker that that seen in the main results.

Table 5.1.3.11.1: Factors associated with a strict switch of regimen, restricted to those who had mutations detected (N=3620)

		Univariable analyses		Multivariable analyses	
		OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**
GSS at time of resistance	<1	1.80 (1.27, 2.53)	0.0001	2.08 (1.41, 3.08)	<0.0001
test	1-1.75	1.91 (1.34, 2.72)		1.71 (1.16, 2.54)	
	2-2.75	1.36 (0.93, 1.98)		1.09 (0.72, 1.64)	
	≥3	1		1	
Drug options remaining	0-3	0.43 (0.35, 0.52)	<0.0001	0.42 (0.31, 0.58)	<0.0001
	4-6	0.72 (0.60, 0.86)		0.73 (0.57, 0.94)	
	7-9	0.74 (0.62, 0.89)		0.77 (0.62, 0.97)	
	≥10	1		1	
Age	Per 10 years older	0.95 (0.88, 1.03)	0.21	1.04 (0.95, 1.14)	0.38
Year of resistance test	≤1999	1	0.30	1	0.65
	1999-2001	0.95 (0.77, 1.18)		0.97 (0.77, 1.23)	
	2002-2003	0.87 (0.71, 1.07)		0.96 (0.75, 1.24)	
	2004-2005	0.97 (0.78, 1.21)		1.14 (0.86, 1.52)	
	2006-2007	0.79 (0.62, 1.02)		1.02 (0.73, 1.42)	
Regimen at resistance test	NNRTI + NRTIs	1	<0.0001	1	<0.0001
	PI/r + NRTIs	0.32 (0.27, 0.39)		0.43 (0.34, 0.54)	

	Single PI + NRTIs	1.09 (0.89, 1.34)		1.35 (1.08, 1.68)	
	NRTIs only	0.61 (0.51, 0.74)		0.64 (0.52, 0.78)	
	Other	0.43 (0.33, 0.54)		0.64 (0.48, 0.84)	
Time between start of ART and resistance test (months)	≤3	2.82 (1.75, 4.53)	<0.0001	1.67 (0.95, 2.94)	0.02
	4 – 24	2.35 (1.90, 2.90)		1.57 (1.18, 2.08)	
	25 – 36	1.61 (1.26, 2.05)		1.20 (0.89, 1.61)	
	37 – 60	1.29 (1.08, 1.56)		1.07 (0.86, 1.35)	
	61 – 84	1.13 (0.93 1.37)		1.00 (0.81, 1.24)	
	>84	1		1	
Ethnicity	White	1	0.004	1	0.99
	Black	1.30 (1.12, 1.52)		1.00 (0.80, 1.26)	
	Other/unknown	1.12 (0.90, 1.40)		0.99 (0.78, 1.26)	
Sex/Exposure	MSM	1	0.01	1	0.74
	Heterosexual male	1.35 (1.10, 1.66)		1.16 (0.90, 1.50)	
	Heterosexual female	1.25 (1.04, 1.50)		1.05 (0.81, 1.37)	
	Other/unknown	1.16 (0.92, 1.47)		1.05 (0.81, 1.36)	
Number of regimens failed before resistance test	Per 1 regimen greater	0.81 (0.78, 0.85)	<0.0001	0.96 (0.89, 1.03)	0.24
CD4 at resistance test cells/mm^3	≤200	1.84 (1.57, 2.16)	<0.0001	2.16 (1.78, 2.62)	<0.0001
	200-350	1.35 (1.14, 1.59)		1.38 (1.15, 1.65)	

	>350	1		1	
	Missing	1.63 (0.85, 3.11)		1.31 (0.66, 2.62)	
VL <50 before resistance test	Yes	0.95 (0.83, 1.08)	0.44	1.29 (1.10, 1.52)	0.002
VL at resistance test	<400 copies/mL	0.32 (0.23, 0.44)	<0.0001	0.35 (0.24, 0.52)	<0.0001
	401-1000 copies/mL	0.68 (0.50, 0.93)		0.68 (0.48, 0.97)	
	1001-10,000 copies/mL	0.71 (0.56, 0.90)		0.68 (0.52, 0.89)	
	10,000-30,000 copies/mL	0.79 (0.61, 1.03)		0.78 (0.58, 1.04)	
	30,000-100,000 copies/mL	0.82 (0.62, 1.08)		0.79 (0.58, 1.05)	
	>100,000 copies/mL	1		1	
	Missing	0.62 (0.44, 0.86)		0.57 (0.40, 0.82)	

5.1.3.12. GSS of new regimen compared to GSS of old regimen

Finally, amongst the patients who did make a switch to their therapy within 4 months of the resistance test result, the GSS of the new regimen was calculated and compared to the GSS of the regimen taken at the time of the resistance test (old regimen). Amongst those who made a strict switch to therapy (n=1834) and had an old regimen GSS of <1, 12% still had a GSS <1 after switching regimens (Table 5.1.3.12.1). Forty-five percent had a new GSS of 1-1.75 and 35% had a new GSS of 2-2.75. Only 7% of patients had a new GSS $\geq$3. Most patients with an old GSS of 1-1.75 had a new GSS of $\geq$2, though I did identify 4 patients who had a new GSS of <1 and 12 patients who had a new GSS of 1-1.75. Amongst patients with an old GSS $\geq$2, most patients had a new GSS at least that of the old regimen. However, a third of patients with an old GSS $\geq$4 had a new GSS <4.

Using the relaxed definition of switching, 30% of patients with an old GSS <1 had a new GSS of <1 after switching therapy (Table 5.1.3.12.2). Of those with an old GSS of 1-1.75, 18% still had a new GSS of 1-1.75. Around 14% of patients with an old GSS of $\geq$2 had a new GSS <2.

Table 5.1.3.12.1: GSS of regimen at resistance test (old regimen) stratified by GSS of regimen switched to (new regimen) (N=1834): strict definition of switching

Overall mean (SD) increase in GSS from old to new regimen: 1.2 (0.9)

			GSS of new regimen n (%)				
		Mean increase	<1	1-1.75	2-2.75	3-3.75	≥4
GSS of old regimen	<1	1.4	103 (12)	384 (45)	304 (35)	64 (7)	2 (0)
	1-1.75	1.5	4 (1)	55 (12)	222 (48)	155 (33)	26 (6)
	2-2.75	1.0	1 (0)	13 (6)	51 (22)	121 (52)	48 (20)
	3-3.75	0.5	0 (0)	2 (1)	16 (7)	114 (51)	91 (41)
	≥4	0.1	0 (0)	1 (2)	1 (2)	18 (31)	38 (65)

Table 5.1.3.12.2: GSS of regimen at resistance test (old regimen) stratified by GSS of regimen switched to (new regimen) (N=2693): relaxed definition of switching

Overall mean (SD) increase in GSS from old to new regimen: 1.0 (1.0)

			GSS of new regimen n (%)				
		Mean increase	<1	1-1.75	2-2.75	3-3.75	≥4
GSS of old regimen	<1	1.2	341 (30)	426 (37)	304 (27)	64 (6)	2 (0)
	1-1.75	1.3	69 (11)	111 (18)	258 (42)	155 (25)	26 (4)
	2-2.75	0.8	31 (8)	20 (6)	89 (25)	166 (47)	48 (14)
	3-3.75	0.4	58 (13)	5 (1)	33 (7)	211 (46)	146 (32)
	≥4	-0.1	12 (9)	3 (2)	3 (2)	32 (25)	80 (62)

5.1.4. Discussion

These analyses were restricted to patients who had a resistance test performed whilst receiving ART, and in whom the result of the resistance test was made available to the clinician within 4 months of the date of the test. An added restriction that patients did not change therapy between the date of the resistance test and the date when the result was received was also enforced. This strict criterion was used in order to assess changes made to therapy based on the outcome of the resistance test, rather than changes made for other reasons, such as tolerability, pill burden and virological failure. I used two different definitions of a switch to therapy since there is no gold-standard definition. The strict definition (starting at least 2 new drugs) is perhaps more likely to be linked with the result of the resistance test as it excludes patients who switched a single drug, perhaps for toxicity reasons. However, clinicians do sometimes switch patients with resistance mutations to single PI (Personal communication, Gazzard) therapy or make switches to only those drugs for which resistance mutations are present. These cases will have been captured in the relaxed definition of a switch.

The definition of a switch impacted considerably on the proportion of tests which were identified as being followed by a switch within 4 months of the resistance test result. Using the strict definition, 36% of tests were followed by a switch, compared to 56% when using the relaxed definition. This suggests a substantial number of patients only made a single change to their regimen within 4 months of the resistance test result. In both cases, patients with mutations detected had an increased likelihood of switching and, in particular, the proportion of patients switching decreased as the GSS of the current regimen increased. Interestingly, the difference in the proportion of patients making a switch using the strict and relaxed definitions was less pronounced if the GSS of the current regimen was low (difference in proportion of patients switching regimen with GSS <1=14%; difference in proportion of patients switching regimen with GSS >3=21%).

A third of patients with low GSS (<1) to the current regimen had only 0-3 future drug options available. This may explain why some patients with low GSS did not switch regimens, and amongst those that did, the reason for the equally low GSS of the new regimen. Those with higher current GSS had a greater number of future drug options available and were hence likely to have experienced fewer previous failures. The number of regimens previously failed was not associated with switching regimen after adjusting for potential confounders, though in univariable analyses,

was associated with a decreased risk of switching. It is likely that the variable consisting of the number of future drug options confounded the relationship between the number of previous regimens failed and switching regime, explaining the lack of significance of the number of previous regimens failed in multivariate analyses.

A low GSS at the time of the resistance test was independently associated with an increased risk of switching, with absolute estimates stronger using the strict definition of switching. These results are in line with findings from other studies (420;495;496) and both national and international guidelines (124;491) which have stated the importance of resistance testing and GSS before switching regimens. The estimates are likely to be lower when using the relaxed definition of switching as GSS may not be the main reason that patients switched therapy – for example, patients may have switched a single drug for toxicity reasons.

The number of drug options available was also significantly associated with switching, with the risk of switching decreasing as the number of drug options decreased. This has also been shown in other studies (420;495;496). Further, in contrast to findings from the ARGENTA trial, I found GSS to be associated with switching regimen, even after adjusting for the number of drug options available (492). In fact, the effect of GSS was stronger after adjusting for the availability of drug options. Patients with low GSS may not switch regimen because of few options, but when the number of options is taken into account, a larger effect of GSS is expected.

Interestingly, I also found patients who had previously attained an undetectable VL were more likely to switch regimen compared to those who had not achieved virological suppression. It is well recognised that adherence is associated with virological suppression (152;498-500) and hence clinicians may be more willing to switch regimens in those patients they perceive to have proven themselves to have been adherent than those with adherence issues. These findings are also in line with those reported by Cingolani et al (492) as discussed above. Patients with higher VLs and lower CD4 counts at the time of the resistance test were also more likely to switch regimen, indicating that together with GSS, VL and CD4 count are key indicators in influencing the decision to switch. This is well recognised and previous analyses from the UK CHIC Study have also shown that these indicators are associated with rapid switching after viral rebound (501). Although I did not restrict these analyses to those who experienced viral rebound, it is likely that the

majority of resistance tests were performed for this reason, and in general resistance tests are only performed if the VL is above 200-500 copies/mL.

The type of regimen being taken at the time of the resistance test was also significantly associated with switching regimens. In particular, tests performed whilst patients were receiving boosted PI regimens were less likely to be followed by a switch whilst those performed amongst patients taking a single PI-based regimen were more likely to be followed by a switch compared to those performed in patients receiving NNRTI- based regimens, even after adjusting for GSS. Although PI/r based regimens are usually recommended as second-line regimens (124), and hence patients receiving these regimens may have fewer treatment options available, this effect remained highly significant after adjusting for both number of prior failures and number of drug options available. In the event that these patients have already failed an NNRTI-based regimen, other NNRTIs are usually not a feasible option (although not necessarily the case with Etravirine), since a single mutation (L100I, Y181C, Y181I, G190A) can cause class-specific resistance. It is also well recognised that resistance accumulation on PI/r-based regimens is quite slow, which may also explain the reluctance in switching patients to other regimens. Single PI-based regimens are sub-optimal in the HAART era and hence clinicians may be more inclined to switch therapy amongst patients receiving such regimens. This is likely to be particularly evident amongst patients who started therapy in earlier calendar years and did not have the full range of ART drugs available that are accessible today.

Patients with a shorter time period between starting ART and having a resistance test performed were at an increased risk of switching. Those who had been on ART for shorter time periods are likely to have a higher number of drug options available, resulting in a higher probability of switching. These patients, and in particular those who had a resistance test performed within 3 months of starting ART, may also have been infected with a resistant virus. Though guidelines state that all newly infected HIV patients should have a resistance test before starting ART, this only became routine around 2004 (127). Patients starting ART in earlier calendar years are unlikely to have had a resistance test performed before starting therapy, and hence may be more likely to switch soon after starting ART, in an attempt to suppress the virus from the outset.

Although the year in which the resistance test was performed was not found to be significantly associated with switching, either using the strict or relaxed definition of switching, there was a significant interaction between calendar year of resistance test and GSS. Patients who had resistance tests performed in later calendar years were more likely to switch if their GSS was 1-1.75 compared to >2. This effect was significant even after adjusting for the number of future drug options available. One explanation for this effect is that drugs in earlier calendar years were less potent, with higher levels of toxicities, so clinicians/patients may have been less willing to switch regimen despite a low GSS.

The association between GSS and probability of switching regimen was weakened, but was still significant when analyses were restricted to those who carried resistance mutations. This is to be expected, given that the overall range of GSS will be smaller when restricting to patients with mutations detected. Interestingly, patients who had failed a greater number of regimens before having a resistance test performed were less likely to switch in univariable analyses but after adjusting for potential confounders, this effect was reversed. Given that the number of future drug options was the main variable which caused this reversal, the most likely explanation for this reversal is that patients with a higher number of prior failures were more likely to have fewer drug options available. However, after adjusting for the number of future drug options available, we would expect a higher probability of switching with a greater number of previous failures for reasons outlined earlier in this Discussion.

When using the strict definition of switching, over 10% of patients with a GSS of <1 still had a GSS of <1 after switching. This increased to over 30% when using the relaxed definition of switching. Though these patients may have had limited future drug options available, it is likely (particularly in the latter case) that patients did not switch solely to increase the number of active agents in their regimen, but instead to either ‘simplify’ their regimen, or to reduce the number of toxic drugs in their regimen. This may be particularly true in later calendar years, when newer more tolerable drugs were readily available.

There are limitations to this analysis. Firstly, my definition of ‘current drug options’ is based on the GSS of available drugs not yet experienced at the date of the resistance test. Of note, I do not have data on previous experimental use of drugs which may have some impact on these analyses. It is also possible that in some

cases, a previous resistance test may have revealed a mutation which has since disappeared from the majority virus, so clinicians may be aware of limitations in drug options that are not captured by my measure. Though statistically, I could have used cumulative genotype results to somewhat deal with this issue, I felt that this was diverting from the initial question I was trying to answer, i.e. whether individual resistance tests are followed by a switch. Clinicians may not have results of previous tests to hand, especially in the case where patients were seen at more than one clinic. Further, tolerability issues, such as previous drug intolerance and cardiovascular/renal toxicities of particular drugs may also factor in decisions to switch. It is also possible that some resistance tests may not have had the date of the result recorded, despite being made available to the clinician. Tests excluded from the analyses may have had some impact on the results seen.

In relation to the data I have available, I have shown that the GSS of the current regimen is a strong predictor of switching regimen within 4 months of the resistance test result, regardless of which definition of switching is used. When using the strict definition of switching, fewer than half of all those with GSS <2 switch within 4 months of the test result, and amongst those that do switch, 12% with an old GSS of <1 continue to have a GSS of <1 after switching therapy. These patients are highly ART-experienced and have few treatment options available. Given that those with fewer options are less likely to switch, these results show that the need for drugs from both existing classes and new classes continues to exist.

5.2. Outcomes of second-line ART when switching from NNRTI to PI/r

5.2.1. Introduction

A 3-drug combination of two NRTIs with an NNRTI is the most widely prescribed regimen for first-line therapy worldwide (124). Despite marked improvements in antiretroviral therapy in recent calendar years, in terms of efficacy, tolerability and adherence, treatment failures continue to occur. After failure on an NNRTI-based regimen, most second-line combinations are PI/r-based. In the case of virological failure of a first-line regimen, current guidelines advise switching to at least 2 active agents (preferably 3), where a drug's activity is predicted based on results from a resistance test and knowledge of the drugs that a patient was receiving during the first-line regimen. However, the potency and high genetic barrier of PI is such that 2 additional fully active nucleoside drugs may not be necessary. PI/r such as lopinavir/ritonavir and darunavir/ritonavir have shown high rates of virologic efficacy as monotherapy (84;409;502), although virological success rates are better for induction-maintenance strategies than when PI/r are used as initial therapy (503). Trials investigating the efficacy of PI/r and single NRTI combinations are planned or ongoing (504).

If second-line ART efficacy is not dependent on NRTI activity then this could have far-reaching implications, in particular for resource-limited settings. Currently, routine viral load monitoring remains inaccessible to many patients in developing countries and hence the diagnosis of virological failure may be delayed resulting in accumulation of drug resistance mutations. NRTI resistance may be of less concern with PI/r-based second-line HAART although this must be balanced by the risks of transmission of resistant virus and resistance to other classes (i.e. NNRTI).

In practice, approaches differ between clinics with respect to how many fully active agents are selected for second-line PI/r based therapy. If two fully active NRTIs are not required for second-line PI/r, savings could be made both in terms of drug cost but also short- and long-term ART-related toxicity. Zidovudine, for example, is one of the few NRTIs that remain fully active after failing NNRTI-based HAART with a 3TC-containing backbone (lamivudine and emtricitabine are now recommended NRTI backbone components in all first-line regimens) so long as no TAMs have developed, but is associated with mitochondrial toxicities including lipoatrophy.

In this Section I investigate whether it is necessary to include two fully active NRTIs in a second-line PI/r-based regimen after first-line NNRTI failure. In particular, I will identify factors associated with failing second-line therapy and focus on the impact of the number of new or fully active NRTI included with second-line therapy on virological success rates.

5.2.2. Methods

5.2.2.1. Inclusion criteria

All HIV-positive patients who commenced first-line NNRTI-based HAART (defined as at least two NRTIs with an NNRTI (and no PI component)) and who subsequently experienced virological failure (defined as HIV-RNA >200 copies/mL after at least 4 months of therapy) were eligible for inclusion if they switched to second-line PI/r-based therapy after failure.

Patients with VLs $\leq$200 copies/mL at date of switch (defined using last VL prior to switching), those with less than 4 months follow-up after the start of second-line therapy and those who failed a new NRTI between failing the first-line NNRTI regimen and starting the second-line PI/r regimen were excluded from this analysis.

5.2.2.2. Definition of virological failure and GSS

When analysing responses to second-line therapy, virological failure was also defined as HIV-RNA >200 copies/mL after 4 months of continuous use of a PI/r. I decided to use only one VL >200 copies/mL to define failure of the second-line regimen (as opposed to two VL >200 copies/mL as in the previous analysis) to be consistent with the definition of failure of first-line regimens. In this analysis, patients who switched regimen before experiencing virological failure were *not* excluded. In order to analyse the impact of the predicted activity level of NRTIs in the second-line regimen, a GSS was calculated for the new regimen based on the Stanford algorithm (version 5.1.2). Patients were assigned a GSS as described in Section 5.1.2.2; drug-susceptible viral strains were assigned a sensitivity score of one, and those with low-level, intermediate, high-level and very high-level resistance were assigned scores 0.75, 0.50, 0.25 and 0 respectively. These sensitivity scores were then summed for each drug in the regimen.

5.2.2.3. Statistical analyses

A Kaplan-Meier analysis was performed to describe time to failure of the second-line regimen and Cox regression to identify factors associated with second-line virological failure. In these initial analyses, patients were not required to have a resistance test performed as it was the number of new drugs started that was the focus of the analysis. Models were adjusted for the following potential confounders: age at start of second-line HAART, time from failing first-line to starting second-line HAART, number of new NRTIs started at second-line HAART, ethnicity, sex/exposure, year of starting second-line HAART, whether the VL was <200

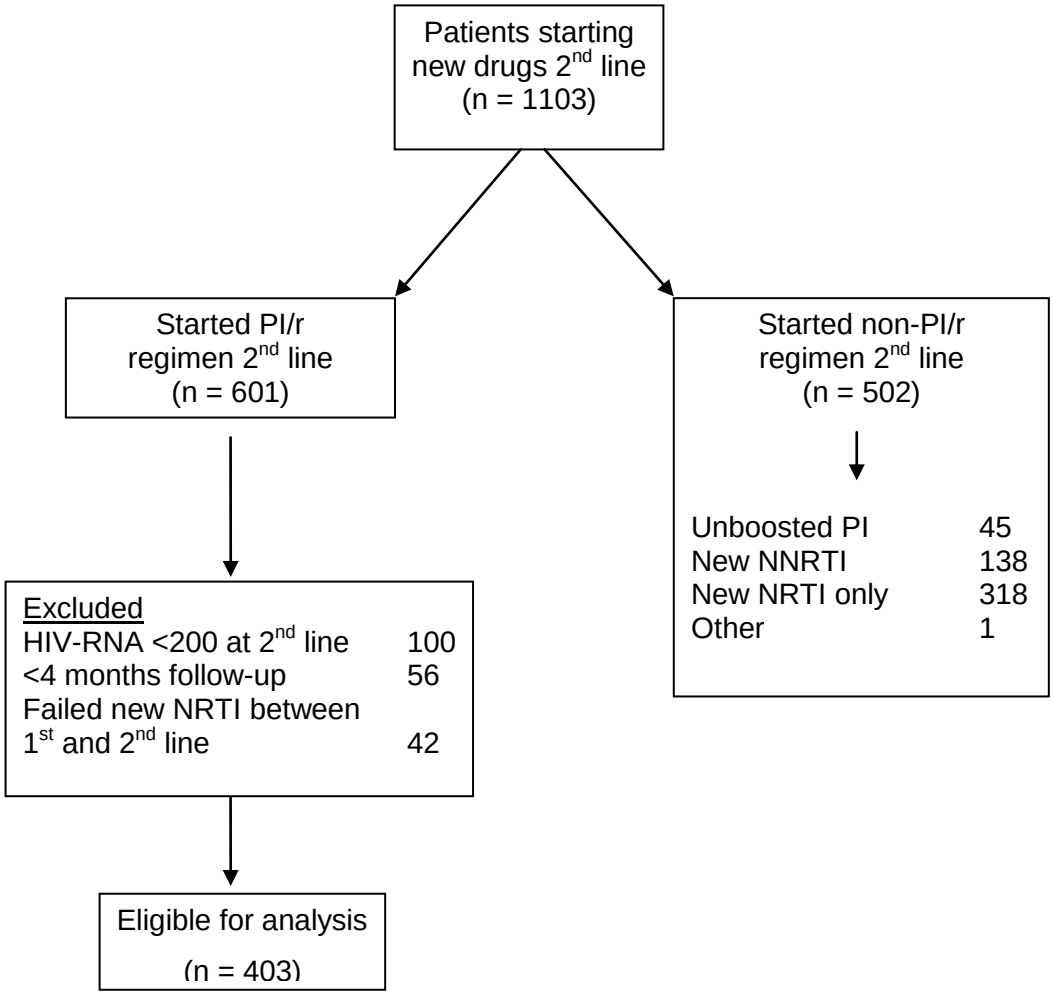
copies/mL between failing first-line and before starting second-line, CD4 at start of second-line HAART (per 50 cells higher) and VL at start of second-line HAART (per 1 log higher). Chi-squared and Mann-Whitney tests were used to identify differences amongst patients who did and did not have a resistance test performed. Logistic regression was used to identify factors associated with having a resistance test performed and Cox regression was used to identify factors associated with virological failure amongst those with resistance tests.

5.2.3. Results

5.2.3.1. Patient disposition

During the study period (January 1999 to December 2007) 9285 subjects commenced NNRTI-based first-line HAART, and of these 1692 (18.2%) experienced virological failure according to the definition outlined above. Only 65% of patients experiencing virological failure started new drugs (n=1103) after the date of failure. Of these, 700 did not meet the inclusion criteria (Figure 5.2.3.1.1); 502 patients started non-PI/r based regimens, 100 patients who did start PI/r-based regimens had VL <200 copies/mL at start of second-line therapy, 56 patients had less than 4 months follow-up after starting second-line PI/r and 42 patients failed a new NRTI after failing their 1st line NNRTI-based regimen and before starting their second-line PI/r-based regimen. This left 403 patients eligible for the analysis. Not all 403 patients had resistance data available; those who did not were removed from analyses requiring resistance data.

Figure 5.2.3.1.1: Patient disposition



5.2.3.2. Baseline characteristics

Baseline characteristics are described in Table 5.2.3.2.1. AZT and 3TC (either in combination with each other or with other NRTIs) were the most frequently received NRTIs at first-line failure, whilst TFV was most frequently received at start of second-line. Over a half of patients started 2 or more NRTIs at second-line HAART. Median (IQR) CD4 cell count at start of second-line therapy was 213 (124, 310) cells/mm^3 and median (IQR) VL 4.4 (3.6, 5.0) log copies/mL. Most patients initiated lopinavir/ritonavir (59.3%) or atazanavir/ritonavir (23.1%) based second-line therapy and a total of 216 (53.6%) patients underwent genotypic resistance testing.

Table 5.2.3.2.1: Characteristics of patients starting second-line PI/r-based therapy

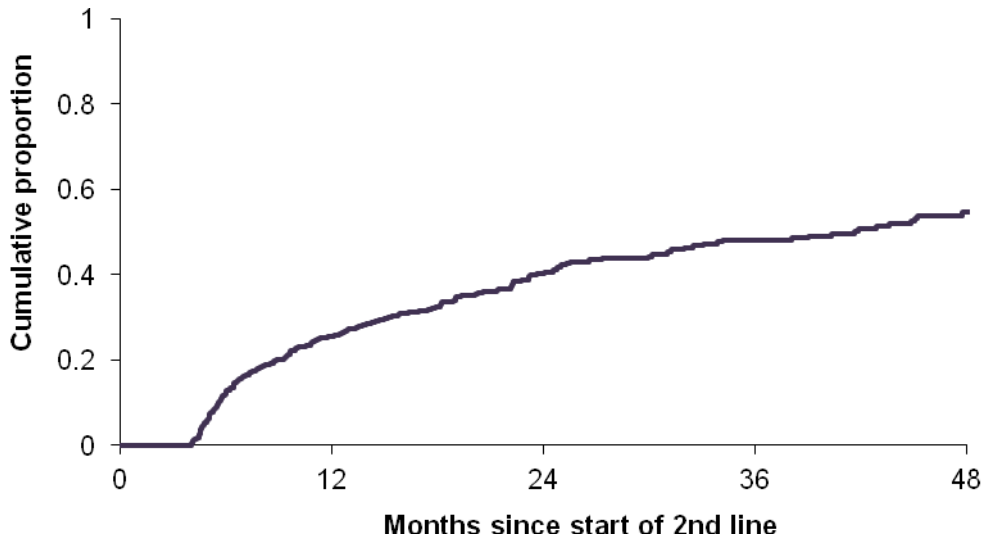
Patients starting second-line PI/r HAART		**403**
Time to failing first-line HAART (months)	Median (IQR)	9.4 (5.3, 20.0)
NRTIs taken at first-line failure n (%)	AZT	216 (53.6)
	DDC	1 (0.2)
	DDI	87 (21.6)
	d4T	62 (15.4
	3TC	308 (76.4)
	ABC	62 (15.4)
	TFV	65 (16.1)
	FTC	26 (6.5)
NRTIs taken at second-line n (%)	AZT	129 (32.0)
	DDC	1 (0.2)
	DDI	134 (33.2)
	d4T	36 (8.9)
	3TC	144 (35.7)
	ABC	143 (35.5)
	TFV	255 (63.3)
	FTC	36 (8.9)
New NRTIs started at second-line n (%)	0	69 (17.1)
	1	113 (28.0)
	$\geq$2	221 (54.8)
Year of starting second-line n (%)	1999-2001	195 (48.4)
	2002-2004	164 (40.7)
	2005-2007	44 (10.9)
Ethnicity n (%)	White	161 (40.0)
	Black	206 (51.1)
	Other	36 (8.9)
Risk group n (%)	MSM	154 (38.2)
	Heterosexual	214 (53.1)
	Other	35 (8.7)
VL <200 between first and second-line HAART n (%)		63 (15.6)

CD4 at start first-line (cells/mm^3)	Median (IQR)	150 (54, 220)
VL at start first-line (log_{10} copies/mL)	Median (IQR)	5.1 (4.6, 5.5)
CD4 at start second-line (cells/mm^3)	Median (IQR)	213 (124, 310)
VL at start second-line (log_{10} copies/mL)	Median (IQR)	4.4 (3.6, 5.0)

5.2.3.3. Factors associated with virological failure of second-line HAART

Of the 403 patients starting second-line PI/r HAART, 181 (44.9%) experienced virological failure, after a median of 42 months (Figure 5.2.3.3.1).

Figure 5.2.3.3.1: Kaplan-Meier estimate of the proportion of patients failing second-line PI/r



Factors associated with virological failure are presented in Table 5.2.3.3.1. In univariable analyses, patients of black ethnicity (HR 1.56 (95% CI: 1.14, 2.13)) compared with white ethnicity), heterosexual risk group (2.03 (1.39, 2.97) and 1.86 (1.27, 2.72) for heterosexual males and females respectively, compared with MSM) and those with higher VLs at start of second-line HAART (1.37 (1.20, 1.56) per 1 log increase) were at an increased risk of virological failure. Patients with higher CD4 counts at start of second-line HAART were less likely to experience virological failure (0.86 (0.81, 0.91) per 50 cells higher).

Factors found from multivariable analyses to be independently associated with virological failure were heterosexual risk group (1.93 (1.17, 3.20); p=0.01 and 2.33 (1.35, 4.02); p=0.002 for heterosexual males and females, respectively, compared with MSM) and VL at start of second-line (1.23 (1.06, 1.42); p=0.01 per 1 log higher). Higher CD4 cell count at start of second-line regimen was associated with a reduced risk of virological failure (0.89 (0.83, 0.94); p<0.001 per 50 cells/mm^3 higher). There was a trend towards increased risk of virological failure of second-line therapy with each month increase between time of failure and switch (1.01 (1.00, 1.03); p=0.07 per month). The number of new NRTIs included in the second-line regimen was not associated with virological outcome; compared to individuals with 2 or more new NRTIs, the hazard ratios for virological failure were 0.83 (0.51, 1.33; p=0.44) and 1.11 (0.79, 1.56; p=0.56) for those receiving 0, or 1 new NRTI, respectively. When number of new NRTIs was analysed as a continuous variable the HR for virological failure was 1.00 per new NRTI (0.82, 1.21; p=0.99).

Table 5.2.3.3.1: Associations between various factors and virological failure from univariable and multivariable analyses

		Univariable		Multivariable	
		HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
Age at start of second-line	Per 10 years older	1.02 (0.83, 1.26)	0.83	1.08 (0.86, 1.34)	0.52
Time from failing first-line to starting second-line HAART	Per month longer	1.01 (0.99, 1.02)	0.35	1.01 (1.00, 1.03)	0.07
Number of new NRTIs started at second-line	0	0.80 (0.51, 1.25)	0.32	0.83 (0.51, 1.33)	0.44
	1	1.07 (0.77, 1.48)	0.70	1.11 (0.79, 1.56)	0.56
	$\geq$2	1	-	1	-
Ethnicity	White	1	-	1	-
	Black	1.56 (1.14, 2.13)	0.01	0.95 (0.60, 1.51)	0.95
	Other	0.61 (0.31, 1.23)	0.17	0.52 (0.24, 1.12)	0.52
Sex/Exposure	MSM	1	-	1	-
	Heterosexual male	2.03 (1.39, 2.97)	0.0003	1.93 (1.17, 3.20)	0.01
	Heterosexual female	1.86 (1.27, 2.72)	0.002	2.33 (1.26, 4.02)	0.002
	Other	1.79 (1.06, 3.03)	0.03	1.59 (0.62, 2.80)	0.11
Year of starting second-line	Per 1 year later	0.94 (0.86, 1.01)	0.10	0.94 (0.86, 1.02)	0.15
VL <200 after failing first-line and before starting second-line		0.86 (0.57, 1.31)	0.47	0.76 (0.47, 1.24)	0.28
CD4 at second-line (cells/mm^3)	Per 50 cells higher	0.86 (0.81, 0.91)	<0.0001	0.89 (0.83, 0.94)	<0.0001
VL at second-line (copies/mL)	Per 1 $\log_{10}$ higher	1.37 (1.20, 1.56)	<0.0001	1.23 (1.06, 1.42)	0.01

5.2.3.4. Resistance testing prior to switch

When comparing patients who did (n=216) and did not (n=187) undergo resistance testing prior to switch, patients who did not have a resistance test had significantly lower median CD4 cell count at virological failure (220 vs. 287 cell/mm^3; p=0.01) and were less likely to have ever achieved viral suppression, defined as VL less than 500 copies/mL, on first-line HAART (43.8% vs. 56.2%; p=0.05) than those who did have a test. Patient characteristics according to resistance testing status are shown in Table 5.2.3.4.1.

Table 5.2.3.4.1: Characteristics of patients stratified by whether or not they had a resistance test after failing first-line HAART and before starting second-line

Resistance test performed		**No**	**Yes**	**P-value**
N		**187**	**216**	
Age at failing first-line (yrs)	Median (IQR)	35 (31, 39)	36 (32, 40)	0.31
Ethnicity n (%)	White	73 (45.3)	88 (54.7)	0.73
	Black	99 (48.1)	107 (51.9)	
	Other	15 (41.7)	21 (58.3)	
Sex/Exposure n (%)	MSM	69 (44.8)	85 (55.2)	0.06
	Heterosexual male	59 (56.7)	45 (43.3)	
	Heterosexual female	47 (42.7)	63 (57.3)	
	Other	12 (34.3)	23 (65.7)	
VL <500 before starting second-line n (%)		137 (43.8)	176 (56.2)	0.05
Year of failing first-line n (%)	1999-2001	54 (49.1)	56 (50.9)	0.16
	2002-2004	90 (49.5)	92 (50.6)	
	2005-2008	43 (38.7)	168 (61.3)	
CD4 at failing first-line (cells/mm^3)	Median (IQR)	220 (124, 349)	287 (168, 390)	0.01
Log VL at failing first-line (copies/mL)	Median (IQR)	4.0 (3.1, 4.8)	3.7 (3.0, 4.5)	0.08

In multivariable analyses, only log VL at first-line failure was associated with having a resistance test performed; patients with higher viral loads were less likely to have

a resistance test performed (OR: 0.80 (95% CI: 0.63, 1.00), p=0.05 per 1 log higher).

5.2.3.5. NRTI GSS and risk of virological failure amongst patients with resistance tests performed

Amongst the 216 patients with resistance tests performed prior to switching regimen, the NRTI GSS for the new regimen was $\leq$1, 1.25-1.75 and $\geq$2 in 15%, 33% and 49% of patients respectively. In univariable analysis, there was no statistical difference in NRTI GSS between patients who failed second-line PI/r therapy and those who achieved and maintained virological suppression (HR for experiencing virological failure: 0.73 (0.39, 1.34) and 0.74 (0.46, 1.17) for patients with GSS $\leq$1 and GSS 1-1.75 respectively, compared to those with a GSS $\geq$2). When NRTI GSS was included in a multivariable analysis adjusted for potential confounders, it was not associated with virological failure: HR for virological failure compared to subjects with GSS ≥2 was 0.73 (p=0.34) and 0.70 (p=0.16) for subjects with NRTI GSS of ≤1 and 1.25-1.75, respectively. When NRTI GSS was included in to the multivariable model as a continuous variable there was, again, no association with virological failure (1.14 (0.76, 1.72) per 1 unit higher).

5.2.4. Discussion

This analysis was performed on a very specific subset of patients, with the aim of investigating whether two fully active NRTIs in a second-line PI/r-based regimen are necessary. In this analysis there was a wide degree of variability in the number of new and active NRTIs started as part of second PI/r line; however, I found no association between the number of new NRTIs, nor the number of fully active NRTIs, and virological failure of a second-line PI/r-based regimen.

These findings are consistent with data presented recently indicating that, amongst individuals with the M184V mutation, and therefore resistance to lamivudine (3TC) and emtricitabine (FTC), outcomes on a PI/r were similar regardless of number of NRTIs (505); in a retrospective observational analysis, patients receiving 3TC/FTC with a PI/r and one other NRTI were as likely to achieve virological suppression as those receiving 2 NRTI + PI/r +/- 3TC/FTC. Possible explanations are the inherent potency of PI/r rendering the number of new or active NRTIs less important. This is supported by the high rates of efficacy when PI/r is issued as monotherapy; any benefit of including active NRTIs in the combination is small in comparison to the efficacy of PI/r alone. LPV/r monotherapy, the most studied single PI/r strategy to date, is associated with higher rates of viral suppression when used as a simplification option in already virologically suppressed patients than as initial therapy. Most patients in this analysis switched directly from failing therapy and had lower viral load levels than patients starting first-line HAART. This may explain the relatively low rates of virological failure found - by 12 months of starting second-line HAART, only a quarter of patients had experienced virological failure.

There is also the possibility that there was a benefit of active NRTIs in terms of efficacy but that this was masked by lower adherence rates in those with more active NRTIs in the regimen. Regimens with at least 2 active NRTIs were more likely to include zidovudine than those with fewer than 2 (39% of regimens with >2 active NRTIs included ZDV compared to 31% of regimens with <2 active NRTIs) and thus side effects may have resulted in lower adherence rates; unfortunately adherence data is not collected in the UK CHIC Study, so it is difficult to evaluate this hypothesis. Although not statistically significant, there was a numerically lower risk of virological failure in patients with NRTI GSS less than 2 compared to those with a GSS of 2 or greater. In order to definitively address the question of the number of nucleosides needed a randomized trial is required, in order to rule out confounding secondary to adherence or other factors.

Notably, of the 1103 individuals who started a new second-line regimen after failing NNRTI-based HAART, 138 started a new NNRTI and 318 started a new NRTI only. In these cases, it may be possible that clinicians were modifying the initial regimen not in relation to virological failure, but perhaps due to toxicity or adherence issues. It is also possible that clinicians may be reluctant for patients to switch to PI/r-based regimens because of adherence issues.

Though these findings do have the limitations of low statistical power, recognised by the wide confidence intervals for the effect of number of new drugs and GSS, ultimately these findings could be of global importance. With scarce second-line ART options in resource-limited settings, minimising the number of agents required for second-line therapy could yield large benefits in terms of toxicity, tolerability, adherence and, of course, cost. However, in resource-rich settings, with access to regular monitoring (including resistance testing), these data support the possible use of second-line PI/r regimens containing fewer than 2 active NRTI.

5.3. Summary

In this Chapter I have shown that the definition of 'switching' impacts considerably on the proportion of patients identified as having switched regimen. However, regardless of using a strict or relaxed definition, the GSS of the current regimen is a strong predictor of whether patients make a switch following the result of a resistance test, even after adjusting for the number of future drug options available. The second Section of this Chapter investigates the need for 3 fully active drugs amongst patients on second-line PI/r-based HAART. The number of new NRTIs and the NRTI GSS was not associated with virological failure of second-line HAART. Although the relaxed definition I used for switching in the first Section initially appeared to be somewhat too broad, results from the second Section suggest that switching a single component, i.e. NNRTI to PI/r can still be beneficial.

Chapter 6: Is a single ALT>200 IU sufficient to define an alanine transaminase (ALT) flare in HIV-positive populations?

In this Chapter I will focus on ALT elevations. Though several factors may contribute to rises in ALT, these rises have also been linked to HAART in various studies. I have chosen to focus on ALT elevations as opposed to other toxicities associated with HAART since there is currently no consensus on what constitutes an ALT flare, i.e. an abnormally high value of ALT. Since ALT is regularly measured in most centres in UK CHIC, it is possible to investigate ALT elevations and distinguish these random fluctuations in ALT level from ALT flares within the CHIC dataset.

6.1. Introduction

Several studies have suggested that HAART may cause a rise in ALT levels (269-275;506-508). ALT elevations range in both severity and duration. The definition of an ALT 'flare' is varied. Some definitions include new ALT elevations which persist, while others refer only to transient elevations which return to baseline or normal levels. Some definitions are based on a single ALT measurement >50 IU (276;509) and others use the definition of Grade 3/4 hepatotoxicity from the AIDS Clinical Trial Group (ACTG) (>5 times upper limit of normal (283)). The extent to which such increases in ALT level are due to immune reconstitution, non-treatment related events, or random fluctuations has not been previously explored.

Chronic infection with hepatitis C virus (HCV) or hepatitis B virus (HBV) is an independent risk factor for liver damage in HIV-positive patients (269;279;283;286-288;510). However, there is no consensus on the relative impact of hepatitis status, HAART use and CD4 count in determining the risk of ALT flares.

The aim of this analysis was to derive a definition of an ALT flare which may be useful in understanding the causes and significance of ALT elevations in the HIV-treated population, by investigating the frequency and time course of ALT elevations in untreated HIV-positive patients without hepatitis co-infection. I used this derived definition to identify factors that were associated with the risk of an ALT flare amongst both naïve and treatment experienced patients. Finally, I compared the strength of these associations with those identified when using a commonly utilized

definition of an ALT flare (ACTG Grade 3 hepatotoxicity) (270;271;279;285;511;512) with the aim of identifying any factors which may be given too little or too much importance when using the common definition of an ALT flare.

6.2. Methods

6.2.1. Patient population

Patients with ALT measurements recorded after 1996 were included in the analyses. Differences between those included and excluded were tested using Chi-squared and Mann Whitney tests.

6.2.2. Justifying the need for an ALT flare definition to be restricted to HAART-naive patients

In the first part of this analysis, I crudely verified the relationship between ALT elevations and HAART status. This was done in order to justify the need for the definition of an ALT flare to be solely based on HAART-naïve patients, i.e. to exclude the impact of HAART on ALT levels. I compared the frequency and duration of ALT elevations using a series of different thresholds (100, 120, 140, 160, 180 and 200 IU) to define an elevation, stratifying analyses by HAART status and HBV and/or HCV status.

Patients were classified in three (non-mutually exclusive) groups: patients who had never had a positive HBV/HCV test (including those who were never tested, i.e. those with negative/unknown HBV/HCV status); patients who had been tested for HBV/HCV co-infection and never had a positive HBV/HCV test (i.e. those with negative HBV/HCV status only); patients who had tested positive to HBV/HCV at least once. Positive HBV status was defined as having a positive HBV surface antigen result.

For each individual, an episode of ALT elevation started when ALT levels first increased above the defined threshold (with the start date estimated by linear interpolation between the date of the first ALT level above the threshold and the date of the preceding ALT measurement to try to account for the issue of interval censoring) and ended when ALT levels fell below the threshold (end date estimated by linear interpolation between the date when ALT levels first fell below the threshold and the date of the preceding ALT measurement). Patients were stratified according to whether or not they had started HAART at the date of the ALT elevation. For those who had not yet started HAART, ALT measurements from Jan 1996 to the earliest of the date of starting HAART and the date of the last ALT measurement were eligible for inclusion.

After patients had started HAART, ALT measurements from the date of starting HAART to the date of the last ALT measurement were eligible for inclusion. If ALT

levels did not fall below the threshold, the episode was censored at the date of the last ALT measurement for patients who did not start HAART and at the earliest of the date of starting HAART and the date of the last ALT measurement for patients who did start HAART. The proportion of ALT elevation episodes that were sustained for two or more consecutive ALT measurements was described.
In these analyses, patients whose first HBV/HCV test was positive were defined as being HBV/HCV-positive from the start of their follow-up.

6.2.3. Defining an ALT flare using data from HAART-naive and HBV/HCV-negative patients

Given the results of the above analysis (reported in Sections 6.3.2 - 6.3.4), all subsequent analyses in relation to defining an ALT flare were restricted to HAART-naïve patients. Poisson regression was used to verify the association between HBV/HCV status and ALT elevation, with follow-up starting from date of first ALT measurement after 1996 and ending at the earliest of date of starting HAART and last ALT measurement. Analyses were then restricted to patients who were HBV and HCV-negative or who had unknown hepatitis status at the time of their ALT measurement. The frequency and duration of ALT elevation was calculated using linear interpolation as described above. The number of episodes lasting 0.5 months or longer, 1 month or longer, 3 months or longer and 6 months or longer was described. As a further summary measure, patient follow-up was split into 3 month intervals and the mean ALT measurement in each 3 month interval was calculated.

A subset of these patients tested positive for hepatitis at a later date, so sensitivity analyses were also performed in which I additionally excluded any patients who had ever had a positive HBV/HCV test and then further excluded patients who had never been tested. Using these results (Section 6.3.7), a definition of an ALT flare, based on untreated, HBV/HCV-negative patients was derived, which was used in all subsequent analyses (2 consecutive ALTs >200 IU, measured >2 weeks apart).

6.2.4. Identifying factors associated with ALT flares

Poisson regression was used to identify factors associated with my newly derived definition of an ALT flare. In these analyses, all patients were included, regardless of treatment and hepatitis status. In patients who did not experience a flare, follow-up was calculated from the date of the first ALT measurement after 1996 and censored at the date of death or the last clinic visit. Amongst patients who experienced an ALT flare, follow-up was calculated from the date of the first ALT measurement after

1996 and ended on the date of experiencing the flare. For those who had a flare which resolved, follow-up began again from the first ALT measurement after resolution of the flare (ALT<200 IU).

The following potential confounders were considered: sex, exposure (MSM, heterosexual, other/unknown), ethnicity (white, black, other/unknown), current CD4 count, current viral load, current age, current calendar year, previous AIDS diagnosis, number of prior of ALT measurements, HCV diagnosis, HBV diagnosis, current HAART regimen (didanosine/stavudine (ddI/d4T) with nevirapine (NVP), ddI/d4T without NVP, NVP without ddI/d4T and 'other') and any ritonavir (RTV) use. These specific regimens were selected as the drugs included have been reported to be associated with ALT flares (270;272;276). The number of prior ALT measurements has also been associated with ALT flares (273); this covariate may be a surrogate for the frequency of attendance or monitoring at a clinical centre. It was hypothesised that positive hepatitis status may impact on CD4 counts and on treatment regimen, and hence interactions between hepatitis status and both treatment and CD4 count were explored and analyses were stratified by these variables where appropriate.

6.2.5. Comparing the derived definition with the definition commonly used in the literature

Poisson regression was also used to identify factors associated with experiencing an ALT flare when categorised using the most common definition in the literature (a single ALT >200 IU). These estimates were compared to those calculated above. The rationale for this comparison was that the RRs obtained using the standard definition may differ to those using the derived definition, due to issues such as monitoring bias.

Finally, the association between ALT flares (fitted as a time updated covariate, current flare yes/no) and all-cause mortality was explored using Kaplan Meier analyses and Cox regression. Again, estimates using both the derived definition and the most commonly found definition in the literature were compared. Patients were followed from the date of the first ALT measurement after 1996 until the date of last clinical follow-up. Analyses were adjusted for the following confounders: current CD4 count, current viral load, current age, ethnicity, exposure group and number of prior ALT flares.

6.3. Results

6.3.1. Differences between those who have an ALT measurement recorded after 1996 and those without a measurement recorded after 1996

ALT measurements recorded after 1996 were available in 19,707 (60.4%) of the total 32,607 patients. Patients without measurements were more likely to be female (51.4% of females had no available ALT measurements compared to 48.6% of females with ALT measurements, $p<0.0001$), of black ethnicity (55.8% had no ALT measurements recorded compared to 44.2% of patients with ALT measurements, $p<0.0001$) and of heterosexual risk group (51.2% had no ALT measurements, compared to 48.8% with ALT measurements, $p<0.0001$). Patients without measurements available were also less likely to have started HAART (31.8% vs. 68.2%) and less likely to have ever had a positive HBV/HCV test (5.7% vs. 94.3%).

Amongst the 19,707 patients who did have available ALT measurements, the median number of measurements per patient was 14 (IQR: 4, 28) at a mean duration of 141 (SD: 149) days apart.

Table 6.3.1.1: Differences between those who did and those who did not have an ALT measurement recorded after 1996

		Patients with ALT measurements after 1996		P-value
		No	**Yes**	
		12900	**19707**	
Sex	Males	8863 (35.8)	15888 (64.2)	<0.0001
	Females	4037 (51.4)	3819 (48.6)	
Ethnicity	White	5981 (31.5)	13008 (68.5)	<0.0001
	Black	5233 (55.8)	4149 (44.2)	
	Other	1686 (39.8)	2550 (60.2)	
Risk group	MSM	4857 (28.6)	12103 (71.4)	<0.0001
	Heterosexual	5297 (51.2)	5056 (48.8)	
	Other	2746 (51.9)	2548 (48.1)	
Started HAART	No	6206 (53.8)	5338 (46.2)	<0.0001
	Yes	6694 (31.8)	14369 (68.2)	
HBV/HCV-positive	No	12761 (42.3)	17398 (57.7)	<0.0001
	Yes	139 (5.7)	2309 (94.3)	

6.3.2. ALT measurements in HAART-naive and HAART-experienced patients who are not known to be HBV or HCV-positive

Table 6.3.2.1 shows the frequency and duration of elevated ALT episodes for a range of different thresholds amongst HAART-naive, HBV/HCV-negative patients. Similar results are shown in Table 6.3.2.2 for HAART-experienced, HBV/HCV-negative patients. In both the untreated and the treated groups, the proportion of patients with at least one ALT measurement above each threshold decreases as the threshold increases. This is also true of the number of episodes above each threshold. Amongst HAART-naive patients, the proportion of patients with at least 2 consecutive measurements above each threshold also decreases as the threshold increases, but this is not true for the HAART-experienced patients.

Although the general trends are similar in both groups, the actual proportions are considerably different. The proportion of patients with at least one ALT measurement above each threshold drops from 10.5% for ALT levels >100 IU to

3.5% for ALT levels >200 IU amongst HAART-naive patients, but these proportions are much higher amongst HAART-experienced patients (22.8% for ALT levels >100 IU and 7.9% for ALT levels >200 IU). Not much variation was seen between the groups in the proportion of episodes in which the ALT level was sustained above the threshold of interest for 2 consecutive measurements. However, as a proportion of the total number of patients under follow-up in each group, HAART-experienced patients were more likely to have 2 consecutive ALT measurements above each threshold than HAART-naive patients (5.5% for ALT levels >100 IU in HAART-naive patients compared to 16.9% in HAART-experienced patients). In the group of HAART-experienced patients there were also a considerably higher maximum number of episodes above each threshold, at around 15-18 for at least one patient, whilst the patient with the maximum number of episodes among HAART-naive patients had only 5 episodes above each threshold. The median duration of episodes was slightly lower in the HAART-naïve group for ALT levels >180 IU, but was higher for ALT levels >180 IU.

Table 6.3.2.1: Frequency and duration of elevated ALT episodes when using a series of different ALT thresholds amongst patients who were HAART-naïve and HBV/HCV-negative or of unknown status (patients who had never tested positive for HBV or HCV) throughout follow-up (N=12564)

ALT threshold (IU)	N (%) patients with ≥ 1 ALT above threshold	No. of episodes	Sustained for ≥2 consecutive measurements N (% of episodes)	No. of episodes of ≥ 2 consecutive measurements per patient Median (Range)	Duration (days) of each episode of ≥ 2 consecutive measurements Median (IQR)
ALT >100	1323 (10.5)	1709	691 (40.4)	1 (1,5)	96 (26, 243)
ALT >120	985 (7.8)	1256	500 (39.8)	1 (1,4)	85 (23, 226)
ALT >140	786 (6.3)	985	378 (38.4)	1 (1,4)	74 (23, 202)
ALT >160	638 (5.1)	788	309 (39.2)	1 (1,3)	73 (20, 193)
ALT >180	529 (4.2)	639	241 (37.7)	1 (1,3)	73 (18, 187)
ALT >200	440 (3.5)	517	196 (37.9)	1 (1,3)	69 (20, 183)

Table 6.3.2.2: Frequency and duration of elevated ALT episodes when using a series of different ALT thresholds amongst patients who were HAART-experienced and HBV/HCV-negative or of unknown status (patients who had never tested positive for HBV or HCV) throughout follow-up (N=11783)

ALT threshold (IU)	N (%) patients with ≥ 1 ALT above threshold	No. of episodes	Sustained for ≥2 consecutive measurements N (% of episodes)	No. of episodes of ≥ 2 consecutive measurements per patient Median (Range)	Duration (days) of each episode of ≥ 2 consecutive measurements Median (IQR)
ALT >100	2689 (22.8)	4925	1995 (40.5)	1 (1,16)	109 (49, 215)
ALT >120	2066 (17.5)	3490	1423 (40.8)	1 (1,15)	93 (42, 189)
ALT >140	1642 (13.9)	2649	1067 (40.3)	1 (1,17)	84 (37, 182)
ALT >160	1321 (11.2)	2058	851 (41.4)	1 (1,17)	81 (33, 165)
ALT >180	1097 (9.3)	1686	688 (40.8)	1 (1,18)	72 (34, 148)
ALT >200	930 (7.9)	1385	563 (40.6)	1 (1,16)	64 (31, 126)

6.3.3. ALT measurements in HAART-naive and HAART-experienced patients who are known to be HBV and HCV-negative

Similar analyses were performed amongst patients who had had at least one HBV and HCV test after 1996. Analyses were restricted to patients who had never had a positive HBV/HCV test result and hence were known to be HBV/HCV-negative. Results of these analyses are shown in Table 6.3.3.1 and Table 6.3.3.2. Although the absolute numbers under follow-up were lower in both groups, the proportions of patients with at least one ALT measurement and with at least two consecutive ALT measurements above each threshold were very similar to those seen in patients of negative or unknown hepatitis status. A considerably higher proportion of HAART-experienced patients experienced ALT elevations than HAART-naive patients and these occurred more frequently amongst the former group. Since the results of this analysis are similar to those obtained from pooling together hepatitis-negative patients and those with unknown status, I believe that to classify not tested patients as HBV/HCV-negative was a reasonable assumption to make.

Table 6.3.3.1: Frequency and duration of elevated ALT episodes when using a series of different ALT thresholds amongst patients who were HAART-naive and known to be HBV/HCV-negative (patients who had had at least one HBV/HCV test and had not ever tested positive) throughout follow-up (N=10094)

ALT threshold (IU)	N (%) patients with ≥ 1 ALT above threshold	No. of episodes	Sustained for ≥2 consecutive measurements N (% of episodes)	No. of episodes of ≥ 2 consecutive measurements per patient Median (Range)	Duration (days) of each episode of ≥ 2 consecutive measurements Median (IQR)
ALT >100	1071 (10.6)	1368	562 (41.1)	1 (1,5)	90 (23, 220)
ALT >120	809 (8.0)	1021	415 (40.6)	1 (1,4)	76 (21, 211)
ALT >140	647 (6.4)	806	315 (38.1)	1 (1,4)	68 (20, 183)
ALT >160	527 (5.2)	643	263 (40.9)	1 (1,3)	68 (18, 180)
ALT >180	433 (4.3)	519	208 (40.1)	1 (1,3)	68 (18, 171)
ALT >200	361 (3.6)	426	170 (39.9)	1 (1,3)	67 (18, 174)

Table 6.3.3.2: Frequency and duration of elevated ALT episodes when using a series of different ALT thresholds amongst patients who were HAART-experienced and known to be HBV/HCV-negative (patients who had had at least one HBV/HCV test and had not ever tested positive) throughout follow-up (N=9952)

ALT threshold (IU)	N (%) patients with ≥ 1 ALT above threshold	No. of episodes	Sustained for ≥2 consecutive measurements N (% of episodes)	No. of episodes of ≥ 2 consecutive measurements per patient Median (Range)	Duration (days) of each episode of ≥ 2 consecutive measurements Median (IQR)
ALT >100	2304 (23.2)	4203	1689 (40.2)	1 (1,16)	109 (49, 210)
ALT >120	1759 (17.7)	2964	1193 (40.2)	1 (1,15)	92 (41, 186)
ALT >140	1408 (14.2)	2251	902 (40.1)	1 (1,17)	84 (37, 178)
ALT >160	1130 (11.4)	1739	728 (41.9)	1 (1,17)	80 (33, 160)
ALT >180	942 (9.5)	1432	598 (41.8)	1 (1,18)	70 (34, 141)
ALT >200	801 (8.1)	1190	493 (41.4)	1 (1,16)	63 (29, 123)

6.3.4. ALT measurements in HAART-naive and HAART-experienced patients who have had a positive HBV/HCV test result

In the analyses presented below, only patients who had tested positive for either HBV or HCV were included. As anticipated, the proportion of patients with either one or two consecutive ALT measurements above each threshold was significantly higher than in the HBV/HCV-negative populations. Again, HAART-experienced patients experienced a greater number of ALT elevations than HAART-naive patients (33.3% with at least one ALT >200 IU compared to 16% in HAART-naive patients). This difference was particularly noticeable when comparing the overall proportion of patients who experienced at least 2 consecutive measurements above each threshold (29.3% for ALT >200 IU compared to 11.4% in HAART-naive patients). The frequency of episodes was similar amongst the two populations whilst episodes appeared to last longer amongst HAART-naive patients than in HAART-experienced patients.

Table 6.3.4.1: Frequency and duration of elevated ALT episodes when using a series of different ALT thresholds amongst patients who are HAART-naive and known to be HBV/HCV-positive (N=1644)

ALT threshold (IU)	N (%) patients with ≥ 1 ALT above threshold	No. of episodes	Sustained for ≥2 consecutive measurements N (% of episodes)	No. of episodes of ≥ 2 consecutive measurements per patient Median (Range)	Duration (days) of each episode of ≥ 2 consecutive measurements Median (IQR)
ALT >100	556 (33.8)	795	420 (52.8)	1 (1,6)	157 (70, 326)
ALT >120	460 (28.0)	654	333 (50.9)	1 (1,5)	144 (63, 280)
ALT >140	391 (23.8)	545	267 (49.0)	1 (1,4)	137 (58, 264)
ALT >160	344 (20.9)	464	226 (48.7)	1 (1,4)	135 (57, 243)
ALT >180	298 (18.1)	397	201 (50.6)	1 (1,3)	115 (56, 224)
ALT >200	267 (16.2)	348	188 (54.0)	1 (1,4)	101 (55, 216)

Table 6.3.4.2: Frequency and duration of elevated ALT episodes when using a series of different ALT thresholds amongst patients who are HAART-experienced and known to be HBV/HCV-positive (N=1791)

ALT threshold (IU)	N (%) patients with ≥ 1 ALT above threshold	No. of episodes	Sustained for ≥2 consecutive measurements N (% of episodes)	No. of episodes of ≥ 2 consecutive measurements per patient Median (Range)	Duration (days) of each episode of ≥ 2 consecutive measurements Median (IQR)
ALT >100	990 (55.3)	2259	1240 (54.9)	1 (1,7)	148 (77, 276)
ALT >120	884 (49.4)	1920	1015 (52.9)	1 (1,8)	129 (69, 240)
ALT >140	799 (44.6)	1635	823 (50.3)	1 (1,6)	121 (67, 214)
ALT >160	713 (39.8)	1410	705 (50.0)	1 (1,7)	102 (60, 187)
ALT >180	652 (36.4)	1231	608 (49.4)	1 (1,5)	94 (54, 174)
ALT >200	596 (33.3)	1072	525 (49.0)	1 (1, 5)	89 (52, 154)

6.3.5. Verifying the relationship between hepatitis status and ALT elevations in HAART-naive patients

Given the above results which clearly show that HAART-experienced patients are more likely to experience ALT elevations, regardless of which ALT threshold is used, the following analyses were restricted to HAART-naive patients only. Rate-ratios for patients who were HCV-positive (compared to HCV-negative) and HBV-positive (compared to HBV-negative) in relation to experiencing 2 or more consecutive ALT measurements above each threshold are shown in Table 6.3.5.1. In these analyses, patients were defined to be HBV/HCV-positive from the start of follow-up if their first HBV/HCV test was positive. In unadjusted analyses, patients who were HCV-positive were 4-5 times more likely to experience ALT elevations than patients who were HCV-negative, regardless of the threshold used. The rate-ratios increased linearly with increasing ALT thresholds. After adjusting for potential confounders, positive HCV status was generally still strongly associated with ALT elevations, though the magnitude of the estimates were smaller than in the unadjusted analyses. In particular, no association was seen between positive HCV status and ALT elevations >200 IU (p=0.26).

Similar results were seen when analysing HBV status and ALT elevations. Higher ALT thresholds were associated with higher rate ratios of experiencing an ALT elevation for positive HBV status (compared to negative), and the magnitude of these estimates, though still highly significant, were smaller after adjusting for potential confounders. In addition, the increase in risk associated with positive HBV status was smaller than that of positive HCV status, regardless of the threshold used.

In sensitivity analyses, the date of positive hepatitis status was taken as the date of the first positive HBV/HCV test result (as opposed to start of follow-up if the first hepatitis test result was positive). In these analyses, the rate-ratios for positive HCV status were slightly higher than those in the previous analyses (Table 6.3.5.2) and a linear trend (rate-ratios increasing as ALT threshold increases) was evident even after adjusting for potential confounders. Positive HCV status, regardless of which threshold was used, was still also highly associated with the risk of experiencing ALT elevations. The HBV results were very similar to those seen in Table 6.3.5.1, again confirming that patients with positive HBV tests were more likely to experience ALT elevations than those with negative HBV tests. This analysis also shows that any bias caused by potential misclassification of person-time in the previous

analyses (defining patients as HBV/HCV-positive if their first test is positive), is likely to be negligible, although results suggest that associations may have been slightly underestimated.

Table 6.3.5.1: Rate-ratios for patients who are HCV-positive (vs. HCV-negative) and HBV-positive (vs. HBV-negative) in relation to predicting ALT elevation (defined as ≥2 consecutive measurements above each threshold) in HAART-naïve patients (n=14242) (Patients defined as HBV/HCV-positive from start of follow-up if first HBV/HCV test is positive)

ALT threshold (IU)	No. of episodes above threshold	Rate-ratios (95% CI[1]) for HCV+ vs. HCV- in predicting ALT rises				Rate-ratios (95% CI[1]) for HBV+ vs. HBV- in predicting ALT rises			
		Unadjusted	P-value	Adjusted	P-value	Unadjusted	P-value	Adjusted	P-value
>100	1111	4.67 (4.02, 5.44)	<0.0001	1.31 (1.06, 1.61)	0.01	2.99 (2.57, 3.48)	<0.0001	1.86 (1.58, 2.19)	<0.0001
>120	833	4.74 (3.99, 5.64)	<0.0001	1.63 (1.31, 2.03)	<0.0001	2.94 (2.47, 3.50)	<0.0001	1.58 (1.31, 1.91)	<0.0001
>140	645	4.72 (3.88, 5.74)	<0.0001	2.10 (1.66, 2.66)	<0.0001	2.80 (2.29, 3.42)	<0.0001	1.65 (1.33, 2.05)	<0.0001
>160	535	5.18 (4.18, 6.42)	<0.0001	1.85 (1.42, 2.40)	<0.0001	2.71 (2.18, 3.38)	<0.0001	1.92 (1.50, 2.45)	<0.0001
>180	442	5.27 (4.19, 6.64)	<0.0001	1.88 (1.39, 2.53)	<0.0001	2.86 (2.26, 3.63)	<0.0001	1.62 (1.24, 2.12)	0.004
>200	384	5.54 (4.34, 7.07)	<0.0001	0.75 (0.69, 1.35)	0.26	3.03 (2.36, 3.89)	<0.0001	0.97 (0.69, 1.35)	0.26

[1] CI: Confidence interval

Table 6.3.5.2: Rate-ratios for patients who are HCV-positive (vs. HCV-negative) and HBV-positive (vs. HBV-negative) in relation to predicting ALT elevation (defined as ≥2 consecutive measurements above each threshold) in HAART-naive patients (n=14242) (Patients defined as HBV/HCV-positive from date of first positive HBV/HCV test result)

ALT threshold (IU)	No. of episodes above threshold	Rate-ratios (95% CI[1]) for HCV+ vs. HCV- in predicting ALT rises				Rate-ratios (95% CI[1]) for HBV+ vs. HBV- in predicting ALT rises			
		Unadjusted	P-value	Adjusted	P-value	Unadjusted	P-value	Adjusted	P-value
>100	1111	5.53 (4.71, 6.48)	<0.0001	1.68 (1.38, 2.04)	<0.0001	2.91 (2.46, 3.43)	<0.0001	1.77 (1.48, 2.11)	<0.0001
>120	833	5.80 (4.85, 6.94)	<0.0001	1.65 (1.32, 2.07)	<0.0001	2.81 (2.32, 3.40)	<0.0001	1.47 (1.19, 1.82)	0.0003
>140	645	6.02 (4.92, 7.37)	<0.0001	2.32 (1.83, 2.96)	<0.0001	2.60 (2.08, 3.24)	<0.0001	1.47 (1.15, 1.88)	0.0002
>160	535	6.54 (5.25, 8.16)	<0.0001	2.20 (1.69, 2.86)	<0.0001	2.46 (1.92, 3.14)	<0.0001	1.72 (1.31, 2.26)	<0.0001
>180	442	6.74 (5.33, 8.54)	<0.0001	2.24 (1.66, 3.01)	<0.0001	2.72 (2.10, 3.53)	<0.0001	1.49 (1.11, 2.00)	0.01
>200	384	7.06 (5.50, 9.07)	<0.0001	3.69 (2.72, 5.00)	<0.0001	2.80 (2.13, 3.69)	<0.0001	0.79 (0.54, 1.14)	0.21

[1] CI: Confidence interval

6.3.6. Moving towards a definition of an ALT flare using data from HAART-naive and HBV/HCV-negative patients

Given the above results, only patients who were HAART-naive and HBV/HCV-negative were included in further analyses to define an ALT flare. Table 6.3.6.1 shows the frequency and duration of ALT elevation episodes in patients who were HAART-naive and HBV/HCV-negative or of unknown status at the time of their ALT measurement. Of the 14,208 patients included in this analysis, 11.3% had at least one ALT >100 IU, whilst 4.1% had at least one ALT >200 IU. 7.2% of patients had 2 consecutive ALT measurements >100 IU and only 2.3% of patients had 2 consecutive ALT measurements >200 IU. 25% of patients had an episode lasting around 4 weeks, whilst the median duration of episodes was around 3 months.

In sensitivity analyses, patients who had ever had a positive HBV or HCV test were excluded. The results of these analyses have been shown previously in Table 6.3.2.1. A slightly smaller proportion of patients experienced ALT elevations above each threshold than those seen in Table 6.3.6.1, suggesting that some patients who tested positive for HBV/HCV after the date of their ALT measurement may have been positive at the time of their ALT measurement but did not have a hepatitis test to confirm this. The median duration of episodes was shorter amongst the subset of patients with subsequent positive HBV/HCV tests excluded. Again, this may be explained by patients in the former analyses not being picked up as HBV/HCV-positive early enough, so in analyses in which patients are known not to be HBV/HCV-positive, the duration of ALT elevations is likely to be shorter.

In the last set of sensitivity analyses, patients who had not had a HBV or HCV test were also excluded (Table 6.3.3.1). Again, this table was shown previously when comparing HAART-naive to HAART-experienced patients. Results were very similar to the above analyses, suggesting that patients with unknown hepatitis status were likely to be HBV/HCV-negative.

Table 6.3.6.1: ALT elevation episodes amongst patients who were HAART-naïve and HBV/HCV-negative or of HBV/HCV unknown status at time of ALT measurements (N=14208)

ALT threshold (IU)	N (%) patients with ≥ 1 ALT above threshold	No. of episodes	Sustained for ≥2 consecutive measurements N (% of episodes)	No. of episodes of ≥ 2 consecutive measurements per patient Median (Range)	Duration (days) of each episode of ≥ 2 consecutive measurements Median (IQR)
ALT >100	1601 (11.3)	2504	857 (34.2)	1 (1,5)	108 (36, 270)
ALT >120	1220 (8.6)	1910	633 (33.1)	1 (1,4)	100 (32, 250)
ALT >140	987 (7.0)	1530	484 (31.6)	1 (1,4)	92 (30, 225)
ALT >160	815 (5.7)	1252	400 (31.9)	1 (1,3)	92 (28, 209)
ALT >180	684 (4.8)	1036	325 (31.4)	1 (1,3)	88 (28, 364)
ALT >200	581 (4.1)	865	277 (32.0)	1 (1,3)	84 (29, 207)

The duration of ALT elevations was analysed in greater detail amongst the 3 groups of patients described in this sub-section. Amongst patients who were not known to be HBV/HCV-positive at the time of their ALT measurement, the proportion of patients experiencing episodes lasting >2 weeks was around 85% (Table 6.3.6.2). The proportion of patients experiencing episodes lasting longer than 1 month, 3 months and 6 months decreased as the ALT threshold increased. For ALT elevations >200 IU, 87.0%, 73.6%, 46.6% and 28.9% of patients experienced episodes lasting longer than 2 weeks, 1 month, 3 months and 6 months respectively.

Amongst patients who had never had a positive HBV/HCV test result, trends were similar to the above analyses, though the proportions calculated were slightly lower (Table 6.3.6.3). Of the 196 ALT elevation episodes >200 IU, 84.2%, 68.4%, 44.9% and 27.6% lasted longer than 2 weeks, 1 month, 3 months and 6 months respectively.
Little difference was seen in the results when analyses were restricted to patients who had had at least one HBV/HCV test (Table 6.3.6.4).

Table 6.3.6.2: Duration of elevations amongst patients who were HAART-naïve and HBV/HCV-negative or of HBV/HCV unknown status at time of ALT measurements (N=14208)

ALT threshold (IU)	No. of episodes[1] above threshold	Episodes lasting >2 weeks N (%)	Episodes lasting >1 month N (%)	Episodes lasting >3 months N (%)	Episodes lasting >6 months N (%)
>100	857	755 (88.1)	663 (77.3)	471 (55.0)	301 (35.1)
>120	633	548 (86.6)	477 (75.4)	335 (52.9)	218 (34.4)
>140	484	416 (85.9)	361 (74.6)	241 (49.8)	152 (31.4)
>160	400	340 (85.0)	290 (72.5)	198 (49.5)	119 (29.8)
>180	325	277 (85.2)	237 (72.9)	154 (47.4)	94 (28.9)
>200	277	241 (87.0)	204 (73.6)	129 (46.6)	80 (28.9)

[1] Defined as 2 consecutive ALT values above each threshold

Table 6.3.6.3: Duration of elevations amongst patients who were HAART-naïve and HBV/HCV-negative or of unknown status throughout follow-up (had never tested positive for HBV/HCV) (N=12564)

ALT threshold (IU)	No. of episodes[1] above threshold	Episodes lasting >2 weeks N (%)	Episodes lasting >1 month N (%)	Episodes lasting >3 months N (%)	Episodes lasting >6 months N (%)
>100	691	602 (87.1)	519 (75.1)	367 (53.1)	234 (33.9)
>120	500	424 (84.8)	359 (71.8)	252 (50.4)	168 (33.6)
>140	387	318 (82.2)	268 (69.3)	178 (46.0)	115 (29.7)
>160	309	256 (82.8)	212 (68.6)	147 (47.6(	90 (29.1)
>180	241	199 (82.6)	165 (68.5)	109 (45.2)	66 (27.4)
>200	196	165 (84.2)	134 (68.4)	88 (44.9)	54 (27.6)

[1] Defined as 2 consecutive ALT values above each threshold

Table 6.3.6.4: Duration of elevations amongst patients who were HAART-naïve and known to be HBV/HCV-negative throughout follow-up (had at least one HBV/HCV test and had never tested positive) (N=10094)

ALT threshold (IU)	No. of episodes[1] above threshold	Episodes lasting >2 weeks N (%)	Episodes lasting >1 month N (%)	Episodes lasting >3 months N (%)	Episodes lasting >6 months N (%)
>100	562	483 (85.9)	413 (73.5)	284 (50.5)	173 (30.8)
>120	415	347 (83.6)	292 (70.4)	201 (48.4)	129 (31.1)
>140	315	261 (82.9)	216 (68.6)	142 (45.1)	86 (27.3)
>160	263	216 (82.1)	175 (66.5)	118 (44.9)	69 (26.2)
>180	208	170 (81.7)	137 (65.9)	91 (43.8)	51 (24.5)
>200	170	143 (84.1)	113 (66.5)	74 (43.5)	43 (25.3)

[1] Defined as 2 consecutive ALT values above each threshold

As a final summary measure, patient follow-up was split into 3-month intervals and the mean ALT measurement in each 3-month and 6-month interval was calculated. The number of patients known to be HBV and HCV-negative, with at least one mean 3-monthly and 6-monthly ALT measurement above each threshold is shown in Table 6.3.6.5.

Table 6.3.6.5: Duration of elevations amongst patients who were HAART-naïve and known to be HBV/HCV-negative throughout follow-up (had at least one HBV/HCV test and had never tested positive) (N=10094)

ALT threshold (IU)	N (%) patients with ≥ 1 ALT above threshold N (%)	Patients with ≥ 1 mean ALT over 3 month period N (%)	Patients with ≥ 1 mean ALT over 6 month period N (%)
>100	1071 (10.6)	641 (6.4)	138 (1.4)
>120	809 (8.0)	451 (4.5)	95 (0.9)
>140	647 (6.4)	360 (3.6)	56 (0.6)
>160	527 (5.2)	288 (2.9)	43 (0.4)
>180	433 (4.3)	230 (2.3)	34 (0.3)
>200	361 (3.6)	187 (1.9)	28 (0.3)

The proportion of patients with a mean ALT measurement sustained over 3 and 6 months decreased as the ALT threshold increased, suggesting that flares of duration longer than 3 months are uncommon. For ALT levels >200 IU, 1.9% and 0.3% of patients had a sustained mean ALT for 3 and 6 months respectively.

6.3.7. Definition of an ALT flare

Amongst the HAART-naïve, HBV/HCV-negative/unknown population, 1601 (11.3%) patients had at least one ALT >100 IU compared to 581 (4.1%) patients with at least one ALT >200 IU. Only 277 (2.3% of the total group) of these patients had 2 consecutive ALT measurements above this level. The majority of these patients (87.0%) had a sustained episode of >2 weeks, though three-quarters of patients had a sustained episode for >1 month. The lower quartile for the duration of these elevations was 29 days, though this was lower in the sensitivity analyses in which only patients who were known to be HBV/HCV-negative were included (18 days). Based on this information, I defined an individual as having an ALT flare if s/he had 2 consecutive ALT measurements >200 IU measured at least 14 days apart. I chose

14 days since 25% of the population had an ALT elevation >200 IU which lasted 18 days (lower quartile of duration of episode), and hence these patients were more likely to have a clinic appointment at 2 weeks, rather than at 18 days apart. Further, almost 90% of patients had a sustained ALT elevation of >2 weeks, suggesting that a gap of 2 weeks between measurements was the minimum that was needed for a stringent definition of an ALT flare.

In sensitivity analyses, I defined an ALT flare as 2 consecutive ALT measurements >200 IU measured at least 30 days apart. This time period was chosen since the lower quartile for the duration of an ALT elevation was around 30 days, regardless of the threshold used. In addition, three-quarters of the patient population had a sustained flare lasting >1 month.

6.3.8. ALT flares (defined as at least 2 ALTs >200 IU, >14 days apart) in the overall population (HAART-naive and HAART-experienced, HBV/HCV-negative and HBV/HCV-positive)

6.3.8.1. Differences between those who experienced an ALT flare and those who did not

Of the 19,707 patients with $\geq$1 ALT measurement available after 1996, 17,634 (89.5%) patients had at least 2 measurements recorded after 1996.

807 (4.6%) patients had at least one ALT-flare using the proposed definition of an ALT flare (2 consecutive measurements >200 IU, >14 days apart). Of those that resolved this first flare, 165 patients (20.4%) experienced at least 2 ALT flares.

Patients who experienced ALT flares were more likely to be male (5.2% of males vs. 1.9% of females experienced an ALT flare, $p<0.0001$), of 'other' risk group (5.5% vs. 2.2% of MSMs and 4.6% of heterosexuals, $p<0.0001$) and of 'other' ethnicity (5.5% vs. 2.1% of white ethnicity and 3.7% of black ethnicity, $p<0.0001$). Patients experiencing ALT flares also had a shorter median duration between ALT measurements (2.2 vs. 2.8 months, $p<0.0001$), were more likely to be HCV-positive (47.0% vs. 3.9% of HCV-negative, $p<0.0001$) and were more likely to be HBV-positive (40.0% vs. 3.9% of HBV-negative, $p<0.0001$) than those who did not experience ALT flares. Furthermore, 5.3% of patients who had started HAART-experienced ALT flares compared to 2.2% of patients who had not yet started HAART ($p<0.0001$).

6.3.8.2. Baseline characteristics of patients who experienced an ALT flare

Baseline characteristics of patients who experienced an ALT flare are shown in Table 6.3.8.2.1. In total, patients contributed 1031 episodes of ALT flares to the analysis. Each episode lasted an average 3.7 months and the median peak ALT measurement recorded during the episode was 531 IU. ALT flares occurred mainly within white males, of MSM risk group, at a median CD4 count and viral load of 363 cells/mm^3 and 368 copies/mL respectively. There was no difference in the duration between ALT measurements amongst those who were HAART-naive and those who had started HAART (2.0 months). 21.9% of patients who had experienced an ALT flare were HCV-positive, whilst 16.7% of patients were HBV-positive. Patients who were HCV-negative had a slightly longer duration between ALT measurements than those who were HCV-positive, though the absolute difference was minimal (2.2 vs. 2.1 months). Almost a third of patients were not receiving any treatment at the time of the ALT flare, whilst another third were receiving regimens not containing d4T/ddI or NVP and a fifth of patients experiencing an ALT flare were receiving d4T/ddI without NVP.

Table 6.3.8.2.1: Characteristics of patients experiencing an ALT flare (2 consecutive ALTs >200 IU, >14 days apart)

Total number of patients	N	17634
No. of patients with flare	N (%)	807 (4.6)
No. of episodes	**N**	**1031**
Age at start of flare (years)	Median (IQR)	39 (34, 44)
CD4 count at start of flare (cells/mm^3)	Median (IQR)	363 (210, 533)
Viral load at start of flare (copies/mL)	Median (IQR)	368 (50, 23400)
Sex N (%)	Males	956 (92.7)
	Females	75 (7.3)
Ethnicity N (%)	White	837 (81.2)
	Black	86 (8.3)
	Other	108 (10.5)
Exposure N (%)	MSM	784 (76.0)
	Heterosexual	122 (11.8)
	Other	125 (12.1)
No. of prior ALT measurements	Median (IQR)	13 (5, 26)
Duration between ALT measurements before starting HAART(months)	Median (IQR)	2.0 (0.8, 3.4)
Duration between ALT measurements after starting HAART(months)	Median (IQR)	2.0 (1.5, 2.7)
HCV-positive[1] prior to flare	N (%)	226 (21.9)
HBV-positive prior to flare	N (%)	172 (16.7)
Duration between ALT measurements before amongst HCV-negative patients (months)	Median (IQR)	2.2 (1.5, 3.0)
Duration between ALT measurements before amongst HCV-positive patients (months)	Median (IQR)	2.1 (1.4, 2.8)
Maximum ALT measurement (IU)	Median (IQR)	531 (361, 962)
Duration of flare (months)	Median (IQR)	3.7 (2.2, 7.0)

AIDS within 6 weeks after flare	Yes	329 (31.9)
HAART regimen	d4T/ddI without NVP	216 (21.0)
	d4T/ddI with NVP	84 (8.2)
	NVP without d4T/ddI	98 (9.5)
	Other regimens	319 (30.9)
	Currently off treatment	314 (30.5)
	Any RTV	279 (27.1)

[1]Defined from date of first positive test

6.3.8.3. Rates of ALT flares

The number of events, person-years (pys) and crude rates of ALT flares for each potential covariate are shown in Table 6.3.8.3.1. The overall rate of ALT flares was 1.02 (95% CI: 0.96, 1.09) /100 pys. This is somewhat lower than the crude risk of an ALT flare shown in Table 6.3.6.1 (277/14208=1.9%). This is to be expected as ALT flares of shorter duration (<14 days) were included in the crude risk calculations. Rates of ALT flares were higher amongst patients of younger age (1.11/100 pys for $\leq$35 years), those with CD4 counts below 200 cells/mm^3 (1.71/100 pys) and those with detectable viral loads (1.23/100 pys). Patients seen in earlier calendar years (1.60/100 pys), those with positive HBV/HCV status (2.02 and 3.74/100 pys respectively) and those on HAART regimens including d4T or DDI (1.56/100 pys) also had higher rates of ALT flares than those seen in the overall population.

The crude rates of ALT-flares stratified by hepatitis and treatment status are shown in Table 6.3.8.3.2. Patients co-infected with HBV/HCV had higher flare rates than those not co-infected, though HBV/HCV-positive HAART-experienced patients had lower rates than HBV/HCV-positive HAART-naive patients. Similar results were seen after removing patients with unknown hepatitis status (Table 6.3.8.3.3). In these analyses, patients of HBV/HCV-negative status had lower ALT flare rates than those seen if patients of unknown hepatitis status were included.

Table 6.3.8.3.1: Events, person-years and crude rates (95% CI) of ALT flares

	Person years	Events	Rate (95% CI)
Total		**1031/100827**	**1.02 (0.96, 1.09)**
Current age (years)	≥35	312/27998	1.11 (0.99, 1.24)
	35-50	490/46203	1.06 (0.97, 1.15)
	>50	229/26626	0.86 (0.75, 0.97)
Current CD4 count	<200	239/14006	1.71 (1.49, 1.92)
(cells/mm^3)	200-349	231/24334	0.95 (0.83, 1.07)
	350-449	227/25725	0.88 (0.77, 1.00)
	≥500	308/35747	0.86 (0.77, 0.96)
	Missing	26/1015	2.56 (1.58, 3.55)
Nadir CD4 count	<50	224/18693	1.20 (1.04, 1.36)
(cells/mm^3)	50-199	362/36864	0.98 (0.88, 1.08)
	200-349	247/25951	0.95 (0.83, 1.07)
	≥350	172/18305	0.94 (0.80, 0.94)
	Missing	26/1015	2.56 (1.58, 3.55)
Current viral load	≤50	353/49852	0.71 (0.63, 0.78)
(copies/mL)	>50	576/46917	1.23 (1.13, 1.33)
	Missing	102/4058	2.51 (2.03, 3.00)
Current calendar year	1996-2000	378/23646	1.60 (1.44, 1.76)
	2001-2004	392/40974	0.96 (0.86, 1.05)
	≥2005	261/36207	0.72 (0.63, 0.81)
Sex	Males	956/84169	1.14 (1.06, 1.21)
	Females	75/16658	0.45 (0.35, 0.55)
Ethnicity	White	837/72433	1.16 (1.08, 1.23)
	Black	86/17610	0.49 (0.38, 0.59)
	Other	108/10785	1.00 (0.81, 1.19)
Exposure N (%)	MSM	784/68372	1.15 (1.07, 1.23)
	Heterosexual	122/22128	0.55 (0.45, 0.65)
	Other	125/10328	1.21 (1.00, 1.42)
No. of prior ALT	<10	427/41124	1.04 (0.94, 1.14)
measurements	10-20	263/27932	0.94 (0.83, 1.06)
	>20	341/31771	1.07 (0.96, 1.19)

HCV status at flare	Positive	223/5965	3.74 (3.25, 4.23)
	Negative	424/56358	0.75 (0.68, 0.82)
	Unknown	384/38505	1.00 (0.90, 1.10)
HBV status at flare	Positive	175/8676	2.02 (1.72, 2.32)
	Negative	535/60924	0.88 (0.80, 0.95)
	Unknown	321/31227	1.03 (0.92, 1.14)
Time since start of HAART	HAART-naive	320/28496	1.03 (0.92, 1.15)
	$\leq$ 3 years	396/29427	1.14 (1.02, 1.26)
	> 3 years	315/42904	0.91(0.82, 1.00)
AIDS diagnosis at time Of flare	Yes	320/25516	1.25 (1.12, 1.39)
	No	711/75311	0.94 (0.88, 1.01)
HAART regimen	No d4T/ddI/NVP	280/33528	0.84 (0.74, 0.93)
	d4T/ddI with NVP	85/5463	1.56 (1.23, 1.89)
	NVP without d4T/ddI	95/10891	0.87 (0.70, 1.05)
	d4T/ddI without NVP	254/19417	1.31 (1.15, 1.47)
	Currently off treatment	317/31528	1.01 (0.90, 1.12)
Any prior RTV experience	Yes	270/21858	1.24 (1.09, 1.38)
	No	761/78970	0.96 (0.90, 1.03)

In sensitivity analyses, patients whose first HBV/HCV test was positive were defined as HBV/HCV-positive from the start of follow-up. Rates of ALT flares stratified by treatment and hepatitis status for these patients are shown in Table 6.3.8.3.4 and Table 6.3.8.3.5. In analyses in which patients of unknown hepatitis status were included, the rates of ALT flares were lower than those seen when positive hepatitis status was defined from the first positive HBV/HCV test. After excluding patients of unknown hepatitis status, rates of ALT flares were similar to those seen in Table 6.3.8.3.3 amongst HBV/HCV-negative patients, though were lower for HAART-naïve HBV/HCV-positive patients and higher for HAART-experienced HBV/HCV-positive patients.

Table 6.3.8.3.2: Events, person-years and rates (95% CI) of ALT flares stratified by hepatitis status (including unknown) and HAART status: Patients defined as HBV/HCV-positive from first date of positive test result

	All	HBV/HCV-negative/unknown		HBV/HCV-positive	
		HAART-naive	HAART-experienced	HAART-naive	HAART-experienced
Events	1031	148	386	165	332
Person-years	100827	24117	63068	3210	10432
Rate (95% CI) /100 person years	1.02 (0.96, 1.09)	0.61 (0.51, 0.71)	0.61 (0.55, 0.67)	5.14 (4.36, 5.93)	3.18 (2.84, 3.52)

Table 6.3.8.3.3: Events, person-years and rates (95% CI) of ALT flares stratified by *known* hepatitis status and HAART status: Patients defined as HBV/HCV-positive from first date of positive test result

	All	HBV/HCV-negative		HBV/HCV-positive	
		HAART-naive	HAART-experienced	HAART-naive	HAART-experienced
Events	689	44	148	165	332
Person-years	58835	10718	34476	3210	10432
Rate (95% CI) /100 person years	1.17 (1.08, 1.26)	0.41 (0.29, 0.53)	0.43 (0.36, 0.50)	5.14 (4.36, 5.93)	3.18 (2.84, 3.52)

Table 6.3.8.3.4: Events, person-years and rates (95% CI) of ALT flares stratified by hepatitis status (including unknown) and HAART status: Patients defined as HBV/HCV-positive from start of follow-up if first test is positive

	All	HBV/HCV-negative/unknown		HBV/HCV-positive	
		HAART-naive	HAART-experienced	HAART-naive	HAART-experienced
Events	1031	126	336	188	404
Person-years	100827	23338	61662	4013	11814
Rate (95% CI) /100 person years	1.02 (0.96, 1.09)	0.54 (0.45, 0.63)	0.55 (0.49, 0.60)	4.69 (4.02, 5.35)	3.42 (3.09, 3.75)

Table 6.3.8.3.5: Events, person-years and rates (95% CI) of ALT flares stratified by *known* hepatitis status and HAART status: Patients defined as HBV/HCV-positive from start of follow-up if first test is positive

	All	HBV/HCV-negative		HBV/HCV-positive	
		HAART-naive	HAART-experienced	HAART-naive	HAART-experienced
Events	761	44	148	188	381
Person-years	61021	10718	34476	4013	11814
Rate (95% CI) /100 person years	1.12 (1.03, 1.20)	0.41 (0.29, 0.53)	0.43 (0.36, 0.50)	4.69 (4.02, 5.35)	3.42 (3.09, 3.75)

6.3.8.4. Factors associated with ALT flares

Table 6.3.8.4.1 shows the unadjusted and adjusted factors associated with experiencing an ALT flare. In univariable analyses, patients aged above 50 years (RR=0.77 (95% CI: 0.65, 0.92) vs. <35 years), those of female sex (0.40 (0.31, 0.50) compared to male sex), of black ethnicity (0.42 (0.34, 0.53) compared to white ethnicity) and of heterosexual risk group (0.48 (0.40, 0.58) compared to MSM) were less likely to experience ALT flares. Current CD4 count was linearly associated with the risk of an ALT flare; patients with lower current CD4 counts were more likely to experience ALT flares than those with higher current CD4 counts (1.98 (1.67, 2.35) for CD4 counts <200 compared to $\geq$500 cells/mm^3). A similar trend was observed with nadir CD4 count. Patients with detectable VLs (1.73 (1.52, 1.98) comparing >50 copies/mL to $\leq$50 copies/mL), those seen in earlier calendar years (2.20 (1.89, 2.60) comparing patients seen in 1996-2000 to those seen after 2005), those with positive HBV and HCV status (2.30 (1.94, 2.72) and 4.97 (4.23, 5.84) respectively) and patients currently receiving d4T/ddI containing regimens (1.86 (1.46, 2.38) and 1.57 (1.32, 1.86) comparing d4T/ddI with NVP and d4T/ddI without NVP to regimens not containing d4T/ddI) were at an increased risk of experiencing an ALT flare. Patients who had had any prior RTV experience were also more likely to experience an ALT flare (1.28 (1.12, 1.47)).

With the exception of nadir CD4 count and current calendar year, all variables remained significantly associated with the risk of an ALT flare after adjusting for potential confounders. A linear association was seen between the nadir CD4 count and the risk of an ALT flare, but this association did not reach statistical significance. Positive HCV status remained highly associated with ALT flares (4.99 (4.18, 5.96)), as did regimens containing d4T/ddI with NVP (1.73 (1.34, 2.24)) and d4T/ddI without NVP (1.22 (1.02, 1.47)). Patients receiving regimens containing NVP without d4T/ddI were also at an increased risk of experiencing an ALT flare (1.50 (1.18, 1.92)). Patients who had been receiving HAART for >3 years were less likely to experience ALT flares (0.62 (0.47, 0.80)) compared to those who were HAART-naive. Patients receiving HAART for <3 years were more likely to experience ALT flares than HAART-naive patients, though this association was not significant in adjusted analyses.

Table 6.3.8.4.1: Factors associated with experiencing an ALT flare (2 consecutive ALTs>200 IU, > 14 days apart) from adjusted and unadjusted Poisson regression analyses

		Unadjusted RR (95% CI)[1]		Adjusted RR (95% CI)	
		RR (95% CI)	**P-value**	**RR (95% CI)**	**P-value**
Current age (years)	≥35	1	-	1	-
	35-50	0.95 (0.83, 1.10)	0.49	0.95 (0.82, 1.11)	0.53
	>50	0.77 (0.65, 0.92)	0.003	0.81 (0.67, 0.97)	0.02
Current CD4 count	<200	1.98 (1.67, 2.35)	<0.0001	1.31 (1.04, 1.65)	0.02
(cells/mm^3)	200-349	1.10 (0.93, 1.31)	0.27	0.92 (0.75, 1.12)	0.41
	350-449	1.02 (0.86, 1.22)	0.79	0.93 (0.78, 1.12)	0.45
	≥500	1	-	1	-
	Missing	2.97 (1.99, 4.43)	<0.0001	Excluded[2]	
Nadir CD4	<50	1.28 (1.05, 1.56)	0.02	0.86 (0.63, 1.17)	0.33
	50-199	1.05 (0.87, 1.25)	0.64	0.92 (0.71, 1.18)	0.51
	200-349	1.01 (0.83, 1.23)	0.90	1.00 (0.80, 1.24)	0.97
	≥350	1	-	1	-
	Missing	2.73 (1.80, 4.12)	<0.0001	Excluded	
Current viral load	≤50	1	-	1	-
(copies/mL)	>50	1.73 (1.52, 1.98)	<0.0001	1.82 (1.54, 2.14)	<0.0001

	Missing	3.55 (2.85, 4.42)	<0.0001	3.50 (2.60, 4.72)	<0.0001
Current calendar year	1996-2000	2.20 (1.89, 2.60)	<0.0001	1.16 (0.98, 1.37)	0.08
	2001-2004	1.33 (1.13, 1.55)	0.0004	1.16 (0.98, 1.37)	0.09
	≥2005	1	-	1	-
Sex	Male	1	-	1	-
	Female	0.40 (0.31, 0.50)	<0.0001	0.49 (0.37, 0.65)	<0.0001
Ethnicity	White	1	-	1	-
	Black	0.42 (0.34, 0.53)	<0.0001	0.64 (0.49, 0.84)	0.001
	Other	0.87 (0.71, 1.06)	0.16	0.96 (0.78, 1.18)	0.72
Exposure	MSM	1	-	1	-
	Heterosexual	0.48 (0.40, 0.58)	<0.0001	0.91 (0.70, 1.17)	0.45
	Other	1.05 (0.87, 1.27)	0.57	0.71 (0.57, 0.88)	0.002
No. of prior ALT	<10	1	-	1	-
measurements	10-20	0.91 (0.78, 1.06)	0.21	1.09 (0.93, 1.28)	0.30
	>20	1.03 (0.90, 1.19)	0.65	1.32 (1.10, 1.55)	0.002
HCV status prior to flare	Negative	1	-	1	-
	Positive	4.97 (4.23, 5.84)	<0.0001	4.99 (4.18, 5.96)	<0.0001
	Unknown	1.33 (1.15, 1.52)	<0.0001	1.01 (0.85, 1.21)	0.90
HBV-positive prior to flare	Negative	1	-	1	-

	Positive	2.30 (1.94, 2.72)	<0.0001	1.73 (1.45, 2.07)	<0.0001
	Unknown	1.17 (1.02, 1.34)	0.03	1.26 (1.06, 1.50)	0.01
Time since start of HAART	HAART-naive	1	-	1	-
	≤ 3 years	1.20 (1.03, 1.39)	0.02	1.14 (0.91, 1.44)	0.26
	> 3 years	0.65 (0.56, 0.76)	<0.0001	0.62 (0.47, 0.80)	0.0003
AIDS diagnosis prior to flare	Yes	1.33 (1.16, 1.52)	<0.0001	1.16 (0.99, 1.36)	0.06
HAART regimen	No d4T/ddI/NVP	1	-	1	-
	d4T/ddI with NVP	1.86 (1.46, 2,38)	<0.0001	1.73 (1.34, 2.24)	<0.0001
	NVP without d4T/ddI	1.04 (0.83, 1.32)	0.71	1.50 (1.18, 1.92)	0.001
	d4T/ddI without NVP	1.57 (1.32, 1.86)	<0.0001	1.22 (1.02, 1.47)	0.03
	Currently off treatment	1.20 (1.03, 1.41)	0.02	0.82 (0.65, 1.04)	0.10
Any prior RTV experience	Yes	1.28 (1.12, 1.47)	0.001	1.58 (1.34, 1.87)	<0.0001

[1] RR: rate-ratios, CI: confidence intervals

[2] Missing categories were excluded due to co-linearity between current and nadir CD4 counts

6.3.8.5. Sensitivity analyses and interactions between hepatitis status and treatment status, and between CD4 status and treatment status

In sensitivity analyses, patients with unknown hepatitis status were excluded from the multivariable regression model. The adjusted rate ratio for positive HCV status was higher (5.99 (4.95, 7.24)) in these analyses, but was lower for positive HBV status (1.53 (1.26, 1.87)). If positive hepatitis status was defined from the start of follow-up amongst patients whose first HBV/HCV test result was positive, rate-ratios for both positive HBV status (2.08 (1.77, 2.44)) and positive HCV status (5.29 (4.48, 6.23)) were higher than those seen in the main analyses.

A significant interaction was identified between known HCV status and current regimen (p=0.01). Analyses were therefore stratified by current regimen and rate-ratios for positive HCV status were identified. Though positive HCV status was associated with the risk of an ALT flare, regardless of which regimen patients were receiving, the rate-ratio was significantly lower for those currently off treatment (4.47 (3.18, 6.28)) than those receiving treatment (RRs ranging from 6.41 to 7.17 depending on which regimen was being received).

In contrast, there was no significant interaction between current CD4 count and current regimen (p>0.15), or between HBV status and current regimen (p=0.31).

6.3.9. ALT flares (defined as at least 2 consecutive ALTs >200 IU, >30 days apart)

In sensitivity analyses, an ALT flare was defined as experiencing at least 2 ALTs >200 IU, at least 30 days apart. Consistent with the result that the longer the duration of sustained elevation, the rarer the event (Table 6.3.6.2), the number of patients experiencing an ALT flare using this definition (N=644, 3.6%), was lower than that seen in Section 6.3.8 in which a flare was defined as at least 2 ALTs >200 IU, >14 days apart (4.6%).

6.3.9.1. Characteristics of patients experiencing and ALT flare; 2 consecutive ALTs >200 IU, >30 days apart

A total of 803 episodes of ALT flares using this definition occurred; characteristics of patients experiencing an ALT flare were generally similar to those seen using the definition in the main analyses (Table 6.3.9.1.1). Patients in this analysis did have a higher VL at the time of the flare (400 vs. 368 copies/mL in the main analysis), were more likely to be HCV-positive (24.2% vs. 21.9% in the main analysis) and, as

expected, had a longer flare duration (median 4.8 vs. 3.7 months in the main analysis) than those patients included in the main analyses.

Table 6.3.9.1.1: Characteristics of patients at the time of each ALT flare

Total number of patients	N	17634
No. of patients with flare	N (%)	644 (3.7)
No. of episodes	**N**	**803**
Age at start of flare (years)	Median (IQR)	39 (34, 44)
CD4 count at start of flare (cells/mm^3)	Median (IQR)	370 (210, 540)
Viral load at start of flare (copies/mL)	Median (IQR)	400 (50, 29200)
Sex N (%)	Males	745 (92.8)
	Females	58 (7.2)
Ethnicity N (%)	White	663 (82.6)
	Black	43 (5.4)
	Other	97 (12.1)
Exposure N (%)	Homosexual	603 (75.1)
	Heterosexual	91 (11.3)
	Other	109 (13.6)
No. of prior ALT measurements	Median (IQR)	13 (5, 26)
Duration between ALT measurements before starting HAART (months)	Median (IQR)	2.0 (0.9, 3.5)
Duration between ALT measurements after starting HAART (months)	Median (IQR)	2.0 (1.5, 2.8)
HCV-positive[1] prior to flare	N (%)	194 (24.2)
HBV-positive prior to flare	N (%)	132 (16.4)
Duration between ALT measurements amongst HCV-negative patients (months)	Median (IQR)	2.3 (1.5, 3.1)
Duration between ALT measurements amongst HCV-positive patients (months)	Median (IQR)	2.1 (1.4, 2.8)
Maximum ALT measurement (IU)	Median (IQR)	521 (357, 945)
Duration of flare (months)	Median (IQR)	4.8 (3.1, 8.0)

AIDS within 6 weeks after flare	Yes	265 (33.0)
HAART regimen	d4T/ddI without NVP	153 (19.1)
	d4T/ddI with NVP	67 (8.3)
	NVP without d4T/ddI	79 (9.8)
	Other regimens	261 (32.5)
	Currently off treatment	243 (30.3)
	Any RTV	218 (27.1)

[1]Defined from date of first positive test

6.3.9.2. Events, person-years and rates (95% CI) of ALT flares

The overall rate of an ALT flare using this definition was 0.80 (0.74, 0.85) / 100 pys. As expected, this was somewhat lower that seen in the main analysis (1.02/100 pys). As before, patients who were HBV/HCV-positive had higher rates of ALT flares than those who were HBV/HCV-negative/unknown, though amongst the positive group, HAART-experienced patients had lower rates than HAART-naïve patients (Table 6.3.9.2.1). There was little difference in the rate of ALT flares between HAART-naive and HAART-experienced patients in the HBV/HCV-negative group. Since the overall rate of an ALT flare was lower in these analyses, the group-specific rates were also lower compared to the main analyses. Crude rates for other variables are shown in Table 6.3.9.2.2. Again, in general, the rates were lower, but were in line with the findings in the main analyses.

Table 6.3.9.2.1: Events, person-years and rates (95% CI) of ALT flares (2 consecutive ALTs >200 IU, >30 days apart) stratified by hepatitis status[1] and HAART status

	All	HBV/HCV-negative/unknown		HBV/HCV-positive	
		HAART-naive	HAART-experienced	HAART-naive	HAART-experienced
Events	803	110	300	129	264
Person-years	100881	24132	63088	3218	10443
Rate (95% CI) /100 person years	0.80 (0.74, 0.85)	0.46 (0.37, 0.54)	0.48 (0.42, 0.53)	4.00 (3.32, 4.70)	2.53 (2.83, 2.53)

[1] Patients defined as positive from first date of positive test result

Table 6.3.9.2.2: Events, person-years and crude rates (95% CI) of ALT flares (2 consecutive ALTs >200 IU, >30 days apart)

	Person years	Events	Rate (95% CI)
Total		**803/100881**	**0.80 (0.74, 0.85)**
Current age (years)	≥35	253/28011	0.90 (0.7, 1.02)
	35-50	392/46227	0.85 (0.76, 0.93)
	>50	158/26642	0.59 (0.50, 0.69)
Current CD4 count (cells/mm^3)	<200	182/14011	1.30 (1.11, 1.49)
	200-349	175/24354	0.72 (0.61, 0.83)
	350-449	178/25738	0.69 (0.59, 0.79)
	≥500	249/35762	0.70 (0.61, 0.78)
	Missing	19/1016	1.87 (1.03, 2.71)
Nadir CD4 count (cells/mm^3)	<50	174/18693	0.93 (0.79, 1.07)
	50-199	279/36883	0.76 (0.67, 0.85)
	200-349	196/25972	0.76 (0.65, 0.86)
	≥350	135/18317	0.74 (0.61, 0.86)
	Missing	19/1016	1.87 (1.03, 2.71)
Current viral load (copies/mL)	≤50	269/49868	0.54 (0.48, 0.60)
	>50	455/46950	0.97 (0.88, 1.06)
	Missing	79/4063	1.94 (1.52, 2.37)
Current calendar year	1996-2000	287/23657	1.21 (1.07, 1.35)
	2001-2004	310/41001	0.76 (0.67, 0.84)
	≥2005	206/36223	0.57 (0.49, 0.65)
Sex N (%)	Males	745/84222	0.89 (0.82, 0.95)
	Females	58/16659	0.35 (0.26, 0.44)
Ethnicity N (%)	White	663/72470	0.92 (0.85, 0.99)
	Black	62/17619	0.35 (0.26, 0.44)
	Other	78/10792	0.72 (0.56, 0.88)
Exposure N (%)	Homosexual	603/68420	0.88 (0.81, 0.95)
	Heterosexual	91/22130	0.41 (0.33, 0.50)
	Other	109/10331	1.06 (0.86, 1.25)
No. of prior ALT measurements	<10	333/41146	0.81 (0.72, 0.90)
	10-20	204/27949	0.73 (0.63, 0.83)

	>20	266/31786	0.84 (0.74, 0.94)
HCV status at flare	Positive	188/5971	3.15 (2.70, 3.60)
	Negative	314/56389	0.56 (0.50, 0.62)
	Unknown	301/38521	0.78 (0.69, 0.87)
HBV status at flare	Positive	130/8690	1.50 (1.24, 1.75)
	Negative	423/60947	0.69 (0.63, 0.76)
	Unknown	250/31244	0.80 (0.70, 0.90)
Time since start of HAART	HAART-naive	245/28519	0.86 (0.75, 0.97)
	$\leq$ 3 years	315/29440	1.07 (0.95, 1.19)
	> 3 years	243/42921	0.57 (0.50, 0.64)
AIDS diagnosis at time of flare	Yes	256/25516	1.00 (0.88, 1.13)
	No	547/75365	0.73 (0.67, 0.79)
HAART regimen	No d4T/ddI/NVP	236/33543	0.70 (0.61, 0.79)
	d4T/ddI with NVP	70/5463	1.28 (0.98, 1.58)
	NVP without d4T/ddI	75/10894	0.69 (0.53, 0.84)
	d4T/ddI without NVP	179/19427	0.92 (0.79, 1.06)
	Currently off treatment	243/31554	0.77 (0.67, 0.87)
Any prior RTV experience	Yes	210/21866	0.96 (0.83, 1.09)
	No	593/79015	0.75 (0.69, 0.81)

6.3.9.3. Factors associated with ALT flares (2 consecutive ALTs >200 IU, >30 days apart)

Table 6.3.9.3.1 shows factors associated with ALT flares in unadjusted and adjusted analyses. In unadjusted analyses, trends were in line with those seen in the main analyses, with estimates generally being very close to those seen in Table 6.3.8.4.1 (albeit less precise). Estimates were slightly stronger for positive HCV status (5.65 (4.72, 6.77) compared to 4.97 (4.23, 5.84) in the main analysis), though the confidence intervals did overlap.

In adjusted analyses, though estimates were in the same direction as those seen in the main analyses, they were generally weaker. However, the rate ratio for positive HCV status was again stronger than that seen in the main analyses (5.53 (4.54, 6.75) vs. 4.99 (4.18, 5.96) in the main analysis).

Table 6.3.9.3.1: Factors associated with experiencing an ALT flare (2 consecutive ALTs>200 IU, > 30 days apart) from adjusted and unadjusted Poisson regression analyses

		Unadjusted RR (95% CI)[1]		Adjusted RR (95% CI)	
		RR (95% CI)	**P-value**	**RR (95% CI)**	**P-value**
Current age (years)	≥35	1	-	1	-
	35-50	0.94 (0.80, 1.10)	0.43	0.90 (0.76, 1.07)	0.23
	>50	0.66 (0.54, 0.80)	<0.0001	0.66 (0.53, 0.82)	0.0001
Current CD4 count	<200	1.87 (1.54, 2.26)	<0.0001	1.16 (0.89, 1.51)	0.26
(cells/mm^3)	200-349	1.03 (0.85, 1.25)	0.75	0.83 (0.66, 1.04)	0.10
	350-449	0.99 (0.82, 1.20)	0.95	0.89 (0.73, 1.09)	0.25
	≥500	1	-	1	-
	Missing	2.69 (1.68, 4.28)	<0.0001	Excluded	
Nadir CD4	<50	1.26 (1.01, 1.58)	0.04	0.92 (0.65, 1.29)	0.62
	50-199	1.03 (0.84, 1.26)	0.80	0.96 (0.72, 1.28)	0.77
	200-349	1.02 (0.82, 1.27)	0.83	1.04 (0.81, 1.34)	0.75
	≥350	1	-	1	-
	Missing	2.54 (1.57, 4.10)	0.0001	Excluded	
Current viral load	≤50	1	-	1	-
(copies/mL)	>50	1.80 (1.55, 2.09)	<0.0001	1.99 (1.65, 2.39)	<0.0001

	Missing	3.60 (2.80, 4.63)	<0.0001	3.74 (2.67, 5.25)	<0.0001
Current calendar year	1996-2000	2.13 (1.78, 2.55)	<0.0001	1.15 (0.90, 0.46)	0.26
	2001-2004	1.33 (1.11, 1.59)	0.002	1.16 (0.96, 1.40)	0.13
	≥2005	1	-	1	-
Sex	Male	1	-	1	-
	Female	0.39 (0.30, 0.51)	<0.0001	0.46 (0.33, 0.64)	<0.0001
Ethnicity	White	1	-	1	-
	Black	0.38 (0.30, 0.50)	<0.0001	0.58 (0.42, 0.79)	0.001
	Other	0.79 (0.62, 1.00)	0.05	0.86 (0.68, 1.10)	0.23
Exposure	Homosexual	1	-	1	-
	Heterosexual	0.47 (0.37, 0.58)	<0.0001	0.90 (0.67, 1.20)	0.47
	Other	1.20 (0.98, 1.47)	0.08	0.78 (0.61, 0.90)	0.03
No. of prior ALT	<10	1	-	1	-
measurements	10-20	0.90 (0.76, 1.07)	0.25	1.09 (0.90, 1.31)	0.37
	>20	1.03 (0.88, 1.21)	0.68	1.34 (1.10, 1.64)	0.004
HCV status prior to flare	Negative	1	-	1	-
	Positive	5.65 (4.72, 6.77)	<0.0001	5.53 (4.54, 6.75)	<0.0001
	Unknown	1.40 (1.20 1.64)	<0.0001	1.12 (0.91, 1.37)	0.29
HBV-positive prior to flare	Negative	1	-	1	-

	Positive	2.16 (1.77, 2.63)	<0.0001	1.63 (1.33, 2.00)	<0.0001
	Unknown	1.15 (0.99, 1.35)	0.07	1.20 (0.98, 1.46)	0.07
Time since start of HAART	HAART-naive	1	-	1	-
	≤ 3 years	1.25 (1.05, 1.47)	0.01	1.18 (0.91, 1.52)	0.22
	> 3 years	0.66 (0.55, 0.79)	<0.0001	0.61 (0.45, 0.82)	0.001
AIDS diagnosis prior to flare	Yes	1.38 (1.19, 1.60)	<0.0001	1.26 (1.06, 1.50)	0.01
HAART regimen	No d4T/ddI/NVP	1	-	1	-
	d4T/ddI with NVP	1.82 (1.39, 2.38)	<0.0001	1.67 (1.26, 2.21)	0.0004
	NVP without d4T/ddI	0.98 (0.75, 1.27)	0.87	1.43 (1.09, 1.87)	0.01
	d4T/ddI without NVP	1.31 (1.08, 1.59)	0.01	1.00 (0.81, 1.23)	0.98
	Currently off treatment	1.09 (0.92, 1.31)	0.32	0.73 (0.56, 0.96)	0.02
Any prior RTV experience	Yes	1.28 (1.09, 1. 05)	0.002	1.60 (1.32, 1.93)	<0.0001

[1] RR: rate-ratios, CI: confidence interval

6.3.10. Comparing the derived definition of an ALT flare to a commonly used definition in the literature (1 ALT >200 IU)

Using the single ALT >200 IU definition for an ALT flare (commonly used in the literature; Chapter 2.2.1), 2280 patients had experienced at least one ALT flare, contributing 3,602 episodes of ALT flares to the analysis. The overall rate of an ALT flare using this definition was 3.60 (3.48, 3.71)/100 pys. This was considerably higher than the rate calculated when using the 2 consecutive ALTs >200 IU, >14 days apart' definition. Table 6.3.10.1 shows factors independently associated with ALT flares when using the 1 ALT >200 IU definition and when using the 2 consecutive ALTs >200 IU, >14 days apart definition. Stronger associations were seen between current CD4 count and ALT flares when using the 1 ALT >200 IU definition (1.75 (1.54, 1.98) comparing CD4 <200 cells/mm^3 to CD4 >500 cells/mm^3 compared to 1.31 (1.04, 1.65) when using the derived definition) and weaker associations were seen between positive HCV status and ALT flares (3.41 (3.07, 3.78) compared to 4.99 (4.18, 5.96) using the derived definition). Weaker associations were also seen between current regimen and ALT flares when using the 1 ALT >200 IU definition.

Given the stronger associations seen between CD4 count and ALT flares when using the 1 ALT >200 IU definition, the frequency of CD4 measurements was calculated for patients with an ALT flare and for those who did not experience an ALT flare (using the 1 ALT >200 IU definition). Patients experiencing an ALT flare had a median 31 (18, 44) CD4 measurements during follow-up, whilst those not experiencing an ALT flare had a median 19 (8, 31) CD4 measurements during follow-up. Since data on patient visits is not collected in the UK CHIC Study, CD4 counts can be used as a surrogate marker for patient visits. This suggests that patients with ALT flares may have been monitored more frequently than those without ALT flares and it is possible that the association between CD4 count and risk of flares using the 1 ALT >200 IU definition could simply be explained by this difference in monitoring.

Table 6.3.10.1: Adjusted rate ratios of ALT flares in the whole population using 2 different definitions of an ALT flare using a Poisson regression model

		1 ALT >200 IU		2 ALTs >200 IU, >14 days apart	
		RR (95% CI)	**P-value**	**RR (95% CI)**	**P-value**
Current age (years)	≥35	1	-	1	-
	35-50	0.93 (0.86, 1.00)	0.01	0.95 (0.82, 1.11)	0.53
	>50	0.74 (0.67, 0.82)	<0.001	0.81 (0.67, 0.97)	0.02
Current CD4 count	<200	1.75 (1.54, 1.98)	<0.0001	1.31 (1.04, 1.65)	0.02
(cells/mm^3)	200-349	1.06 (0.94, 1.18)	0.27	0.92 (0.75, 1.12)	0.41
	350-449	0.95 (0.86, 1.05)	0.79	0.93 (0.78, 1.12)	0.45
	≥500	1	-	1	-
Nadir CD4	<50	0.95 (0.80, 1.12	0.51	0.86 (0.63, 1.17)	0.33
	50-199	0.95 (0.83, 1.10)	0.52	0.92 (0.71, 1.18)	0.51
	200-349	1.02 (0.90, 1.15)	0.79	1.00 (0.80, 1.24)	0.97
	≥350	1	-	1	-
Current viral load	≤50	1	-	1	-
(copies/mL)	>50	1.55 (1.42, 1.70)	<0.0001	1.82 (1.54, 2.14)	<0.0001
	Missing	2.88 (2.47, 3.37)	<0.0001	3.50 (2.60, 4.72)	<0.0001
Current calendar year	1996-2000	1.59 (1.42, 1.79)	<0.0001	1.16 (0.98, 1.37)	0.08

	2001-2004	1.34 (1.22, 1.48)	<0.0001	1.16 (0.98, 1.37)	0.09
	≥2005	1	-	1	-
Sex	Male	1	-	1	-
	Female	0.62 (0.54, 0.71)	<0.0001	0.49 (0.37, 0.65)	<0.0001
Ethnicity	White	1	-	1	-
	Black	0.69 (0.60, 0.78)	<0.0001	0.64 (0.49, 0.84)	0.001
	Other	0.94 (0.84, 1.05)	0.28	0.96 (0.78, 1.18)	0.72
Exposure	MSM	1	-	1	-
	Heterosexual	1.08 (0.95, 1.23)	0.24	0.91 (0.70, 1.17)	0.45
	Other	0.91 (0.82, 1.02)	0.11	0.71 (0.57, 0.88)	0.002
No. of prior ALT	<10	1	-	1	-
measurements	10-20	1.21 (1.11, 1.32)	<0.0001	1.09 (0.93, 1.28)	0.30
	>20	1.61 (1.46, 1.77)	<0.0001	1.32 (1.10, 1.55)	0.002
HCV status prior to flare	Negative	1	-	1	-
	Positive	3.41 (3.07, 3.78)	<0.0001	4.99 (4.18, 5.96)	<0.0001
	Unknown	1.04 (0.95, 1.14)	0.40	1.01 (0.85, 1.21)	0.90
HBV-positive prior to flare	Negative	1	-	1	-
	Positive	1.64 (1.49, 1.81)	<0.0001	1.73 (1.45, 2.07)	<0.0001
	Unknown	1.17 (1.07, 1.28)	0.001	1.26 (1.06, 1.50)	0.01

Time since start of HAART	HAART-naive	1	-	1	-
	≤ 3 years	1.10 (0.97, 1.24)	0.12	1.14 (0.91, 1.44)	0.26
	> 3 years	0.63 (0.55, 0.72)	<0.0001	0.62 (0.47, 0.80)	0.0003
AIDS diagnosis prior to flare	Yes	1.20 (1.11, 1.31)	<0.0001	1.16 (0.99, 1.36)	0.06
HAART regimen	No d4T/ddI/NVP	1	-	1	-
	d4T/ddI with NVP	1.40 (1.22, 1,61)	<0.0001	1.73 (1.34, 2.24)	<0.0001
	NVP without d4T/ddI	1.18 (1.03, 1.35)	0.02	1.50 (1.18, 1.92)	0.001
	d4T/ddI without NVP	1.06 (0.97, 1.17)	0.21	1.22 (1.02, 1.47)	0.03
	Currently off treatment	0.87 (0.77, 0.98)	0.02	0.82 (0.65, 1.04)	0.10
Any prior RTV experience	Yes	1.35 (1.23, 1.48)	0.001	1.58 (1.34, 1.87)	<0.0001

6.3.10.1. Association between ALT flares and mortality

In total, 1666 (9.5%) patients died after having previously had at least one ALT measurement. Patients were stratified according to whether or not they experienced an ALT flare using both the derived definition (2 consecutive ALTs >200 IU, >14 days apart) and the standard definition (1 ALT>200 IU). Kaplan-Meier plots stratified by whether or not patients had a flare are shown in Figure 6.3.10.1.1 and in Figure 6.3.10.1.2. When using the derived definition of an ALT flare, no difference in mortality rates were seen amongst those who did experience an ALT flare and those who did not (log-rank p=0.86). Mortality rates at 1 year, 3 years, 5 years and 10 years after the first ALT measurement were 3.2%, 6.2%, 8.6% and 14.6% respectively for those who did not experience an ALT flare and 0.5%, 4.9%, 8.0% and 15.5% respectively for those who did experience an ALT flare.

However, when using the standard definition of an ALT flare found in the literature (1 ALT >200 IU), patients who experienced ALT flares were found to be at increased risk of death than those who did not experience an ALT flare (p<0.0001). Mortality rates at 1 year, 3 years, 5 years and 10 years after the first ALT measurement for these patients were 3.1%, 5.9%, 8.2% and 13.8% respectively for those who did not experience an ALT flare and 3.2%, 7.5%, 10.9% and 19.1% respectively for those who did experience an ALT flare.

The association between current ALT flares (time-updated) and death was explored using Cox regression. In unadjusted analyses, patients with an ALT flare using the standard definition of 1 ALT >200 IU were over 3 times more likely to die than those who did not have an ALT flare (HR=3.73 (2.92, 4.76)). However, when using the derived definition, the hazard ratio was reduced to 1.97 (1.12, 3.48). After adjusting for potential confounders, using the standard definition of an ALT flare, patients experiencing ALT flares were still at an increased risk of death than those without ALT flares (2.10 (1.62, 2.72)). Using the derived definition of an ALT flare, this association was no longer significant (1.27 (0.71, 2.26)).

Interestingly, monitoring bias did not appear to explain the difference in hazard ratios seen above. Patients who died did have fewer CD4 counts than those who did not die (median 8 vs. 19) though this can be explained by the longer follow-up time in the latter period. The median number of CD4 counts per year was similar in both groups (4.0 (3.1, 5.0) and 4.0 (2.1, 5.9) amongst those who did not die and those who died respectively). The absolute number of ALT measurements was also similar

amongst those who did not die (16 (67, 29)) and those who died (15 (6, 32)). However, the difference in the average number of ALT measurements per year in the two groups suggested that monitoring bias may partly explain the difference in hazard-ratios for death when using different definitions of ALT flares. Patients who did not die had a median 4 (2, 5) ALT measurements per year, whilst those who did die had a median 7 (4, 17) measurements per year. This suggests that the sickest patients (i.e. those who eventually died) were being monitored more frequently and were hence more likely to have a single ALT >200 IU recorded.

Figure 6.3.10.1.1: Kaplan Meier estimates of the proportion of patients who died after their first ALT measurement, stratified by whether or not they had a flare (2 ALTs >200 IU, >14 days apart)

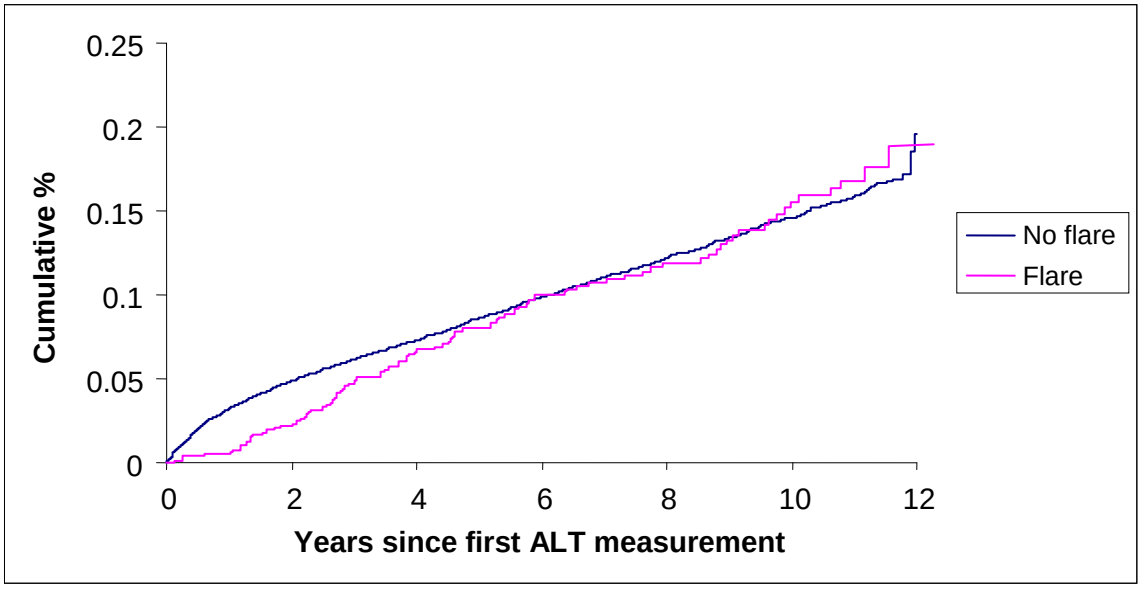
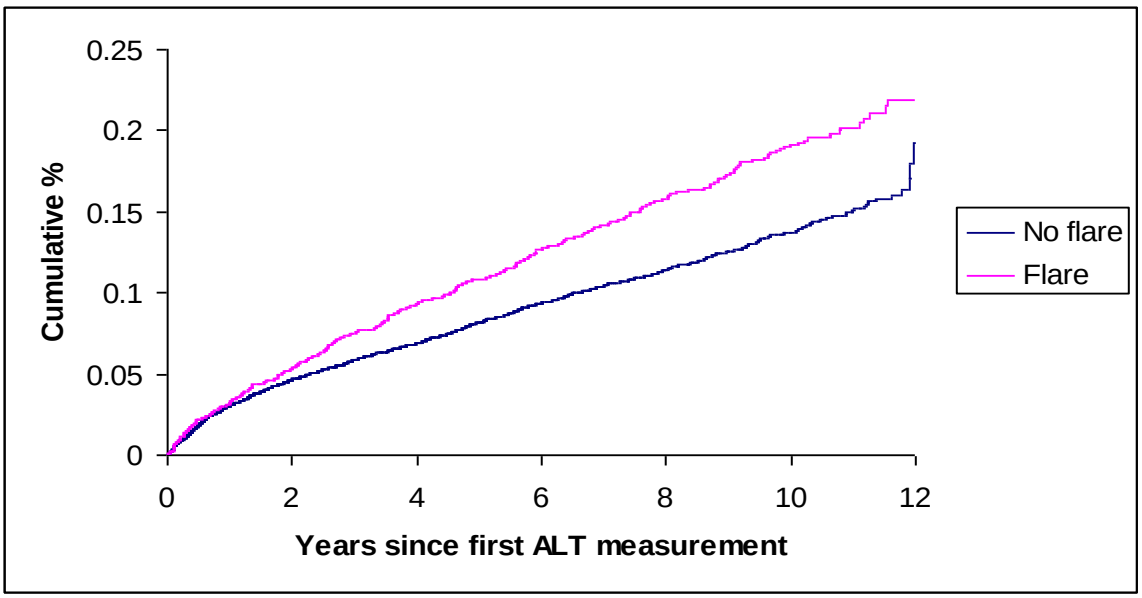


Figure 6.3.10.1.2: Kaplan Meier estimates of the proportion of patients who died after their first ALT measurement, stratified by whether or not they had a flare (1 ALT >200 IU)



6.4. Discussion

Though one ALT measurement above 200 IU is frequently used as an indication of an ALT rise in the HIV treated population, it is not known whether such an increase is a genuine reason for concern, or whether it is a random fluctuation, possibly due to non-treatment related events/chance alone. In this Chapter, I first justified the need for the definition of an ALT flare to be derived from untreated HBV/HCV-negative patients. HAART-experienced patients, and those with positive HBV/HCV status experienced greater ALT rises than HAART-naive patients and this was confirmed by a series of descriptive analyses. Using data from untreated HBV/HCV-negative patients, I established that up to 4.1% (depending on how HBV/HCV-positive is defined) of patients also experience single ALT values above this limit (200 IU). Furthermore, patients may experience such rises on multiple occasions, suggesting that rather than reflecting a genuine adverse effect of treatment, many of these episodes may be due to another cause or may be random ALT fluctuations.

The derived definition of an ALT flare of 2 consecutive measurements >200 IU, at least 2 weeks apart is more stringent than the standard definition of only 1 ALT >200 IU and hence it is less likely that random fluctuations will be defined as flares. The definition based on confirmed values above a threshold is likely to be more accurate than that based on a single value. The 2 week duration was chosen for several reasons outlined in sub-section 6.3.7, but it also mirrors clinical practice, to the extent that the occurrence of such laboratory abnormalities is likely to prompt an early repeat test. Using this definition, I found that 4.6% of all patients, including those who were HAART-experienced and/or HBV/HCV-positive, with at least two ALT measurements experienced one or more ALT flares. Cicconi et al. in 2007 reported a slightly higher percentage (5.2%), most likely due to both their different definition of an ALT flare (>3.5 times the baseline ALT value) and the higher prevalence of HCV-positive patients in Italy (287). Many studies focus only on treatment-experienced patients; using the derived definition, 5% of treatment-experienced patients had an ALT flare. Whilst this was somewhat in line with estimates from some studies (281;308;316), it was lower than that reported in others (275;285;315).

Patients who experienced an ALT flare in these analyses were generally males, of white ethnicity and MSM risk group, in line with the demographics of the CHIC dataset as a whole. There was no difference in the duration between ALT measurements amongst HAART-naive and HAART-experienced patients,

suggesting that the decision to perform an ALT test was not dependent on whether or not patients had started HAART. However, ALT tests were performed more often on HCV-positive patients than HCV-negative patients, possibly reflecting more frequent monitoring in the HCV-positive population.

Interestingly, crude rates of ALT flares amongst those co-infected with HBV/HCV were lower amongst treatment-experienced patients than those who were treatment naïve. One possible reason for this is that naïve patients tend to have lower CD4 counts and this, in turn, may be associated with a higher risk of liver disease (305). Though there was no significant interaction between treatment status and CD4 count, it is possible that there was not enough power to detect such an interaction and there may have been a stronger association between CD4 count and ALT flares amongst HAART-naive patients than HAART-experienced patients. Other studies have reported such an association between CD4 count and liver disease, albeit using different definitions of liver disease (312;313). Low CD4 counts have also been reported to be associated with death from liver disease (310). A second possible explanation is that ART experienced patients may have been exposed to drugs such as lamivudine and emtricitabine which are known to have activity against HBV, which may reduce the apparent impact of HBV on ALT levels.

In multivariable analyses, patients taking NVP (either with ddI/d4T or without) and those taking RTV were at increased risk of ALT flares, consistent with results from other studies (272;276). Both positive HCV and HBV status were associated with ALT flares and this too has been well documented (269;270;286). However, unlike findings reported by Cicconi et al (287) a significant interaction between hepatitis status and current regimen was identified. A stronger association between positive HCV status and ALT flares was seen amongst patients receiving treatment, regardless of which ART drugs were being received, than amongst those currently not receiving treatment. Though this does suggest that the effect of HAART on ALT flares is stronger amongst the HCV-positive patient population than the HCV-negative population, a third of HCV-positive patients were not receiving any treatment at the time of the ALT flare, which may also impact on this finding.

Patients who were on treatment for longer periods of time were less likely to have ALT flares, unlike Martinez et al. who found the risk of an ALT flare was increased by 10% (p=0.02) for each additional year since starting treatment (275). This difference is likely to be explained by the inclusion criteria applied in the latter study;

patients were required to have started NVP-based HAART and NVP was found to be a risk factor for ALT flares in our study.

The risk of an ALT flare was lower amongst patients of older age, those with undetectable viral loads and those with higher current CD4 counts. These findings are consistent with other studies (313-315). Though the effect of nadir CD4 count was significant in univariable analyses, it ceased to be signifcant after adjustment for current CD4 count, suggesting the latter is a stronger predictor of ALT flares. The effect of calendar year was also not signficant after adjusting for other variables, albeit there being a trend towards a higher risk of ALT flares in earlier calendar years. This is likely to be due to improved toxicities of antiretroviral drugs in later calendar years, and the reduced usage of drugs such as d4T and ddI which are known to be associated with ALT flares.

Patients with a higher number of prior ALT measurements were at an increased risk of an ALT flare. These patients were likely to have been closely monitored in anticipation of ALT rises and were perhaps also more likely to be HCV-positive, given the shorter duration between ALT measurements amongst HCV-positive patients discussed earlier in this Section.

In sensitivity analyses, a more stringent definition of an ALT flare was applied, such that the duration between the 2 consecutive ALTs >200 IU was required to be at least 30 days apart. Although trends were generally in line with those seen in the main analysis, a stronger association was observed between HCV-positive status and ALT flares. This implies that HCV-positive patients experience flares of longer duration than HCV-negative patients, and therefore perhaps of greater severity.

The main limitation of these analyses is that it is not possible to control for unmeasured confounding, such as that resulting from alcohol consumption, which has been reported as predictive of ALT flares (307). The UK CHIC dataset also does not yet have information on other non-HIV related drugs that may have an impact on ALT levels, such as acetaminophen. However, this is unlikely to affect the conclusions when comparing the different definitions of an ALT flare. Comparing the derived definition of an ALT flare to that usually reported in the literature highlights the importance of differentiating between ALT flares and isolated ALT elevations. The stronger relationships seen between CD4 count and ALT flares when using a wider, less stringent definition of an ALT flare may lead to unnecessary concern by

both clinicians and patients when analysing CD4 count, whilst the role of treatment regimen, viral load and hepatitis status may not be given enough significance.
In particular, these issues accentuate the need to consider monitoring bias when analysing outcomes such as ALT flares, as associations between known risk factors may be under- or over-estimated if using a definition based on a single ALT value. In these analyses, patients with ALT flares had a higher number of CD4 measurements during follow-up than those without ALT flares, suggesting that patients with flares were likely to be more closely monitored than those without flares. Hence, the probability of patients having a lower CD4 count at the time of the flare is likely to be greater amongst patients who do experience ALT rises.

All-cause mortality was only significantly associated with ALT flares when using the 1 ALT >200 IU definition, and not when using the derived definition. This was true when categorising patients as ever experiencing a flare/not experiencing a flare, as well as when analysing flares as a time-updated variable. Given that a single ALT value >200 IU was strongly associated with mortality, further work is required to assess the reasons why the derived definition was not as strong a predictor of mortality as the standard definition of an ALT flare. In hindsight, I would have performed the mortality analyses in the first instance, i.e. exploring differing definitions of ALT flares as shown in the first part of this chapter as predictors of mortality. This would result in a definition of an ALT flare which was focussed around a strong clinical endpoint. However, the current method used does have the advantage of detailed descriptive analyses. Analyses on all-cause mortality are likely to lack specificity, resulting in weaker associations with variables such as ALT. Data on all-cause mortality are poor; causes of death are often not provided and hence it is difficult to distinguish liver-related mortality from other causes. Another issue with analyses surrounding all-cause mortality is that if ALT levels are elevated, clinicians are likely to intervene and therefore prevent death. Hence death itself is unlikely to capture all liver associated problems. Finally, the number of deaths in the UK CHIC cohort is relatively low and therefore it is likely that all-cause mortality analyses are lacking in power.

In conclusion, the findings presented in this chapter do suggest that in cases where new solitary ALT measurements >200 IU are measured, a second test should be performed to confirm the measurement. The use of two consecutive ALTs >200 IU, measured >2 weeks apart, may more reliably identify the causes and significance of

ALT elevations and may prove more useful in exploring liver function tests abnormalities.

6.5. Summary

The definition of an ALT flare in the current literature varies, though there is emphasis on a definition based on a single ALT >200 IU. In this chapter, I have shown that such a definition may not represent a genuine ALT flare, but rather random fluctuations in ALT. Using data from HAART-naïve, HBV/HCV-negative patients, I derived an alternative definition of an ALT flare; 2 consecutive ALT measurements >200 IU, at least 2 weeks apart. Factors associated with this proposed definition were compared to those associated with the standard definition in the literature. CD4 count was found to be a stronger predictor of ALT flares using the standard definition, whilst weaker associations were seen between treatment regimen, current viral load and hepatitis status and ALT flares, compared to those when using the derived definition. The association between ALT flares and all-cause mortality was also accentuated when using the standard definition of an ALT flare.

Chapter 7: The impact of increased duration of viral suppression, prior treatment failures and prior treatment interruptions on viral rebound rates amongst patients with viral suppression

7.1. Introduction

The ultimate goal of HAART is to fully suppress HIV replication, currently judged as attaining a plasma viral load of less than 50 copies/mL, as this minimizes the risk of resistance evolution and results in the greatest potential for immune recovery (482). Viral rebound is often associated with emergence of HIV with resistance (260;513). With accumulating regimen failures, the attainment of full viral suppression becomes more difficult (514), and failure to achieve this can result in immunological decline (515). Factors associated with an increased rate of viral rebound include pre-HAART use of NRTIs as single or dual therapy (413;414;417;418;430;516), use of particular antiretroviral drugs (422;430) and poor adherence to medication (412). Lower rates of viral rebound have also been reported with increased duration of virological suppression (413;414;517). In the EuroSIDA cohort in 2,444 patients, the overall rate of viral rebound during the first six months after initial full suppression was 33.5/100 pys follow-up, compared with 8.6/100 pys in those who were suppressed for greater than 24 months (430).

Whilst the number of patients interrupting treatment may have declined since findings of an increased risk of clinical progression in patients who interrupt therapy (449), patients continue to consider the option of temporarily discontinuing treatment, largely with the aim of minimising the unwanted side effects and/or inconvenience of HAART. It has been shown that patients who have interrupted HAART have a raised risk of drug resistance and increased immuno-suppression (439;440;455;459;460;518-521), though many patients are able to suppress viral load upon restarting therapy (445;450;451).

However, the risk of subsequent viral rebound in these patients has not been previously explored. If resistant virus has emerged following treatment interruptions this may have been archived in latently infected cells and might only emerge at some later time point when such cells become activated. The association between pre-HAART use of nucleoside mono or dual therapy and a raised risk of rebound in people with viral suppression is likely to be due to such a mechanism.

In this Chapter, I will investigate the frequency of treatment interruptions amongst patients who have started HAART, and will determine whether prior episodes of treatment interruptions are associated with a raised risk of viral rebound in individuals who have attained virological suppression. I will also determine whether the rate of viral rebound decreases with increasing duration of viral suppression regardless of prior failure and, if so, whether rebound rates in patients who have previously failed one or more regimens ultimately decline to levels as low as those seen in patients receiving first-line therapy if they are able to re-suppress viral load on a new regimen.

7.2. Methods

7.2.1. Proportion of time spent on treatment amongst all patients and description of treatment interruptions amongst patients who had started HAART

The proportion of follow-up time spent on treatment was calculated for all patients, by dividing the time spent on treatment by the number of days patients were under follow-up. These analyses were stratified by calendar year in order to distinguish whether the results of the SMART Study had influenced the proportion of time spent receiving treatment. Amongst patients who had started HAART, the number of interruptions, the regimen interrupted (NNRTI/PI/r/PI/Other) and the regimen restarted were described. The viral load and CD4 count at time of interruption was also calculated.

7.2.2. Selection of patients for inclusion in viral rebound analysis

In the main analysis, all patients who achieved a viral load of $\leq$50 copies/mL while receiving HAART (defined as any treatment combination including three or more drugs) were eligible to enter the analyses. Differences between patients receiving HAART and achieving an undetectable viral load and those receiving HAART and not achieving an undetectable viral load were described using Chi-squared and Mann Whitney tests.

Patients were followed from the date when their viral load first fell below 50 copies/mL while on HAART until the time of viral rebound (defined as two consecutive viral load values $\geq$400 copies/mL or one viral load value $\geq$400 copies/mL followed by starting two or more new drugs). Patient follow-up was censored (i.e. patients were removed from the risk set for the analysis) prior to this point if the patient discontinued or reduced HAART to fewer than three drugs, or on the date of the patient's last viral load. Patients who experienced viral rebound or whose follow-up was censored on discontinuation/reduction of HAART re-entered the analysis if they subsequently re-suppressed their viral load to $\leq$50 copies/mL. The rate of viral rebound was calculated by dividing the total number of events (viral rebounds) by the person time spent in a particular category.

7.2.3. Defining failure history at the start of each period of suppression

At the start of each period of viral suppression, the patient's viral failure history was assessed and the patient was categorised as having experienced virological failure to 0, 1, 2, 3, or 4 or more prior regimens. Patients who were antiretroviral naïve

when they started HAART and those with pre-HAART NRTI exposure were included. Any treatment taken before January 1996 counted as having failed one regimen, regardless of viral load. From January 1996, having a viral load of ≥400 copies/mL, whilst receiving any treatment and not having been off treatment in the previous 4 months counted as a regimen failure. Each time a viral load of ≥400 copies/mL was obtained whilst being on a regimen containing at least one drug (and having been on that drug for over 4 months) that the patient had not previously failed was counted as an additional regimen failure. Patients who had temporarily been removed from the risk set after discontinuing HAART were not classified as having failed that regimen as long as they had not met the definition of failure at the time of discontinuation; thus, if these patients subsequently re-entered the analysis their number of failed regimens remained unchanged. Patient follow-up in the analysis was stratified according to the length of time a patient's viral load had been suppressed in the current episode of viral suppression; for individuals re-entering the risk set after treatment discontinuation/reduction, the clock was reset such that this length started once again from zero. Thus each patient could contribute multiple viral suppression episodes to the analysis. The allocation of pys is further described in Figure 7.2.4.1.

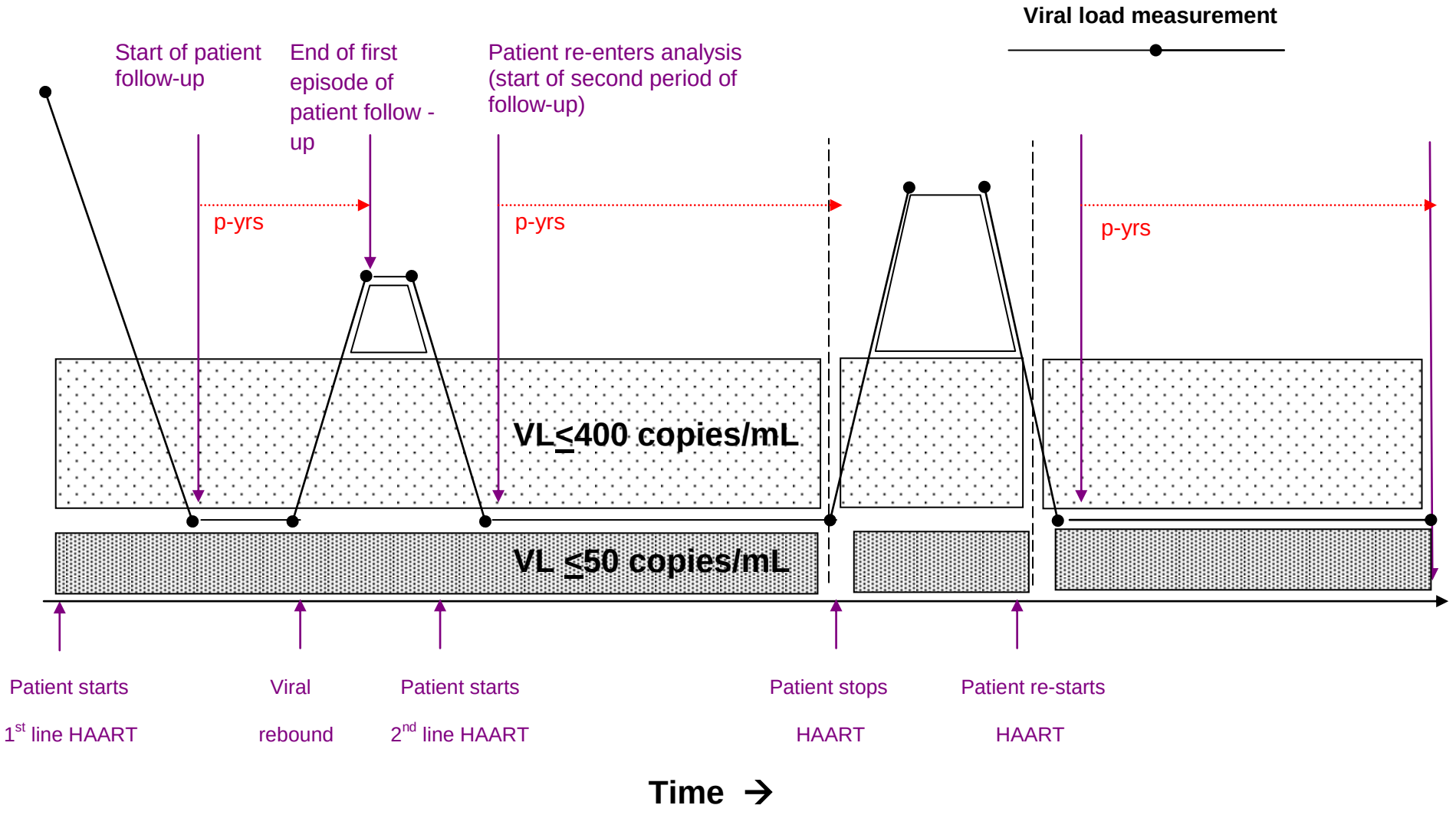
7.2.4. Statistical methods

The relationship between the duration of each viral suppression episode, calendar year of entry into a viral suppression episode and number of regimens previously failed was analysed by stratifying the crude rates of viral rebound by the number of regimens previously failed and the duration of viral suppression/calendar year of entry into a viral suppression episode. Poisson regression was used to determine the impact of the duration of viral suppression, the number of previous regimens failed and the number of prior treatment interruptions (discontinuation of all therapy for at least 2 weeks, followed by restart of therapy) on viral rebound. In addition to the total number of prior interruptions, the regimen interrupted/restarted and the viral load at time of interruption (i.e. the number of interruptions that had previously occurred whilst the viral load was detectable, undetectable or not known) were also considered as potential predictors of viral rebound. The following potential predictors were also considered: current antiretroviral regimen (backbone NRTIs and 'third' drug), calendar year, age, ethnicity (white, black African or other), sex, risk group (MSM, heterosexual, other), viral load at initiation of ART, time since initiation of ART and CD4 count at the start of the period of viral suppression. An interaction

variable between number of previous regimens failed and duration of suppression was also incorporated in the model.

As each person could contribute more than one period of follow-up (and viral rebound) to the analysis, rate ratios were also estimated using generalized estimating equations to fit univariable and multivariable Poisson regression models, allowing for multiple events in the same individuals.

Figure 7.2.4.1: Example of allocation of person-years of follow-up for a hypothetical individual

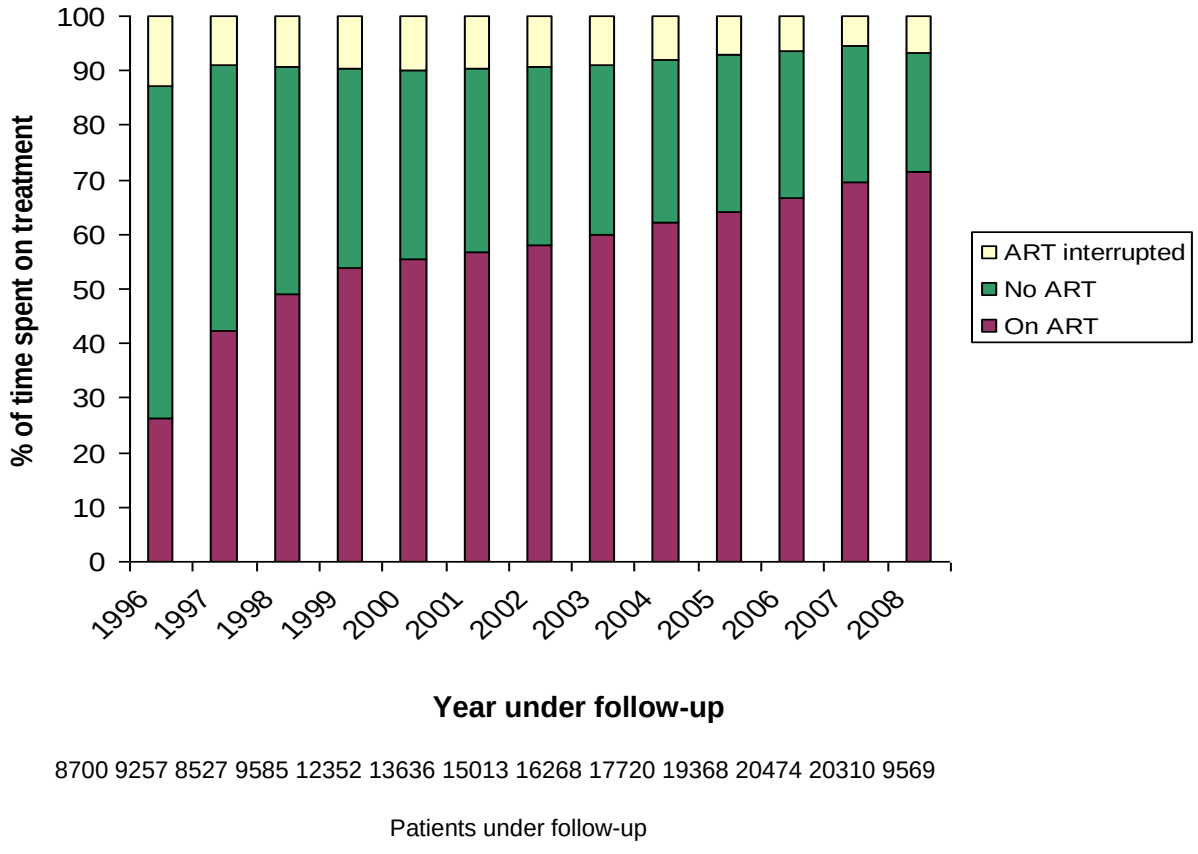


7.3. Results

7.3.1. Time spent on treatment in the whole population

The proportion of time patients spent on treatment each year is shown in Figure 7.3.1.1. In 1996, only a quarter of time under follow-up was spent on treatment. This increased to almost 70% in 1999 but dropped to 55% in the year 2000. The results of the SMART trial were published in 2006, and though time spent on treatment has increased since then, this increase was actually evident from 2000 onwards. In 2008, over 70% of time under follow-up was spent receiving treatment. The proportion of patients under follow-up who had not yet started treatment decreased from 60.8% in 1996 to 21.9% in 2008. The proportion of patients who had interrupted treatment also decreased over time and stood at 6.5% in 2008.

Figure 7.3.1.1: Proportion of time under follow-up each year spent on treatment, not yet started treatment and treatment interruptions



7.3.2. Treatment interruptions amongst patients who had started HAART

Amongst the 20,036 patients who had started HAART between 1996 and 2008, a quarter (n=4936, 24.6%) interrupted treatment at least once whilst under follow-up.

The total number of interruptions recorded was 7,801. The median number of interruptions per patient was 1 (range: 1, 13) and the median duration of each treatment interruption was just under 5 months (4.8 (IQR: 2.0, 12.0)). Characteristics of patients at the time of each interruption, together with details of regimen interrupted and restarted are shown in Table 7.3.2.1. Patients interrupted treatment for the first time at a median 1.2 years after starting HAART and subsequent interruptions were a median 1.6 years apart. A higher proportion of interruptions took place between 2000-2003, after which the proportions of interruptions decreased. Most interruptions were made by patients of male sex, MSM exposure and white ethnicity. The median age at interruption was 38 years.

Of those who interrupted therapy, over a third of patients interrupted an NNRTI-based regimen (of whom 36.1% restarted the same regimen and 29.6% restarted a PI/r-based regimen). Over a quarter of patients (26.7%) interrupted a PI/r-based regimen (of whom 28.9% restarted the same regimen and 12.9% restarted an NNRTI-based regimen). Treatment interruptions were made whilst patients' viral loads were a median 1154 copies/mL and CD4 counts were a median 300 cells/mm^3. Patients restarted therapy with a median viral load of 63,400 copies/mL and a median CD4 count of 190 cells/mm^3.

Table 7.3.2.1: Characteristics of patients at start of each treatment interruption

Patients who interrupted treatment at least once		**4936**
Total number of treatment interruptions N (%)		**7801 (100.0)**
Time from start of HAART to interruption (years)	Median (IQR)	2.1 (0.7, 4.3)
Regimen interrupted n (%)	NNRTI	2897 (37.1)
Restarted same regimen n (%)		897 (31.0)
Restarted n (%)	*NNRTI*	*1635 (56.4)*
	PI/r	*857 (29.6)*
	PI	*122 (4.2)*
	Other	*283 (9.8)*
Regimen interrupted n (%)	PI/r	2080 (26.7)
Restarted same regimen n (%)		601 (28.9)
Restarted n (%)	*NNRTI*	*269 (12.9)*
	PI/r	*1456 (70.0)*
	PI	*105 (5.1)*
	Other	*250 (12.0)*
Regimen interrupted n (%)	PI	1335 (17.1)
Restarted same regimen n (%)		196 (14.7)
Restarted n (%)	*NNRTI*	*407 (30.5)*
	PI/r	*337 (25.2)*
	PI	*364 (27.3)*
	Other	*227 (17.0)*
Regimen interrupted n (%)	Other	1489 (19.1)
Restarted same regimen n (%)		225 (11.7)
Restarted n (%)	*NNRTI*	*365 (24.5)*
	PI/r	*449 (30.1)*
	PI	*116 (7.8)*
	Other	*559 (37.5)*
Year of interruption n (%)	1996-1999	2042 (26.2)
	2000-2003	3221 (41.3)
	2004-2008	2538 (32.5)
Number of viral load measurements between	Median (IQR)	1 (1,3)

interruption and restart		
Viral load at interruption (copies/mL)	Median (IQR)	1154 (50, 38080)
Viral load at restart (copies/mL)	Median (IQR)	63400 (10000,203481)
Number of CD4 count measurements between interruption and restart	Median (IQR)	2 (1,3)
CD4 at interruption (cells/mm^3)	Median (IQR)	300 (160, 475)
CD4 at restart (cells/mm^3)	Median (IQR)	190 (93, 300)
Sex n (%)	Males	5810 (74.5)
	Females	1991 (25.5)
Ethnicity n (%)	White	4813 (61.7)
	Black	2337 (30.0)
	Other	651 (8.4)
Exposure n (%)	MSM	4318 (55.4)
	Heterosexual	2620 (33.6)
	Other	863 (11.1)
Age at interruption (years)	Median (IQR)	38 (33, 43)

7.3.3. Differences between those included and excluded from viral rebound analyses

17,743/20,036 (88.6%) patients who started HAART achieved VL ≤50 copies/mL at a median 6.1 (6.0, 6.3) months after initiation of therapy. Although differences amongst patients who did and did not achieve VL ≤50 copies/mL were statistically significant, the absolute differences were of limited clinical significance (Table 7.3.3.1).

Compared to patients who started HAART and did not achieve VL ≤50 copies/mL, patients with VL ≤50 copies/mL after starting HAART were more likely to be of male sex (88.9% of males achieved VL ≤50 copies/mL compared to 87.6% of women, p=0.001), of MSM exposure (90.3% of MSMs achieved VL ≤50 copies/mL compared to 88.8% of heterosexuals and 79.4% of those from 'other' exposure groups, p<0.0001) and had lower viral loads at HAART initiation (58,600 copies/mL compared to 64,729 copies/mL, p=0.01). Patients who achieved VL ≤50 copies/mL after starting HAART also had marginally higher CD4 counts at start of HAART (190 vs. 180 cells/mm^3, p=0.002) and were more likely to start HAART on an NNRTI-based regimen than those who did not achieve VL ≤50 copies/mL after starting HAART (91.4% of patients starting HAART with an NNRTI-based regimen achieved VL ≤50 copies/mL compared to 85.4% of those who started HAART on PI/r-based regimens, p<0.0001). Finally, patients starting HAART in 2000-2003 appeared to be more likely to achieve VL ≤50 copies/mL after starting HAART than those starting HAART before 2000 and after 2003. Though the absolute difference was small, one possible explanation for this is that patients starting HAART in later calendar years may not have enough follow-up time to achieve VL ≤50 copies/mL.

Table 7.3.3.1: Differences between those who started HAART and achieved VL ≤50 copies and those who didn't achieve VL ≤50 copies

		VL >50 copies/mL	VL ≤50 copies/mL	P-value
		2293	**17743**	
Sex	Males	1681 (11.1)	13405 (88.9)	0.001
	Females	612 (12.4)	4338 (87.6)	
Ethnicity	White	1332 (11.2)	10582 (88.8)	0.17
	Black	702 (11.6)	5362 (88.4)	
	Other	259 (12.6)	1799 (87.4)	
Exposure	MSM	1056 (9.7)	9830 (90.3)	<0.0001
	Heterosexual	770 (11.2)	6117 (88.8)	
	Other	467 (20.6)	1796 (79.4)	
Year of starting HAART	1996-1999	975 (12.9)	6566 (87.1)	<0.0001
	2000-2003	542 (8.8)	5625 (91.2)	
	2004-2008	776 (12.3)	5552 (87.7)	
VL at start of HAART	Median (IQR)	64729 (10319, 224100)	58600 (9754, 198000)	0.01
CD4 at start of HAART	Median (IQR)	180 (63, 297)	190 (94, 290)	0.002
1st line regimen	NNRTI	945 (8.6)	10061 (91.4)	<0.0001
	PI/r	526 (14.6)	3089 (85.4)	
	PI	591 (15.2)	3302 (84.8)	
	Other	231 (15.2)	1291 (84.8)	

7.3.4. Characteristics of patients included in the viral rebound analyses

The 17,743 patients included in the viral rebound analyses contributed a total of 22,965 unique viral suppression episodes (median 1 episode, range 1 to 11 per person). Most patients included in the analysis were of male sex, white ethnicity and MSM risk group. A quarter of patients had a CD4 count below 200 cells/mm^3 at the start of the viral suppression episode and 39.2% of patients had failed at least one regimen before entering the viral suppression episode. Of the 22,965 viral

suppression episodes, 5,198 (22.6%) were in individuals who had interrupted therapy prior to the start of the episode. The characteristics of the patients included in the analysis at the start of each viral suppression episode, stratified according to whether or not the patient had previously interrupted therapy, are shown in Table 7.3.4.1. In general, patients who had interrupted therapy were more likely to be of white ethnicity, to have attained viral suppression in later calendar years and had failed a greater number of previous regimens than those who did not interrupt therapy prior to the viral suppression episode. Patients who had interrupted therapy were also more likely to have received mono/dual therapy before receiving HAART.

Of the 8,024 treatment interruptions that occurred prior to the start of the viral suppression episode (Table 7.3.4.2), 37.5% were interruptions of NNRTI-based regimens, 23.3% of PI/r-based regimens and 19.9% of single PI-based regimens. The remaining 19.3% of treatment interruptions occurred amongst patients receiving 'other' regimens, for example, NRTI only regimens and regimens containing both PIs and NNRTIs. Of the 3,009 NNRTI interruptions, 27.1% ended with the patient restarting the same NNRTI and the same proportion (27.1%) ended with the patient restarting a different NNRTI. A slightly higher proportion of patients (29.2%) restarted therapy with a PI/r-based regimen. Of the 1,870 PI/r interruptions, 25.9% ended with the patient restarting the same PI/r, 38.8% ended with patients starting a different PI/r and 15.7% ended with patients starting an NNRTI-based regimen. The median duration of each treatment interruption was 5.7 (IQR: 2.5, 13.0) months and treatment interruptions were initiated at a median CD4 count of 281 (152, 457) cells/mm^3 and median viral load of 2150 (56, 32,000) copies/mL. Patients restarted treatment with a median CD4 count of 190 (97, 290) cells/mm^3 and median viral load of 67,900 (16,221, 192,036) copies/mL.

Table 7.3.4.1: Patient characteristics at the start of each viral suppression episode, stratified by whether or not patients had had prior treatment interruptions

			Treatment interruption prior to start of viral suppression episode	
		Total	**Yes**	**No**
N		22965	5198	17767
Age (years)	Median (IQR)	38 (33, 44)	39 (35, 45)	38 (33, 44)
CD4 count (cells/mm^3)	<200	5679 (24.7)	1324 (25.5)	4355 (24.5)
	200-349	7086 (30.9)	1605 (30.9)	5481 (30.9)
	350-499	5183 (22.6)	1203 (23.1)	3980 (22.4)
	≥500	4478 (19.5)	1044 (20.1)	3434 (19.3)
	Missing	539 (2.4)	22 (0.4)	517 (2.9)
Ethnicity, n (%)	White	13866 (60.4)	3288 (63.3)	10578 (59.5)
	Black	6872 (29.9)	1488 (28.6)	5384 (30.3)
	Other	2227 (9.7)	422 (8.1)	1805 (10.2)
Risk group, n (%)	MSM	12928 (56.3)	3032 (58.3)	9896 (55.7)
	Heterosexual	7805 (34.0)	1668 (32.1)	6137 (34.5)
	Other	2232 (9.7)	498 (9.6)	1734 (9.8)

Male sex, n (%)		17334 (75.5)	3861 (74.3)	13473 (75.8)
Year of viral suppression, n (%)	1996-2000	6343 (27.6)	933 (18.0)	5410 (30.5)
	2001-2004	9069 (39.5)	2250 (43.3)	6819 (38.4)
	2005 onwards	7553 (32.9)	2015 (38.8)	5538 (31.2)
Number of previous regimens failed, n (%)	0	13951 (60.8)	1655 (31.8)	12296 (69.2)
	1	4112 (17.9)	1364 (26.2)	2748 (15.5)
	2	2353 (10.3)	935 (18.0)	1418 (8.0)
	3	1358 (5.9)	600 (11.5)	758 (4.3)
	4	677 (3.0)	361 (6.9)	316 (1.8)
≥5		514 (2.2)	283 (5.4)	231 (1.3)
Receipt of mono/dual therapy prior to HAART, n (%)		6284 (27.4)	2108 (40.6)	4176 (23.5)
Suppression episode	1	17743 (77.3)	2053 (39.5)	15690 (88.3)
	2	2176 (17.0)	2176 (41.9)	1719 (9.7)
	3	966 (4.2)	682 (13.1)	284 (1.6)
	≥4	361 (1.6)	287 (5.5)	74 (0.4)

Table 7.3.4.2: Patient characteristics at the time of each treatment interruption (TI)

		N (%)
Total number of treatment interruptions[1] N (%)		8024 (100)
Number of interruptions	Median (range)	1 (1, 11)
Treatment status at start and end of interruption		
Interrupted NNRTI regimen N (%)		3009 (37.5)
	Restarted same NNRTI regimen	814 (27.1)
	Restarted different NNRTI regimen	816 (27.1)
	Restarted PI/r regimen	880 (29.2)
	Restarted PI regimen	168 (5.6)
	Restarted Other regimen	331 (11.0)
Interrupted PI/r regimen N (%)		1870 (23.3)
	Restarted same PI/r regimen	485 (25.9)
	Restarted different PI/r regimen	725 (38.8)
	Restarted PI regimen	118 (6.3)
	Restarted NNRTI regimen	294 (15.7)
	Restarted Other regimen	248 (13.3)
Interrupted PI regimen N (%)		1600 (19.9)
	Restarted same PI regimen	234 (14.6)
	Restarted different PI regimen	191 (11.9)
	Restarted PI/r regimen	367 (22.9)
	Restarted NNRTI regimen	542 (33.9)
	Restarted Other regimen	266 (16.6)
Interrupted Other regimen N (%)		1545 (19.3)
	Restarted same Other regimen	225 (14.6)
	Restarted different Other regimen	330 (21.4)
	Restarted PI/r regimen	419 (27.1)
	Restarted PI regimen	135 (8.7)
	Restarted NNRTI regimen	436 (28.2)
Duration of TI (months)	Median (IQR)	5.7 (2.5, 13.0)
CD4 count at start of TI (cells/mm^3)	Median (IQR)	281 (152, 457)

CD4 count at end of TI (cells/mm^3)	Median (IQR)	190 (97, 290)
Number of CD4 counts between interrupting and restarting ART	Median (IQR)	2 (1, 4)
Viral load at start of TI (copies/mL)	Median (IQR)	2150 (56, 32000)
Viral load at end of TI (copies/mL)	Median (IQR)	67900 (16221, 192036)
Number of VL measurements between interrupting and restarting treatment	Median (IQR)	2 (1, 4)

7.3.5. Person-years, events and rates

Patients were followed for a median 66,053 pys. In total 4,078 viral rebound events occurred over follow-up, giving an overall rebound rate of 6.17 (95% CI: 5.98, 6.36) per 100 pys. Table 7.3.5.1 shows the number of events and pys, together with the viral rebound rate and 95% CI for a range of demographic and other variables. The viral rebound rate was higher amongst females (compared to males), those of black ethnicity (compared to white ethnicity) and those of 'other' risk groups (compared to MSM). Patients who had received mono/dual therapy prior to HAART, those who were under follow-up in earlier calendar years and those with lower CD4 counts also had higher viral rebound rates. The rate of viral rebound linearly increased as the number of regimens previously failed increased, as the number of previous viral rebounds increased and as the number of prior treatment interruptions increased. The rebound rate also varied considerably according to the NRTI combination and the 'third' drug used in regimens. Patients receiving d4T/ddI had the highest rebound rate, whilst those receiving FTC/TFV NRTI combinations had the lowest rebound rate. In terms of the 'third' drug used in regimens, patients receiving unboosted SQV had the highest rebound rates and those receiving EFV had the lowest rebound rates. Viral rebound rates decreased as the time since start of HAART increased and as the duration of viral suppression increased.

Table 7.3.5.1: Events, person-years and rates (95% CI) of viral rebound, stratified by demographic and other variables

		Events/ person- years	Rate (95% CI)
Total		**4078/66053**	**6.17 (5.98, 6.36)**
Sex	Male	3052/52459	5.82 (5.61, 6.02)
	Female	1026/13594	7.55 (7.09, 8.01)
Ethnicity	White	2442/43260	5.65 (5.42, 5.87)
	Black	1299/16893	7.69 (7.27, 8.11)
	Other	337/5901	5.71 (5.10, 6.32)
Risk group	MSM	2274/40895	5.56 (5.33, 5.79)
	Heterosexual	1388/19691	7.05 (6.68, 7.42)
	Other	416/5467	7.61 (6.88, 8.34)
Mono/dual therapy	No	2309/45097	5.12 (4.91, 5.33)
received	Yes	1769/20956	8.44 (8.05, 8.83)
Calendar year	1996-2000	1109/8181	13.56 (12.76, 14.35)
	2001-2004	1697/27265	6.22 (5.93, 6.52)
	2005 onwards	1272/30607	4.16 (3.93, 4.38)
Number of regimens	0	1409/35708	3.95 (3.74, 4.15)
previously failed	1	992/14489	6.85 (6.42, 7.27)
	2	741/8257	8.97 (8.33, 9.62)
	3	502/4397	11.42 (10.42, 12.42)
	4	239/2081	11.49 (10.03, 12.94)
	>5	195/1122	17.38 (14.94, 19.82)
Current CD4 count	<200	826/6449	12.81 (11.93, 13.68)
(cells/mm^3)	200-349	1156/15056	7.68 (7.24, 8.12)
	350-499	962/17443	5.51 (5.17, 5.86)
	>500	1113/26899	4.14 (3.90, 4.38)
	Missing	21/207	10.15 (5.81, 14.48)
Number of prior	0	2901/54407	5.33 (5.14, 5.53)
treatment interruptions	1	758/8615	8.80 (8.17, 9.42)
	2	258/2069	12.47 (10.95, 13.99)
	>3	161/961	16.75 (14.17, 19.34)
NRTI combination	AZT/3TC	966/19918	4.85 (4.54, 5.16)
	AZT/DDI	123/1414	8.70 (7.16, 10.24)

	d4T/3TC	589/6350	9.28 (8.53, 10.03)
	d4T/ddI	350/2416	14.49 (12.97, 16.00)
	AZT/ABC	30/370	8.11 (5.21, 11.01)
	TFV/3TC	224/5602	4.00 (3.48, 4.52)
	ABC/3TC	220/7443	2.96 (2.57, 3.35)
	TFV/DDI	231/2395	9.65 (8.40, 10.89)
	FTC/TFV	167/6284	2.66 (2.25, 3.06)
	Other	1178/13861	8.50 (8.01, 8.98)
“Third” drug	EFV	478/18077	2.64 (2.41, 2.88)
	NVP	796/15766	5.05 (4.70, 5.40)
	SQV	76/449	16.93 (12.12, 20.73)
	IDV	178/1829	9.73 (8.30, 11.16)
	NFV	450/3678	12.23 (11.10, 13.37)
	RTV	39/485	8.04 (5.52, 10.56)
	AZV	28/365	7.67 (4.83, 10.51)
	IDV/r	97/1119	8.67 (6.94, 10.39)
	SQV/r	265/3120	8.49 (7.47, 9.52)
	LPV/r	497/7373	6.74 (6.15, 7.33)
	AZV/r	217/3986	5.44 (4.72, 6.17)
	ABC	629/7275	8.65 (7.97, 9.32)
	ETR	4/114	3.51 (0.96, 8.98)
	Other	325/2417	13.45 (11.98, 14.91)
Time since start of HAART	<1.0 year	585/7331	7.98 (7.33, 8.63)
	1.1-3.0 years	1348/18576	7.26 (6.87, 7.64)
	3.1-5.0 years	938/15682	5.98 (5.60, 6.36)
	>5.0 years	1207/24464	4.93 (4.66, 5.21)
Duration of viral suppression	<1.0 year	2232/19975	11.17 (10.71, 11.64)
	1.1-3.0 years	1260/22865	5.51 (5.21, 5.81)
	3.1-5.0 years	386/12989	2.97 (2.68, 3.27)
	>5.0 years	200/10223	1.96 (1.69, 2.23)
Number of previous viral rebounds	0	3199/58212	5.50 (5.30, 5.69)
	1	666/6586	10.11 (9.34, 10.88)
	2	163/1035	15.75 (13.33, 18.17)
	3	37/186	19.89 (13.48, 26.30)
	>4	13/35	37.14 (19.78, 63.51)

7.3.6. Number of regimens previously failed and duration of viral suppression/calendar year of entry into viral suppression episode

Table 7.3.6.1 shows the rates of viral rebound according to both the number of regimens previously failed and duration of viral suppression. Within the first year of viral suppression, the rate of viral rebound was 6.57/100 pys (95% CI: 6.11, 7.03) in patients who had not failed treatment previously, rising to 29.38/100 pys (24.21, 34.56) in patients who had failed five or more regimens. Viral rebound rates decreased progressively with increased duration of viral suppression despite one or more previous episodes of failure. In patients who had failed one or more antiretroviral combinations and remain suppressed after 5 years, rebound rates fell to levels nearly as low as those seen in patients who had never experienced virological failure. After 1-3 years of suppression the rate of rebound was 3.31/100 pys (95% CI: 3.00, 3.63) in naïve and 13.11/100 pys (95% CI: 9.51, 16.71) in patients who had failed $\geq$5 regimens; and after $\geq$5 years, the corresponding rebound rates were 1.54/100 pys (95% CI: 1.16, 1.92) and 3.64/100 pys (95% CI: 0.99, 9.31).

The association between the number of regimens previously failed and the calendar year of entering an episode of viral suppression was also investigated (Table 7.3.6.2). Although rebound rates increased as the number of regimens previously failed increased, regardless of calendar year, viral rebound rates decreased progressively with later calendar year, despite one or more previous episodes of failure.

Table 7.3.6.1: Events/ person-years and rates (95% CI) stratified by number of regimens previously failed and duration of viral suppression

	Duration of viral suppression			
Number of regimens previously failed	**≤ 1 year**	**1-3 years**	**3-5 years**	**≥ 5 years**
0	783/11914 *6.57 (6.11, 7.03)*	430/12978 *3.31 (3.00, 3.63)*	132/6662 *1.98 (1.64, 2.32)*	64/4153 *1.54 (1.16, 1.92)*
1	523/3739 *13.99 (12.79, 15.19)*	324/4713 *6.87 (6.13, 7.62)*	92/3063 *3.00 (2.39, 3.62)*	53/2973 *1.78 (1.30, 2.26)*
2	394/2104 *18.73 (16.88, 20.58)*	221/2606 *8.48 (7.36, 9.60)*	73/1738 *4.20 (3.24, 5.16)*	53/1810 *2.93 (2.14, 3.72)*
3	268/1220 *21.97 (19.34, 24.60)*	162/1488 *10.89 (9.21, 12.56)*	53/881 *6.02 (4.40, 7.64)*	19/807 *2.35 (1.42, 3.68)*
4	140/578 *24.22 (20.21, 28.23)*	72/691 *10.42 (8.01, 12.83)*	20/444 *4.50 (2.53, 6.48)*	7/370 *1.89 (0.76, 3.90)*
≥ 5	124/422 *29.38 (24.21, 34.56)*	51/389 *13.11 (9.51, 16.71)*	16/201 *7.96 (4.55, 12.93)*	4/110 *3.64 (0.99, 9.31)*

Table 7.3.6.2: Events, person-years and rates (95% CI) stratified by number of regimens previously failed and year of entry of viral suppression episode

	Year of entering an episode		
Number of regimens previously failed	**1996-2000**	**2001-2004**	**2005-2008**
0	326/3513 *9.28 (8.27, 10.29)*	601/14341 *4.19 (3.86, 4.53)*	482/17853 *2.70 (2.46, 2.94)*
1	311/2337 *13.31 (11.83, 14.79)*	400/6055 *6.61 (5.96, 7.25)*	281/6096 *4.61 (4.07, 5.15)*
2	264/1466 *18.01 (15.84, 20.18)*	288/3600 *8.00 (7.08, 8.92)*	189/3192 *5.92 (5.08, 6.77)*
3	146/636 *22.96 (19.23, 26.68)*	221/1943 *11.37 (9.87, 12.87)*	135/1818 *7.43 (6.17, 8.68)*
4	46/175 *26.29 (18.69, 33.88)*	105/909 *11.55 (9.34, 13.76)*	88/997 *8.83 (6.98, 10.67)*
≥ 5	16/53 *30.19 (15.40, 44.98)*	82/418 *19.62 (15.37, 23.86)*	97/652 *14.88 (11.92, 17.84)*

7.3.7. Factors associated with viral rebound

In univariable analyses (Table 7.3.7.1), patients who had interrupted treatment at least once prior to their viral suppression episode had an 89% greater chance of experiencing viral rebound than those who had no prior treatment interruptions. When the number of prior treatment interruptions was fitted as a categorical variable (0, 1, 2, ≥3 prior treatment interruptions), the relative rate of viral rebound increased as the number of interruptions increased; compared to those with no prior treatment interruptions, rate-ratios for patients with 1, 2 and >3 prior treatment interruptions were 1.65 (95% CI: 1.77, 2.03), 2.34 (2.06, 2.66) and 3.14 (2.68, 3.68) respectively. Rate-ratios also increased linearly as the number of previous regimens failed increased (4.40 (3.79, 5.11) comparing failing ≥5 regimens to 0 regimens) and decreased linearly as the duration of viral suppression increased (5.71 (4.94, 6.60) comparing patients whose viral load was suppressed for ≤1 year to >5 years). Females (compared to males), patients of black ethnicity (compared to white ethnicity), of heterosexual/other risk group (compared to MSM) of younger age and those who had received mono/dual therapy before starting HAART were more likely to experience viral rebound. Patients under follow-up in later calendar years, those with higher current CD4 counts, with no prior AIDS diagnosis and patients who had experienced fewer episodes of viral rebound in the past were less likely to experience viral rebound. The regimen that patients were currently receiving was also a strong predictor of viral rebound. In particular, patients receiving d4T/ddI as their NRTI-backbone were 3 times as likely to experience viral rebound than those receiving AZT/3TC and those receiving unboosted SQV as the 'third' drug were over 6 times as likely to experience viral rebound than those receiving EFV.

In multivariable analyses, categorical variables were fitted as continuous variables if a linear effect was evident in univariable analyses. After adjusting for potential confounders, patients who had interrupted therapy at least once before their viral suppression episode had a 30% higher risk of experiencing viral rebound than those who had no prior treatment interruptions (Table 7.3.7.1). When the number of treatment interruptions was fitted as a categorical variable, compared to those who had no treatment interruptions, the rate-ratios for patients with 1, 2 and ≥3 prior treatment interruptions were 1.22 (1.12, 1.33), 1.46 (1.28, 1.67) and 1.62 (1.39, 1.92) respectively (Figure 7.3.7.1).

Patients with a higher number of previous regimens failed (1.27 (1.23, 1.31) per additional regimen failed) were at an increased risk of viral rebound, whilst those who maintained viral suppression for longer periods of time were at a reduced risk of

viral rebound (0.80 (0.79, 0.82) per 1 year higher). Patients of black ethnicity (compared to white ethnicity) were also at an increased risk of viral rebound, though sex and risk group were no longer significantly associated with viral rebound. Patients of older age, those seen in later calendar years and those with lower CD4 counts were still at a reduced risk of viral rebound, after adjusting for other confounders. Compared to receiving an AZT/3TC NRTI combination, patients receiving AZT/DDI, d4T/3TC, d4T/ddI and TFV/DDI had a higher risk of viral rebound whilst those receiving ABC/3TC and FTC/TFV had a lower risk of viral rebound. Other than etravirine (taken by only 136 patients), patients receiving any other third drug were at increased risk of viral rebound compared to patients receiving EFV.

The test for interaction between number of past regimens failed and time under suppression was statistically significant with a p-value of 0.01. Thus, although rebound rates were higher in those with a greater number of previously failed regimens, this effect waned with increased duration of suppression. The relative rate of rebound, estimated separately after stratifying by the number of regimens failed according to time with viral suppression is shown in Table 7.3.7.1. Patients who had remained virologically suppressed for less than one year had a 27% (1.27 (1.22–1.33)) increased chance of viral rebound per extra regimen failed. This decreased to 16% (1.16 (1.00, 1.34) in those who remain suppressed for >5 years.

Figure 7.3.7.1: Adjusted rate-ratios (and 95% CIs) according to the number of prior treatment interruptions

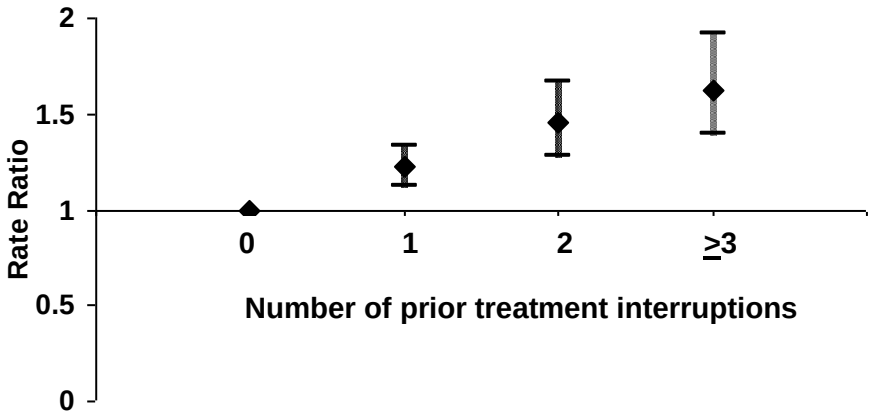


Figure 7.3.7.2: Adjusted rate-ratios (and 95% CIs) for number of previous regimens failed (per 1 regimen higher), stratified by years under viral suppression

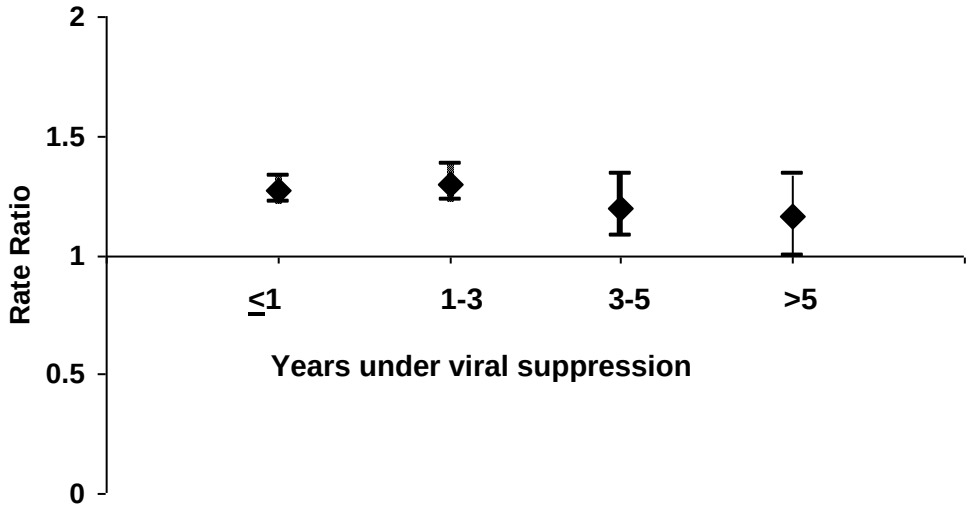


Table 7.3.7.1: Factors associated with viral rebound from univariable and multivariable analyses

		Univariable		Multivariable	
		RR (95% CI)	**P-value**	**RR (95% CI)**	**P-value**
Prior treatment interruption	No	1	<0.0001	1	<0.0001
	Yes	1.89 (1.77, 2.03)		1.30 (1.20, 1.40)	
Number of prior	0	1	<0.0001	Co-linear with	
treatment interruptions	1	1.65 (1.52, 1.79)		'prior treatment	
	2	2.34 (2.06, 2.66)		Interruption'	
	>3	3.14 (2.68, 3.68)			
Number of regimens	0	1	<0.0001	1.27 (1.23, 1.31)	<0.0001
previously failed	1	1.74 (1.60, 1.88)		Per extra regimen	
	2	2.27 (2.08, 2.49)		failed	
	3	2.89 (2.61, 3.20)			
	4	2.91 (2.65, 3.34)			
	>5	4.40 (3.79, 5.11)			
Duration of viral	<1.0 year	5.71 (4.94, 6.60)	<0.0001	0.80 (0.79, 0.82)	<0.0001
Suppression	1.1-3.0 years	2.82 (2.43, 3.27)		Per 1 year higher	
	3.1-5.0 years	1.52 (1.28, 1.80)			
	>5.0 years	1			

Sex	Male	1	-	1	0.61
	Female	1.30 (1.21, 1.39)	<0.0001	0.98 (0.88, 1.08)	
Ethnicity	White	1	<0.0001	1	<0.0001
	Black	1.36 (1.27, 1.46)		1.29 (1.17, 1.43)	
	Other	1.01 (0.90, 1.13)		1.00 (0.89, 1.12)	
Risk group	MSM	1	<0.0001	1	0.09
	Heterosexual	1.27 (1.19, 1.36)		1.08 (0.97, 1.20)	
	Other	1.37 (1.23, 1.52)		1.13 (1.01, 1.27)	
Current age (years)	<35	1	<0.0001	0.76 (0.73, 0.79)	<0.0001
	35-45	0.73 (0.68, 0.79)		Per 10 years older	
	>45	0.47 (0.43, 0.51)			
Mono/dual therapy	No	1	<0.0001	1	0.14
received	Yes	1.65 (1.55, 1.75)		0.94 (0.86, 1.02)	
Calendar year	1996-2000	3.26 (3.01, 3.54)	<0.0001	0.95 (0.93, 0.97)	<0.0001
	2001-2004	1.50 (1.39, 1.61)		Per 1 year later	
	2005 onwards	1			
Current CD4 count	<200	3.10 (2.83, 3.39)	<0.0001	1.83 (1.67, 2.02)	<0.0001
(cells/mm^3)	200-349	1.86 (1.71, 2.01)		1.37 (1.26, 1.49)	
	350-499	1.33 (1.22, 1.45)		1.15 (1.05, 1.25)	

	>500	1		1	
	Missing	2.45 (1.59, 3.78)		1.32 (0.86, 2.04)	
NRTI combination	AZT/3TC	1	<0.0001	1	<0.0001
	AZT/DDI	1.79 (1.49, 2.16)		1.39 (1.15, 1.68)	
	d4T/3TC	1.91 (1.73, 2.12)		1.23 (1.10, 1.37)	
	d4T/ddI	2.99 (2.64, 3.38)		1.59 (1.40, 1.82)	
	AZT/ABC	1.67 (1.16, 2.40)		1.42 (0.98, 2.05)	
	TFV/3TC	0.82 (0.71, 0.95)		0.92 (0.79, 1.07)	
	ABC/3TC	0.61 (0.53, 0.71)		0.86 (0.74, 1.00)	
	TFV/DDI	1.99 (1.72, 2.30)		1.22 (1.04, 1.43)	
	FTC/TFV	0.55 (0.46, 0.65)		0.75 (0.63, 0.90)	
	Other	1.75 (1.61, 1.91)		1.16 (1.05, 1.28)	
“Third” drug	EFV	1	<0.0001	1	<0.0001
	NVP	1.91 (1.70, 2.14)		1.87 (1.67, 2.10)	
	SQV	6.40 (5.02, 8.15)		3.32 (2.59, 4.27)	
	IDV	3.68 (3.10, 4.37)		2.07 (1.72, 2.49)	
	NFV	4.63 (4.07, 5.26)		2.88 (2.51, 3.30)	
	RTV	3.04 (2.19, 4.21)		1.68 (1.20, 2.34)	
	AZV	2.90 (1.98, 4.25)		2.35 (1.60, 3.45)	
	IDV/r	3.28 (2.64, 4.08)		1.78 (1.42, 2.23)	

	SQV/r	3.21 (2.76, 3.73)		2.01 (1.72, 2.36)	
	LPV/r	2.55 (2.25, 2.89)		1.57 (1.38,1.80)	
	AZV/r	2.06 (1.75, 2.42)		1.72 (1.45, 2.05)	
	ABC	3.27 (2.90, 3.68)		2.29 (2.02, 2.59)	
	ETR	1.33 (0.50, 3.56)		0.48 (0.18, 1.29)	
	Other	5.08 (4.42, 5.85)		2.85 (2.45, 3.31)	
Time since start of	<1.0 year	1.62 (1.47, 1.79)	<0.0001	-	-
HAART	1.1-3.0 years	1.47 (1.36, 1.59)			
	3.1-5.0 years	1.21 (1.11, 1.32)			
	>5.0 years	1			
Prior AIDS diagnosis	Yes	1.22 (1.14, 1.30)	<0.0001	1.03 (0.96, 1.10)	0.39
Number of previous	0	1	<0.0001	1.12 (1.05,1.20)	0.001
viral rebounds	1	1.84 (1.69, 2.00)		Per 1 previous	
	2	2.87 (2.45, 3.35)		rebound higher	
	>3	4.13 (3.12, 5.46)			

7.3.8. Association between duration of treatment interruption and viral rebound

Though the median duration of a treatment interruption was just under 6 months, 2,956 (36.8%) interruptions were shorter than 3 months and a further 1,676 (20.9%) lasted for between 3 and 6 months. The remaining 3,392 (42.3%) interruptions were longer than 6 months. The risk of viral rebound increased linearly as the number of interruptions increased, for all durations of treatment interruptions. In univariable analyses, compared to those who had never interrupted therapy for less than 3 months (i.e. those who interrupted therapy for longer than 3 months), patients who made 1 interruption that was shorter than 3 months had a 77% higher risk of viral rebound (1.77 (1.61, 1.95)), whilst those who had interrupted therapy at least twice for less than 3 months were over twice as likely to experience viral rebound (2.13 (1.75, 2.58)). Compared to patients who had never interrupted therapy for 3-6 months, patients who had 1 previous treatment interruption for this period of time were twice as likely to experience viral rebound (2.05 (1.83, 2.30)), whilst those who had 2 or more previous interruptions were 3 times as likely to experience viral rebound (3.07 (2.35, 4.06)). Finally, patients who had interrupted therapy once for >6 months had a 74% increased risk of viral rebound compared to those who had never interrupted therapy for >6 months (1.74 (1.58, 1.91)), and those who had interrupted therapy 2 or more times had a 2.6 times increased risk of viral rebound (2.60 (2.14, 3.14)).

After adjusting for potential confounders, including demographics, number of regimens previously failed and the number of treatment interruptions of different durations, all the above estimates were still significantly associated with viral rebound (Table 7.3.8.1).

In sensitivity analyses, the total duration of treatment interruptions was calculated. For example, a patient who had interrupted treatment on two occasions for 3 months and 6 months respectively had a total duration of 9 months of treatment interruption. In univariable analyses, the risk of viral rebound increased by 5% per 3 month increase in total duration of prior interruptions (1.05 (1.04, 1.06)). Though this estimate was weaker after adjusting for potential confounders, it still remained statistically significant (1.03 (1.02, 1.04)).

Table 7.3.8.1: Independent associations between duration of treatment interruptions and viral rebound (obtained by fitting 3 separate models)

		RR (95% CI)	P-value
Number of prior treatment	0	1	0.005
interruptions lasting <3 months	1	1.18 (1.06, 1.30)	
	>2	1.15 (0.94, 1.41)	
Number of prior treatment	0	1	<0.0001
interruptions lasting 3 -6 months	1	1.32 (1.17, 1.48)	
	>2	1.49 (1.13, 1.99)	
Number of prior treatment	0	1	<0.0001
interruptions lasting >6 months	1	1.27 (1.15, 1.41)	
	>2	1.47 (1.21, 1.80)	

7.3.9. Viral load at time of prior treatment interruptions

The number of treatment interruptions was also considered after stratification by the viral load at the time of the interruption. The total number of interruptions at an undetectable viral load (≤400 copies/mL) was 2,207 (27.5%), whilst 3,250 (40.5%) interruptions were made when the viral load was above 400 copies/mL. In univariable analyses, patients who interrupted treatment once with a detectable viral load were almost twice as likely to experience viral rebound than those who had never interrupted therapy with a detectable viral load (1.97 (1.80, 2.14)). This estimate increased to 2.77 (2.34, 3.25) amongst patients who had interrupted therapy at least twice whilst having a detectable viral load. Patients interrupting therapy once with an undetectable viral load were 36% more likely to experience viral rebound (1.36 (1.21, 1.54)) compared to those who had never interrupted therapy with an undetectable viral load and this increased to 52% for those who had interrupted therapy at least twice with an undetectable viral load (1.52 (1.12, 2.08)). Interestingly, 2,567 (32.0%) interruptions to therapy were made without having a viral load measure recorded at time of interruption. Patients with 1 prior interruption had a 79% higher risk of viral rebound compared to those with no prior interruptions with missing viral loads, whilst those with at least 2 prior interruptions were over 3 times as likely to experience viral rebound. In multivariable analyses, interrupting treatment at an undetectable viral load was no longer significantly associated with viral rebound (1.07 (0.94, 1.21) and 0.92 (0.67, 1.26) comparing 1 prior treatment interruption and 2 or more prior treatment interruptions at an undetectable viral load to no prior treatment interruptions at an undetectable viral load, p=0.50). However, patients who had one prior interruption at a detectable viral load had a 28% higher risk of viral rebound compared to those who had no prior treatment interruptions at a detectable viral load (1.28 (1.16, 1.41)), whilst those who had 2 or more prior interruptions at a detectable viral load had a 49% higher risk of viral rebound (1.49 (1.25, 1.78)) ($p<0.0001$). Patients with missing viral loads at treatment interruption were also more likely to experience viral rebound compared to those without any prior interruptions whilst the viral load was unknown (1.24 (1.11, 1.39) and 1.76 (1.44, 2.15) for one interruption and 2 or more interruptions respectively, compared to no interruptions whilst the viral load was unknown).

Table 7.3.9.1: Independent associations between viral load at treatment interruption and viral rebound (obtained by fitting 3 separate models)

		RR (95% CI)	P-value
Number of prior treatment interruptions at VL ≤400 copies/mL	0	1	0.50
	1	1.07 (0.94, 1.21)	
	>2	0.92 (0.67, 1.26)	
Number of prior treatment interruptions at VL >400 copies/mL	0	1	<0.0001
	1	1.28 (1.16, 1.41)	
	>2	1.49 (1.25, 1.78)	
Number of prior treatment interruptions at a missing VL	0	1	<0.0001
	1	1.24 (1.11, 1.39)	
	>2	1.76 (1.44, 2.15)	

7.3.10. Regimen and viral load at time of prior treatment interruptions

The regimen at the time of the treatment interruption was initially fitted in a separate model to the models assessing the viral load at treatment interruption (Table 7.3.10.1). In univariable analyses, the risk of viral rebound increased with the number of prior treatment interruptions for those receiving NNRTI-, PI/r- and PI-based regimens. The strongest effect was seen amongst patients taking PI/r-based regimens; patients with 2 or more prior interruptions of a PI/r-based regimen were over 3 times as likely to experience viral rebound compared to those with no prior interruptions of a PI/r based regimen. These effects remained statistically significant after adjusting for potential confounders. Patients with 2 or more interruptions of an NNRTI-based regimen had a 46% increased risk of viral rebound compared to those with no prior interruptions, whilst those interrupting a PI/r-based regimen on 2 or more occasions had a 54% higher risk of viral rebound. Patients interrupting a single PI-based regimen on 2 or more occasions had a 20% higher risk of viral rebound compared to those with no prior interruptions of a single PI-based regimen. In multivariable analyses, the number of interruptions remained linearly associated with viral rebound. Again, the strongest effect was seen amongst patients who had interrupted a PI/r regimen on more than 2 occasions (1.54 (1.20, 1.98) compared to no prior interruptions of a PI/r-based regimen).

Given that a detectable viral load at interruption was significantly associated with viral rebound, this variable was fitted in the same model as the regimen interrupted

variable. After adjusting for the regimen interrupted, having a detectable viral load at the time of the treatment interruption was no longer associated with the risk of viral rebound, whilst interrupting NNRTI or PI/r based regimens remained significantly associated with the risk of viral rebound (Table 7.3.10.1). However, these analyses may have been confounded with the regimen that patients subsequently restarted. Thus, sub-group analyses were performed separately among those who were receiving a NNRTI-based regimen at the time of viral suppression and those who were receiving a PI/r-based regimen and focused on prior treatment interruptions taken whilst the viral load was detectable (Table 7.3.10.2).

Table 7.3.10.1: Univariable and multivariable estimates of the impact of regimen and viral load at interruption on risk of viral rebound

		Univariable		Multivariable	
		RR (95% CI)	**P-value**	**RR (95% CI)**	**P-value**
Model 1: Regimen at interruption					
Number of NNRTI-based	0	1	<0.0001	1	0.002
regimen interruptions	1	1.65 (1.49, 1.82)		1.18 (1.03, 1.36)	
	≥2	2.71 (2.21, 3.32)		1.46 (1.17, 1.82)	
Number of PI/r-based	0	1	<0.0001	1	0.0004
regimen interruptions	1	1.96 (1.73, 2.23)		1.24 (1.07, 1.45)	
	≥2	3.31 (2.61, 4.20)		1.54 (1.20, 1.98)	
Number of PI-based	0	1	<0.0001	1	0.03
regimen interruptions	1	1.78 (1.59, 1.98)		1.20 (1.04, 1.38)	
	≥2	2.30 (1.67, 3.17)		1.23 (0.88, 1.72)	
Number of ‘Other’-based	0	1	<0.0001	1	0.44
regimen interruptions	1	0.55 (0.51, 0.59)		0.94 (0.82, 1.08)	
	≥2	1.42 (1.06, 1.89)		1.12 (0.84, 1.51)	
Model 2: Detectable VL and regimen at interruption					
Number of interruptions	0			1	0.16

at detectable viral load	1	1.11 (0.99, 1.25)	
	≥2	1.14 (0.90, 1.44)	
Number of NNRTI-based	0	1	0.04
regimen interruptions	1	1.13 (0.97, 1.32)	
	≥2	1.37 (1.07, 1.75)	
Number of PI/r-based	0	1	0.01
regimen interruptions	1	1.20 (1.02, 1.41)	
	≥2	1.47 (1.11, 1.93)	
Number of PI-based	0	1	0.18
regimen interruptions	1	1.15 (0.99, 1.34)	
	≥2	1.17 (0.83, 1.64)	
Number of ‘Other’-based	0	1	0.62
regimen interruptions	1	0.95 (0.82, 1.10)	
	≥2	1.07 (0.79, 1.46)	

Among those receiving a NNRTI-based regimen, those who had previously interrupted at least one NNRTI regimen at a detectable viral load had a 71% increased rate (adjusted RR 1.71 (1.17, 2.50)) of viral rebound compared to those patients who had never interrupted therapy. The rate of rebound among patients who had interrupted only non-NNRTI-based regimens at detectable viral load levels was only 18% higher (1.18 (0.86, 1.61)) compared to that in patients who had never previously interrupted therapy and was not statistically significant. In contrast, among patients who were currently on a PI/r-based regimen, those who had previously interrupted a PI/r-based regimen with a detectable viral load had a 59% increased rate (1.71 (1.17, 2.50)) of viral rebound compared to those patients who had never interrupted therapy. Patients in this group who had interrupted only non-PI/r-based regimens at a detectable viral load had a rate of viral rebound that was 18% (1.18 (0.98, 1.41)) higher than that among patients who had never interrupted therapy.

Table 7.3.10.2: Independent estimates from sub group analyses: Patients currently receiving NNRTI based regimens and those currently receiving PI/r based regimens

	Univariable		Multivariable	
	RR (95% CI)	**P-value**	**RR (95% CI)**	**P-value**
Patients currently on an NNRTI regimen				
No prior interruptions	1	<0.0001	1	0.06
At least one interruption of a NNRTI regimen at a detectable viral load	2.41 (1.67, 3.48)		1.71 (1.17, 2.50)	
No interruption of a NNRTI at a detectable viral load, but interruption of other regimens at a detectable viral load	1.96 (1.46, 2.64)		1.18 (0.86, 1.61)	
No interruption at a detectable viral load, but at least one interruption at an undetectable viral load	1.14 (0.82, 1.59)		1.11 (0.79, 1.56)	
Patients currently on an PI/r regimen				
No prior interruptions	1	<0.0001	1	0.004
At least one interruption of a PI regimen at a detectable viral load	2.14 (1.68, 2.73)		1.59 (1.23, 2.05)	
No interruption of a PI at a detectable viral load, but interruption of other regimens at a detectable viral load	1.42 (1.20, 1.68)		1.18 (0.98, 1.41)	
No interruption at a detectable viral load, but at least one interruption at an undetectable viral load	1.07 (0.84, 1.36)		0.98 (0.77, 1.25)	

7.4. Discussion

Following the publication of the SMART trial (522) it has been anticipated that the proportion of follow-up time spent on treatment may rise. In these analyses, I have shown that the proportion of time spent on treatment has been increasing steadily in the last 10 years. In this dataset, information is only available up until early 2008 and hence it is likely that the proportion of time spent on treatment has continued to rise. Further, as the results of the SMART trial are incorporated into treatment guidelines, it is possible that the rise in the proportion of time spent on treatment may be at a higher rate than previously seen. However, the proportion of new patients who are diagnosed early may also impact on these results, i.e. the proportion of time spent on treatment may decrease if a larger number of patients under follow-up are diagnosed early and do not start treatment until their CD4 counts are below guideline recommendations.

However, amongst those who have started HAART, a quarter of patients interrupted all treatment for greater than two weeks at least once whilst under follow-up. Interestingly, the proportion of interruptions decreased in later calendar years. This may be explained by a greater awareness of the consequences of interrupting treatment, especially in light of the results from the SMART trial. Amongst those who interrupted therapy, a higher proportion of patients (36%) interrupted an NNRTI regimen than a PI/r regimen (27%). This is not surprising given that NNRTI-based regimens are the most commonly used regimens within the CHIC cohort. A third of patients restarted the same NNRTI regimen, suggesting that the interruption amongst these patients was not due to viral rebound or resistance to the NNRTI, but rather just a break from therapy. However, 30% of patients did restart a PI/r-based regimen, suggesting that these patients may have had resistance to the NNRTI in their regimen. Patients were still being monitored whilst on a treatment break, evident from the number of CD4 counts and viral loads during the interruption.

Though previous studies have reported the short to medium-term effects of interrupting therapy (439;445;450;455;459;460;518-520;522), the long-term effects of treatment interruptions among those who subsequently achieve an undetectable viral load has not previously been investigated. Further, many studies have identified factors associated with viral rebound, but no study to date has specifically focussed on the effect of the duration of viral suppression on the rate of viral rebound in relation to the antiretroviral regimens previously failed. These analyses included patients who are representative of the UK CHIC population as a whole as

most patients were male, of white ethnicity and MSM risk group. Patients who had interrupted treatment prior to the viral suppression episode had similar demographics and CD4 counts to those without prior interruptions but were more likely to have received mono/dual therapy before receiving HAART and were also more likely to have failed regimens before entering the viral suppression episode. This suggests that prior interruptions were not simply a break in treatment, but were due to resistance mutations surfacing and hence a change in antiretroviral therapy being implemented. This is further supported by the fact that only a quarter of patients restarted the same regimen at the end of their treatment interruption. Patients who had previously interrupted while their viral load was detectable (>400 copies/mL) had up to a 49% higher chance of rebounding, even after controlling for the duration of viral suppression. In contrast, there was no evidence to suggest that interrupting therapy at a viral load of $\leq$400 copies/mL was associated with a raised risk of viral rebound

Other studies focusing on rates of viral suppression after interrupting therapy (455;520) have demonstrated that interruption of treatment at an undetectable viral load is generally associated with a lower rate of suppression compared to continuous therapy. It should be noted that these analyses focus solely on those patients with a suppressed viral load while on therapy. In particular, patients who have previously interrupted therapy must have re-attained viral suppression on restarting therapy to be included in these analyses. Thus, a selected group of patients with better adherence who may be expected to have better long-term outcomes may have been included.

The results in this Chapter show that the rebound rate increases as the number of prior treatment interruptions with detectable viral load increases. These findings may be a consequence of the higher risk of resistance evolution in patients who had previously interrupted HAART(459). Resistance mutations may have been archived during the period when drug levels were low immediately after interruption, as well as in the period before interruption when the viral load was detectable. Yerly et al reported that the M184V/I mutation is frequently selected during repeated treatment interruptions (460), consistent with results from Schweighardt et al, who concluded that repeated treatment interruptions of certain antiretroviral drug regimens may lead to the development of drug resistance (521). It is also possible that prior detectable viral load while on therapy, with the consequent evolution of resistance, is the main reason for the association, rather than the interruption itself**.** These hypotheses

would be consistent with our findings of a higher rate of viral rebound in those who had previously interrupted an NNRTI with a detectable viral load and then restarted the same class of drug, which appeared to be larger than the corresponding level of increased risk for those who had interrupted a PI/r among those on a PI/r, and which may be a consequence of the longer half-life of the NNRTI class.
A lower rate of archived resistance is expected in patients who interrupt their therapy when their viral load levels are undetectable due to the reduced opportunity for replication of virus in the presence of sub-optimal drug levels. The findings of a lack of association between interruption of therapy at undetectable viral loads and subsequent viral rebound are consistent with this.

However, these findings might also be explained by confounding by adherence, in that patients who have previously interrupted HAART may be those who are generally less adherent to HAART and hence are more likely to experience viral rebound, either due to the emergence of resistance or to the presence of sub-optimal drug levels. If so then the explanation for the lack of relationship between treatment interruptions at undetectable viral loads and viral rebound may be that those patients who interrupted treatment at undetectable viral loads and then managed to re-suppress their viral load on restarting treatment may generally have better adherence levels than those who interrupt at a detectable viral load and may therefore be more likely to maintain viral suppression.

In these analyses, I have also shown that though viral rebound rates increased with the number of regimens previously failed they declined progressively with increased duration of viral suppression. Within the first year of viral suppression, the rate of rebound ranged from 6.57/100 pys in patients on first-line therapy, to 1.54/100 pys in patients who had failed five or more regimens. However, this large difference in viral rebound rate observed between those on first-line and heavily treatment-experienced patients was substantially reduced with increasing time with suppression and there appeared to be little difference in the rebound rate observed in patients who remained suppressed for >5 years.

These results showing that viral rebound rates decrease progressively with increased duration of viral suppression are consistent with data from other observational cohorts (413;414;429). This is likely to be due to a selection effect, whereby patients who are able to suppress their virus for the longest periods tend to be those who are most adherent to therapy and those who experience fewest drug

toxicities. Further, these patients are likely to be those who achieve the highest plasma drug concentrations and have the fewest pre-existing drug resistance mutations. It has also been reported that patients who are able to suppress their viral load to <50 copies/mL for longer durations of time, are likely to experience even lower viral load decreases, to <3 copies/mL (523).

Other factors associated with viral rebound are consistent with those reported in other studies. In accordance with the results of a previous study (478), these results indicate that a lower baseline CD4 count was a significant predictor of viral rebound. One potential explanation is that levels of some drugs are not maintained at optimal levels in those with low CD4 counts, due to poorer drug absorption or other factors.
The finding that black ethnicity was associated with an increased risk of viral rebound is consistent with other studies (414;422). This may, in part, be related to socio-economic issues. Older age was found to be a significant predictor of a better response. In other areas of medicine, older age is generally associated with poorer adherence to therapy. However, the converse appears to be the case in HIV, and younger age has been associated with poorer responses to therapy (423). The reasons for these associations are unclear, however social or behavioural factors may play an important role. Calendar year was also associated with an increased risk of viral rebound, whereby patients commencing therapy in earlier calendar years were at increased risk of viral rebound. These patients are more likely to have had prior exposure to NRTI mono or dual therapy and have accumulated mutations associated with drug resistance. Further, in recent calendar years, antiretroviral drugs have improved considerably and are now associated with fewer toxicities. Hence patients starting HAART in later calendar years may be more adherent, hence reducing the risk of viral rebound.

Current drug regimen was also a strong predictor of viral rebound. In particular, patients receiving d4T/ddI as an NRTI combination were at an increased risk of viral rebound when compared to patients receiving AZT/3TC. Both d4T and DDI have been associated with peripheral neuropathy and pancreatitis and hence it is possible that patients receiving these drugs may not be as adherent as those receiving other NRTI backbone combinations. The newer NRTI drug combinations (i.e. those with fewer toxicities and less pill burden), for example ABC/3TC and FTC/TFV were associated with a reduced risk of viral rebound, further supporting this explanation. With regards to the ‘third’ drug in the regimen, patients receiving PIs (single or ritonavir-boosted) were at an increased risk of viral rebound compared to those

receiving EFV. One possible explanation for this is that PI-based therapy is now generally used as second-line therapy and hence patients receiving these regimens are likely to have failed previous regimens. Patients receiving NVP were also at an increased risk of viral rebound compared to those receiving EFV. Although other studies have reported similar results (100;102;103;430;524), 2NN, a large randomised trial comparing EFV and NVP did not verify this finding (104). EFV was, however, seen as favourable when analyses were restricted to patients who had taken at least one dose of the drug they were assigned to.

It is important to note that in these analyses in particular, unmeasured confounding, such as that due to adherence, has not been controlled for. Adherence is likely to play a key part in both the decision to interrupt treatment as well as the risk of subsequent viral rebound. Furthermore, findings of a non-significant relationship between the number of treatment interruptions taken whilst the viral load was undetectable and viral rebound, should be interpreted cautiously, given the relatively small number of such interruptions.

The results in this Chapter show that there may be long term effects of treatment interruption that are present even if viral suppression is subsequently achieved. Among those with suppressed viral load on HAART, those who have previously interrupted therapy while having a raised viral load may be at increased risk of subsequent viral rebound and hence should be monitored regularly. It is important that the potential negative long-term effects of interrupting therapy (both in terms of the risk of clinical events and long-term viral failure) are taken into account when the decision to interrupt is being made, even if the short-term effects appear to be minimal. Amongst patients who do achieve viral suppression, previous failure is associated with a higher rate of viral rebound, but the likelihood of rebound declines with the duration of suppression. Achievement of viral suppression in a patient with previous multiple failures is followed by a period of high risk of rebound that requires careful management. Both clinicians and patients can be encouraged that regardless of previous treatment failure, rebound rates fall to levels approaching those of patients on first-line therapy after 5 years of suppressive therapy.

7.5. Summary

Although the proportion of time spent on treatment has increased over the last 10 years, a quarter of patients starting HAART are likely to interrupt treatment at some point whilst under follow-up. Though some of these interruptions are simply a

treatment break, most patients restart a different regimen. Patients interrupting therapy whilst their viral load is detectable, who subsequently restart ART and achieve viral suppression are more likely to experience viral rebound than those with viral suppression who have not previously interrupted ART. However, patients maintaining viral suppression for long periods of time are less likely to experience viral rebound, regardless of both the number of prior treatment interruptions and the number of regimens previously failed.

Chapter 8: Association between laboratory abnormalities and mortality

8.1. Introduction

Though patients with HIV who are successfully treated now have near normal life expectancies as a result of antiretroviral treatment, adverse events associated with therapy are of growing concern. Table 1.6.1 in Chapter 1 lists common and rare side effects associated with each of the antiretroviral drugs available.

Results from the Swiss HIV Cohort Study (525) indicate that patients with laboratory abnormalities are at increased risk of mortality; those with clinical abnormalities are more likely to either change or discontinue treatment. However, these results were based on a cross-sectional analysis and hence the effect of adverse events over time on mortality and treatment modification could not be assessed. Further, in the Swiss Study, an overall score was calculated by summing up individual scores (calculated by assigning 4 points to each serious abnormality, 3 points to each severe abnormality, 2 points to each moderate abnormality and 1 point to each mild abnormality, based on the Table for Grading Severity of Adult Adverse Experiences (283)) of 11 adverse laboratory parameters (haemoglobin, absolute neutrophil count, platelet count, creatinine, urate, alanine aminotransferase, alkaline phosphatase, bilirubin, amylase, creatinine phosphokinase and lactate). This did not allow distinction between those abnormalities which may have contributed more to the overall score (and therefore overall risk) than other abnormalities.

In this Chapter, I investigate the association between laboratory abnormalities and mortality. In addition to applying the cut-offs used in the Table for Grading Severity of Adult Adverse Experiences to score laboratory abnormalities, I will explore several other methods of assigning a score to these abnormalities and choosing appropriate cut-offs for these scores to represent high risk. In addition to the laboratory markers discussed above, I will also include CD4 and viral load in all analyses as both are well recognised predictors of mortality.

8.2. Methods

8.2.1. Laboratory data checks

Although laboratory data received from centres is checked and cleaned before the dataset is made available for analysis, the number of different tests performed and the centre-specific decision to perform these tests results in a highly variable database of laboratory tests. Some centres provide very little or no laboratory data at all, whilst others provide data on a wide range of toxicity measures. Centres may also provide laboratory data infrequently in one calendar period, but routinely in another. In order to describe these irregularities and hence reduce the impact of selection bias (i.e. centres providing laboratory measures in only those patients with abnormal values or in those perceived to be at high risk), I calculated the proportion of patients with laboratory measurements in each calendar year and at each CHIC centre. Further, the proportion of patients seen at each centre with laboratory measurements available was stratified by calendar year in order to identify any centre-specific irregularities. Based on these results, years in which the proportion of patients with laboratory measures were very low, and centres at which these variables were not measured routinely on all patients, were excluded from the analysis.

Differences between the number of laboratory measurements and the duration between measurements before and after starting HAART were described. When analysing the number of laboratory measurements prior to starting HAART, follow-up began on date of entry to CHIC and ended at the earliest date of starting HAART and 31st December 2007. When analysing the number of laboratory markers after starting HAART, follow-up began on date of starting HAART and ended on 31st December 2007.

Factors associated with having each laboratory measurement in the years 2000 (start of follow up, see Section 8.2.2) and 2007 (last complete year of follow up) were identified. These latter analyses were performed in order to highlight any potential biases that may occur in later analyses (deriving a laboratory score). Separate models were fitted for each laboratory measurement and adjusted for the following potential predictors: ethnicity, risk-group, age at start of year, sex, CD4 count at start of year, VL at start of year, and prior laboratory measurement (none, abnormal, normal, with measurements being defined as abnormal using the Table for Grading the Severity of Adverse Events (283)). Analyses were also adjusted for

whether or not patients had started HAART in the year in question to capture the possibility of more frequent monitoring around the time of HAART initiation.

8.2.2. Inclusion criteria

For patients to be included in the main analyses, they must have started HAART in or after the year 2000. This time point was chosen since monitoring practices have changed considerably since the early HAART era, as have treatment strategies and the type of patient under follow-up (in the early HAART era, patients were more likely to have experienced mono/dual NRTIs before starting HAART). Patients were also required to have at least one laboratory measurement within 2 years of starting HAART (the first of each laboratory measurement in this period was defined as the 'baseline' measurement), including at least one CD4 count and one viral load measurement, as these variables have been consistently shown to be associated with mortality. Laboratory measurements considered are shown in Section 8.2.3. Patients who died within 3 months of first being seen at a CHIC clinic were excluded.

Differences between patients who started HAART and had laboratory measurements available and those who did not have laboratory measurements available (and were hence excluded from the analyses) were investigated using Chi-squared and Mann Whitney tests.

8.2.3. Deriving a laboratory score to predict mortality

Several methods were used to derive a laboratory score that could efficiently predict mortality within 2 years of starting HAART. The following laboratory markers were included in the analyses: alanine aminotransferase (ALT), albumin (ALB), amylase (AMY), aspartate transaminase (AST), alkaline phosphatase (ALK), bilirubin (BIL), cholesterol (CHL), creatinine (CRE), creatinine phosphokinase (CPK) gamma glutamyl transpeptidase (GGT), glucose (GLU), haemoglobin (HAE), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TRI) and urea (URE).
Patients who were under follow up for at least two years, or had died within 2 years of starting HAART, were included in the analyses. I used a validation method to assess the accuracy of the derived scores. The dataset was randomly partitioned into a training set (70% of the data) and a validation set (the remaining 30% of the data); all analyses were initially performed on the training set and then validated on the validation set. Harrell's C-statistic was used to assess the ability of the score to discriminate between those who died and those who were alive after 2 years of

starting HAART. A value of greater than 0.5 suggests that the score is able to discriminate between the two groups, with values close to 1 indicating better discriminatory ability. Patients were also stratified according to the deciles of the score distributions; observed and expected numbers of deaths in each strata were compared with the Hosmer-Lemeshow statistic ($p>0.05$ indicates no evidence of lack of fit). These statistics were also performed on the validation sets to assess model adequacy.

Only baseline laboratory measurements at start of HAART were considered. The different approaches taken to derive a score are outlined below.

8.2.3.1. Stratifying baseline laboratory measurements by quintiles (Approach A)

In the first instance, I split the distribution of each laboratory variable into quintiles and calculated the proportion of deaths in each stratum. For most variables, a score of 1 was assigned to the lowest stratum and 5 to the highest. The exceptions to this were ALB, HAE and HDL, where a high measurement is considered better than a lower measurement. Hence a score of 1 was assigned to the highest stratum and 5 to the lowest for these three variables. The total score was calculated by adding up the individual scores for each laboratory measurement. Patients with missing baseline laboratory measurements were assigned a score of 1 for each measurement (i.e. these patients were considered to have a low risk). The overall score was split into quintiles and associations between this score and mortality was assessed using logistic regression.

8.2.3.2. Using continuous baseline laboratory measures (Approach B)

These analyses were similar to those described above. However, the laboratory measures were not split into categorical variables, but rather, the continuous baseline measures were used instead. The estimates from a multivariable logistic regression model were used to derive an overall laboratory score to predict mortality. Sensitivity and specificity of the chosen score were calculated. Patients with missing baseline laboratory measures were excluded from these analyses.

8.2.3.3. Principal component analysis (Approach C)

Principal component analysis is a method of reducing the number of variables included in a regression model. Variables which are correlated to some degree are reduced into a smaller number of principal components which account for most of the variance in the observed variables. For example, all variables which measure

liver function may be correlated into one new variable. A linear combination of weighted observed variables forms a principal component. Ideally, the first component extracted accounts for a large amount of total variance and each subsequent component accounts for progressively smaller amounts of variance. In these analyses, I retained all components which cumulatively accounted for at least 70% of the total variance. Other methods of selecting the components to keep included the following:

1. Retain all components with an eigenvalue >1
2. The scree test – plot eigenvalues and look for a 'break' between the components. Retain all components that appear before the break
3. Remove any variables which 'load' significantly onto more than one component. In these analyses, a loading is defined as significant if its absolute value is greater than 0.40. Variables which significantly load onto more than one variable are not measuring either component efficiently.
4. Ensure there are at least 3 variables which 'load' on to a component, i.e. each component is made up of at least 3 variables which share the same conceptual meaning

Further details of how to select principal components can be found on the following website: http://support.sas.com/publishing/pubcat/chaps/55129.pdf).

I initially performed these analyses on only those patients who had all baseline laboratory measures available. I then excluded AMY and GLU from the analyses as a large proportion of patients had these measurements missing. In the final step, I also excluded HDL and LDL from the analyses in an attempt to increase the number of patients in the analysis.

8.2.3.4. Replicating the Swiss score (Approach D)

In these analyses, the Swiss toxicity score (525) was replicated using the CHIC dataset. ALT, ALK, ALB, AMY, BIL, CRE, HAE and URE were used to derive the score. Though lactate, CPK, neutrophil count and platelet count were also used in the Swiss score, these variables are not routinely collected in UK CHIC and hence were not used to derive the overall score.

Each laboratory measure was stratified and scored according to the Table for Grading Severity of Adult Adverse Experiences. Serious severe abnormalities were a given a score of 4, severe abnormalities a score of 3, moderate abnormalities a score of 2 and mild abnormalities a score of 1. Scores were summed to provide an overall score. Logistic regression was used to assess the association between this

overall score and death. Patients who did not have baseline laboratory values measured, and those with measures that did not meet the criteria to be defined as having a 'mild' toxicity were given a score of 0 for each particular laboratory variable. The rationale for assigning a score of 0 to those without laboratory values measured was that patients who reported no adverse events may have been less likely to have a laboratory measurement. Hence, though not always the case, it is likely that their laboratory values were in the normal range. In sensitivity analyses, only patients with all baseline laboratory measures were included.

8.2.3.5. Six-month risk of mortality

The approaches described above aim to predict the long-term risk of mortality.
I used two of these approaches to derive a score for the short-term risk of mortality, i.e. a six month risk of mortality. These were Approach B (using continuous baseline measures) and Approach D (replicating the Swiss score). In the former method, all available laboratory markers were used (not just baseline) and a score was derived using the estimates of the regression model. In the latter method, a score was assigned to the time-updated laboratory variables and this score was fitted in a regression model. I did not use Approach A as splitting the laboratory measures into quintiles did not give a convincing significant association between the overall score and mortality. The same was true for Approach C (principal component analysis).

In order to use the continuous measures at baseline approach to assess short-term mortality, patient follow-up was split into 6-month periods. The association between laboratory measurements at the start of each period and mortality within 6 months was assessed using logistic regression, taking into account multiple records from each patient (by using generalised estimating equations). Significant variables in the multivariable model were used to derive a score as previously described.

In the second method, using Approach D, the Swiss score was updated at the start of each 6-month interval. Again, logistic regression was used to assess the association between the overall score and mortality.

8.3. Results

8.3.1. Laboratory data checks

8.3.1.1. Proportion of patients with laboratory measurements in each calendar year

CD4 and VL are routinely measured markers and were hence analysed separately from the laboratory markers discussed below. The proportion of patients with CD4 counts in each year of follow-up steadily increased from 69% in 1996 to 86% in 2007 (Table 8.3.1.1.1). Though the proportion of patients under follow-up with recorded VL measurements was low in 1996 (27%), in 1997 it was 71% and this increased to 93% in 2007.

Table 8.3.1.1.1: Proportion of patients with CD4 and VL measurements stratified by year under follow-up

Year under follow-up	Number of patients under follow-up	% of patients with at least one measurement in each year	
		CD4	VL
1996	8791	69	27
1997	9330	73	71
1998	10327	78	79
1999	11342	81	81
2000	12441	83	78
2001	13740	85	81
2002	15111	86	83
2003	16381	87	86
2004	17816	87	87
2005	19468	88	88
2006	20570	91	93
2007	20451	91	93

The proportions of patients with at least one laboratory measurement in each year, stratified by year of follow-up, are shown in Table 8.3.1.1.2. Generally, over 50% of patients had at least one laboratory measurement. However, the proportion of patients with specific laboratory measurements was highly variable across all calendar years and there was little evidence to suggest that a higher proportion of patients had laboratory measurements in later calendar years as may have been expected. In most years, over 50% of patients had the following measurements: ALT, ALB, ALK, BIL, CRE, and HAE. Around 40% of patients had CHL, TRI and URE measured, and around 30% of patients had HDL, LDL, AMY and GLU measured. Less than 30% of patients had AST, CPK, LAC and GGT measured in most calendar years.

Table 8.3.1.1.2: Proportion of patients with laboratory measurements stratified by year under follow-up

Year	Patients under follow-up	% of patients with at least one laboratory measurement in each year								
	N	Any measure	ALT	ALB	AMY	AST	ALK	BIL	CHL	CPK
1996	8791	48	41	38	10	17	44	44	2	2
1997	9330	50	43	40	10	14	47	47	4	2
1998	10327	55	48	45	18	13	53	53	23	2
1999	11342	59	52	45	33	12	57	57	41	2
2000	12441	71	63	53	41	12	68	68	51	3
2001	13740	72	65	56	42	13	70	70	56	4
2002	15111	73	65	58	41	13	71	71	51	4
2003	16381	73	65	61	38	15	71	71	44	4
2004	17816	66	57	57	32	14	63	63	51	4
2005	19468	55	47	53	19	13	54	54	46	5
2006	20570	55	46	53	18	13	53	53	47	6
2007	20451	51	43	50	16	8	50	50	42	5

Table 8.3.1.1.2 continued

Year	Patients under follow-up	% of patients with at least one laboratory measurement in each year								
	N	CRE	GGT	GLU	HAE	HDL	LAC	LDL	TRI	URE
1996	8791	44	17	8	47	0	0	0	2	32
1997	9330	46	20	13	49	1	0	1	4	30
1998	10327	52	21	20	53	3	0	3	20	38
1999	11342	56	22	23	57	5	2	5	37	42
2000	12441	65	25	26	70	12	17	11	47	53
2001	13740	64	28	30	71	17	20	16	51	54
2002	15111	71	27	35	72	23	9	22	48	57
2003	16381	71	28	35	72	27	3	27	44	58
2004	17816	63	25	35	65	28	9	28	51	47
2005	19468	54	22	34	55	39	4	38	45	41
2006	20570	53	21	33	54	43	1	41	46	42
2007	20451	50	17	30	50	41	1	31	41	45

8.3.1.2. Proportion of patients with laboratory measurements at each clinical centre

The proportion of patients with laboratory measurements in each calendar year was lower than anticipated, particularly in later calendar years. Since the denominator used was all patients under follow-up, I next stratified the proportion of patients with laboratory measurements by clinical centre to identify any centres which did not provide laboratory measurements. These proportions are shown in Table 8.3.1.2.1. Although the proportions do differ by laboratory marker, i.e. some centres provide data on some laboratory markers and not on others, it was apparent that four centres provided very little data on laboratory markers at all (centres 107, 109, 110 and 111). Infact, it is extremely likely that the laboratory data provided from these 4 centres was actually from patients who had been seen at more than one clinic and hence did have some laboratory measurements on their records (i.e. recorded by another clinic which did provide laboratory data to CHIC).

Given that patients seen at these clinics contributed to the proportions in Table 8.3.1.1.2, I recalculated the proportion of patients with a laboratory measurement in each calendar year, after removing all patients seen at any of the above 4 centres (Table 8.3.1.2.2). I also restricted analyses to the year 2000 onwards for the reasons outlined in Section 8.2.2. The proportion of patients with at least one laboratory measurement increased from around 50% (before excluding the 4 centres) to above 60%. However, this proportion appeared to decline from 2000 (80%) to 2007 (62%). This decline was evident in all laboratory variables.

To investigate this further, I stratified patients by both the year of follow-up and the clinical centre they were attending. Tables showing the proportion of patients at each centre with laboratory measurements stratified by calendar year are provided in Appendix A. Whilst a decline in the proportion of patients having specific laboratory measurements from 2000 to 2007 was evident in some centres, the overall proportion of patients with at least one laboratory measurement increased in all but one centre. In Centre 103 however, the proportions of patients with any laboratory markers measured in the years 2000-2007 were 75%, 76%, 76%, 77%, 54%, 20%, 16% and 8% respectively. Hence it was apparent that from 2004 onwards, we had not received the full laboratory dataset from this centre. This contributed wholly to the overall decline in proportions seen in Table 8.3.1.2.2. Hence, in all further analyses, patient follow-up was censored at December 2003 amongst patients seen at Centre 103.

Table 8.3.1.2.1: Proportion of patients with toxicity measurements at each centre

Centre	Patients under follow-up	% of patients with at least one laboratory measurement in each year								
	N	ALT	ALB	AMY	AST	ALK	BIL	CHL	CPK	CRE
101	2137	82	83	47	9	84	84	79	15	83
102	4361	90	90	74	24	90	90	80	33	90
103	8438	66	54	50	19	66	66	46	1	66
104	4750	82	82	24	5	82	82	71	8	81
105	2945	20	87	79	2	87	87	78	1	86
106	3023	92	92	4	91	92	92	83	1	92
107	2879	9	8	5	3	9	9	8	1	9
108	915	87	84	78	8	87	87	52	78	87
109	1304	7	7	3	3	7	7	6	1	7
110	1013	5	6	2	2	6	6	5	1	6
111	842	3	3	2	1	3	3	3	0	3

Table 8.3.1.2.1 continued

Centre	Patients under follow-up	% of patients with at least one laboratory measurement in each year							
	N	GGT	GLU	HAE	HDL	LAC	LDL	TRI	URE
101	2137	12	78	84	78	12	78	73	84
102	4361	44	78	91	63	9	52	80	68
103	8438	29	10	66	9	34	9	46	65
104	4750	26	61	85	68	11	68	71	82
105	2945	86	43	86	58	17	58	78	66
106	3023	91	86	91	77	24	76	83	92
107	2879	4	6	9	6	2	6	8	8
108	915	2	2	87	0	7	30	30	71
109	1304	5	6	7	5	2	5	6	7
110	1013	3	4	6	5	1	5	5	5
111	842	2	2	3	2	1	2	3	3

Table 8.3.1.2.2: Proportion of patients with toxicity measurements stratified by year under follow-up (excluding patients seen at centres 107, 109, 110, 111 and years 1996-1999)

Year	Patients under follow-up	% of patients with at least one laboratory measurement in each year								
	N	Any	ALT	ALB	AMY	AST	ALK	BILI	CHL	CPK
2000	10884	80	63	53	41	12	68	68	51	3
2001	11922	82	65	56	42	13	70	70	56	4
2002	13007	83	65	58	41	13	71	71	51	4
2003	14044	83	65	61	38	15	71	71	44	4
2004	15087	76	57	57	32	14	63	63	51	4
2005	16089	65	47	53	19	13	54	54	46	5
2006	16792	66	46	53	18	13	53	53	47	6
2007	16448	62	43	50	16	8	50	50	42	5

Table 8.3.1.2.2. continued

Year	Patients under follow-up	% of patients with at least one laboratory measurement in each year							
	N	GGT	GLU	HAE	HDL	LAC	LDL	TRI	URE
2000	12441	71	63	53	41	12	68	68	51
2001	13740	72	65	56	42	13	70	70	56
2002	15111	73	65	58	41	13	71	71	51
2003	16381	73	65	61	38	15	71	71	44
2004	17816	66	57	57	32	14	63	63	51
2005	19468	55	47	53	19	13	54	54	46
2006	20570	55	46	53	18	13	53	53	47
2007	20451	51	43	50	16	8	50	50	42

8.3.2. Frequency, duration and value of laboratory measurements pre and post HAART

The frequency and duration of laboratory measurements stratified according to HAART status (pre-HAART and post-HAART) amongst the 24,166 patients eligible for analyses are shown in Table 8.3.2.1. For all laboratory markers, a higher number of measurements were observed post-HAART than pre-HAART. This rise was particularly evident for the liver function tests ALT (median number of measurements increased from 4 whilst not receiving HAART to 16 after receiving HAART), ALB (3 to 13), and ALK (4 to 17). The median number of BIL and HAE measurements also increased substantially in the post-HAART period.

The duration between measurements was shorter after patients had started HAART for all toxicity measurements, other than AST (duration was longer post-HAART) and URE, for which the median duration between measurements was the same, though the upper quartile was shorter than that seen in the pre-HAART period (3.4 vs. 4 months).

The value of most laboratory markers was higher post-HAART than pre-HAART. The exceptions to this were ALT, AMT and AST. Amongst the markers which did increase after the start of HAART, the biggest increase was seen for ALK and GGT.

Table 8.3.2.1: Frequency and duration of laboratory measurements before and after starting HAART

	Number of measures per patient (Median (IQR))		Duration (months) between measurements (Median (IQR))		Value of measurement (Median (IQR))	
Laboratory marker	**Pre-HAART**	**Post-HAART**	**Pre-HAART**	**Post-HAART**	**Pre-HAART**	**Post-HAART**
ALT	4 (2, 8)	16 (7, 28)	2.4 (0.7, 4.2)	2.2 (0.9, 3.3)	29 (20, 45)	24 (17, 37)
ALB	3 (1, 8)	13 (5, 25)	2.4 (0.7, 4.1)	2.3 (0.9, 3.4)	41 (37, 44)	44 (41, 46)
AMY	2 (1, 4)	8 (3, 15)	3.2 (1.8, 6.4)	2.9 (1.6, 4.4)	70 (51, 95)	65 (49, 89)
AST	3 (1, 8)	7 (2, 20)	1.5 (0.4, 3.4)	2.0 (0.8, 3.3)	30 (24, 40)	26 (21, 34)
ALK	4 (1, 8)	17 (7, 29)	2.4 (0.7, 4.1)	2.2 (0.2, 3.3)	72 (59, 90)	85 (69, 106)
BIL	3 (1, 8)	17 (7, 29)	2.5 (0.7, 4.2)	2.2 (0.9, 3.3)	8 (6, 11)	8 (6, 11)
CHL	2 (1, 4)	10 (4, 17)	3.4 (2.1, 6.4)	3.0 (1.8, 4.2)	4 (3, 5)	5 (4, 5)
CRE	3 (1, 8)	17 (7, 28)	2.3 (0.5, 4.1)	2.1 (0.8, 3.3)	80 (70, 90)	81 (72, 91)
GGT	2 (1, 6)	6 (2, 17)	2.5 (0.7, 4.2)	2.4 (0.8, 3.7)	31 (18, 60)	39 (25, 79)
GLU	2 (1, 4)	9 (4, 18)	2.9 (0.9, 5.3)	2.8 (1.4, 4.1)	5 (4, 5)	5 (4, 5)
HAE	4 (2, 9)	17 (8, 29)	2.2 (0.6, 3.9)	2.1 (0.8, 3.3)	13 (12, 14)	14 (12, 15)
HDL	2 (1, 4)	8 (4, 14)	3.2 (1.8, 6.0)	3.0 (1.8, 4.6)	1 (1, 1)	1 (1, 2)
LDL	2 (1, 3)	6 (3, 13)	3.3 (1.8, 6.0)	3.0 (1.8, 4.3)	2 (2, 3)	3 (2, 3)
TRI	2 (1, 4)	10 (5, 17)	3.4 (2.1, 6.4)	3.0 (1., 4.2)	1 (1, 2)	1 (1, 2)
URE	3 (1, 8)	11 (4, 23)	2.1 (0.5, 4.0)	2.1 (0.7, 3.4)	4 (3, 5)	5 (4, 6)

8.3.3. Factors associated with having a laboratory measurement

Factors associated with having a laboratory measurement in the year 2000 were identified and compared to those associated with having a laboratory measurement in 2007. Separate models were fitted for each laboratory measurement. Since CD4 count and VL were routinely measured since 2000 (Table 8.3.1.1.1), separate models were not fitted for these markers. However, models for all other laboratory markers were adjusted for both CD4 count and VL.

In univariable analyses, having started HAART was positively associated with having a laboratory measurement in 2000 for all laboratory markers, apart from ALT and URE. The strongest association was seen for ALB (OR=1.53 (95% CI: 1.34, 1.75)). These associations remained significant after adjusting for potential confounders (ethnicity, risk, sex, age at start of year, CD4 at start of year, VL at start of year, status of prior laboratory measurement (normal, abnormal, missing)) – the strongest association between having started HAART and having a laboratory measurement was seen for TRI (2.87 ((2.46, 3.36)). These associations also remained significant when the outcome was changed to having a laboratory measurement in 2007. However, in these analyses, the strongest associations between having started HAART and having a laboratory measurement were seen for AMY (2.85 (2.45, 3.31)).

Having a prior abnormal laboratory measurement (defined using the Table for Grading the Severity of Adverse Events (283)) was also independently associated with a higher chance of having a laboratory measurement in both 2000 and 2007. This was true for all laboratory measurements, other than ALB and HDL cholesterol. In both 2000 (0.43 (0.37, 0.49)) and 2007 (0.39 (0.33, 0.45)), having a prior abnormal ALB measurement was associated with a reduced risk of having an ALB measurement. A prior abnormal HDL measurement was not significantly associated with having a HDL measurement in 2000 (1.07 (0.76, 1.53)).

The associations between black ethnicity and having a laboratory measurement were similar in 2000 and 2007, with the exception of AMY and URE. Patients of black ethnicity had a lower risk of having an AMY measurement in 2000 (0.69 (0.58, 0.83)) compared to those of white ethnicity, but a higher risk of having an AMY measurement in 2007 (1.16 (1.01, 1.34)). Similarly, patients of black ethnicity had a lower risk of having an URE measurement in 2000 (0.81 (0.68, 0.96)) compared to those of white ethnicity, but a higher risk of having an URE measurement in 2007

(1.23 (1.09, 1.40)). Lipids, such as CHL and TRI were less likely to be measured in females in 2000; this association remained significant in multivariable analyses. However, in 2007, no association was seen between gender and lipid measurements.

Interestingly, the independent associations between heterosexual risk group (compared to MSM) and laboratory measurements differed considerably from 2000 to 2007. In 2000, patients of heterosexual risk group were less likely to have the following laboratory measurements: CHL (0.72 (0.59, 0.88)), TRI (0.69 (0.57, 0.84)) and URE (0.71 (0.59, 0.86)). No association was seen between heterosexual risk group and the following laboratory measurements: ALT, AMY, ALK, BIL, GLU, HAE, HDL and LDL. However, other than GLU and HDL, for all laboratory markers, heterosexual risk group increased the chances of having a laboratory measurement in 2007. Weak associations were seen between age and having a laboratory measurement both in 2000 and 2007 – older age was significantly associated with having a GLU, HDL, LDL and TRI measurement in 2007.

Finally, in univariable analyses, longer duration of follow-up was significantly associated with having a laboratory measurement in the year 2000 for most laboratory markers, the exceptions being HDL and LDL. Interestingly, this was not the case in 2007. In univariable analyses, longer duration of follow-up was associated with a decreased likelihood of having most laboratory markers measured in 2007. In multivariable analyses, both in 2000 and 2007, longer duration of follow-up was generally associated with a decreased likelihood of having a laboratory measurement. Adjustment for a prior abnormal measurement was the main variable which caused the effect of duration of follow-up to be reversed in 2000.

Table 8.3.3.1: Factors associated with having a laboratory measurement in 2000 from multivariable logistic regression

		ALT	ALB	AMY	ALK	BIL	CHL	CRE
		Odds Ratio (95% CI)						
Ethnicity	White	1	1	1	1	1	1	1
	Black	0.72 (0.57, 0.90)	1.31 (1.10, 1.56)	0.69 (0.58, 0.83)	1.33 (0.99, 1.78)	1.35 (1.01, 1.81)	1.32 (1.10, 1.58)	1.07 (0.82, 1.38)
	Other	1.29 (1.00, 1.68)	1.20 (1.00, 1.43)	0.90 (0.75, 1.08)	1.10 (0.82, 1.46)	1.08 (0.81, 1.44)	1.18 (0.97, 1.43)	0.91 (0.70, 1.17)
Risk	MSM	1	1	1	1	1	1	1
	Heterosexual	0.84 (0.66, 1.07)	1.56 (1.29, 1.89)	1.04 (0.86, 1.26)	1.17 (0.85, 1.61)	1.16 (0.84, 1.58)	0.72 (0.59, 0.88)	1.34 (1.01, 1.78)
	Other	0.88 (0.77, 1.00)	0.69 (0.33, 0.76)	0.82 (0.75, 0.90)	0.87 (0.75,1.02)	0.87 (0.74, 1.01)	1.22 (1.11, 1.33)	0.78 (0.68, 0.90)
Age	Per 10 years higher	1.11 (1.02, 1.21)	1.01 (0.95, 1.08)	1.04 (0.98, 1.12)	1.08 (0.97, 1.19)	1.07 (0.97, 1.19)	1.10 (1.02, 1.18)	1.09 (0.99, 1.19)
Started HAART	Yes vs. No	1.96 (1.61, 2.39)	1.64 (1.41, 1.92)	2.70 (2.31, 3.15)	2.57 (1.98, 3.35)	2.54 (1.96, 3.30)	2.79 (2.38, 3.27)	2.85 (2.27, 3.58)
Sex	Female vs. male	0.96 (0.76, 1.22)	0.86 (0.71, 1.05)	0.71 (0.58, 0.85)	0.77 (0.56, 1.05)	0.81 (0.59, 1.10)	0.68 (0.60, 0.82)	0.75 (0.57, 1.00)
CD4 at start of year	Per 50 cells higher	1.02 (1.00, 1.03)	0.99 (0.98, 1.00)	0.97 (0.96, 0.98)	1.00 (0.99, 1.02)	1.00 (0.98, 1.02)	0.97 (0.96, 0.98)	0.99 (0.98, 1.01)
VL at start of year	Per 1 log higher	1.20 (1.13, 1.27)	1.15 (1.10, 1.20)	0.93 (0.89, 0.97)	1.11 (1.03, 1.19)	1.12 (1.05, 1.20)	0.87 (0.83, 0.91)	1.04 (0.98, 1.11)
Status of prior	Normal	1	1	1	1	1	1	1
laboratory measurement	Abnormal	1.79 (1.35, 2.39)	0.43 (0.37, 0.49)	1.67 (1.10, 2.54)	2.42 (1.49, 3.93)	2.45 (1.75, 3.45)	2.14 (1.74, 2.63)	2.09 (1.18, 3.69)
(Within 3 years)	No measurement	0.06 (0.05, 0.07)	0.20 (0.18, 0.23)	0.06 (0.05, 0.07)	0.07 (0.06, 0.09)	0.09 (0.07, 0.11)	0.14 (0.12, 0.17)	0.05 (0.04, 0.06)
Duration of follow-up at start of year	Per 1 year increase	0.89 (0.86, 0.93)	0.94 (0.91, 0.97)	0.98 (0.95, 1.01)	0.93 (0.89, 0.98)	0.93 (0.89, 0.98)	1.02 (0.99, 1.06)	0.90 (0.86, 0.94)

Table 8.3.3.1 continued

		GLU	HAE	HDL	LDL	TRI	URE
		Odds Ratio (95% CI)					
Ethnicity	White	1	1	1	1	1	1
	Black	1.01 (0.84, 1.21)	1.26 (0.91, 1.74)	0.89 (0.69, 1.14)	0.91 (0.70, 1.18)	1.66 (1.39, 1.99)	0.81 (0.68, 0.96)
	Other	1.73 (1.44, 2.07)	0.90 (0.67, 1.21)	0.67 (0.51, 0.89)	0.68 (0.51, 0.91)	1.44 (1.19, 1.75)	0.78 (0.65, 0.93)
Risk	MSM	1	1	1	1	1	1
	Heterosexual	0.88 (0.72, 1.07)	1.35 (0.95, 1.91)	0.96 (0.73, 1.25)	1.00 (0.76, 1.30)	0.69 (0.57, 0.84)	0.71 (0.59, 0.86)
	Other	0.83 (0.76, 0.90)	1.00 (0.86, 1.17)	1.08 (0.96, 1.22)	1.06 (0.94, 1.20)	1.38 (1.26, 1.51)	1.51 (1.38, 1.64)
Age	Per 10 years higher	1.12 (1.05, 1.20)	1.05 (0.94, 1.18)	1.07 (0.98, 1.17)	1.02 (0.93, 1.11)	1.05 (0.98, 1.12)	1.08 (1.01, 1.16)
Started HAART	Yes vs. No	2.32 (1.99, 2.71)	1.93 (1.46, 2.55)	1.62 (1.34, 1.96)	1.63 (1.34, 1.99)	2.87 (2.46, 3.36)	1.53 (1.31, 1.80)
Sex	Female vs. male	0.82 (0.68, 1.00)	0.80 (0.57, 1.13)	0.69 (0.53, 0.91)	0.71 (0.54, 0.94)	0.78 (0.64, 0.94)	0.94 (0.78, 1.13)
CD4 at start of year	Per 50 cells higher	1.00 (0.99, 1.01)	1.00 (0.98, 1.02)	0.98 (0.96, 1.00)	0.98 (0.97, 1.00)	0.98 (0.97, 0.99)	1.01 (1.00, 1.02)
VL at start of year	Per 1 log higher	1.07 (1.03, 1.12)	1.22 (1.13, 1.31)	1.18 (1.11, 1.24)	1.19 (1.12, 1.26)	0.88 (0.84, 0.92)	1.21 (1.16, 1.26)
Status of prior	Normal	1	1	1	1	1	1
laboratory measurement	Abnormal	1.73 (1.42, 2.12)	1.39 (0.80, 2.43)	1.07 (0.76, 1.53)	1.95 (1.36, 2.82)	3.28 (2.13, 5.05)	3.18 (2.20, 4.60)
(Within 3 years)	No measurement	0.07 (0.06, 0.08)	0.10 (0.08, 0.12)	0.03 (0.02, 0.04)	0.04 (0.03, 0.05)	0.09 (0.08, 0.10)	0.10 (0.08, 0.11)
Duration of follow-up at start of year	Per 1 year increase	0.90 (0.87, 0.93)	0.94 (0.89, 0.99)	0.91 (0.87, 0.95)	0.90 (0.87, 0.94)	1.03 (0.99, 1.06)	0.87 (0.84, 0.90)

Table 8.3.3.2: Factors associated with having a laboratory measurement in 2007 from multivariable logistic regression

		ALT	ALB	AMY	ALK	BIL	CHL	CRE
		Odds Ratio (95% CI)						
Ethnicity	White	1	1	1	1	1	1	1
	Black	0.68 (0.59, 0.78)	1.24 (1.06, 1.46)	1.16 (1.01, 1.34)	1.19 (1.02, 1.38)	1.23 (1.05, 1.43)	1.51 (1.32, 1.72)	1.16 (1.00, 1.35)
	Other	1.34 (1.16, 1.55)	1.30 (1.10, 1.52)	1.51 (1.31, 1.74)	1.29 (1.11, 1.50)	1.25 (1.07, 1.45)	1.39 (1.21, 1.58)	1.28 (1.10, 1.49)
Risk	MSM	1	1	1	1	1	1	1
	Heterosexual	1.32 (1.13, 1.54)	1.43 (1.20, 1.71)	1.23 (1.05, 1.43)	1.57 (1.32, 1.86)	1.57 (1.32, 1.86)	1.18 (1.01, 1.36)	1.56 (1.31, 1.84)
	Other	0.82 (0.76, 0.89)	0.70 (0.64, 0.77)	0.61 (0.57, 0.65)	0.68 (0.63, 0.75)	0.70 (0.64, 0.76)	0.87 (0.81, 0.93)	0.68 (0.63, 0.75)
Age	Per 10 years higher	1.01 (0.96, 1.06)	1.00 (0.95, 1.06)	0.99 (0.94, 1.05)	0.99 (0.93, 1.04)	0.99 (0.94, 1.05)	1.04 (0.99, 1.09)	0.98 (0.93, 1.03)
Started HAART	Yes vs. No	1.36 (1.15, 1.61)	1.85 (1.51, 2.28)	2.85 (2.45, 3.31)	1.67 (1.37, 2.04)	1.78 (1.46, 2.17)	2.24 (1.90, 2.63)	1.67 (1.37, 2.04)
Sex	Female vs. male	1.26 (1.09, 1.46)	1.31 (1.09, 1.56)	0.99 (0.86, 1.14)	1.17 (0.99, 1.39)	1.25 (1.05, 1.48)	0.92 (0.79, 1.06)	1.17 (0.99, 1.39)
CD4 at start of year	Per 50 cells higher	1.03 (1.02, 1.04)	1.01 (1.00, 1.02)	1.01 (0.99, 1.02)	1.01 (1.01, 1.02)	1.02 (1.01, 1.03)	1.00 (0.99, 1.01)	1.02 (1.01, 1.03)
VL at start of year	Per 1 log higher	1.19 (1.14, 1.24)	1.19 (1.14, 1.25)	1.14 (1.09, 1.19)	1.15 (1.10, 1.20)	1.16 (1.11, 1.21)	1.04 (1.00, 1.08)	1.15 (1.10, 1.20)
Status of prior	Normal	1	1	1	1	1	1	1
laboratory measurement	Abnormal	2.96 (2.65, 3.29)	0.39 (0.33, 0.45)	1.68 (1.41, 2.01)	1.45 (1.14, 1.84)	3.18 (2.82, 3.58)	2.66 (2.41, 2.94)	1.67 (1.36, 2.05)
(Within 3 years)	No measurement	0.08 (0.07, 0.09)	0.05 (0.04, 0.05)	0.09 (0.08, 0.10)	0.10 (0.09, 0.11)	0.13 (0.12, 0.15)	0.17 (0.15, 0.18)	0.10 (0.09, 0.11)
Duration of follow-up at start of year	Per 1 year increase	0.82 (0.81, 0.83)	0.80 (0.78, 0.81)	0.89 (0.88, 0.90)	0.80 (0.79, 0.82)	0.80 (0.79, 0.81)	0.86 (0.85, 0.87)	0.80 (0.79, 0.81)

Table 8.3.3.2 continued

		GLU	HAE	HDL	LDL	TRI	URE
		Odds Ratio (95% CI)					
Ethnicity	White	1	1	1	1	1	1
	Black	0.88 (0.76, 1.02)	1.07 (0.92, 1.24)	1.49 (1.29, 1.72)	1.20 (1.06, 1.35)	1.51 (1.32, 1.73)	1.44 (1.27, 1.63)
	Other	1.50 (1.130, 1.73)	1.25 (1.07, 1.45)	1.39 (1.20, 1.61)	0.60 (0.53, 0.68)	1.40 (1.23, 1.60)	1.23 (1.09, 1.40)
Risk	MSM	1	1	1	1	1	1
	Heterosexual	1.00 (0.85, 1.17)	1.51 (1.27, 1.78)	1.19 (1.01, 1.40)	1.03 (0.89, 1.18)	1.21 (1.04, 1.41)	1.21 (1.05, 1.39)
	Other	0.97 (0.89, 1.05)	0.67 (0.61, 0.73)	0.83 (0.77, 0.90)	1.03 (0.96, 1.10)	0.84 (0.78, 0.91)	0.93 (0.87, 1.00)
Age	Per 10 years higher	1.10 (1.04, 1.16)	0.99 (0.94, 1.04)	1.17 (1.10, 1.23)	1.10 (1.05, 1.15)	1.11 (1.06, 1.17)	0.97 (0.93, 1.02)
Started HAART	Yes vs. No	2.64 (2.25, 3.11)	1.77 (1.44, 2.17)	2.49 (2.10, 2.95)	2.59 (2.24, 3.01)	2.34 (1.99, 2.77)	1.32 (1.12, 1.54)
Sex	Female vs. male	1.15 (0.99, 1.35)	1.09 (0.92, 1.29)	0.95 (0.81, 1.11)	1.04 (0.92, 1.19)	0.97 (0.84, 1.12)	0.96 (0.83, 1.10)
CD4 at start of year	Per 50 cells higher	1.02 (1.01, 1.03)	1.02 (1.01, 1.03)	1.00 (0.99, 1.01)	0.99 (0.98, 0.99)	1.01 (1.00, 1.02)	1.01 (1.00, 1.01)
VL at start of year	Per 1 log higher	1.10 (1.06, 1.15)	1.18 (1.12, 1.23)	0.99 (0.95, 1.03)	0.93 (0.90, 0.97)	1.00 (0.96, 1.04)	1.05 (1.01, 1.09)
Status of prior	Normal	1	1	1	1	1	1
laboratory measurement	Abnormal	2.83 (2.47, 3.25)	1.82 (1.43, 2.31)	1.36 (1.20, 1.55)	1.90 (1.72, 2.11)	2.46 (2.03, 2.99)	3.17 (2.74, 3.66)
(Within 3 years)	No measurement	0.06 (0.05, 0.06)	0.08 (0.07, 0.09)	0.05 (0.05, 0.06)	0.16 (0.14, 0.17)	0.09 (0.08, 0.10)	0.73 (0.65, 0.81)
Duration of follow-up at start of year	Per 1 year increase	0.92 (0.01, 0.93)	0.80 (0.79, 0.81)	0.88 (0.87, 0.89)	0.91 (0.90, 0.92)	0.86 (0.85, 0.88)	0.86 (0.85, 0.87)

8.3.4. Patients included in the main analyses

All patients who had at least one laboratory marker measured after starting HAART and after the year 2000 were eligible for inclusion in the main analyses. After excluding patients seen at any of the 4 centres without toxicity data (n=6038), those who died within 3 months of first being seen (n=348), patients seen at Centre 103 after 2004 (n=2385) and patients who started HAART before 2000 (n=14753), 9083 patients were left in the dataset.

Patients without CD4 counts and VLs within 2 years after starting HAART were also excluded (n=1391), and finally, those without a laboratory measurement within 2 years after starting HAART were removed from the dataset (n=258). The total number of patients included in the analyses was 7,434 (22.8% of the whole CHIC dataset).

I assessed differences between those patients who started HAART and did have laboratory measurements available after the start of HAART, and those who started HAART without laboratory measurements available (Table 8.3.4.1). This latter group was chosen as the comparison group to those included in the analyses as the focus of this analysis was on patients starting HAART without laboratory measurements available. Only differences in ethnicity and risk group were of statistical significance.

Patients included in the main analyses mostly started HAART on NNRTI-based regimens (66.8%) and were mostly naïve to therapy at start of HAART (88.6%). The median duration of follow-up was 2.5 (1.1, 4.3) years, and median CD4 and log VL were 216 (120, 350) cells/mm^3 and 4.6 (4.1, 5.2) copies respectively.

Among this subset with at least one laboratory measurement, over 70% of patients had at least one measure of most laboratory variables within 2 years of starting HAART (the exceptions being AMY, AST, GLU, HDL and LDL). However, only 57% of patients had an AMY measure, 23% had an AST measure and 62% had a GLU measure (Table 8.3.4.2). The median number of measurements varied according to each laboratory variable. ALK, BIL, CRE and HAE were most frequently measured (Median: 11 measurements), whilst AMY, AST and GLU were least frequently measured. The median duration between measurements was similar for all measures, at 2-3 months.

Table 8.3.4.1: Differences between those who started HAART with laboratory measurements and those who started HAART without laboratory measurements available after starting HAART

		Laboratory measurements after HAART		P-value
		No	**Yes**	
N		258	7434	
Sex N (%)	Male	198 (76.7)	5384 (72.4)	0.13
	Female	60 (23.3)	2050 (27.6)	
Ethnicity N (%)	White	159 (61.6)	4067 (54.7)	0.03
	Black	65 (25.2)	2466 (33.2)	
	Other	34 (13.2)	901 (12.1)	
Exposure N (%)	MSM	146 (56.6)	3765 (50.7)	0.004
	Heterosexual	75 (29.1)	2878 (38.7)	
	Other	37 (14.3)	791 (10.6)	
Year of starting HAART N (%)	2000-2002	110 (42.6)	3096 (41.7)	0.36
	2003-2004	76 (29.5)	1973 (26.5)	
	2005-2007	72 (27.9)	2365 (31.8)	
Age at HAART (years)	Median (IQR)	37 (32, 42)	37 (32, 43)	0.90
CD4 at HAART (cells/mm^3)	Median (IQR)	215 (120, 339)	216 (120, 350)	0.98
VL at HAART (log copies/mL)	Median (IQR)	4.6 (4.1, 5.2)	4.6 (4.1, 5.2)	0.70

Table 8.3.4.2: Description of laboratory measurements amongst patients included in the analyses

Laboratory measure-ment	Patients with at least 1 measure after HAART N (%)	Number of measures per patient (Median (IQR)	Duration between measurements (Median (IQR)) (months)
ALT	6314 (84.9)	8 (3, 17)	2.3 (0.9, 3.3)
ALB	6900 (92.8)	9 (3, 17)	2.3 (0.9, 3.4)
AMY	4252 (57.2)	1 (0, 6)	2.8 (1.5, 4.1)
AST	1687 (22.7)	0 (0, 0)	2.1 (0.9, 3.2)
ALK	7314 (98.4)	11 (5, 19)	2.3 (0.9, 3.3)
BIL	7313 (98.4)	11 (5, 18)	2.3 (0.9, 3.3)
CHL	6317 (85.0)	6 (2, 12)	3.0 (1.6, 4.0)
CRE	7286 (98.0)	11 (5, 18)	2.3 (0.9, 3.3)
GLU	4570 (61.5)	2 (0, 9)	2.8 (1.3, 3.9)
HAE	7402 (99.6)	11 (5, 19)	2.2 (0.9, 3.3)
HDL	4529 (60.9)	3 (0, 9)	3.0 (1.6, 4.1)
LDL	4395 (59.1)	3 (0, 8)	3.0 (1.6, 4.2)
TRI	6129 (82.5)	6 (2, 12)	3.0 (1.6, 4.1)
URE	6308 (84.9)	6 (2, 14)	2.2 (0.8, 3.4)
CD4	7434 (100.0)	10 (5, 17)	2.8 (1.6, 3.6)
VL	7434 (100.0)	10 (5, 17)	2.8 (1.4, 3.7)

8.3.5. Association between laboratory measurements and mortality within 2 years of starting HAART

Of the 7434 patients with at least one toxicity measurement after starting HAART, 6121 (82.3%) patients had at least two years follow-up from start of HAART. Amongst these patients, 151 (2.5%) died within two years of starting HAART (and after having at least one laboratory measurement). Several methods were used to derive a score, based on the baseline laboratory measurements (the first measurements within 2 years of starting HAART), to predict mortality.

8.3.5.1. Scoring laboratory measurements according to quintiles of laboratory values (Approach A)

In this crude analysis, laboratory values were split into quintiles and the number of deaths occurring in each quintile was calculated. A score of 1 was assigned to the first quintile, 2 to the second, 3 to the third, 4 to the fourth and 5 to the fifth for most laboratory measurements. The exceptions to this were ALB, HAE, HDL and CD4 count, for which the scoring system was reversed (5 assigned to first quintile, 4 to second etc) to allow for a low value being of more concern than a high value. Patients with missing baseline values were given a score corresponding to the optimal value (1 for most laboratory measures and 5 for ALB, HAE, HDL and CD4). Table 8.3.5.1.1 shows the proportion of deaths occurring in each quintile, of each laboratory variable.

Table 8.3.5.1.1: Number of deaths within 2 years of starting HAART stratified by quintiles of laboratory measure

Laboratory measure at start of HAART		N	Deaths N (%)
ALT IU	0-17	1113	25 (2.2)
	18-24	1082	25 (2.3)
	25-32	1013	23 (2.3)
	33-48	1037	26 (2.6)
	>48	1011	29 (3.4)
	Missing	865	23 (1.7)
ALB g/L	0-37	1349	87 (6.4)
	38-40	1085	23 (2.1)
	41-42	1038	13 (1.3)
	43-45	1357	12 (1.1)
	>45	844	6 (0.9)
	Missing	448	10 (2.5)
AMY U/L	0-48	773	23 (3.0)
	49-62	707	20 (2.8)
	63-77	733	14 (1.9)
	78-100	721	24 (3.3)
	>100	731	20 (2.7)
	Missing	2456	50 (2.0)
ALK U/L	0-59	1283	17 (1.3)

	60-70	1227	15 (1.2)
	71-82	1152	20 (1.7)
	83-100	1165	25 (2.1)
	>100	1197	72 (6.0)
	Missing	97	2 (2.0)
BIL mg/dL	0-5	1716	35 (2.0)
	6	800	12 (1.5)
	7-8	1330	37 (2.8)
	9-11	1055	24 (2.3)
	>11	1123	41 (3.7)
	Missing	97	2 (2.1)
CHL mmol/L	0-3.7	1109	30 (2.7)
	3.8-4.3	1122	24 (2.1)
	4.4-4.8	932	13 (1.4)
	4.9-5.5	1028	21 (2.0)
	>5.5	1020	14 (1.4)
	Missing	910	49 (5.4)
CRE mmol/L	0-69	1294	46 (3.6)
	70-77	1175	25 (2.1)
	78-85	1252	25 (4.4)
	86-94	1146	24 (2.1)
	>94	1138	29 (2.5)
	Missing	116	2 (1.7)
GLU mmol/L	0-4.3	904	12 (1.3)
	4.4-4.7	784	13 (1.7)
	4.8-5.0	673	15 (2.2)
	5.1-5.6	738	9 (1.2)
	>5.6	677	23 (3.4)
	Missing	2345	79 (3.4)
HAE gm/dL	0-11.0	1234	63 (5.1)
	11.1-12.4	1256	36 (2.9)
	12.5-13.5	1287	22 (1.7)
	13.6-14.4	1157	17 (1.5)
	>14.4	1175	13 (1.1)
	Missing	12	0 (0.0)

HDL mmol/L	0-0.9	822	13 (1.6)
	1.0-1.1	801	13 (1.6)
	1.2-1.3	732	11 (1.5)
	1.4-1.5	530	7 (1.3)
	>1.5	710	7 (1.0)
	Missing	2526	100 (4.0)
LDL mmol/L	0-1.9	810	10 (1.2)
	2.0-2.3	616	14 (2.3)
	2.4-2.8	778	12 (1.5)
	2.9-3.4	715	7 (1.0)
	>3.4	627	7 (1.1)
	Missing	2575	101 (3.9)
TRI mmol/L	0-1.00	1110	19 (1.7)
	1.10-1.35	916	16 (1.7)
	1.36-1.80	1071	15 (1.4)
	1.81-2.50	969	21 (2.2)
	>2.50	992	29 (2.9)
	Missing	1063	51 (4.8)
URE mmol/L	0-3.4	1081	32 (3.0)
	3.5-4.2	1121	24 (2.1)
	4.3-4.8	907	17 (1.9)
	4.9-5.7	1039	23 (2.2)
	>5.7	1026	38 (3.7)
	Missing	947	17 (1.8)
CD4 count (cells/mm^3)	0-98	1228	57 (4.6)
	99-180	1268	28 (2.2)
	181-250	1224	21 (1.7)
	251-393	1179	14 (1.2)
	>393	1222	31 (2.5)
VL (log copies/mL)	0-3.9	1188	34 (2.9)
	4.0-4.4	1303	33 (2.5)
	4.5-4.9	1168	20 (1.7)
	5.0-5.4	1312	31 (2.4)
	>5.4	1150	33 (2.9)

Equal groups may not be observed as tied values are assigned the same quintile

There was evidence of a decrease in the proportion of deaths as the values of ALB, HAE and HDL increased, and an increase in the proportion of deaths as the values of ALT, ALK and GLU increased, though there were no other obvious associations between laboratory measurement quintiles and death. Scores were summed; the median total score was 42 (IQR: 37, 46) (minimum possible score=15 and maximum possible score=75). The total score was split into quintiles and fitted as a covariate in logistic regression models, in which the outcome was mortality by 2 years after starting HAART (Table 8.3.5.1.2). In univariable analyses, a linear relationship was observed between the total score and death – patients with a higher total score were at a greater risk of mortality. Though this linear relationship was also broadly evident after adjusting for potential confounders, in particular, age at start of HAART, associations were weaker than those seen in univariable analyses.

Table 8.3.5.1.2: Univariable and multivariable odds ratios (OR) for score quintiles from logistic regression

Quintile	Score	N	Dead	Univariable OR	Multivariable OR
1	13-31	1249	18 (1.4)	0.40 (0.23, 0.72)	0.48 (0.27, 0.87)
2	32-34	1327	24 (1.8)	0.51 (0.30, 0.86)	0.60 (0.35, 1.02)
3	35-37	1392	25 (1.8)	0.50 (0.30, 0.85)	0.57 (0.34, 0.97)
4	38-41	940	33 (3.5)	1	1
5	>41	1213	51 (4.2)	1.21 (0.77, 1.89)	1.08 (0.69, 1.71)

The c-statistic from the multivariable model was 0.72 and the Hosmer-Lemeshow p-value was 0.41, indicating that there was no evidence of a lack of fit of the model.

In sensitivity analyses, only those patients who had *all* laboratory measurements available at baseline were included (n=811). Ten patients (1.2%) died within 2 years of starting HAART in this subset. Again the total score was split into quintiles – the second and third quintile was merged as no deaths occurred in the third quintile.
No association was seen between total score and mortality in univariable analyses (Table 8.3.5.1.3), though there was evidence of an increased risk of mortality for patients with very low and very high scores. Of note, this analysis had very low power, with only ten deaths occurring in this population.

Table 8.3.5.1.3: Univariable odds ratios (OR) for score quintiles amongst patients who had all baseline laboratory measurements available (N=811)

Quintile	Score	N	Dead	Univariable OR
1	15-33	203	5 (2.5)	3.86 (0.45, 33.41)
2 and 3	34-40	322	1 (0.3)	0.48 (0.03, 7.67)
4	41-43	154	1 (0.7)	1
5	>43	132	3 (2.3)	3.56 (0.37, 34.62)

8.3.5.2. Using baseline laboratory measures (continuous variables) to derive a cut-off score (Approach B)

In these analyses, the training set (70% of the randomly selected data) was used in the first instance (n=4302). Laboratory measures were fitted into regression models as continuous variables. Patients without any baseline laboratory measurements were excluded from these analyses. The multivariable analyses included data from 2284 patients. This was considerably higher than the number of patients included in the sensitivity analyses in Section 8.3.5.1 (number of patients with all laboratory measurements=811) as only 8/13 laboratory measurements were included in the multivariable analyses (Table 8.3.5.2.1). The odds ratios for mortality within 2 years of starting HAART from univariable and multivariable regression models are shown in Table 8.3.5.2.1. In univariable analyses, a 1 unit increase in ALB, CHL and HAE was associated with a reduced risk of mortality. An increase in ALK, BIL, GLU, TRI and URE was associated with an increased risk of mortality. After adjusting for potential confounders, including CD4 count and VL (as these variables are of known clinical significance) ALB was still associated with a decreased risk of mortality, whilst TRI was associated with an increased risk of mortality.

The overall score was calculated by summing the significant parameter estimates from the logistic regression model (i.e. the estimated logarithms of the ORs). CD4 count and VL were also included in this score. Using this method, the median score was -4.65 and the c-statistic of the model was 0.81.

Two approaches were taken to choose a cut-off value for the score. In the first instance, differences in the mean score amongst those who died (-3.90) and those who did not die within 2 years of starting HAART (-4.65) were assessed using a t-test ($p<0.0001$). Given this significant result, I used the mean score of those who did die as a cut-off value. The number of patients experiencing mortality stratified by the

Table 8.3.5.2.1: Odds ratios (OR) for baseline laboratory measures (continuous) in relation to death within 2 years of starting HAART from univariable and multivariable

Laboratory measurement	Univariable		Multivariable	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**
ALT (10 unit increase)	1.00 (0.99, 1.03)	0.99	-	
ALB (1 unit increase)	0.85 (0.82, 0.87)	<0.0001	0.90 (0.84, 0.96)	0.002
AMY (10 unit increase)	1.02 (0.98, 1.05)	0.36	-	
ALK (10 unit increase)	1.02 (1.01, 1.03)	0.0002	1.02 (1.00, 1.04)	0.12
BIL (10 unit increase)	1.17 (1.06, 1.29)	0.003	1.04 (0.86, 1.26)	0.69
CHL (1 unit increase)	0.78 (0.62, 0.97)	0.03	0.81 (0.62, 1,07)	0.15
CRE (10 unit increase)	0.99 (0.92, 1.06)	0.72	-	
GLU (1 unit increase)	1.16 (1.03, 1.30)	0.02	1.12 (0.96, 1.31)	0.14
HAE (1 unit increase)	0.72 (0.65, 0.78)	<0.0001	0.89 (0.74, 1.08)	0.24
HDL (1 unit increase)	0.57 (0.25, 1.32)	0.19	-	
LDL (1 unit increase)	0.76 (0.52, 1.11)	0.16	-	
TRI (1 unit increase)	1.15 (1.03, 1.29)	0.01	1.21 (1.02, 1.44)	0.03
URE (1 unit increase)	1.09 (1.03, 1.16)	0.004	0.96 (0.86, 1.07)	0.43
CD4 count (50 cells increase)	1.00 (0.99, 1.00)	0.10	1.00 (0.99, 1.00)	0.47
VL (1 log increase)	1.02 (0.85, 1.23)	0.84	1.41 (0.98, 2.02)	0.06

mean score of those who died (-3.90) is shown in Table 8.3.5.2.2. The sensitivity of the -3.90 cut off was 60.5% and specificity was 80.8%.

Table 8.3.5.2.2: Number of patients experiencing mortality within 2 years of starting HAART, stratified by mean score of those who died (-3.90)

	Mortality within 2 years of starting HAART	
Overall score> -3.90	**Yes**	**No**
Yes	26	431
No	17	1810
Total	43	2241

In an alternative method of choosing a cut-off, I used the 10th percentile of the score amongst those who died (-5.68). This value was chosen as it would allow most patients with a score above this threshold to be correctly classified as having died. The sensitivity of this cut-off was 97.7%, but this was at the cost of the specificity, which was 10.1%.

The observed and expected number of deaths (stratified by score decile) in the training and validation datasets are shown in Table 8.3.5.2.3. Generally, the observed and expected deaths were well matched. The 10th decile was the exception, with the expected number being lower than the observed number in the training set but higher than the observed number in the validation set, though the absolute difference was small. The Hosmer-Lemeshow p-value was >0.05 in the training dataset, indicating the there was no strong evidence of a lack of fit. However, in the validation dataset, the p-value was 0.04, suggesting that the model did not fit as well as it did in the training dataset.

Table 8.3.5.2.3: Expected and observed number of deaths stratified by score deciles

	Training dataset			Validation dataset		
Deciles	**N**	**Expected**	**Observed**	**N**	**Expected**	**Observed**
1	228	1	1	105	0	1
2	230	1	0	80	0	1
3	222	1	0	104	1	2
4	233	2	2	112	1	2
5	224	2	3	70	1	0
6	238	3	1	94	1	2
7	222	3	5	98	1	1
8	230	4	5	95	2	3
9	231	6	7	103	3	1
10	226	13	19	104	6	1
All	**2284**	**36**	**43**	**965**	**16**	**14**
Hosmer-Lemeshow p-value		**0.45**			**0.04**	

8.3.5.3. Principal component analyses (Approach C)

In the first instance, only those patients with all baseline laboratory measures in the training dataset were included (n=560, 13.0%). I did not attempt to include CD4 and VL in the principal component analyses as both these variables have been shown to be independently associated with mortality and hence I felt they should be covariates in regression models in their own right. Using the eigenvalue $\geq$1 criterion, 6 components were retained. The scree plot showed a large break between factors 3 and 4, implying that only the first 3 components should be retained. However, only 43% of the variance was accounted for with just the first 3 components – 7 components were needed to account for at least 70% of the variance. Components 6 and 7 had eigenvalues of <1 and hence only 5 components were retained, accounting for 61% of the total variance. Variables significantly loading onto each component are shown below. HDL was not assigned to a specific component as it loaded significantly onto more than one component.

Component 1: CHL, LDL
Component 2: ALB, AMY, HAE
Component 3: CRE, URE
Component 4: GLU, TRI
Component 5: ALT, ALK, BIL

Although variables which are likely to be correlated have been grouped into the same component, 3 components had fewer than 3 variables. This did not meet the original selection criteria (outlined in Section 8.2.3.3) and suggests that the solution is not satisfactory or interpretable. Hence, I did not validate this solution on the validation set.

I then excluded AMY and GLU from the analysis as a high proportion of patients had either one or both of these variables missing. The total number of patients with all baseline measures available increased to 2018 (46.9%). Five components were retained using the eigenvalue $\geq$1 criterion and the cumulative variance of these 5 components was just over 70% (70.3%). There was also evidence of a break between the 5th and 6th component in the scree plot. ALK loaded significantly onto more than one component and was hence not assigned to any single component. Variables significantly loading onto each factor are shown below:

Component 1: CHL, LDL
Component 2: ALB, HAE
Component 3: CRE, URE
Component 4: ALT, BIL
Component 5: HDL, TRI

Again, though similar variables have been grouped together, there are fewer than 3 variables associated with each component, suggesting that this solution is also unsatisfactory. For this reason, the validation dataset was not used to validate the solution.

Finally, I also removed HDL and LDL from the analysis to increase the number of patients with baseline measures available. This increased the number of patients in the dataset to 2623 (61.0%). Four components were retained in using the eigenvalue $\geq$1 criterion, though 5 components were needed to account for over 70% of the total variance. The scree plot showed a gap between the 4th and 5th

component and hence I decided to retain only 4 components, despite the cumulative variance accounting for only 60.9% of the total variance. The variables associated with each of the four factors are shown below. Again, ALK loaded significantly onto more than one component and was hence not assigned to any single component.

Component 1: ALB, HAE
Component 2: ALT, BIL
Component 3: CHL, TRI
Component 4: CRE, URE

Again, most components are associated with only 2 variables. Similar variables were grouped together, and despite this solution not being optimum (since fewer than 3 variables are associated with most components), I decided to proceed with assigning scores to patients based on the above components.

The components are a linear equation of all the standardised laboratory variables (denoted by the prefix 'STD_'). To standardise a variable, the mean of the variable is subtracted from it (resulting in a mean of 0) and then the variable is divided by its standard deviation (resulting in a standard deviation of 1). Equations for each component (extracted from the principal component analysis) are given below:

Component 1: (-0.02*STD_ALT) + (0.52*STD_ALB) + (-0.26*STD_ALK) + (0.09*STD_BIL) + (0.01*STD_CHL) + (0.01*STD_CRE) + (0.52*STD_HAE) + (-0.02*STD_TRI) + (0.001*STD_URE)

Component 2: (0.55*STD_ALT) + (-0.02*STD_ALB) + (0.32*STD_ALK) + (0.53*STD_BIL) + (-0.10*STD_CHL) + (0.004*STD_CRE) + (0.11*STD_HAE) + (0.04*STD_TRI) + (-0.01*STD_URE)

Component 3: (-0.03*STD_ALT) + (0.02*STD_ALB) + (0.16*STD_ALK) + (-0.08*STD_BIL) + (0.60*STD_CHL) + (-0.13*STD_CRE) + (0.01*STD_HAE) + (0.56*STD_TRI) + (0.10*STD_URE)

Component 4: (-0.005*STD_ALT) + (-0.02*STD_ALB) + (-0.06*STD_ALK) + (0.02*STD_BIL) + (-0.07*STD_CHL) + (0.72*STD_CRE) + (0.02*STD_HAE) + (0.05*STD_TRI) + (0.62*STD_URE)

Each component has larger multiples of the variables it is associated with than the other variables in the equation. For example, in Component 1, ALB and HAE are both weighted by 0.52, whilst all the other variables are weighted by lower values.

Amongst the 2623 patients included in this analysis, 49 (1.9%) patients died within 2 years of starting HAART. Table 8.3.5.3.1 shows the odds ratios in relation to death from univariable and multivariable analyses for each of the four components.
In univariable analyses, only Component 1 (ALB, HAE) was significantly associated with mortality. Hence only this Component was retained in multivariable analysis. After adjusting for potential confounders, Component 1 remained significantly associated with mortality. The c-statistic of the model was 0.77 indicating a good ability of the score to discriminate between those who died and those who did not. The significant estimates of the multivariable regression model were used to derive an overall score as previously described.

Table 8.3.5.3.1: ORs for components derived from principal component analysis in relation to mortality within 2 years of starting HAART

	Univariable		**Multivariable[1]**	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**
Component 1	0.55 (0.45, 0.67)	<0.0001	0.49 (0.38, 0.62)	<0.0001
Component 2	1.10 (0.91, 1.32)	0.32	Excluded	
Component 3	1.08 (0.82, 1.40)	0.59	Excluded)	
Component 4	1.07 (0.93, 1.22)	0.36	Excluded)	

[1]Adjusted for ethnicity, risk group, viral load at start of HAART, age at start of HAART, regimen, CD4 at start of HAART and year of starting HAART

The median score was -4.60. Table 8.3.5.3.2 shows the observed and expected values stratified by score deciles, in both the training set and the validation set. These values are well matched and the Hosmer-Lemeshow p-value is >0.05, indicating no evidence of a lack of fit.
The mean score amongst those who did die was -3.63 and hence patients who had a score greater than this were assumed to be at a greater risk of mortality. The sensitivity and specificity of this cut-off (using the training set) were 49.0% and 87.7% respectfully. If the lower 10^{th} percentile of those who died was used to derive a cut off (-4.86), the sensitivity was considerably higher at 93.9% but the specificity dropped to 37.2%.

Table 8.3.5.3.2: Expected and observed number of deaths stratified by regression quintiles

	Training dataset			Validation dataset		
Deciles	**N**	**Expected**	**Observed**	**N**	**Expected**	**Observed**
1	264	1	0	95	0	1
2	260	1	1	117	1	2
3	268	2	1	98	1	1
4	252	2	3	115	1	0
5	272	2	2	110	1	1
6	257	3	3	116	1	1
7	261	3	6	119	2	0
8	262	4	5	105	2	3
9	265	6	7	124	3	2
10	262	17	21	130	8	9
All	**2623**	**42**	**49**	**887**	**19**	**20**
Hosmer-Lemeshow p-value		**0.70**			**0.99**	

8.3.5.4. Replicating the Swiss score (Approach D)

In the final approach, the scoring method used in the Swiss study (525) was replicated using the UK CHIC dataset. The same criteria and cut-points were used (see Appendix B for cut-points), though four measures of toxicity that were used in the Swiss study were not routinely collected in UK CHIC and were hence excluded from the analyses (lactate, neutrophil count, platelet count and CPK). Only patients with at least one other laboratory measure available at baseline were included (n=6121). Scores were assigned according to the Table for Grading Severity of Adult Adverse Experiences and the overall score was calculated by summing the individual scores for each measure. Patients with missing baseline laboratory measures and those with measures below the cut-off for mild toxicity were given a score of 0.

The median score per individual was 1 (0, 2). In univariable analyses, a linear relationship was seen between the overall score and mortality – as the score

increased, so did the risk of mortality. The score was then fitted as a continuous variable – for a unit increase in score, the risk of mortality was 31% higher.
After adjusting for potential confounders, the score remained significantly associated with the risk of mortality (1.31 (1.23, 1.39) per 1 unit increase). The c-statistic of the model was 0.75 and the Hosmer-Lemeshow p-value 0.60, indicating no evidence of a lack of fit.

In sensitivity analyses, only those patients with all laboratory measures used to compile this score were included (n=2527). Results of the multivariable analyses were similar to those seen above – a one unit increase in score was associated with a 28% increase in the risk of mortality (1.28 (1.18, 1.38)).

Table 8.3.5.4.1: Association between replicated Swiss score (derived from measures of ALT, ALK AMY, BIL, CRE, HAE and URE) and mortality

			Univariable		Multivariable	
Score	**N**	**Dead**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
0	2489	27 (1.1)	1			
1	2056	39 (1.9)	1.76 (1.08, 2.89)	0.02		
2	883	41 (4.6)	4.44 (2.72, 7.27)	0.001		
>2	693	44 (6.4)	6.18 (3.80, 10.06)	<0.0001		
Continuous	6362	151	1.31 (1.23, 1.39)	<0.0001	1.28 (1.18, 1.38)	<0.0001

8.3.5.5. Comparison of approaches A-D

A comparison of the four approaches is shown in Table 8.3.5.5.1. The sensitivity and specificity of Approaches A and D could not be calculated as a cut-off was not chosen. The c-statistic for Approach A was the smallest of the four approaches, though the absolute difference was marginal. No approaches showed evidence of lack of fit. The sensitivity of the chosen cut-off was highest using Approach B, but this approach also had the lowest specificity.

Table 8.3.5.5.1: Comparison of approaches A-D

	A	B	C	D
N	6121	4302	2623	2527
Score cut-off	-	-3.90	-3.63	
c-statistic	0.72	0.81	0.77	0.75
Hosmer-Lemeshow	0.41	0.45	0.70	0.60
Sensitivity	-	60.5%	49.0%	
Specificity	-	80.8%	87.7%	

8.3.6. Short-term association between laboratory measurements and mortality (mortality within 6 months of laboratory measurements)

To measure the short-term association between laboratory and death, follow-up was split into 6-month periods. In total, there were 53829 distinct periods of follow-up, in which 218 deaths occurred. The number of follow-up periods with laboratory measures available and the median measure at start of follow-up (+/- 3 months) are shown in Table 8.3.6.1. With the exception of GLU, HDL and LDL, at least 60% of the periods of follow-up had laboratory measures available at the start of follow-up.

The association between each laboratory measure at the start of the period (continuous) and death was analysed using logistic regression (Approach B). This approach was initially chosen since Approach A did not yield a convincing significant association between laboratory scores and mortality within 2 years of starting HAART. It was not possible to perform the principal component analysis for time-updated variables (and nor did the principal component analysis approach work effectively since fewer than 3 variables were associated with most components), whilst the replication of the Swiss score is discussed towards the end of this Section.

Table 8.3.6.1: Median laboratory measures in each period of follow-up

	Number of periods of follow-up in which a measure is available N (%)	Median (IQR)
ALT	46662 (86.7)	26 (18, 39)
ALB	48974 (91.0)	43 (40, 46)
AMY	35747 (66.4)	64 (48, 87)
ALK	52348 (97.2)	81 (66, 101)
BIL	52342 (97.2)	8 (6, 10)
CHL	45256 (84.1)	5 (4, 6)
CRE	52034 (96.7)	82 (72, 92)
GLU	31857 (59.2)	5 (4, 5)
HAE	53026 (98.5)	14 (13, 15)
HDL	28505 (53.0)	1 (1, 2)
LDL	28814 (53.5)	3 (2, 3)
TRI	43999 (81.7)	2 (1, 2)
URE	46104 (85.6)	5 (4, 6)
CD4	52801 (98.1)	390 (260, 546)
VL	52524 (97.6)	50 (50, 100)

Unadjusted and adjusted odds ratios for each laboratory measure are shown in Table 8.3.6.2. All measures apart from AMY and GLU were significantly associated with death in univariable analyses. After adjusting for potential confounders, only ALB, HAE and URE were significantly associated with death (URE was of borderline significance). Patients of older age (1.45 (1.09, 1.90) per 10 years higher) were also at an increased risk of mortality. Interestingly, the CD4 count at the start of the period was not associated with death (0.95 (0.87, 1.04)), though the viral load at the start of the period was of borderline significance (1.21 (0.96, 1.53) per 1 log copies/mL higher).

A score was derived using the parameter estimates from the logistic regression model, as previously described. Only significant and borderline significant variables in the multivariable model were included in the score. Hence, ALB, HAE, URE and age at start of period were used to derive the score. The median score overall was -6.60. Amongst those who died, the mean score was -5.23 and hence patients with a score of -5.23 were presumed to be at an increased risk of mortality. The sensitivity of this score was 44.3% and the specificity was 93.9%.

Table 8.3.6.2: Associations between laboratory measures and death during 6-month periods

	Univariable		Multivariable[1]	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**
ALT[2]	1.01 (1.00, 1.02)	0.01	1.00 (1.00, 1.01)	0.36
ALB[3]	0.85 (0.84, 0.86)	<0.0001	0.91 (0.88, 0.94)	<0.0001
AMY[2]	0.99 (0.96, 1.03)	0.69	Excluded	-
ALK[2]	1.04 (1.03, 1.05)	<0.0001	1.01 (0.99, 1.03)	0.41
BIL[2]	1.23 (1.19, 1.30)	<0.0001	1.09 (0.95, 1.26)	0.21
CHL[3]	0.62 (0.53, 0.72)	<0.0001	0.67 (0.34, 1.31)	0.24
CRE[2]	1.02 (1.01, 1.03)	0.003	0.97 (0.91, 1.03)	0.28
GLU[3]	1.08 (1.01, 1.16)	0.23	Excluded	-
HAE[3]	0.59 (0.56, 0.63)	<0.0001	0.75 (0.66, 0.86)	<0.0001
HDL[3]	0.37 (0.22, 0.64)	0.0004	1.04 (0.75, 2.40)	0.93
LDL[3]	0.68 (0.53, 0.87)	0.002	1.34 (0.75, 2.40)	0.32
TRI[3]	1.07 (0.99, 1.17)	0.09	1.06 (0.84, 1.34)	0.63
URE[3]	1.15 (1.12, 1.18)	<0.0001	1.11 (0.99, 1.26)	0.08
CD4[4]	0.79 (0.76, 0.82)	<0.0001	0.95 (0.87, 1.04)	0.28
VL[5]	1.52 (1.39, 1.67)	<0.0001	1.21 (0.96, 1.53)	0.10
Ethnicity				
White	1	-	1	-
Black	0.68 (0.49, 0.93)	0.02	0.38 (0.13, 1.15)	0.09
Other	1.00 (0.66, 1.53)	0.98	0.73 (0.32, 1.64)	0.44
Risk group				
MSM	1	-	1	-
Heterosexual	1.14 (0.84, 1.55)	0.39	1.04 (0.40, 2.72)	0.94
Other	3.53 (2.51, 4.95)	<0.0001	1.92 (0.89, 4.16)	0.10
Regimen at start of 6 month period				
NNRTI	1	-	1	-
PI	1.48 (1.04, 2.14)	0.03	1.41 (0.70, 2.84)	0.34
Other reg	2.10 (1.55, 2.83)	<0.0001	1.70 (0.88, 3.27)	0.11
Age	1.05 (1.03,1.06)	<0.0001	1.44 (1.09, 1.90)	0.01

[1]Adjusted for ethnicity, exposure and regimen at start of 6 month period; [2]Per 10 unit increase; [3]Per 1 unit increase; [4]Per 50 cells increase; [5]Per 1 log increase

In sensitivity analyses, Approach D (replication of the Swiss score) was extended to include time-updated variables. As in the above approach, follow-up was split into 6 month-periods and scores were assigned according to the laboratory measures at the start of the 6 month period. The cut-offs used for each laboratory marker were identical to those used in Approach D (283). Patients with missing measurements at the start of the 6 month period and those with measurements not meeting the minimum to be defined as a 'mild toxicity' were given a score of 0.

The median score across the 6 month periods was 0 (0, 1). The proportion of patients experiencing mortality increased as the overall score increased, from 0.2% for those with a score of 0 to 1.4% for those with a score of >2.

The unadjusted and adjusted odds ratios for overall score in relation to mortality are shown in Table 8.3.6.3. In univariable analyses, patients with a score of $\geq$2 were over 5 times more likely to experience mortality than those with a score of 1. These results remained significant in multivariable analyses. A one unit increase in score was associated with a 41% increase in the risk of mortality.

Table 8.3.6.3: Association between time-updated Swiss score and mortality

Overall score	6-monthly periods	Dead n (%)	Univariable	Multivariable
0	28521	58 (0.2)	0.71 (0.48, 1.05)	
1	14913	43 (0.3)	1	
2	4638	35 (0.8)	2.63 (1.68, 4.11)	
>2	5757	82 (1.4)	5.00 (3.45, 7.24)	
Continuous	53829	218 (0.4)	1.45 (1.40, 1.51)	1.41 (1.35, 1.47)

[1]Adjusted for ethnicity, exposure, current CD4, current viral load, current regimen, current age, sex and year of starting of HAART

8.3.7. Comparing long vs. short term risk of mortality

I used the continuous laboratory measures at baseline and the Swiss scoring system to predict mortality initially within 2 years of starting HAART, and then to consider the short term risk to mortality (within 6 months). In the former method, the estimates of the regression model were used to derive a score, whereas in the latter method, a score was assigned to the laboratory measure before entering it into the regression model. The differences in the scores in relation to short-term and long-term risk of mortality are discussed below.

8.3.7.1. Continuous baseline toxicity measures

The median score for the 2-year risk of mortality (long-term) was -4.65 and the cut-off relating to an increased risk in mortality was -3.90, whilst for the 6-month risk of mortality, the median score was lower, at -6.60, as was the cut-off relating to an increased risk of mortality (-5.23). The variables required for the derivation of the score and the weights of each variable are shown below.

Table 8.3.7.1.1: Variables contributing to long-term and short-term risk to mortality scores

Long-term risk		Short-term risk	
Variable	**Weight**	**Variable**	**Weight**
Intercept	-4.84		-4.89
ALB	-0.10	ALB	-0.10
TRI	0.19	HAE	-0.28
Heterosexual	0.27	URE	0.11
Other risk	1.23	Current age	0.37
Age	0.44		
CD4	-0.002		
VL	0.34		

ALB contributed equal weighting to both long-term and short-term risk of mortality. However, whilst TRI also contributed to the long-term risk, it did not contribute to the short term risk. HAE and URE on the other hand contributed to the short-term risk score but did not contribute to the long-term risk score. Age contributed to both risk scores, whilst CD4, VL and risk group contributed only to the long-term risk score.

8.3.7.2. Swiss score replication

The median score using this approach was 1 when analysing the risk to mortality within 2 years of starting HAART. However, when taking into account time-updated variables, the median score per individual was 0. A one unit increase in score was associated with a 28% increased risk of long-term mortality using the Swiss score, and a 41% increased risk of short-term mortality.

8.4. Discussion

The median number of laboratory measurements per patient was notably higher after HAART initiation than before, for all measurements. This is not surprising given the known side-effects of HAART, but another explanation for this finding may be that patients who have started HAART are more likely to be under follow-up than those who haven't. Hence the opportunity to perform these laboratory tests will be greater amongst the former group. Furthermore, patients who have started HAART are likely to be older than those not receiving HAART - patients of older age may also be more likely to have laboratory tests performed as some variables are age-dependent, even in the HIV-negative population. The duration between measurements was also generally shorter amongst the HAART-experienced population and this is likely to be attributable to the same reasons. Having started HAART was positively associated with having a laboratory measurement in multivariable analyses, further supporting the above points. A prior abnormal measurement was associated with having further measurements, which is expected. Patients with abnormal measurements are likely to be re-tested to confirm the measurement and are also likely to be monitored more frequently as clinicians attempt to deal with the abnormalities.

Patients who had started HAART but had no laboratory measurements available within 2 years after the start of HAART were more likely to be of 'other' exposure group and of white ethnicity. Patients classified into 'other' exposure groups may be those who generally have limited data recorded. Although these differences were statistically significant, the absolute values of the differences were small and hence, clinically, were unlikely to be of significance. Most of the 7434 patients with laboratory measurements available after the start of HAART were typical of the CHIC dataset as a whole – male patients of white ethnicity, MSM risk group, who started HAART on NNRTI-based regimens. Over 70% of patients had at least one measure of most laboratory variables after starting HAART, though this was considerably lower for AST (23%). ALT and AST are similar measures of liver function and whilst ALT is primarily found in the liver, AST is also found in the brain, pancreas, heart and lungs. ALT is therefore a more specific indicator of liver function and, for this reason, clinics may prefer to perform just an ALT test, rather than AST as well. Furthermore, BHIVA guidelines on monitoring practice state that ALT and/or AST tests should be performed when starting therapy and hence both tests may not be deemed necessary by the clinician (526) . Interestingly, these guidelines also state that all laboratory measurements listed in Section 8.3.1 should be routinely

measured. Even amongst those who were included in the main analyses, around 40% of patients did not have GLU, HDL or LDL measured within 2 years of starting HAART.

The association between abnormal values of these laboratory markers and morbidity and/or mortality in HIV-positive patients is well recognised. High ALT levels suggest liver tissue damage whilst low ALB has been shown to be associated with mortality (38;41;527). Abnormal AMY values are indicative of pancreatitis; common HIV drugs such as ddI have been reported to cause elevated AMY levels (401;404). TDF increases the concentration of ddI in the blood and so patients receiving this drug may also be at an increased risk of pancreatitis (402;403). TDF has also been associated with elevated ALK (528). Abnormalities in ALK may signify obstructed bile flow and liver complications. Elevated BIL levels have been reported in patients receiving IDV (75;396-399) and are also linked to anaemia. Low haemoglobin, in turn, is associated with an increased risk of mortality (38;356). Dyslipidemia is an important risk factor for cardiovascular disease and is commonly observed in HIV-positive patients (529). Patients receiving ART have been shown to be at increased risk of myocardial infarction (530). Both HIV itself, and the antiretrovirals used to treat HIV, have been linked to kidney damage. CRE and URE are used to measure kidney function. Whilst it is possible that other factors may contribute to the abnormal measurements seen amongst patients on HAART, it is likely that HAART itself does play a major role. This was demonstrated in earlier analyses which showed that most laboratory measurements were higher amongst patients receiving HAART than amongst those who had not yet received HAART.
Given the number of laboratory markers that may be associated with mortality, there is a need for distinction between those abnormalities which may contribute more to mortality than other abnormalities.

To derive a score relating laboratory measurements to mortality, several approaches were used. In some approaches, the score was derived by assigning a weight to the raw laboratory measurements. The overall score was then calculated by summing up the individual weights and the odds ratio of the overall score was used to assess the association between the score and mortality risk. In other approaches, laboratory variables in their raw form were included in regression models and the estimates from the regression models were used to derive a score. Both methods have previously been used to derive scores (525;531-535).

For the first method, Approach A, values of laboratory measurements were split into quintiles and a score of 1-5 assigned to each quintile. The sum of these individual scores were calculated for each individual and fitted into a regression model. Only a very weak association was seen between overall score and mortality. This was a very crude method of assigning scores and despite a c-statistic of >0.5, this method does not take account of any clinical implications of the quintiles. Consequently, no association was seen between the overall score and mortality. In Approach D, methods used in the Swiss HIV Cohort study were used to assign a score to the raw toxicity measures (525). The Table for Grading Severity of Adult Adverse Experiences was used to stratify each toxicity measurement into mild, moderate, severe and very severe and a score of 1 (mild) to 4 (very severe) was assigned to each measurement. Patients who did not meet the minimum criteria to be assigned to the 'mild' category and those with missing laboratory measurements were given a score of 0. These cut-offs were based on the 'normal' value of each toxicity and hence were of clinical significance. The overall score was also statistically significant in relation to mortality. The main issue with this score was that it was reliant on a large number of toxicity measurements, which as the CHIC records have shown, are not always available. In the replicated score in this chapter, I did not initially restrict analyses to only those patients who had all toxicity measurements available. Instead, patients with a missing value of any toxicity were given a score of 0. This however, may also be problematic, since those with missing values may not always have normal values. Patients who have reported no adverse effects may be less likely to have a laboratory test performed, and whilst in most cases it is likely that their laboratory values are in the normal range, this may not always be the case.

For approach B, scores were assigned using estimates from regression models.
I fitted the continuous laboratory variables into regression models, and hence excluded all patients with missing baseline values. The score derived included only ALB and TRI. A potential bias with this method is that only patients who had all laboratory measurements available at baseline were included and were therefore either from centres who regularly monitored these toxicities, or were patients who had prior abnormal values of these measures and were hence being closely monitored. I used the mean score of those who did experience mortality to define the cut off associated with an increased risk of mortality; this score had a sensitivity of 61% and specificity of 81%. In an alternative method of choosing a cut-off, I used the 10^{th} percentile of the score amongst those who died. The sensitivity of this cut-off was 97.7%, but this was at the cost of the specificity, which was 10.1%.

Another method of choosing a cut off is adding 2 standard deviations to the mean score of those who did not experience mortality (536;537). Only 2.5% of patients would have a score greater than this cut-off and in some cases it may be assumed that those who do have a score greater than this cut-off are at an increased risk of experiencing the event in question. However, in this situation, though the cut-off would have a high specificity, it would be at the cost of the sensitivity, as many patients who did experience mortality would not have a score above the cut-off. Hence, instead, I used the mean score of those who died as the cut-off. This would ensure that around 50% of the population would be correctly identified, though the specificity may not be optimal. Approach B had the disadvantage of a smaller dataset and the biases associated with including only those patients with laboratory measures available. Further, fitting laboratory measurements as continuous variables also involves the assumption of a linear association. However, Approach B did have the advantage of using the raw values of the laboratory measurements and hence not relying on other statistical procedures which may introduce further biases into deriving a score.

The final method I used in deriving a score was principal component analyses. The main advantage of this method was that it would considerably reduce the number of variables fitted into a regression model, whilst still taking into account each variable's contribution to the outcome. The main issue with this method however, was that only patients with a measurements available could be included in the analysis. Only 13% of patients had all measurements available, whilst 47% had all but AMY and GLU available. I felt that 47% was still too small a subset and hence I also excluded HDL and LDL from the analysis, leaving 61% of patients available for analysis. The 9 laboratory variables left were reduced to 4 components but only 1 of these 4 components remained significantly associated with mortality in multivariable analysis. This component was primarily weighted by ALB and HAE. A score was derived using this component, together with all other significant estimates of the multivariable model; the sensitivity of this score was 49% and specificity 88%. HAE was not used to derive the score in any of the earlier methods, though ALB did contribute to the score when using the continuous toxicity variables. This method had the best balance of sensitivity and specificity, though did have the disadvantage of being difficult to implement in a clinical setting. Each component consisted of weighted laboratory variables and so not only would patients require values for all laboratory measurements, but the score calculation was also more complicated than

the other approaches. Hence, to use this method, the score calculation would have to be programmed on an existing computer system or smart-phone.

Scores associated with the short-term (6-month) risk to mortality using continuous baseline measures included HAE and URE, which did not contribute to the long-term risk of mortality. This suggests that current HAE and URE levels are strong predictors of death, but measures at baseline are not associated with mortality over the longer term. Conversely, TRI levels at baseline did predict long-term mortality, though current values of either measurement were not associated with death. Patients with high baseline measures of TRI may be more prone to certain lifestyle/dietary habits, which may explain this finding. These variables may be surrogates for other confounding factors which have not been adjusted for. For example, it may be possible that a patient with high TRI is also overweight and hence has other medical issues contributing to mortality. When the Swiss score was replicated to include time-updated variables, similar findings to those in the fixed variable analyses were seen. However, the association between the score and mortality was more pronounced when analysing short term risk of mortality compared to the long term risk of mortality. This can be explained by the fact that the laboratory measurements are more likely to be accurate for a short-term follow up period, rather than a long-term follow-up period.

Initial checks on the laboratory data in UK CHIC revealed important discrepancies. AST, CPK, GGT and LAC were not routinely collected within the CHIC clinics, whilst only some centres provided data on GLU, HDL and LDL. Four centres provided very limited laboratory data and were hence excluded from the analyses. The proportion of patients with laboratory measurements in one centre declined from 2004 onwards, and hence follow-up from this centre was censored at Dec 2003. These checks are extremely important in analyses such as those shown in this Chapter, as discrepancies are likely to impact on the any score which may be derived. Laboratory tests are used to identify abnormalities with the liver, lipid levels and kidney functions. HAART has been associated with such abnormalities and hence the monitoring of such values is important amongst those receiving treatment. In particular, patients receiving NVP, d4T and ddI may be at an increased risk of liver injury (270;272;276) and hence it is important to monitor ALT, ALK, BIL, ALB and GGT levels. GGT, in particular, was not included in these analyses as it was not routinely monitored in the UK CHIC cohort. IDV and TDF have been associated with chronic kidney disease (339) and patients receiving these drugs should be

monitored closely for abnormal CRE and URE levels. Patients receiving ART for long periods of time have been reported to be at an increased risk of myocardial infarction (530) and hence lipid levels should also be monitored routinely, in particular, HDL, LDL and TRI levels.

All-cause mortality is one of several outcomes that a score can be used to predict. As discussed in the last Chapter, it does has the limitation of having low specificity; the associations between all-cause mortality and laboratory markers are likely to be weakened as a result of patients dying from causes such as suicide or other non-HIV-related illnesses. With hindsight, it may have been more appropriate to consider an outcome such as treatment interruption, as well as all-cause mortality. Abnormal laboratory markers are likely to be strongly associated with interrupting treatment (and in particular, interrupting the drug associated with the laboratory abnormality), and in most cases, the interruption is likely to be the result of greater than 1 laboratory abnormality. Hence, this outcome may have resulted in a better comparison between the approaches I used. Other studies have successfully calculated scores to predict treatment modification (525).

Deriving a score to accurately predict mortality is difficult, given the many variables that could potentially contribute to the score, the diversity of the causes of death and the various methods in both assigning a score and a cut-off point to identify patients of high-risk. The score must take into account both clinical and statistical issues; in a real life setting where it is unlikely that a patient will have all laboratory measurements available, it is of little use having a score which relies on a full laboratory medical record. Hence, though replication of the Swiss score did result in a positive association with mortality, in these analyses, where 0 was assigned to those who did not have a measurement available, it may not necessarily be the best method to derive a score. Rather, a method in which only 2 or 3 laboratory variables are needed is preferred. The score derived using the principal component analyses is likely to be difficult to implement in practice. For these reasons, using the score derived from the time-updated continuous variables is likely to be the best of the approaches discussed above. Only the latest ALB, HAE and URE values are needed to predict the risk of mortality and all three variables have been associated with drug related toxicities. ALB is a measure of liver function and patients receiving drugs such as NVP, d4T and ddI have been shown to be at an increased risk of liver injury (270;272;276). The association between ZDV and anaemia has been well documented (363-365) and even when used as part of combination therapy, ZDV

use has been shown to be a risk factor for anaemia (366). DdI and d4T have also been associated with low haemoglobin levels, though not as frequently or as strongly as ZDV (367;368). DdI, together with 3TC and PIs has also been associated with an increased risk of hyperuricemia (390;391), whilst patients receiving TDF may be at an increased risk of hypouricemia (392;393).

Finally, ALB, HAE and URE are routinely measured in clinical practice, and the method used to derive the score also has the advantages of being both statistically and clinically applicable.

8.5. Summary

The aim of this Chapter was to derive a score to predict mortality. Several methods were used and discussed, each of which had advantages and disadvantages. The replication of the Swiss score confirmed findings reported from the Swiss HIV Cohort Study and developing this score, in particular, to include time-updated variables was one of the better approaches. However, in order to implement this approach, all laboratory measurements were required to avoid the biases of assigning a score of 0 to missing measurements. For these reasons, together with the disadvantages discussed above of other scoring methods, I feel using the short-term mortality score derived from the continuous baseline measures approach, in which only ALB, HAE and URE are required, is the optimum scoring strategy. Further, a 2-year risk score may be more relevant to the general population than the HIV-positive population. HIV-positive patients are seen frequently at clinical centres and hence a 6-month risk score is likely to be more relevant for this population.

Chapter 9: Concluding remarks

9.1. Summary of main findings

The introduction of HAART around 1996 has transformed HIV from a terminal disease, to one which can be well managed with treatment. Therapy has advanced significantly since HAART was first introduced, both in terms of pill-burden and adverse events associated with it. However, almost 15 years after HAART was first introduced, interlinked barriers (resistance, drug toxicity, adherence) to successful treatment still exist in the developed world.

In Chapter 2 I extensively reviewed the literature associated with some of these barriers to successful treatment. Resistance to therapy, both transmitted and that which has developed whilst receiving therapy, is a major concern amongst HIV-positive individuals. There is evidence to suggest that transmitted resistance is actually increasing, whilst a lack of adherence amongst those on therapy has resulted in resistance to current drugs being received. One likely reason for lack of adherence is the adverse events still associated with particular antiretroviral drugs. Hepatotoxicity, especially, is an ongoing concern for patients. The differing definitions of hepatotoxicty make it difficult to differentiate between what may be normal elevations in liver function markers (such as ALT), elevations due to hepatitis co-infection and abnormal rises due to HAART. HAART is also associated with abnormal values in a number of other markers, such as haemoglobin, creatinine and urea. A consequence of lack of adherence is viral rebound. Patients may switch treatments or take a treatment break altogether due to the adverse events associated with therapy and this in turn is likely to result in viral rebound. The literature on the association between treatment interruptions and viral rebound favours continuous therapy, though little information is available on the effect of treatment interruptions on viral rebound amongst patients who were able to suppress their viral load after interruption.

The methodology of the thesis was described in Chapter 3. This included the processes involved in establishing the UK CHIC Study and the linkages to the UK HIV Drug Resistance Database. I summarised trends over time in markers of clinical success; the proportion of white MSMs under follow-up had decreased over time, whist those of black ethnicity and heterosexual risk group had increased. This is

likely to be due to an increase in immigration from sub-saharan Africa for a period of time. Three-quarters of patients were receiving HAART in 2007, and of these, 84% had an undetectable viral load.

The barriers to successful treatment start with transmitted resistance and hence Chapter 4, the impact of transmitted drug-resistance on treatment selection and outcome of first-line HAART, was the first of the Results chapters. In this Chapter, I showed that over 9% of patients starting first-line therapy have evidence of resistance mutations and over 5% of patients have resistance to drugs in their initial HAART regimen. This resulted in 3% of patients having a GSS <3 to their first HAART regimen. These patients were less likely to achieve virological suppression, though no association was found between viral rebound and GSS <3, nor CD4 increase of >50 cells/mm^3 and GSS <3. NRTI resistance appeared to be compensated for by use of a PI/r-based regimen. The contribution of NRTI resistance to virological failure is an important finding and use of PI-based regimens alone is often not enough to reduce the risk of virological failure. Hence other methods to improve virological outcomes amongst patients with transmitted NRTI resistance need to be explored.

Drug-resistance after receiving ART was explored in Chapter 5. The proportion of patients who switched regimen within 4 months of a resistance test result was considerably lower than anticipated, even if using a relatively relaxed definition of switching (56%). However, those with mutations detected were more likely to switch therapy, and this number increased as the GSS of the current regimen decreased. Of concern, a third of patients with a GSS <1 had only 0-3 drug options available – this is likely to explain the relatively low proportion of patients switching regimen after having a resistance test performed. In analyses restricted to only those with detectable mutations, GSS was still found to be an independent predictor of switching regimen. In the second part of this Chapter, the need for two fully active NRTIs in a second-line PI/r based regimen was investigated. No association was seen between the number of new NRTIs, nor the NRTI GSS and risk of virological failure of second-line HAART; the benefit of including active NRTIs in second-line regimens appeared to be small in comparison to the efficacy of PI/r alone.

In Chapter 6, a second barrier to successful treatment was explored – ALT flares. Flares are associated with particular drugs and are likely to result in either a switch in regimen, or a break in treatment altogether. This can cause resistance to ART

drugs which was the focus of Chapter 5. The existing definition of an ALT flare was questioned and a new definition, based on HAART-naïve, HBV/HCV-negative patients was derived; 2 ALTs >200 IU, >2 weeks apart. This new definition is more stringent than the present most commonly used definition (1 ALT >200 IU) and hence it is less likely that random fluctuations in ALT will be defined as ALT flares. Using this definition, 4.6% of patients were identified as having an ALT flare. Stronger relationships were seen between CD4 count and the risk of an ALT flare when using the standard definition compared to the derived definition, which may lead to unnecessary concern by clinicians when analyzing CD4 count. Also, the significance of regimen, viral load and hepatitis status appeared to be underestimated using the standard definition. This Chapter also highlighted the need to consider monitoring bias when analyzing outcomes such as ALT flares, as definitions based on single values are particularly prone to be affected by this.

In Chapter 7, the issues surrounding treatment interruption were investigated. This relates to both Chapters 5 and 6, since interrupting treatment can be due to toxicities associated with particular drugs, such as ALT flares, whilst also being a cause of the first barrier to successful treatment – resistance to ART drugs.
A quarter of patients had interrupted therapy before achieving viral suppression and the number of prior interruptions was linearly associated with a raised risk of viral rebound. In particular, patients who interrupted therapy with a detectable VL were at a higher risk of viral rebound, whilst no association was seen between risk of viral rebound and interrupting therapy whilst the VL was undetectable. Another important finding from this Chapter is the association between duration of viral suppression, number of regimens previously failed and risk of viral rebound. Patients with longer durations of viral suppression were less likely to experience viral rebound, whilst those with a greater number of previous failed regimens were at an increased risk of viral rebound. A significant interaction was seen between duration of suppression and number of regimens previously failed, suggesting that though patients with a higher number of previous regimens failed were at an increased risk of viral rebound, this risk decreased as the duration of suppression increased.

Finally, in Chapter 8, the association between laboratory abnormalities and mortality was investigated by deriving a score to characterise these abnormalities. Toxicity to drugs is a main barrier of successful treatment in the developed world and is a likely cause of both treatment interruptions (discussed in Chapter 7) and resistance (discussed in Chapter 5). Several methods of assigning a score were explored,

whilst an existing method was reproduced. For a number of methods, all laboratory measures were needed for each patient, but for the vast majority of patients these were not available. The method which I felt was the most appropriate was based on the estimates from a regression model in which the current continuous laboratory measurements were fitted. Only three laboratory measures were needed to calculate the score (ALB, HAE and URE), all of which are routinely measured and have been linked to specific drug toxicity. This method eliminates the need for a full laboratory medical record and is both clinically and statistically viable.

9.2. Relevance and limitations of main findings

In this thesis, I have focused on the main barriers to successful treatment in the developed world. Resistance and toxicity to drugs limit the potential success of ART, whilst treatment interruptions are both a cause and consequence of these barriers. In clinical practice, whilst resistance testing before starting HAART is now mandatory, not all patients start HAART with a fully active regimen. This finding needs to be investigated further. Also of concern, is that only a small proportion of resistance tests after starting treatment are followed by a switch in treatment. The reasons as to why the resistance tests were performed, and more importantly, why the results of these tests were not acted upon, also need further investigation. Both these findings have serious potential consequences for patient well-being and raise questions about optimum clinical practice.

After failure of a first-line NNRTI-regimen, it is of popular belief that the second-line regimen should consist of 2 fully active NRTIs and a PI/r. However, I have shown that the role of the NRTIs is questionable and should be investigated further on a larger dataset, or perhaps in collaboration with other cohort studies. Though the CHIC dataset used in these analyses consisted of over 32,000 patients, only 403 patients met the strict inclusion criteria used to investigate the role of NRTIs in second-line regimens.

I believe the findings in Chapter 7 are of key clinical importance. Patients continue to interrupt treatment, despite clinical advice against treatment interruptions. These patients should be warned of the risks of interrupting therapy, and in particular, have knowledge of the fact that suppressed viral load after treatment interruption is not sufficient on its own to outweigh the risk of interruption. Instead, longer duration of suppression is a key protective factor against viral rebound and this is only achievable through maintenance of high levels of adherence. The positive finding

that this is also true for patients who have failed a high number of previous regimens should be stressed as it provides motivation for adherence to the current regimen.

The relevance of a score to predict mortality is important in a clinical setting. There are many laboratory markers that are measured after patients start HAART, and whilst a high proportion of patients have some of these markers measured, it is of concern that markers such as HDL, TRI and GGT are not routinely measured. Though most of these markers may be used to predict morbidity, measurement of a smaller number of markers, such as ALB, HAE, and URE, are enough to predict short-term mortality. This is of importance in settings in which not all makers can be measured, perhaps for financial or patient convenience reasons.

These analyses have generally been performed with established statistical methods on firm endpoints and hence can easily be reproduced. Though UK CHIC does contain information from most of the large HIV clinics in the UK, there is the limitation that some large centres, such as those located in Manchester and Birmingham, and others in London, are not yet part of UK CHIC. Given the likely number of HIV-positive patients seen at these clinics, it is possible that the results are biased towards those patients seen at the large London clinics, who may have a different demographic profile to those seen elsewhere in the UK. There is a drive towards including additional centres in UK CHIC and hence in future research, analyses should be less affected by such biases.
It would be interesting to replicate some analyses, in particular, those performed in Chapters 4 and 5 in other datasets which are not UK-based in order to compare results.

Another potential limitation with this research is that it was based on a cohort study, in which there is inevitably unmeasured confounding which by definition cannot be controlled for. In particular, given the focus of this research was barriers to successful treatment, the role of adherence would have been very interesting to explore. Unfortunately, information on adherence is not collected in UK CHIC and hence surrogate markers, such as CD4 count measurements were used instead. Though a randomized controlled trial (RCT) would be the desirable gold standard for answering most research questions, it is not always the most viable option. For example, in the second Section of Chapter 5, I was only able to include 403 patients. Recruiting patients to a trial to answer the question set out in Chapter 5 would have been extremely difficult, given the strict inclusion criteria. Further, RCTs

are expensive as intensive follow-up is required. Therefore, patients are often observed for only short periods of time and so some clinical outcomes, such as death, may not be available. A particular limitation of RCTs in relation to analysing toxicities associated with HAART is that abnormal laboratory markers are usually not the primary endpoint of a RCT. Within the HIV field, the majority of trials are designed to evaluate the efficacy of a new drug and hence are unlikely to be powered to detect differences in prevalence of abnormal laboratory markers (538;539). Hence, the role of cohort studies in HIV research is of extreme importance, and though cohort studies do have limitations, there are often statistical methods which are able to at least partly correct for any known biases.

In conclusion, based on analyses performed in this thesis, I believe that the full potential of ART is yet to be achieved. The results of resistance tests need to be acted upon effectively and counselling on treatment interruptions needs to be prioritised. Toxicity of ART drugs is improving, but those with abnormal laboratory measures should have repeat tests to confirm the measurement is not just a random independent fluctuation. It is difficult to choose cut-off points for these laboratory measurements to define an abnormal measurement, and the score proposed in Chapter 8, though needing validation can be used as an overall predictor of all-cause mortality. In relation to abnormal laboratory measurements and mortality, collection of information on just three markers would ease the workload of the clinician and would reduce the inconvenience of the patient and hence the score should be verified in order to be put into practice.

Appendix A: Proportion of patients with laboratory measurements stratified by year under follow-up and centre

	Year under follow-up							
Centre 101	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**
N under follow-up	653	738	852	963	1068	1186	1296	1385
Any laboratory measurement (%)	83	81	83	83	78	84	91	93
ALT (%)	82	79	82	82	77	82	90	91
ALB (%)	79	78	80	81	77	83	91	92
AMY (%)	76	71	57	31	11	8	6	7
AST (%)	5	2	3	3	2	1	2	5
ALK (%)	82	80	83	82	77	82	91	92
BIL (%)	82	80	83	82	77	82	91	92
CHL (%)	74	74	77	76	73	70	77	78
CPK (%)	5	3	4	3	3	5	5	4
CRE (%)	78	79	83	83	77	82	91	92
GGT (%)	5	5	5	6	6	4	5	3
GLU (%)	67	69	74	74	69	69	76	77
HAE (%)	83	80	83	83	78	83	91	92
HDL (%)	70	70	74	75	70	69	77	78
LAC (%)	10	9	8	6	5	3	1	1
LDL (%)	66	66	72	75	70	67	75	75
TRI (%)	6	6	36	76	73	68	75	76
URE (%)	82	79	82	82	76	82	90	92

	Year under follow-up							
Centre 102	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**
N under follow-up	1917	2048	2201	2349	2511	2641	2703	2756
Any laboratory measurement (%)	82	84	85	84	82	82	86	85
ALT (%)	79	82	82	82	81	81	83	83
ALB (%)	76	79	80	80	80	81	84	84
AMY (%)	54	59	61	60	60	60	64	62
AST (%)	6	7	8	12	15	15	15	16
ALK (%)	80	82	83	82	82	81	84	84
BIL (%)	80	82	83	82	82	81	84	84
CHL (%)	69	71	72	71	75	75	80	80
CPK (%)	7	9	8	10	10	18	19	17
CRE (%)	79	80	82	81	81	81	84	84
GGT (%)	19	28	21	24	25	23	17	17
GLU (%)	62	67	70	71	75	77	78	80
HAE (%)	80	82	83	81	81	81	84	84
HDL (%)	3	4	5	6	6	73	80	80
LAC (%)	5	5	3	1	3	5	2	2
LDL (%)	2	4	5	6	6	71	69	12
TRI (%)	68	70	71	71	75	75	80	80
URE (%)	28	23	37	44	23	43	59	63

	Year under follow-up							
Centre 103	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**
N under follow-up	3702	3993	4224	4477	4794	5097	5327	5427
Any laboratory measurement (%)	**75**	**76**	**76**	**77**	**54**	**20**	**16**	**8**
ALT (%)	73	74	74	75	51	19	15	7
ALB (%)	31	31	35	46	33	19	15	8
AMY (%)	62	61	61	61	40	9	6	2
AST (%)	7	8	8	10	8	4	4	2
ALK (%)	74	75	75	76	52	19	16	8
BIL (%)	74	75	75	76	52	19	16	8
CHL (%)	66	65	40	11	33	16	13	7
CPK (%)	0	0	0	1	0	1	1	1
CRE (%)	73	74	74	76	52	20	16	8
GGT (%)	17	20	19	19	10	7	6	3
GLU (%)	4	5	11	7	7	7	7	5
HAE (%)	74	76	75	76	52	20	16	8
HDL (%)	2	4	5	6	8	8	8	7
LAC (%)	43	52	19	3	22	7	0	0
LDL (%)	2	3	6	6	8	8	8	6
TRI (%)	66	64	39	11	33	16	13	7
URE (%)	73	74	73	74	50	18	15	7

	Year under follow-up							
Centre 104	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**
N under follow-up	1911	2129	2341	2568	2763	2951	3101	3236
Any laboratory measurement (%)	78	89	90	90	89	88	89	90
ALT (%)	68	81	84	82	80	80	79	88
ALB (%)	65	79	82	80	80	80	80	89
AMY (%)	20	19	15	14	15	4	2	3
AST (%)	3	4	4	4	3	3	3	2
ALK (%)	69	81	85	82	81	80	80	89
BIL (%)	69	81	84	82	81	80	80	88
CHL (%)	34	53	55	60	61	65	68	66
CPK (%)	4	0	4	3	3	3	3	2
CRE (%)	52	47	83	82	81	80	80	89
GGT (%)	13	17	18	17	12	7	7	5
GLU (%)	27	47	47	43	44	45	41	41
HAE (%)	77	87	89	90	89	88	88	89
HDL (%)	26	46	51	58	58	64	68	66
LAC (%)	4	6	7	5	4	2	2	1
LDL (%)	24	44	49	56	57	63	66	64
TRI (%)	33	53	54	60	61	65	68	66
URE (%)	67	80	84	82	80	80	79	88

	Year under follow-up							
Centre 105	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**
N under follow-up	958	1134	1303	1438	1552	1653	1755	1732
Any laboratory measurement (%)	80	79	79	80	84	84	87	91
ALT (%)	14	13	12	12	11	11	12	11
ALB (%)	76	75	75	78	83	84	86	91
AMY (%)	55	66	71	71	76	62	59	49
AST (%)	1	1	1	1	2	1	1	1
ALK (%)	79	78	78	79	84	84	86	91
BIL (%)	79	78	78	79	83	83	86	91
CHL (%)	57	68	72	73	79	78	83	86
CPK (%)	0	0	0	0	0	1	1	0
CRE (%)	79	77	78	79	83	83	86	90
GGT (%)	75	72	72	75	79	80	82	86
GLU (%)	15	16	15	16	20	10	4	4
HAE (%)	79	78	79	80	84	84	86	91
HDL (%)	4	8	9	11	20	31	61	78
LAC (%)	13	16	9	5	4	5	4	2
LDL (%)	4	8	9	11	20	30	59	75
TRI (%)	57	68	72	72	79	78	82	86
URE (%)	18	18	18	19	20	22	24	87

	Year under follow-up							
Centre 106	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**
N under follow-up	1298	1419	1619	1767	1891	2016	2035	1335
Any laboratory measurement (%)	87	89	90	90	91	90	92	80
ALT (%)	85	88	89	89	87	89	91	78
ALB (%)	84	87	88	89	90	90	91	78
AMY (%)	4	4	4	3	2	1	1	2
AST (%)	80	83	84	84	85	85	87	70
ALK (%)	85	88	89	90	90	90	91	78
BIL (%)	85	88	89	90	90	90	91	78
CHL (%)	58	65	79	87	89	89	91	76
CPK (%)	0	0	0	0	0	1	0	0
CRE (%)	85	87	89	90	90	90	91	78
GGT (%)	79	82	82	85	85	86	87	70
GLU (%)	59	58	74	86	87	88	89	74
HAE (%)	83	88	89	89	90	89	89	77
HDL (%)	24	36	67	85	87	88	91	76
LAC (%)	12	13	12	8	7	5	2	0
LDL (%)	23	32	64	83	85	87	90	72
TRI (%)	57	64	79	87	89	89	91	76
URE (%)	85	88	89	89	89	89	91	78

	Year under follow-up							
Centre 108	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**
N under follow-up	445	461	467	482	508	545	575	577
Any laboratory measurement (%)	87	88	90	90	92	94	95	99
ALT (%)	87	88	90	90	91	94	95	99
ALB (%)	78	80	82	80	72	83	82	91
AMY (%)	67	75	67	61	64	57	60	68
AST (%)	1	1	0	0	0	0	3	9
ALK (%)	87	88	90	90	91	94	95	99
BIL (%)	87	88	90	90	91	93	95	99
CHL (%)	20	20	34	22	19	22	23	21
CPK (%)	42	63	62	56	60	60	74	77
CRE (%)	86	88	90	90	92	93	95	99
GGT (%)	0	0	0	0	0	0	0	0
GLU (%)	0	0	0	0	0	0	1	1
HAE (%)	87	88	89	90	92	93	95	99
HDL (%)	0	0	0	0	0	0	1	1
LAC (%)	1	2	2	3	2	1	1	1
LDL (%)	0	0	3	9	9	15	17	17
TRI (%)	21	20	25	3	3	2	2	2
URE (%)	34	38	56	34	36	40	37	38

Appendix B: Table for Grading Severity of Adverse Experiences

DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
PUBLISH DATE: DECEMBER, 2004

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
HEMATOLOGY	***Standard International Units are listed in italics***			
Absolute CD4+ count – **Adult and Pediatric > 13 years** (HIV NEGATIVE ONLY)	300 – 400/mm^3 *300 – 400/µL*	200 – 299/mm^3 *200 – 299/µL*	100 – 199/mm^3 *100 – 199/µL*	< 100/mm^3 *< 100/µL*
Absolute lymphocyte count – **Adult and Pediatric > 13 years** (HIV NEGATIVE ONLY)	600 – 650/mm^3 *0.600 x 10^9 – 0.650 x 10^9/L*	500 – 599/mm^3 *0.500 x 10^9 – 0.599 x 10^9/L*	350 – 499/mm^3 *0.350 x 10^9 – 0.499 x 10^9/L*	< 350/mm^3 *< 0.350 x 10^9/L*
Absolute neutrophil count (ANC)				
Adult and Pediatric, > 7 days	1,000 – 1,300/mm^3 *1.000 x 10^9 – 1.300 x 10^9/L*	750 – 999/mm^3 *0.750 x 10^9 – 0.999 x 10^9/L*	500 – 749/mm^3 *0.500 x 10^9 – 0.749 x 10^9/L*	< 500/mm^3 *< 0.500 x 10^9/L*
Infant*†, 2 – ≤ 7 days	1,250 – 1,500/mm^3 *1.250 x 10^9 – 1.500 x 10^9/L*	1,000 – 1,249/mm^3 *1.000 x 10^9 – 1.249 x 10^9/L*	750 – 999/mm^3 *0.750 x 10^9 – 0.999 x 10^9/L*	< 750/mm^3 *< 0.750 x 10^9/L*
Infant*†, 1 day	4,000 – 5,000/mm^3 *4.000 x 10^9 – 5.000 x 10^9/L*	3,000 – 3,999/mm^3 *3.000 x 10^9 – 3.999 x10^9/L*	1,500 – 2,999/mm^3 *1.500 x 10^9 – 2.999 x 10^9/L*	< 1,500/mm^3 *< 1.500 x 10^9/L*
Fibrinogen, decreased	100 – 200 mg/dL *1.00 – 2.00 g/L* OR 0.75 – 0.99 x LLN	75 – 99 mg/dL *0.75 – 0.99 g/L* OR 0.50 – 0.74 x LLN	50 – 74 mg/dL *0.50 – 0.74 g/L* OR 0.25 – 0.49 x LLN	< 50 mg/dL *< 0.50 g/L* OR < 0.25 x LLN OR Associated with gross bleeding
Hemoglobin (Hgb)				
Adult and Pediatric ≥ 57 days (HIV POSITIVE ONLY)	8.5 – 10.0 g/dL *1.32 – 1.55 mmol/L*	7.5 – 8.4 g/dL *1.16 – 1.31 mmol/L*	6.50 – 7.4 g/dL *1.01 – 1.15 mmol/L*	< 6.5 g/dL *< 1.01 mmol/L*
Adult and Pediatric ≥ 57 days (HIV NEGATIVE ONLY)	10.0 – 10.9 g/dL *1.55 – 1.69 mmol/L* OR Any decrease 2.5 – 3.4 g/dL *0.39 – 0.53 mmol/L*	9.0 – 9.9 g/dL *1.40 – 1.54 mmol/L* OR Any decrease 3.5 – 4.4 g/dL *0.54 – 0.68 mmol/L*	7.0 – 8.9 g/dL *1.09 – 1.39 mmol/L* OR Any decrease ≥ 4.5 g/dL *≥ 0.69 mmol/L*	< 7.0 g/dL *< 1.09 mmol/L*
Infant*†, 36 – 56 days (HIV POSITIVE OR NEGATIVE)	8.5 – 9.4 g/dL *1.32 – 1.46 mmol/L*	7.0 – 8.4 g/dL *1.09 – 1.31 mmol/L*	6.0 – 6.9 g/dL *0.93 – 1.08 mmol/L*	< 6.00 g/dL *< 0.93 mmol/L*

* Values are for term infants.

† Use age and sex appropriate values (e.g., bilirubin), including preterm infants.

DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
PUBLISH DATE: DECEMBER, 2004

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Infant*†, 22 – 35 days (HIV POSITIVE OR NEGATIVE)	9.5 – 10.5 g/dL *1.47 – 1.63 mmol/L*	8.0 – 9.4 g/dL *1.24 – 1.46 mmol/L*	7.0 – 7.9 g/dL *1.09 – 1.23 mmol/L*	< 7.00 g/dL *< 1.09 mmol/L*
Infant*†, 1 – 21 days (HIV POSITIVE OR NEGATIVE)	12.0 – 13.0 g/dL *1.86 – 2.02 mmol/L*	10.0 – 11.9 g/dL *1.55 – 1.85 mmol/L*	9.0 – 9.9 g/dL *1.40 – 1.54 mmol/L*	< 9.0 g/dL *< 1.40 mmol/L*
International Normalized Ratio of prothrombin time (INR)	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 3.0 x ULN	> 3.0 x ULN
Methemoglobin	5.0 – 10.0%	10.1 – 15.0%	15.1 – 20.0%	> 20.0%
Prothrombin Time (PT)	1.1 – 1.25 x ULN	1.26 – 1.50 x ULN	1.51 – 3.00 x ULN	> 3.00 x ULN
Partial Thromboplastin Time (PTT)	1.1 – 1.66 x ULN	1.67 – 2.33 x ULN	2.34 – 3.00 x ULN	> 3.00 x ULN
Platelets, decreased	100,000 – 124,999/mm^3 *100.000 x 10^9 – 124.999 x 10^9/L*	50,000 – 99,999/mm^3 *50.000 x 10^9 – 99.999 x 10^9/L*	25,000 – 49,999/mm^3 *25.000 x 10^9 – 49.999 x 10^9/L*	< 25,000/mm^3 *< 25.000 x 10^9/L*
WBC, decreased	2,000 – 2,500/mm^3 *2.000 x 10^9 – 2.500 x 10^9/L*	1,500 – 1,999/mm^3 *1.500 x 10^9 – 1.999 x 10^9/L*	1,000 – 1,499/mm^3 *1.000 x 10^9 – 1.499 x 10^9/L*	< 1,000/mm^3 *< 1.000 x 10^9/L*
CHEMISTRIES	***Standard International Units are listed in italics***			
Acidosis	NA	pH < normal, but ≥ 7.3	pH < 7.3 without life-threatening consequences	pH < 7.3 with life-threatening consequences
Albumin, serum, low	3.0 g/dL – < LLN *30 g/L – < LLN*	2.0 – 2.9 g/dL *20 – 29 g/L*	< 2.0 g/dL *< 20 g/L*	NA
Alkaline Phosphatase	1.25 – 2.5 x ULN†	2.6 – 5.0 x ULN†	5.1 – 10.0 x ULN†	> 10.0 x ULN†
Alkalosis	NA	pH > normal, but ≤ 7.5	pH > 7.5 without life-threatening consequences	pH > 7.5 with life-threatening consequences
ALT (SGPT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
AST (SGOT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
Bicarbonate, serum, low	16.0 mEq/L – < LLN *16.0 mmol/L – < LLN*	11.0 – 15.9 mEq/L *11.0 – 15.9 mmol/L*	8.0 – 10.9 mEq/L *8.0 – 10.9 mmol/L*	< 8.0 mEq/L *< 8.0 mmol/L*
Bilirubin (Total)				
Adult and Pediatric > 14 days	1.1 – 1.5 x ULN	1.6 – 2.5 x ULN	2.6 – 5.0 x ULN	> 5.0 x ULN

* Values are for term infants.

† Use age and sex appropriate values (e.g., bilirubin), including preterm infants.

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LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Infant*†, ≤ 14 days (non-hemolytic)	NA	20.0 – 25.0 mg/dL *342 – 428 µmol/L*	25.1 – 30.0 mg/dL *429 – 513 µmol/L*	> 30.0 mg/dL *> 513.0 µmol/L*
Infant*†, ≤ 14 days (hemolytic)	NA	NA	20.0 – 25.0 mg/dL *342 – 428 µmol/L*	> 25.0 mg/dL *> 428 µmol/L*
Calcium, serum, high (corrected for albumin)				
Adult and Pediatric ≥ 7 days	10.6 – 11.5 mg/dL *2.65 – 2.88 mmol/L*	11.6 – 12.5 mg/dL *2.89 – 3.13 mmol/L*	12.6 – 13.5 mg/dL *3.14 – 3.38 mmol/L*	> 13.5 mg/dL *> 3.38 mmol/L*
Infant*†, < 7 days	11.5 – 12.4 mg/dL *2.88 – 3.10 mmol/L*	12.5 – 12.9 mg/dL *3.11 – 3.23 mmol/L*	13.0 – 13.5 mg/dL *3.245 – 3.38 mmol/L*	> 13.5 mg/dL *> 3.38 mmol/L*
Calcium, serum, low (corrected for albumin)				
Adult and Pediatric ≥ 7 days	7.8 – 8.4 mg/dL *1.95 – 2.10 mmol/L*	7.0 – 7.7 mg/dL *1.75 – 1.94 mmol/L*	6.1 – 6.9 mg/dL *1.53 – 1.74 mmol/L*	< 6.1 mg/dL *< 1.53 mmol/L*
Infant*†, < 7 days	6.5 – 7.5 mg/dL *1.63 – 1.88 mmol/L*	6.0 – 6.4 mg/dL *1.50 – 1.62 mmol/L*	5.50 – 5.90 mg/dL *1.38 – 1.51 mmol/L*	< 5.50 mg/dL *< 1.38 mmol/L*
Cardiac troponin I (cTnI)	NA	NA	NA	Levels consistent with myocardial infarction or unstable angina as defined by the manufacturer
Cardiac troponin T (cTnT)	NA	NA	NA	≥ 0.20 ng/mL OR Levels consistent with myocardial infarction or unstable angina as defined by the manufacturer
Cholesterol (fasting)				
Adult ≥ 18 years	200 – 239 mg/dL *5.18 – 6.19 mmol/L*	240 – 300 mg/dL *6.20 – 7.77 mmol/L*	> 300 mg/dL *> 7.77 mmol/L*	NA
Pediatric < 18 years	170 – 199 mg/dL *4.40 – 5.15 mmol/L*	200 – 300 mg/dL *5.16 – 7.77 mmol/L*	> 300 mg/dL *> 7.77 mmol/L*	NA
Creatine Kinase	3.0 – 5.9 x ULN†	6.0 – 9.9 x ULN†	10.0 – 19.9 x ULN†	≥ 20.0 x ULN†
Creatinine	1.1 – 1.3 x ULN†	1.4 – 1.8 x ULN†	1.9 – 3.4 x ULN†	≥ 3.5 x ULN†
Glucose, serum, high				
Nonfasting	116 – 160 mg/dL *6.44 – 8.88 mmol/L*	161 – 250 mg/dL *8.89 – 13.88 mmol/L*	251 – 500 mg/dL *13.89 – 27.75 mmol/L*	> 500 mg/dL *> 27.75 mmol/L*
Fasting	110 – 125 mg/dL *6.11 – 6.94 mmol/L*	126 – 250 mg/dL *6.95 – 13.88 mmol/L*	251 – 500 mg/dL *13.89 – 27.75 mmol/L*	> 500 mg/dL *> 27.75 mmol/L*

* Values are for term infants.

† Use age and sex appropriate values (e.g., bilirubin), including preterm infants.

DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS PUBLISH DATE: DECEMBER, 2004

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Glucose, serum, low				
Adult and Pediatric ≥ 1 month	55 – 64 mg/dL *3.05 – 3.55 mmol/L*	40 – 54 mg/dL *2.22 – 3.06 mmol/L*	30 – 39 mg/dL *1.67 – 2.23 mmol/L*	< 30 mg/dL *< 1.67 mmol/L*
Infant*†, < 1 month	50 – 54 mg/dL *2.78 – 3.00 mmol/L*	40 – 49 mg/dL *2.22 – 2.77 mmol/L*	30 – 39 mg/dL *1.67 – 2.21 mmol/L*	< 30 mg/dL *< 1.67 mmol/L*
Lactate	< 2.0 x ULN without acidosis	≥ 2.0 x ULN without acidosis	Increased lactate with pH < 7.3 without life-threatening consequences	Increased lactate with pH < 7.3 with life-threatening consequences
LDL cholesterol (fasting)				
Adult ≥ 18 years	130 – 159 mg/dL *3.37 – 4.12 mmol/L*	160 – 190 mg/dL *4.13 – 4.90 mmol/L*	≥ 190 mg/dL *≥ 4.91 mmol/L*	NA
Pediatric > 2 - < 18 years	110 – 129 mg/dL *2.85 – 3.34 mmol/L*	130 – 189 mg/dL *3.35 – 4.90 mmol/L*	≥ 190 mg/dL *≥ 4.91 mmol/L*	NA
Lipase	1.1 – 1.5 x ULN	1.6 – 3.0 x ULN	3.1 – 5.0 x ULN	> 5.0 x ULN
Magnesium, serum, low	1.2 – 1.4 mEq/L *0.60 – 0.70 mmol/L*	0.9 – 1.1 mEq/L *0.45 – 0.59 mmol/L*	0.6 – 0.8 mEq/L *0.30 – 0.44 mmol/L*	< 0.60 mEq/L *< 0.30 mmol/L*
Pancreatic amylase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN
Phosphate, serum, low				
Adult and Pediatric > 14 years	2.5 mg/dL – < LLN *0.81 mmol/L – < LLN*	2.0 – 2.4 mg/dL *0.65 – 0.80 mmol/L*	1.0 – 1.9 mg/dL *0.32 – 0.64 mmol/L*	< 1.00 mg/dL *< 0.32 mmol/L*
Pediatric 1 year – 14 years	3.0 – 3.5 mg/dL *0.97 – 1.13 mmol/L*	2.5 – 2.9 mg/dL *0.81 – 0.96 mmol/L*	1.5 – 2.4 mg/dL *0.48 – 0.80 mmol/L*	< 1.50 mg/dL *< 0.48 mmol/L*
Pediatric < 1 year	3.5 – 4.5 mg/dL *1.13 – 1.45 mmol/L*	2.5 – 3.4 mg/dL *0.81 – 1.12 mmol/L*	1.5 – 2.4 mg/dL *0.48 – 0.80 mmol/L*	< 1.50 mg/dL *< 0.48 mmol/L*
Potassium, serum, high	5.6 – 6.0 mEq/L *5.6 – 6.0 mmol/L*	6.1 – 6.5 mEq/L *6.1 – 6.5 mmol/L*	6.6 – 7.0 mEq/L *6.6 – 7.0 mmol/L*	> 7.0 mEq/L *> 7.0 mmol/L*
Potassium, serum, low	3.0 – 3.4 mEq/L *3.0 – 3.4 mmol/L*	2.5 – 2.9 mEq/L *2.5 – 2.9 mmol/L*	2.0 – 2.4 mEq/L *2.0 – 2.4 mmol/L*	< 2.0 mEq/L *< 2.0 mmol/L*
Sodium, serum, high	146 – 150 mEq/L *146 – 150 mmol/L*	151 – 154 mEq/L *151 – 154 mmol/L*	155 – 159 mEq/L *155 – 159 mmol/L*	≥ 160 mEq/L *≥ 160 mmol/L*
Sodium, serum, low	130 – 135 mEq/L *130 – 135 mmol/L*	125 – 129 mEq/L *125 – 129 mmol/L*	121 – 124 mEq/L *121 – 124 mmol/L*	≤ 120 mEq/L *≤ 120 mmol/L*
Triglycerides (fasting)	NA	500 – 750 mg/dL *5.65 – 8.48 mmol/L*	751 – 1,200 mg/dL *8.49 – 13.56 mmol/L*	> 1,200 mg/dL *> 13.56 mmol/L*
Uric acid	7.5 – 10.0 mg/dL *0.45 – 0.59 mmol/L*	10.1 – 12.0 mg/dL *0.60 – 0.71 mmol/L*	12.1 – 15.0 mg/dL *0.72 – 0.89 mmol/L*	> 15.0 mg/dL *> 0.89 mmol/L*

* Values are for term infants.

† Use age and sex appropriate values (e.g., bilirubin), including preterm infants.

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PUBLISH DATE: DECEMBER, 2004

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
URINALYSIS	***Standard International Units are listed in italics***			
Hematuria (microscopic)	6 – 10 RBC/HPF	> 10 RBC/HPF	Gross, with or without clots OR with RBC casts	Transfusion indicated
Proteinuria, random collection	1 +	2 – 3 +	4 +	NA
Proteinuria, 24 hour collection				
Adult and Pediatric ≥ 10 years	200 – 999 mg/24 h *0.200 – 0.999 g/d*	1,000 – 1,999 mg/24 h *1.000 – 1.999 g/d*	2,000 – 3,500 mg/24 h *2.000 – 3.500 g/d*	> 3,500 mg/24 h *> 3.500 g/d*
Pediatric > 3 mo - < 10 years	201 – 499 mg/m^2/24 h *0.201 – 0.499 g/d*	500 – 799 mg/m^2/24 h *0.500 – 0.799 g/d*	800 – 1,000 mg/m^2/24 h *0.800 – 1.000 g/d*	> 1,000 mg/ m^2/24 h *> 1.000 g/d*

http://www.ucdmc.ucdavis.edu/clinicaltrials/documents/DAIDS_AE_GradingTable_FinalDec2004.pdf

Appendix C: Published research

Impact of transmitted drug-resistance on treatment selection and outcome of first-line Highly Active Antiretroviral Therapy (HAART).**(Chapter 4)**
Bansi L, Geretti AM, Dunn D, Hill T, Green H, Fearnhill E, Gazzard B, Nelson M, Porter K, Phillips A, Sabin C; UK Collaborative Group on HIV Drug Resistance and UK Collaborative HIV Cohort (CHIC) Study. J Acquir Immune Defic Syndr. 2010 Apr;53(5):633-9.

The impact of HIV drug resistance testing on changes to treatment **(Chapter 5)**
Loveleen Bansi, Colette Smith, Andrew Phillips, Stuart Kirk, Anna-Maria Geretti, Margaret Johnson, Nicola Mackie, Frank Post, Brian Gazzard, David Dunn, and Caroline Sabin on behalf of the UK Collaborative HIV Cohort (CHIC) Study and the UK Collaborative Group on HIV Drug Resistance (HDRD). AIDS, 2011; *In press*

Is 1 alanine transaminase >200 IU enough to define an alanine transaminase flare in HIV-infected populations? A new definition derived from a large cohort study **(Chapter 6)**
Bansi L, Turner J, Gilson R, Post F, Gazzard B, Leen C, Anderson J, Porter K, Hill T, Fisher M, Ainsworth J, Pillay D, Johnson M, Winston A, Orkin C, Easterbrook P, Phillips A, Sabin C; UK Collaborative HIV Cohort Study. J Acquir Immune Defic Syndr. 2009 Nov 1;52(3):391-6.

Are previous treatment interruptions associated with higher viral rebound rates in patients with viral suppression? **(Chapter 7)**
Bansi LK, Benzie AA, Phillips AN, Portsmouth S, Hill T, Leen C, Schwenk A, Johnson M, Anderson J, Gilson R, Easterbrook P, Gazzard B, Fisher M, Orkin C, Porter K, Pillay D, Taylor GP, Walsh JC, Sabin CA; UK Collaborative HIV Cohort (UK CHIC) Study. AIDS. 2008 Jan 30;22(3):349-56.

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