Switch & Simplification Of Antiretroviral Therapy

Imperial College, London, Department of Medicine

Thesis submitted for the degree of MD (Res)

Dr Laura Jane Waters

July 2013

Abstract

The advent of combined antiretroviral therapy (cART) has transformed HIV care such that most individuals can expect a normal or near normal life expectancy. Managing HIV is increasingly focused on the prevention and treatment of non-infectious co-morbidities and dealing with drug-related side effects and toxicities, the main reasons for switching cART. Switching cART can be complicated by pharmacokinetic (PK) interactions and modern, potent agents may allow us to move away from the standard 'three active drugs' mantra.

This thesis examines the pharmacokinetic impact of switching directly from efavirenz, widely used for initial therapy, to etravirine and to maraviroc. The impact of efavirenz to etravirine switch on central nervous system side effects was also investigated in a double-blind trial. In addition the value of pre-treatment tropism testing to guide susceptibility to maraviroc is explored. Finally a large cohort analysis explored the importance of active nucleoside analogues in a boosted protease inhibitor based, second line regimen for patients who had failed first line non-nucleoside reverse transcriptase inhibitor therapy.

The pharmacokinetic studies confirmed a prolonged induction effect of efavirenz and a novel finding that this induction effect varies according to which new agent is used. The tropism analysis showed that tropism switch during suppressive therapy is uncommon thus supporting the use of pre-treatment testing as a guide to future switch. Individuals with ongoing central nervous symptoms on efavirenz experienced significant improvement in switch to etravirine, the first time this has been proven in a randomised, blinded trial; furthermore significant lipid improvements were observed. Finally the cohort analysis suggested that the number of new or active nucleosides in a second line boosted PI-based combination does not affect virological outcomes; simpler second line regimens should be considered and could provide several advantages over more complex choices.

Acknowledgements

Most importantly, I would like to acknowledge and thank all the patients and volunteers who participated in the studies described herein. Secondly I would like to thank the excellent administration, regulatory, nursing and medical staff at St Stephen's Research; particular gratitude to Dr Marta Boffito, lead research clinician, and Dr Akil Jackson, senior research fellow whose dedication, advice and sharing have proved invaluable. Special thanks to my supervisor, Professor Brian Gazzard and to Dr Mark Nelson, whose wisdom and experience are endlessly inspiring. I would also like to thank Dr Loveleen Bansi, Professor Andrew Phillips, Professor David Back, Dr Martin Fisher, Ms Nicky Perry and Mr Chris Higgs for their hard work and support. Finally thank you to my family and husband Andrew.

Declaration

The work described in this thesis was undertaken by Dr Laura Waters under the supervision of Professor Brian Gazzard at Chelsea & Westminster Hospital, London. Where work has been undertaken jointly my role is documented. Pharmacokinetic analyses were performed by the team at The University of Liverpool under the supervision of Professor David Back. Statistical analyses were performed by St Stephen's Research staff (Laura Waters, Akil Jackson, Marta Boffito and Sundhiya Mandalia), University of Liverpool and, for chapter 5, Dr Loveleen Bansi and Professor Andrew Phillips.

Copyright

The copyright of this thesis rests with the author and is made available under a Creative Commons Attribution Non-Commercial No Derivatives licence. Researchers are free to copy, distribute or transmit the thesis on the condition that they attribute it, that they do not use it for commercial purposes and that they do not alter, transform or build upon it. For any reuse or redistribution, researchers must make clear to others the licence terms of this work

Contents

Abstract	2
Acknowledgements, declaration & copyright	3
Abbreviations	5
Chapter 1: General introduction	7
Chapter 2: Materials and Methods	24
Chapter 3: The pharmacokinetics of switching efavirenz to etravirine in healthy volunteers & the efficacy and safety of switching efavirenz to etravirine in HIV-positive patients with CNS symptoms	36
Chapter 4: The evolution of co-receptor tropism during viral suppression & the pharmacokinetics, efficacy and safety of switching efavirenz to maraviroc in HIV-positive patients	65
Chapter 5: Switching from a failing 1st line, NNRTI-based regimen to a boosted protease inhibitor: how many nucleoside analogues are necessary?	82
Chapter 6: General conclusions	95
Summary	102
Abstract presentations & publications	103
References	106

Abbreviations

3TC	Lamivudine
ABC	Abacavir
ACTG	AIDS Clinical Trials Group
AE	Adverse event
AIDS	Acquired immune deficiency syndrome
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase
ARV	Antiretroviral
ATV/r	Atazanavir/ritonavir
AUC	Area under the curve
AUC0-12	Area under the curve from 0 to 12 hours
AUC0-24	Area under the curve from 0 to 24 hours
AZT	Zidovudine
BHIVA	British HIV Association
BID	Twice daily
BMI	Body mass index
cART	Combined antiretroviral therapy
CCR5	C-C Chemokine Receptor 5
Cmax	Maximum concentration
Cmin	Minimum concentration
Ctrough	Trough concentration
CI	Confidence interval
CNS	Central nervous system
CV	Coefficient of variation
CVD	Cardiovascular disease
CYP	Cytochrome P450
CXCR4	C-X-C chemokine receptor type 4
DAIDS	Division of AIDS
ddC	Zalcitabine
ddI	Didanosine
DNA	Deoxy-ribonucleic acid
DRV/r	Darunavir/ritonavir
DS	Delayed switch
EACS	European AIDS Clinical Society
ECG	Electrocardiogram
EFV	Efavirenz
EI	Entry inhibitor
ETR	Etravirine
FDC	Fixed dose combination
FTC	Emtricitabine
GGT	Gamma-glutamyl transferase
GM	Geometric mean
GMR	Geometric mean ratio
GSS	Genotypic sensitivity score
HADS	Hospital Anxiety and Depression Scale
HDL	High density lipoprotein
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography

IC90	Concentration required to inhibit 90% of virus
INI	Integrase inhibitor
IQR	Interquartile range
IS	Immediate switch
IUD	Intrauterine device
LDL	Low density lipoprotein
LPV/r	Lopinavir/ritonavir
MDRD	Modification of Diet in Renal Disease
M-MASRI	Modified Medication Adherence Self-Report Inventory
MVC	Maraviroc
NICE	National Institute for Clinical Excellence
NRTI	Nucleoside reverse transcriptase inhibitor
NNRTI	Non- nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
OBR	Optimised background regimen
PBMC	Peripheral blood mononuclear cell
PI	Protease inhibitor
PI/r	Ritonavir-boosted PI
PK	Pharmacokinetics
QD	Once daily
RAL	Raltegravir
RNA	Ribonucleic acid
RPV	Rilpivirine
SD	Standard deviation
SPC	Summary of Product Characteristics
t½	Half-life
Tmax	Time to Cmax
TDF	Tenofovir
TG	Triglycerides
TOPS	The Over Volunteering Prevention System
UK CHIC	United Kingdom Collaborative HIV Cohort
VF	Virological failure
WOCBP	Women of childbearing potential

Chapter 1:

General Introduction

Chapter 1: Introduction

1.1 Hypotheses & aims

1.2 Antiretroviral therapy

- 1.2.1 Impact of antiretroviral therapy
- 1.2.2 Consensus guidelines
- 1.2.3 Adverse events and toxicity
- 1.2.4 Switching treatment

1.3 Efavirenz

- 1.3.1 Efficacy and toxicity profile
- 1.3.2 Mechanism of action & pharmacokinetics
- 1.3.3 Switching from efavirenz

1.4 Etravirine

- 1.4.1 Efficacy and toxicity profile
- 1.4.2 Mechanism of action & pharmacokinetics

1.5 Maraviroc

- 1.5.1 Efficacy and toxicity profile
- 1.5.2 Mechanism of action & pharmacokinetics
- 1.5.3 Tropism evolution & determination

1.1 Hypotheses & aims

In the modern era of combination antiretroviral therapy (cART) most treated HIV positive individuals can expect a high chance of virological and immunological success. True virological failure is relatively uncommon and the majority of treatment switches are secondary to problems with tolerability and toxicity associated with cART. However complex drug-drug interactions and variable drug half-lives necessitate caution when switching therapy. Finally modern potent agents may facilitate a move away from the standard three drug paradigm and therefore reduce the risk of drug-related morbidities.

This thesis will examine the following hypotheses:

- Switching directly from efavirenz will alter concentrations of newer antiretrovirals metabolised by the cytochrome P450 system and the induction effect of efavirenz will vary for different drugs.
- Switching from efavirenz to an alternative agent in the absence of prior drug resistance will not compromise virological efficacy.
- Switching from efavirenz to etravirine will improve central nervous system symptoms (in symptomatic patients) and improve lipids without compromising adherence.
- Increasing maraviroc dose for 2 weeks after switching to efavirenz will result in maintenance of therapeutic maraviroc concentrations throughout the early dosing period and can be guided by pre-cART tropism determination.
- Individuals starting a boosted protease inhibitor based regimen after first line treatment failure do not require two additional active drugs.

1.2 Antiretroviral Therapy

1.2.1 Impact of antiretroviral therapy

The first antiretroviral, a nucleoside reverse transcriptase inhibitor (NRTI) was licensed in 1987. Although short-term improvement in HIV markers (CD4 cell count and, later, plasma HIV-RNA) and clinical events was observed with NRTI monotherapy, and to a greater extent dual therapy, this was not sustained due to the rapid emergence of drug resistance [Mayers

et al. 1994]. It was not until the mid 1990s, with the development of a second drug class, protease inhibitors (PI) and the combination of PI with two NRTI that sustained HIV suppression and immune reconstitution could be achieved [Collier *et al*. 1996]. Around 2000 the concept of PI boosting, the use of a sub-therapeutic dose of the PI ritonavir to pharmacologically 'boost' PI concentrations by inhibiting cytochrome mediated drug metabolism, was introduced [Kilby *et al*. 2000. Boosted PI (PI/r) are now standard of care; most PI are not effective without boosting and unboosted options used only occasionally. PI were quickly followed by non-nucleoside reverse transcriptase inhibitors (NNRTI) and, more recently, entry inhibitors (EI) and integrase inhibitors (INI). EI comprise two subclasses: fusion inhibitors (rarely used) and drugs that block a cell surface co-receptor, C-C Chemokine Receptor 5 (CCR5). The advent of combined antiretroviral therapy or 'cART' (which remains typically two NRTI plus one drug from another class) transformed HIV from an almost inevitably terminal condition to one where an HIV-positive individual, who experiences sufficient and sustained immune reconstitution can expect a near normal life expectancy [Lewden *et al*. 2007]. Recent UK data demonstrates that amongst those HIV-positive patients who start cART at a CD4 200-350 cells/mm^3, like expectancy close to that of the general population (40.5 years at the age of 35 vs 43.5 and 46.9 years in men and women in the general population, respectively) [May *et al*. 2011]. Considering current UK guidelines recommend starting at CD4 <350 cells/mm^3 (ideally close to this), optimal treatment should yield even better outcomes. HIV positive individuals will be spending more time on cART for the following reasons:

- Improved life expectancy [Lewden *et al*. 2007; May *et al*. 2011; Nakagawa *et al*. 2012]
- Earlier treatment initiation [BHIVA guidelines 2012; DHHS guidelines 2011; Thompson *et al.* 2012]
- Earlier diagnosis (UK data shows the proportion of new HIV diagnoses with a CD4 <350 cells/mm^3 is declining) [HPA 2011 Report]
- In most situations cART should not be interrupted once started [BHIVA guidelines 2012]

It is therefore crucial to optimise and individualise cART in order to maximise adherence and minimise tolerability & toxicity issues. High levels of adherence are required for successful treatment outcomes; recent clinical trials show significantly worse outcomes amongst sub-optimally adherent individuals (defined as adherence <95%) on NNRTI- and PI/r-based first line regimens [Cohen *et al*. 2012; Nelson *et al.* 2010]

1.2.2 Consensus guidelines

Current consensus British [BHIVA guidelines 2012] and European [EACS guidelines v6] guidelines recommend a two NRTI 'backbone' plus an NNRTI, a PI/r or an INI. In the UK one of the most commonly prescribed initial regimen is based on efavirenz (EFV), a first generation NNRTI and until this year EFV was preferred over other drugs [BHIVA guidelines 2012; Gazzard *et al*. 2008]. Essentially the different preferred regimens in current national guidelines demonstrate similar efficacy (defined as the proportion of all treated subjects achieving viral suppression at 48 weeks or later) but vary in terms of tolerability and short- and long-term toxicity profiles. The recently updated British HIV Association (BHIVA) guidelines state: *The presence or future risk of co-morbidities and potential adverse effects need to be considered in the choice of antiretroviral drugs in individual patients* [BHIVA guidelines 2012].

1.2.3 Adverse events and toxicity

There have been marked improvements in the side effect profile of antiretrovirals over time. Earlier drugs were associated with high rates of nausea [Combivir SPC], diarrhoea [Pulido *et al*. 2009] and other toxicities including renal stones [Famularo *et al.* 2000], peripheral neuropathy [Pahuja *et al*. 2012] and pancreatitis [Manfredi *et al*. 2008]. Modern agents generally have far better adverse event profiles and discontinuations for adverse events are relatively uncommon in clinical trials; recent first-line trials report adverse event driven discontinuation rates of 2-7% at 48 weeks [Cohen *et al*. 2012; Ortiz *et al*. 2008; Molina *et al*. 2008]. However, toxicity remains the major reason for cART switch in clinical cohorts [Elzi *et al*. 2010; Boyle *et al*. 2012]. In the Swiss HIV Cohort, almost 1 in 4 patients modify first line cART secondary to toxicity, most commonly due to gastrointestinal side effects followed by rash and central nervous system (CNS) events. A recent London cohort analysis showed almost half the patients who modified cART (all lines of therapy) over an 18 month period did so for toxicity – 1 in 4 of the toxicity switches were from EFV and almost 80% of these were for CNS toxicity (see 1.3).

Cardiovascular disease is the major cause of mortality in the UK [ONS Mortality Statistics 2010] and an increasingly important cause of death amongst HIV-positive patients [DAD Study Group 2010]. Individuals with HIV appear to have a higher risk of cardiovascular disease (CVD) including myocardial infarction [Triant *et al*. 2007; Durand *et al*. 2011; Islam *et*

al. 2012], sudden cardiac death [Tseng *et al*. 2012], peripheral vascular disease [Ye *et al*. 2010] and thromboembolic events including stroke [Ovbiagele *et al*. 2011]. Cross-sectional cohorts suggest that HIV is an equivalent risk factor to diabetes [Triant *et al*. 2007] and studies of surrogate markers, such as carotid intimal medial thickness (cIMT) support this [Grunfeld *et al*. 2009]. However, all of these results should be interpreted with caution as there are many potential confounders including smoking [Saves *et al*. 2003]. Antiretrovirals may also impact cardiovascular risk either indirectly (such as via impact on lipids) or possibly directly. Some cohorts show a relationship between specific antiretrovirals and the risk of myocardial infarction after adjustment for lipids [DAD Study Group 2010]. One drug that has been implicated in some studies but not others is the NRTI abacavir (ABC). Whether ABC really does increase CVD risk is controversial but there are studies suggesting possible mechanisms including an impact on platelet aggregation [Baum *et al*. 2011; Satchell *et al*; 2011]; data on ABC and endothelial function are conflicting [Hileman *et al*. 2012; Rasmissen *et al*. 2011]. Because of the high, and likely increasing, burden of CVD in HIV-positive people the metabolic impact of cART is of ever-growing importance. Newer agents in general have less impact in lipids and, where data is available, markers of insulin sensitivity than older drugs. Amongst PI, atazanavir/ritonavir (ATV/r) and darunavir/ritonavir (DRV/r) are associated with smaller increases in cholesterol and triglycerides than lopinavir/ritonavir (LPV/r) [Ortiz *et al*. 2008; Molina *et al*. 2008]. ATV/r [Daar *et al*. 2011], rilpivirine (RPV) [Cohen *et al*. 2012], raltegravir (RAL) [Lennox *et al*. 2009], and maraviroc (MVC) [Cooper *et al*. 2010] all yield smaller lipid elevations than EFV although only MVC demonstrated a better total to high density lipoprotein (HDL) cholesterol ratio than EFV [DeJesus *et al*. 2008]. European guidelines recommend first considering a change of ART and *then* lipid-lowering medication in individuals with dyslipidaemia and high cardiovascular risk [EACS guidelines v6].

As highlighted already, high-levels of adherence are necessary for long-term efficacy and side effects are a common reason for late and missed doses [Duran *et al*; 2001]; in addition concerns about side effects is associated with reduced adherence [Cooper *et al*. 2011]. Even mild symptoms can have a marked effect on quality of life and, potentially, adherence [Mannheimer *et al*. 2008]. The National Institute for Clinical Excellence (NICE) guidance on adherence support recommends that where side effects are a problem "*consider adjusting the dosage, switching to another combination or other strategies such as changing the dose timing or formulation"* [Nunes *et al*. 2009]. National HIV guidelines emphasise that

"*switching individual components of an ART regimen may well improve adherence and tolerability, but should not be at the cost of virological efficacy*" [BHIVA guidelines 2012]. Detailed switch data is discussed in section 1.2.4.

Importantly pill burden may impact on adherence. There are several studies of antiretroviral pill burden demonstrating improved adherence with a once daily compared with more than once daily treatment regimens [Parienti *et al*; 2009]. In addition, switching to a single table regimen was associated with better adherence (self-report) in one study [Airoldi *et al*. 2010] but not another [Dejesus *et al*. 2009]. A meta-analysis on fixed dose combinations (FDC) across different disease areas favoured FDC but only one of nine trials was of HIV therapy [Bangalore *et al*; 2007]. In general there is a lack of robust evidence to show that the number of pills in a once daily combination impact adherence rates. In particular there is no data to show whether side effects or pill burden have the greatest impact on adherence – we therefore hypothesised that individuals with CNS toxicity on EFV will maintain high adherence rates when switching to a higher pill burden, non-EFV-based regimen.

1.2.4 Switching treatment

There are relatively few studies that examine the safety and efficacy of different interventions to manage side effects. The decision to treat toxicity or switch to an alternative depends on many factors including: side effect severity, temporal relationship with drug(s) and availability/acceptability of alternative antiretrovirals and/or other interventions. There can be reluctance amongst patients and clinicians to switch therapy in the setting of good efficacy but most switch studies do not show a negative effect on treatment efficacy. Within class switches are usually undertaken to address issues of tolerability or toxicity [BHIVA guidelines 2012] and, in the absence of background resistance, are generally safe. Most NRTI switch studies have examined switch from thymidine analogues (zidovudine or stavudine) to the more modern NRTI (tenofovir (TDF) or ABC) in patients with thymidine-related side effects. These studies have generally shown improved side effects with no loss of virological or immunological control [Moyle *et al*. 2006; Fisher *et al*. 2009], as did a study that switched patients with hyperlipidaemia from ABC/lamivudine (3TC) to TDF/emtricitabine (FTC) [Moyle *et al*. 2010]. Switch studies within the PI class similarly show maintained viral suppression [Mallolas *et al*. 2009; Moyle *et al*. 2012; Gatell *et al*. 2007]. Switch studies within the NNRTI class are scarce; a cohort study describing patients with CNS toxicity switching from EFV to nevirapine (NVP) showed improvement in

neuropsychiatric side effects and lipids with maintained viral suppression [Ward *et al*. 2006]. In a single arm study switching patients suppressed on TDF/FTC/EFV to TDF/FTC/RPV (both as FDC) all subjects (n=49) maintained viral suppression at week 12 [Cohen *et al*. 2011]. Our study, investigating switch from EFV to etravirine (ETR) is described in chapter 3. Between class switch studies include three trials switching treatment-experienced patients on suppressive cART from PI/r to RAL [Eron *et al*. 2010; Vispo *et al*. 2010; Martinez *et al*. 2010] two of which showed that previous NRTI resistance increased the risk of virological failure on switching to RAL compared with continuing the PI/r [Eron *et al*. 2010; Vispo *et al*. 2010]; this highlights the importance of prior treatment experience and resistance when considering switch. Prior treatment failure is also associated with virological failure when switching from PI/r- to NNRTI-based treatment [Martinez *et al*. 2003]. An analysis of French Hospital Database on HIV, demonstrated that patients with viral suppression on a first-line boosted PI-based maintained viral suppression on switch to EFV- or NVP-based treatment but that there was an excess of virological failures if the switch was to an ABC-based, that is NRTI only, combination [Bommenal *et al*. 2011]. A meta-analysis of PI (boosted and unboosted) to NVP switch trials showed non-inferiority in terms of viral suppression but more liver-related discontinuations in those switching to NVP compared with those staying on a PI [Ena *et al*. 2008]. Our work to investigate switch from EFV to the CCR5 antagonist MVC is described in chapter 4.

1.3 Efavirenz

EFV is a first generation NNRTI that is a recommended initial treatment option across national and international guidelines [BHIVA guidelines 2012; DHHS guidelines 2001; EACS guidelines v6; Thompson *et* al. 2012; WHO guidelines 2010]. No third agent, in combination with two NRTI, has demonstrated superior virological efficacy to EFV for first-line treatment although several have exhibited non-inferiority including ATV/r [Daar *et al*. 2011], RAL [Lennox *et al*. 2009], RPV [Cohen *et al*. 2012] and MVC [Cooper *et al*. 2010]. However, EFV is associated with a number of limitations including CNS toxicity, hyperlipidaemia and a low genetic barrier to resistance.

1.3.1 Efficacy and toxicity profile

EFV is commonly associated with CNS toxicity and although this is transient for many patients, tending to resolve in the first 2-4 weeks of treatment [Sustiva SPC]. The evidence regarding longer-term side effects is conflicting. One retrospective study demonstrated no

difference in neuropsychometric testing or self-reported neuro-psychiatric symptoms between individuals treated with EFV or NVP-based ART [von Giesen HJ *et al*. 2003].Other studies show, however, that some patients do experience ongoing symptoms [Maggiolo 2009] and chronic low-grade CNS toxicity may be underestimated. Higher levels of stress, anxiety and abnormal dreams were reported in patients on stable EFV-containing cART compared with matched controls [Rihs *et al*. 2006] and long-term (184 week) follow-up of a subset of patients from the ACTG5095 study showed EFV was associated with bad dreams and anxiety compared with baseline [Clifford *et al*. 2009] however, the same study showed that after week 4, in this controlled trial, neurological symptoms in EFV and non-EFV recipients did not differ after week 4. Clifford *et al* also showed that by neuropsychometric testing there were not detectable differences between those who received EFV and those who did not. The potentially chronic nature of EFV-related CNS toxicity for some is highlighted by the fact that a proportion individuals who switch off EFV for CNS symptoms do so more than 12 months after starting EFV [Scourfield *et al*. 2012; Zhou *et al*. 2012].

Most modern drugs including the NNRTI ETR [Katlama *et al*. 2009] and RPV [Cohen *et al*. 2012], the integrase inhibitor RAL [Lennox *et al*. 2009], the CCR5 antagonist MVC [Cooper *et al*. 2010] and the protease inhibitor ATV/r [Daar *et al*. 2011] show significantly less neuropsychiatric toxicity than EFV in head to head trials. Of note, more granular date on CNS toxicity is infrequently presented and where is has been, fro the ECHO and THRIVE studies comparing EFV with RPV, although overall the rate of psychiatric and neurological adverse events are significantly greater for EFV, after 4 weeks the incidence of new side effects is low in both arms and the point prevalence similar in both groups [Mills *et al*. 2013]. In practice most clinicians would switch an EFV-treated patient with troublesome or persistent CNS side effects to an alternative agent; alternatives include symptomatic management (such as hypnotics) and EFV dose reduction [Fayet Mello *et al*. 2011]. However, there is a paucity of data to guide treatment switch in this setting; common sense would suggest that switching individuals with EFV-related side effects away from EFV to any reasonable non-EFV alternative should improve those side effects but it is important to have evidence to support this, particularly in the light of the pharmacokinetic effects of EFV (see 1.3.3).

EFV is associated lipid elevations; increases in total cholesterol of 10-20% have been observed healthy volunteers and In first-line clinical trials, total cholesterol, HDL-cholesterol,

and triglycerides increased over 48 weeks of treatment (by up to 31%, 34% and 49%, respectively) [Sustiva SPC]. The magnitude of changes in lipid levels varies according to NRTI backbone [Sax *et al*. 2011].

EFV has a low genetic barrier to resistance. The most common treatment-emergent resistance associated mutation at virological failure is the K103N which occurred in up to 90% of cases in the early EFV trials [Sustiva SPC]. EFV resistance will results in high-level cross-resistance but susceptibility to the newer NNRTI, ETR and RPV, will often be maintained; the K103N in isolation does not affect these drugs but accumulation of additional mutations may confer resistance. In a recent trial comparing EFV with RPV, 54% of EFV failures developed treatment emergent NNRTI mutations [Cohen *et al*. 2012] although all retained susceptibility to ETR and RPV.

1.3.2 Mechanism of action & pharmacokinetics

EFV, in common with many antiretrovirals, is metabolised by the hepatic cytochrome P450 system and also induces some cytochrome P450 (CYP) isoenzymes, particularly CYP3A4 [Hariparsad *et al*. 2004]. This induction effect may reduce concentrations of drugs that are metabolized by CYP3A4 such as steroid hormones [Sevinsky *et al*. 2011], methadone [Kharasch *et al*. 2012], and other antiretrovirals [Dailly *et al*. 2006].

Importantly EFV has a long and variable half-life ($t_{½}$) of 40-55 hours at steady state [Smith *et al*. 2001] and therapeutic concentrations have been measured up to several weeks after drug intake cessation in some patients [Taylor *et al*. 2007]. This must be taken into account when switching directly from EFV to an alternative antiretroviral.

1.3.3 Switching from efavirenz

The induction effect of EFV and its long half life mean that even after cessation of EFV intake, induction can persist for days or weeks if a drug that is metabolized by CYP3A4 is commenced. Thus there is risk of sub-therapeutic plasma concentrations of the new drug during this period; depending on the characteristics of that drug and the magnitude of the induction effect, dose adjustment may be required.

Before discussing the impact of drug-drug interactions on drug plasma concentrations it is important to review the different terms and cut-offs used to describe therapeutic drug levels. For a drug to exert a therapeutic effect then adequate (ie therapeutic) concentrations of that drug must be achieved. Most assessments of drug concentrations and their impact on viral replication are based on *in vitro* studies which yield the drug concentrations required to inhibit a certain proportion of viral replication – this most commonly cited are the concentrations required to inhibit 50% or 90% of viral replication, the IC50 and IC90, respectively. The higher the minimum concentration of an antiretroviral drug is above the IC then the lower the risk of reaching sub-therapeutic concentrations and consequent virological rebound. Therefore, when assessing the impact of one drug on the plasma concentrations of another, it is the impact on Cmin that is that most important to estimate the likely impact, if any, on efficacy.

However, the use of *in vitro* data has limitations including assay variability, inability to account for active drug metabolites and the fact that for at least some ARVs it is the

intracellular, not the plasma, concentrations that are crucial to drug activity. Wherever possible the in vivo data should be used –this is usually described as the 'effective concentration', the amount of drug required to inhibit 50% or 90% of virus *in vivo*, the EC50 and EC90, respectively, or the minimum effective concentration (MEC). Unfortunately EC data it no available for many drugs and is usually based on monotherapy studies therefore cannot account for the synergy achieved by using drug *combinations* [Montaner *et al*. 2001].

For most ARVs, the IC90 is the most widely available figure and the one used to estimate the clinical relevance of any impact on Cmin.

When initiating NVP a lead-in period followed by dose escalation at 2-4 weeks is recommended to reduce the risk of rash [Viramine SPC] but a pharmacokinetic study of patients switching from EFV to NVP concluded that immediate full-dose NVP should be used to avoid sub-therapeutic NVP concentrations [Winston *et al.* 2004]. Lower RPV concentrations are observed when switching from EFV; in a healthy volunteer study RPV concentrations were lower when switching from EFV but, other than the minimum concentration (C_{min}), were normal by day 28 [Crauwels *et al*. 2012]. In HIV-positive patients switching from EFV to RPV, RPV reached target concentrations by day 14 and EFV concentrations exceeded the concentration required to inhibit 90% of wild-type virus (IC_{90}) for 4 weeks; all patients maintained an undetectable viral load at week 12 [Mills *et al*. 2011]. As such the reduction in RPV concentrations is not considered to be clinically significant. Based on pharmacological principles no dose adjustment is felt to be necessary when switching from EFV to a PI/r or to RAL [BHIVA guidelines 2012]. Although co-administration of EFV and RAL resulted in a small (21%) reduction in RAL C_{min} [Iwamoto *et al*. 2008], therefore a similar short-term effect could be anticipated when switching, this does not necessitate any alteration in RAL dose. Data on the pharmacokinetics of switching from EFV to ETR or MVC are described in chapters 3 and 4, respectively.

1.4 Etravirine

1.4.1 Efficacy and toxicity profile

ETR is a second-generation NNRTI that is licensed for treatment-experienced HIV-positive patients in combination with a PI/r and other antiretrovirals. ETR registration was based on the DUET trials which compared etravirine with placebo, each with an optimised background regimen (OBR) including DRV/r, in more than 1200 NNRTI- and PI/r-experienced individuals.

At week 48 significantly more ETR-treated patients were undetectable (61% vs. 40%, on placebo; p <0.0001) [Katlama *et al*. 2009]; the majority of patients maintained viral suppression out to week 96 [Katlama *et al*. 2010]. Adverse events were similar in the two treatment groups except rash, which was significantly more frequent in the ETR group (19% vs. 11%, on placebo; p <0.0001), occurring primarily in the second week of treatment; lipid parameters were similar [Katlama *et al*. 2009].

Although not licensed for first-line use ETR was compared with EFV in a double-blind, placebo-controlled trial (The SENSE Study) in 157 patients [Gazzard *et al*. 2011]. The study was powered for CNS toxicity rather than virological efficacy and at week 48 significantly more patients on EFV had ongoing CNS side effects (21.5% vs. 6.3% on ETR; p<0.011). ETR demonstrated non-inferior virological efficacy (76% vs 74% on EFV; p <0.05). There were no 'signature' adverse events or laboratory abnormalities in the ETR arm and overall fewer ETR-treat subjects experienced grade 2-4 clinical adverse event (27% vs 42% on EFV). Of note the ETR dose used in this first-line study was 400mg once daily (QD) rather than the licensed dose of 200mg twice daily (BID) (see 1.3.2). From a metabolic perspective ETR was associated with significantly smaller rises in total-, HDL- and low density lipoprotein (LDL)-cholesterol, and triglycerides (TG) than EFV and there were fewer grade 3/4 lipid elevations in the ETR arm [Fatkenheuer *et al*. 2012]. ETR has a higher genetic barrier to resistance than EFV [Yeni *et al*. 2010]; in SENSE there were seven virological failures on EFV, three of whom developed new NRTI or NNRTI resistance, compared with four virological failures on ETR none of who had emergent mutations [Gazzard *et al*. 2011]. Based on its resistance profile and the efficacy results of DUET and SENSE, using ETR 'off licence' as a first-line or switch option, without a PI/r, is reasonable.

1.4.2 Mechanism of action & pharmacokinetics

The pharmacokinetics of ETR in HIV-infected patients at the recommended dosage (200mg BID) demonstrate moderate inter-subject variability. ETR should be taken following a meal as plasma concentrations are significantly lower in fasted conditions. It is highly protein bound (99.9%) and has an elimination half-life of 30-40 hours. ETR is metabolised by CYP isoenzymes 3A, 2C9 and 2C19; in addition it induces CYP3A and inhibits CYP2C9, 2C19 and is a mild inhibitor of P-glycoprotein. For patients with renal impairment or mild to moderate hepatic impairment no dose adjustment is required and pharmacokinetics are unaffected by ethnicity, gender, age and weight [Scholler-Gyure *et al*. 2009]. In DUET studies no

relationship was observed between ETR pharmacokinetics and efficacy or safety [Kakuda *et al*. 2010].

Although licensed twice-daily, ETR 400mg QD results in similar ETR exposure in HIV-positive patients as the recommended dose [DeJesus *et al*. 2010]. Co-administration of EFV with ETR reduces ETR AUC by 41% and maximum concentration (C_{max}) by 18% [Baede *et al*. 2002] supporting the need to investigate EFV to ETR switch in a formal pharmacokinetics study.

1.5 Maraviroc

1.5.1 Efficacy and toxicity profile

MVC is the only currently licensed drug from the CCR5 antagonist drug class. It is licensed for treatment-experienced HIV-positive individuals with only CCR5-tropic virus detectable (see 1.4.3) in combination with other antiretrovirals [Celsentri SPC]. In the United States it is also licensed for initial therapy [Selzentry FDA]. MVC was compared with placebo (each combined with OBR) in the MOTIVATE studies, two large, double-blind, placebo-controlled trials in patients with triple-class experience and/or resistance. A significantly greater proportion of MVC recipients achieved viral suppression (42-47% vs 16-18% on placebo) [Gulick *et al*. 2008]. The frequency of adverse events was similar in the two groups except there was a higher rate of cardiovascular events on MVC – no events in 111 patient years on the placebo arm of MOTIVATE compared with 11 events in 609 patient years on MVC. Results adjusted for duration of drug exposure have not been presented and all patients who had a cardiovascular event had pre-existing risk [BHIVA guidelines 2012]. MVC was also studied in treatment-naïve individuals against EFV, both with a backbone of Combivir (zidovidine/3TC), in the MERIT study. Using a non-inferiority margin of 10% MVC was non-inferior to EFV using the <400 copies/ml viral load cut-off but failed to reach non-inferiority when a <50 copies/ml analysis was used [Saag *et al*. 2007]. Since this study was performed a more sensitive tropism assay has become routinely available and a *post hoc* re-analysis of MERIT showed that 15% of the patients with apparent R5-tropic virus actually had non-R5 virus. When the analysis was restricted to those individuals with R5-tropic virus by the more sensitive assay, MVC did achieve non-inferiority compared to EFV [Sax *et al*. 2008]. Of note, a subanalysis in the original MERIT Study of individuals with a baseline viral load below 100,000 copies/ml demonstrated only a small numerical difference between MVC and EFV recipients with 69.6% and 71.6% respectively achieving a viral load less than 50 copies/ml at 48 weeks. Broadly, individual adverse events occurred at similar frequencies in the two

arms although abnormal dreams, dizziness and rash were all less common on MVC. In addition median lipid changes were greater in the EFV arm, correlating with a lower predicted risk of cardiovascular disease for MVC recipients [DeJesus *et al*. 2008]; to date this is unique for a head to head study against EFV as other drugs have shown smaller increases in individual lipid parameters but no difference in terms of total:HDL cholesterol ratio. Due to its unique mechanism of action and the greater CD4 rise observed on MVC in head to head studies, it has been studied as an intensification strategy. Although no improvements in CD4 have been observed when MVC is added to suppressive therapy, improvements in immune activation and the latent reservoir have been observed [Gilliam *et al*. 2010; Wilkin *et al*. 2012], immune activation [Gilliam *et al*. 2010] and latent infection [Gutierrez *et al*. 2011]. MVC was associated with postural hypotension in healthy volunteer studies but at concentrations higher than those that would be achieved with the licensed doses [Celsentri SPC].

Overall MVC is non-inferior to EFV and demonstrates a favourable adverse event profile. Although there was a signal for increased cardiovascular events in the MOTIVATE studies, this was not adjusted for duration of drug exposure and, based on this and the impact of MVC on lipids, is unlikely to be clinically significant.

1.5.2 Mechanism of action & pharmacokinetics

HIV gains entry into target cells through a three stage process [Waters *et al*. 2008]:

1. Virus binding to cell surface CD4: gp120 (a viral surface protein) binds to the CD4 receptor which induces a conformational change and exposes an additional binding site. This allows another part of gp120 (the third hypervariable or V3 loop) to interact with one of two cell surface receptors – CCR5 or CXCR4.

   uHIV binds to CCR5 or C-X-C chemokine receptor type 4 (CXCR4): this induces a further conformational change in gp41 (a second HIV surface protein) and activates the fusion process.
2. HIV and target cell fusion.

The chemokine receptors CCR5 and CXCR4 are transmembrane receptors that are activated by a variety of chemokines and form a network of interaction fundamental to human physiology [Waters *et al*. 2008]. There are other chemokine receptors that can support HIV entry *in vitro* but only CCR5 and CXCR4 are used *in vivo*. In terms of co-receptor there are essentially three types of HIV:

1. CCR5 utilising (R5-tropic)
2. CXCR4 utilising (X4-tropic)
3. Dual or mixed (D/M)

Dual-tropic HIV can utilise either co-receptor but a mixture of R5- and X4-tropic virus is more common; most assays cannot distinguish dual from mixed and for practical purposes the two are considered together. R5-tropic HIV strains form the bulk of transmitted virus [Alkhatib *et al*. 1996] and R5-tropism predominates in early disease. Cross-sectional cohort studies demonstrate that D/M and X4-tropic HIV strains are uncommon in ART-naïve populations, ≤20% and <1%, respectively; these proportions increase to approximately 40% and ≤5%, respectively, in treatment-experienced individuals [Waters *et al*. 2008]. A previous analysis of over 550 stored samples from our HIV cohort demonstrated that the majority of samples were R5-tropic: 81.1% of samples from treatment-naïve subjects and 77.6% of samples from treatment-experienced individuals demonstrated R5 tropism [Moyle *et al*. 2005]. Less than 1% harboured pure X4-tropic virus, the majority of non-R5 samples being D/M-tropic.

MVC absorption is variable and median peak plasma concentrations are attained at 2 hours following single oral doses of 300 mg. The pharmacokinetics of oral MVC are not dose proportional; the absolute bioavailability of a 100 mg dose is 23% and is predicted to be 33% at 300 mg [Celsentri SPC]. Co-administration of MVC 300 mg with a high fat breakfast reduces MVC concentrations by about a third, but as there were no food restrictions in the efficacy trials, consequently there are no recommended food restrictions [Celsentri SPC].

Human and *in vitro* studies show MVC is metabolized mainly by the CYP system to inactive metabolites; *in vitro* studies demonstrate that CYP3A4 is the major enzyme responsible for MVC metabolism. Usually around 20% of MVC is excreted renally but this may increase up to 70% in the presence of metabolic inhibitors; in this situation renal impairment could increase MVC exposure. MVC metabolism is reduced by pure CYP3A4 inhibitors (most PI) and increased by CYP3A4 inducers including some NNRTI (such as EFV). Dose adjustments are required when MVC is co-administered with these drugs (see 4.1). MVC is also a substrate for the efflux transporter P-glycoprotein [Celsentri SPC].

1.5.3 Tropism evaluation

As aforementioned, MVC is licensed only patients who only have evidence of R5-tropic virus. There are two types of tropism assay: phenotypic and genotypic. Phenotypic tropism testing

(Trofile is the most common and was used in the MOTIVATE and MERIT trials) uses assays based on cells expressing CCR5 or CXCR4 receptors to identify which receptor an individual's virus can bind to. Phenotypic tests require detectable plasma HIV-RNA (usually at least 500 copies/ml) so cannot be performed in patients who have achieved viral suppression. Our study investigating the use of phenotypic tropism testing on stored plasma samples with detectable HIV-RNA is described in chapter 4 and, along with other studies, supports the use of stored sample testing to guide switch to MVC in patients with viral suppression. However, there are other disadvantages to phenotypic tropism testing including cost, turnaround time and availability. Over recent years evidence has accumulated to support the use of genotypic testing as an alternative to phenotyping. Genotypic testing relies on the fact that co-receptor tropism is determined by genetic sequences encoding the viral gp120. Particularly the highly variable and flexible V3 loop [Briggs *et al*. 2000]. Various alogorithms have been developed to interpret genetic sequencing of this region of the viral genome; some of these incorporate clinical data such as nadir CD4 cell count and percentage. The most widely studied is the geno2pheno alogorithm [Thielen *et al*. 2012]. Genotypic testing is cheaper, faster, more widely available and as accurate at predicting virological response to MVC on both treatment-naïve [McGovern, Dong *et al*. 2010] and treatment-experienced [McGovern, Thielen *et al*. 2010] patients as phenotypic assays. Genotypic tropism testing can also be performed on cellular HIV-DNA meaning that it can be used in patients with viral suppression [Svicher *et al*. 2012; Verhofstede *et al*. 2011].

Chapter 2:

Materials & Methods

Chapter 2: Materials and Methods

2.1 Ethical and regulatory approval

2.2 Cohorts

2.2.1 St Stephen's Research healthy volunteer group

2.2.2 HIV patient cohorts

2.2.3 The UK Collaborative HIV Cohort (UKCHIC) Study

2.3 Subject selection

2.3.1 EFV to ETR pharmacokinetic switch study in healthy volunteers

2.3.2 EFV to ETR switch study in EFV-treated, HIV-positive patients

2.3.3 Tropism evolution cohort

2.3.4 EFV to MVC switch study in EFV-treated, HIV-positive patients

2.3.5 UK CHIC analysis of second line PI/r based cART after failure of a first - line NNRTI-based regimen

2.4 Laboratory methods

2.4.1 Assessment of immunological and virological markers

2.4.2 Assessment of plasma drug concentrations

2.5 Assessment of central nervous system toxicity

2.6 Statistical analyses

Chapter 2: Materials and Methods

2.1 Ethical and regulatory approval

All research was conducted according to the principles of the International Conference on Harmonisation for Good Clinical Practice (ICH-GCP). For each trial the UK National Research Ethics Service (NRES) supplied ethical approval followed by local approval from site Research and Development offices (Chelsea & Westminster NHS Foundation Trust and, for the EFV to ETR switch study in HIV-positive patients, Imperial NHS Trust and Brighton & Sussex University Hospitals NHS Trust). National ethics approval for individual studies is outlined below:

Chapter 3:

(i) Healthy volunteer EFV to ETR switch study, approved by NRES, ref 07/H0706/69; EudraCT number 2007-003540-29.

(ii) EFV to ETR switch study in HIV-positive patients, approved by NRES, ref 08/H0706/25; EudraCT number 2008-000674-20.

Chapter 4:

(i) Tropism evolution study: anonymised retrospective analysis of surplus, stored samples, as such no ethics approval required.

(ii) EFV to MVC switch study in HIV-positive patients, approved by NRES, ref 09/H0706/74; EudraCT number 2009-014696-40.

Chapter 5:

Approval for the UK CHIC Study, granted by NRES (reference number 00/7/47). No written informed patient consent was required as the study was based in anonymised, routinely collected clinical data.

2.2 Cohorts

2.2.1 St Stephen's Research healthy volunteer group

HIV-negative male and female volunteers aged between 18 and 65 years were selected through the St Stephen's Research healthy volunteer database or through advertisements placed in The Metro newspaper (a free daily newspaper available widely across London and

other cities) in accordance with St Stephen's AIDS Trust standard operating procedure 'Advertising for Trial Subjects'. In order to prevent over-volunteering all subjects were screened via The Over Volunteering Prevention System (TOPS); in addition volunteer details were cross-checked against our local, secure database.

2.2.2 Chelsea & Westminster HIV patient cohort

HIV-infected adults attending the Kobler Clinic (Chelsea & Westminster HIV Outpatient Department) who were potentially eligible for the outlined studies were invited to participate by their regular clinic doctor or the research team. For the EFV to ETR switch study (chapter 3) in HIV-positive patients, eligible subjects from the HIV Outpatient Departments at St Mary's Hospital, London and The Lawson Unit, Brighton were also invited to take part. All interested subjects were invited for a discussion of the study and provided with written information; all were given at least 24 hours to consider the study and time to ask questions before consenting.

2.2.3 The UK Collaborative HIV Cohort (UKCHIC) Study

The UK Collaborative HIV Cohort (CHIC) Study, is a large observational cohort formed from a collaboration of some of the largest HIV clinics in the UK. Participating centres provide routinely collected data on all adult patients (≥16 years) attending for care since 1996; 12 HIV clinical centres contribute currently (11 at the time of our analysis – see chapter 5). Collected data includes demographic information, clinical events (HIV- and AIDS-related events, deaths, and, more recently, 'non-HIV-related' events), antiretroviral use and HIV surrogate markers (CD4 counts and HIV RNA levels). Currently, the cohort includes more than 30,000 anonymous and de-linked records of patients who have attended for HIV care. The UK HIV Drug Resistance Database collates the vast majority of genotypic resistance tests conducted as part of routine care in the UK and is linked to UK CHIC

A UK CHIC analysis of second line therapy outcomes (Chapter 5) was proposed by Dr Laura Waters, Dr David Asboe and Dr Anton Pozniak; this was submitted to and approved by the UK CHIC Study Steering Committee and the UK HIV Drug Resistance Database Committee in 2008.

2.3 Subject selection

2.3.1 EFV to ETR pharmacokinetic switch study in healthy volunteers

Male and non-pregnant, non-lactating female subjects were identified using procedures described in section 2.2.1. Volunteers were eligible for enrolment if they provided written informed consent, were aged between 18 and 65 years and had a body mass index (BMI) between 18 and 35 kg/m2. Women of child-bearing potential were required to use a suitable contraceptive method during the study and for one month after study completion. Full inclusion and exclusion criteria are listed in section 3.2.2.1.

2.3.2 EFV to ETR switch study in EFV-treated, HIV-positive patients

HIV-positive subjects attending for care at Chelsea & Westminster, St Mary's or Brighton were identified through clinic meetings, virtual clinics and clinic staff, and invited to participate. Eligible adults were required to be on EFV-containing HAART (EFV plus two NRTI) for at least 12 weeks with continued CNS toxicity, with viral suppression and a CD4 cell count more than 50 cells/mm^3. Women of child-bearing potential were required to use effective contraception for the duration of the study.

2.3.3 Tropism evolution cohort

At Chelsea & Westminster data including demographics, disease characteristics, treatment history and surrogate markers (including CD4 cell count and HIV-RNA) are entered on a prospectively collected database. We used this database to identify patients who achieved viral suppression on ART (defined as continuous therapy for at least 12 weeks) and then had an interruption of therapy for reasons other than virological failure. Data was anonymised and, where available, stored frozen plasma was anonymised and sent for phenotypic tropism testing.

2.3.4 EFV to MVC switch study in EFV-treated, HIV-positive patients

HIV-positive adult males and non-pregnant, non-lactating females attending for care at Chelsea & Westminster were identified at clinic meetings, virtual clinic or by clinic staff, and were invited to participate. Eligible subjects were on a first-line regimen of two NRTI with EFV, a CD4 cell count > 50 cells/mm^3, a viral load < 50 copies/mL for at least 12 weeks and R5-tropic virus on either a stored pre-treatment plasma sample by phenotypic testing or at screen by genotypic testing. There was no requirement for ongoing CNS symptoms.

2.3.5 UK CHIC analysis of second line PI/r based cART after failure of a first line NNRTI-based regimen

The UK CHIC dataset for this analysis includes information on 32,607 patients seen at 11 clinical centres up to the end of 2008. We identified HIV-infected adults who commenced first-line NNRTI-based CART (defined at least two NRTI with an NNRTI), subsequently experienced virological failure (defined as HIV-RNA greater than 200 copies/ml after at least 4 months of therapy) and then switched to PI/r-based therapy 2nd line.

2.4 Laboratory methods

2.4.1 Assessment of immunological and virological markers

For the EFV to ETR switch study in HIV-positive patients, immunological and virological markers process and measured according to each site's routine procedures; each laboratory was required to provide written documentation of accreditation. For Chelsea & Westminster participants in the HIV-positive EFV to ETR switch study and for the EFV to MVC switch study, CD4 and CD8 counts and percentages were measured by the Imperial College pathology service in the Department of Immunology and HIV-RNA quantification was performed via Imperial College Department of Virology using the viral load assay in routine clinical use at the time of each trial.

2.4.2 Assessment of plasma drug concentrations

All plasma drug concentrations were measured by the Pharmacology Research Laboratories at the University of Liverpool. At each scheduled blood draw for pharmacokinetic testing, approximately 6ml of whole blood was collected into one Lithium heparin Vacutainer® blood collection tube for each assay. Samples were centrifuged for 10 minutes at 1200 g at 4^{o}C within 180 minutes then plasma aliquoted equally into four 2.0ml PP tubes with skirted base (Sarstedt®) and stored at -20^{o}C or below. Two plasma samples were sent for pharmacokinetic analysis and two stored as back-up.

Specific details regarding pharmacokinetic analyses are described subsequently.

2.5 Assessment of central nervous system toxicity

In all the prospective studies patients were questioned about symptoms and side effects at every visit. These were documented, graded 1-4 (according to The ACTG Division of AIDS

(DAIDS) grading scale) and causality assessed by the principle investigator or a co-investigator. Trials often report the frequency of grade 2-4 clinical adverse events affecting a certain proportion of subjects (eg >2% or >5%). For smaller studies, such as those described in this thesis, it is reasonable to describe all moderate or greater adverse events deemed at last possible related to study drug. For the EFV to ETR switch study in HIV-positive patients we collected more detailed neuropsychiatric symptom data. As there are few studies with CNS side effect end-points there was a lack of precedent on which to base the CNS end-points.

To ensure as many CNS events as possible were detailed we developed a symptom-specific questionnaire based on: (a) side effects listed in the Summary of Product Characteristics (SPC) for EFV and, (b) side effects of importance selected by the research team based on their expertise and experience; these are detailed in section 3.2.2.2.

Where these symptoms were listed in the DAIDS system they were graded accordingly; where they were not we graded based on DAIDS, from 0 (absent) to 4 depending on impact in normal functioning:

- **Grade 1:** Causing no or minimal interference with usual social & functional activities
- **Grade 2:** Causing greater than minimal interference with usual social & functional activities
- **Grade 3:** Causing inability to perform usual social & functional activities
- **Grade 4:** Life threatening or causing inability to perform basic self-care functions

We also reviewed tools used in previous HIV treatment studies and selected the Hospital Anxiety and Depression Scale (HADS), a validated questionnaire [Zigmond *et al*. 1983] which was developed to assess patients with physical illness. It is useful in identifying individuals who need a more in-depth assessment [Herrmann 1997]. The HADS has been studied in a variety of medical settings including HIV [Savard *et al*. 1998] and performs well in assessing the symptom severity in somatic, psychiatric and primary care patients as well as in the general population [Bjelland *et al*. 2002]. The HADS is reported to give be valuable as a psychological screening tool, for group comparisons and in studies correlating depressive and anxiety symptoms with aspects of disease and quality of life [Herrmann 1997]. The HADS is also sensitive to changes during the course of diseases and in response to different interventions and has been shown to predict both psychosocial and physical outcomes

[Herrmann 1997]. Limitations include the fact that HADS was not developed to be a diagnostic tool and lack of validation data in people living with HIV. It does however, demonstrate similar sensitivity and specificity as other screening tools including GHQ [O'Rourke *et al*. 1998], BDI and PHQ-9 [Applied Health Sciences 2011]. Importantly it has been shown (within primary care) to demonstrate a similar magnitude of change over time as compared with other questionnaires [Applied Health Sciences 2011].

The HADS is essentially composed of 14 questions, which are divided into depression and anxiety sub-categories (table 2.1).

Table 2.1: The Hospital Anxiety & Depression Scale; 'A' items pertain to anxiety, 'D' to depression

I feel tense or 'wound up' (A):			**I feel as if I am slowed down (D):**	
I feel tense or 'wound up' (A):			**I feel as if I am slowed down (D):**	
Most of the time			Nearly all the time	
A lot of the time			Very often	
Occasionally			Sometimes	
Not at all			Not at all	

I still enjoy the things I used to enjoy (D):			**I get a sort of frightened feeling like 'butterflies' in the stomach (A):**	
Definitely as much			Not at all	
Not quite as much			Occasionally	
Only a little			Quite often	
Hardly at all			Very often	

I get a sort of frightened feeling as if something awful is going to happen (A):			**I have lost interest in my appearance (D):**	
Very definitely and quite badly			Nearly all the time	
A lot of the time			Very often	
Occasionally			Sometimes	
Not at all			Not at all	

I can laugh and see the funny side of things (D):			**I feel restless as if I have to be on the move (A):**	
As much as I always could			Very much indeed	
Not quite as much now			Quite a lot	
Definitely not so much now			Not very much	
Not at all			Not at all	

Worrying thoughts go through my mind (A):			**I look forward with enjoyment to things (D):**	
A great deal of the time			As much as I ever did	
A lot of the time			Rather less than I used to	
From time to time, not too often			Definitely less than I used to	
Only occasionally			Hardly at all	

I feel cheerful (D):			**I get sudden feeling of panic (A):**	
Not at all			Not at all	
Not often			Not often	
Sometimes			Sometimes	
Most of the time			Most of the time	

I can sit at ease and feel relaxed (A):			I can enjoy a good book or television programme (D):	
Definitely			Often	
Usually			Sometimes	
Not often			Not often	
Not at all			Very seldom	

Finally, in addition to regular questioning regarding missed and late doses of study medication, participants were required to complete an adherence questionnaire at each visit – Modified Medication Adherence Self-Report Inventory (M-MASRI) (table 2.2). M-MASRI was developed for use, and validated in HIV-positive populations; it correlates with both electronic pill cap measurement and virological outcome [Walsh *et al*. 2002]. In a meta-analysis of different self-report questionnaires across a variety co medical conditions, the M-MASRI performed well [Shi *et al*. 2010]. In a head-to head randomised trial of two PI/r-based regimens (Truvada + DRV/r vs Truvada + LPV/r), adherence assessed by M-MASRI was significantly associated with virological outcome on LPV/r [Nelson *et al*. 2010].

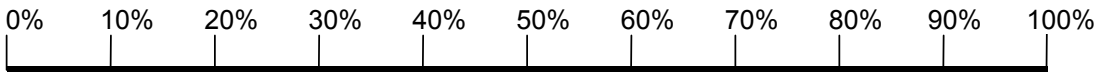
Table 2.2: The Modified Medication Adherence Self-Report Inventory (M-MASRI)

We understand that many people on anti-HIV medications find it difficult to take them regularly. We would like to know HOW MUCH you have taken DURING THE PAST 30 DAYS of the following HIV medication:	
Name (+ other Name(s)) and Description:	
Number of pills per "dose" (= per intake):	
Number of "doses" (intakes) per day:	
<u>This questionnaire is completely confidential</u>. Under no circumstances will your answers be shown to your doctor or anyone else involved in your care.	

We would like you to show us ***HOW MUCH*** of the Medication above you have taken ***DURING THE LAST 30 DAYS*.** We would be surprised if this was 100%.

Put a cross on the line below at the point showing ***<u>your best guess</u>*** about ***HOW MUCH*** of **the Medication above** you have taken ***DURING THE LAST 30 DAYS.***

E.g. **0%** means you **haven't taken any dose** of the Medication above, **50%** means you **have taken half of the prescribed doses** of the Medication above and **100%** means you **have taken every single dose of the Medication above.**



2.6 Statistical analyses

Details of the statistical methods used for each study are described in the relevant chapters. Statistical analyses for the pharmacokinetic analyses were performed by The University of Liverpool team, Laura Waters, Marta Boffito and Akil Jackson. The non-pharmacokinetic results of the switch studies were analysed by Laura Waters and Sundhiya Mandalia. The UK CHIC analysis plan was formulated by Laura Waters, Loveleen Bansi and Andrew Phillipps

with LB and AP performing that actual analysis. Specific details of the analyses performed are described in the appropriate chapters.

Chapter 3:

The pharmacokinetics of switching efavirenz to etravirine in healthy volunteers

&

The efficacy and safety of switching efavrienz to etravirine in HIV-positive patients with CNS symptoms

Chapter 3: The pharmacokinetics, safety & efficacy of switching from efavirenz to etravirine based ART

3.1 Introduction

3.2 Methods

3.2.1 Healthy volunteer study

3.2.1.1 Subject selection & screening

3.2.1.2 Study methodology

3.2.1.3 Pharmacokinetic analysis

3.2.1.4 Data analysis

3.2.2 HIV-positive patient study

3.2.2.1 Subject selection & screening

3.2.2.2 Study methodology

3.2.2.3 Data analysis

3.3 Results

3.3.1 Healthy volunteers

3.3.1.1 Pharmacokinetics

3.3.1.2 Safety and tolerability

3.3.2 HIV-positive patients

3.3.2.1 Central nervous system symptoms

3.3.2.2 Hospital anxiety and depression scale

3.3.2.3 Safety and tolerability

3.3.2.4 Immunological and virological efficacy

3.3.2.5 Adherence

3.4 Discussion

3.4.1 Healthy volunteers

3.4.2 HIV-positive patients

3.1 Introduction

As discussed previously, timely switch of antiretroviral therapy is an important intervention for managing drug-related toxicity. Although there is a relative paucity of data, European guidelines recommend within class switching to manage drug-related side effects [EACS guidelines v6], so when switching from EFV, another NNRTI would be the preferred option. British guidelines do not express such a preference; they recommend that in patients on suppressive cART regimens, consideration is given to differences in side effect profile, drug–drug interactions and drug resistance patterns before switching any antiretroviral (ARV) component and recommend against switching a boosted PI to either a NNRTI or an INI as the third agent in patients with prior NRTI resistance mutations [BHIVA guidelines 2012]. If side effects are a problem the British guidelines give to options:

1) Discuss benefits, and long-term effects and options for dealing with side effects
2) Consider adjusting the dosage, switching to another combination or other strategies such as changing the dose timing or formulation [BHIVA guidelines 2012].

In terms of alternative NNRTI options, NVP use is limited by CD4 restrictions for initial therapy (<400 cells/mm^3 and <250 cells/mm^3 for men and women, respectively) although this no longer applies for individuals switching with an undetectable viral load. However, there remains a background risk of cutaneous and/or hepatic toxicity when switching to NVP, even in the context of viral suppression. ETR is licensed for use in treatment-experienced patients, in combination with a PI/r, so its use as a 3^{rd} agent switch option is off licence. Finally RPV is a recently licensed second generation NNRTI that is licensed for use only in treatment-naïve individuals with a viral load less than 100.000 copies/ml.

ETR is generally well tolerated and in particular had lower reported rates of CNS toxicity than EFV (each with a Truvada backbone) as initial therapy [84], although is unlicensed for this line of treatment. In the treatment-naïve study, 21.5% on EFV still reported neuropsychiatric symptoms at week 48 compared with 6.3% on ETR (p=0.001). In highly treatment-experienced patients, rates of nervous system and psychiatric AE were similar when ETR was compared with placebo in, each in combination with optimised background therapy [Katlama *et al*. 2009]. CNS symptoms are one of the most common reasons for modifying cART [Elzi *et al*. 2010] and chronic neuropsychiatric symptoms are more common in EFV-treated individuals [Rihs *et al*. 2006; Clifford *et al*. 2009]. ETR has a higher genetic barrier to resistance than first-generation NNRTI and, although licensed for twice daily

administration, has pharmacokinetic properties supporting once daily dosing [Scholler-Gyure, Kakuda, De Smedt *et al*. 2007]. In the treatment-naïve study comparing ETR with EFV, ETR was also administered once daily and although the study was not powered for efficacy, response rates to ETR were numerically similar to EFV [Gazzard *et al*. 2011]. Animal studies have shown a low risk of teratogenesis and the major toxicity associated with ETR in clinical trials has been skin rash; this is usually self-limiting and not related to previous NNRTI rash [Intelence SPC].

ETR could offer a switch option for individuals with EFV-related toxicity but due to the long half-life of EFV and its potential impact on ETR concentrations it is important to understand the pharmacokinetics of direct EFV to ETR switch. Although ETR is associated with fewer CNS adverse events when compared head-to-head with EFV, there is a lack of data guiding switch in EFV-treated individuals with chronic CNS toxicity.

This chapter describes two studies examining EFV to ETR switch:

1. A pharmacokinetic study in healthy volunteers to determine the impact of switching directly from EFV to ETR on ETR concentrations over a 2-week period. The aim of the study was to describe the nature and magnitude of any interaction between EFV and ETR in this scenario and to provide guidance when switching from EFV to ETR in routine clinical practice.
2. A subsequent trial in HIV-infected individuals with ongoing CNS toxicity on an EFV-containing regimen. In this double blind, placebo-controlled trial, patients were randomised to switch to ETR from EFV either immediately or after 12 weeks; from week 12 to 24 all participants received open-label ETR (with their usual NRTI backbone). The aim of this study was to describe the efficacy of a switch from EFV to ETR in terms of CNS toxicity in order to ascertain whether or not this could be a safe and feasible switch option for individuals suffering EFV-related side effects.

3.2 Methods

3.2.1 Healthy volunteer study

3.2.1.1 Subject selection & screening

HIV-negative healthy volunteers were contacted as previously described (see section 2.3.1). Male and non-pregnant, non-lactating female subjects were eligible for enrolment if they provided written informed consent and met the following criteria:

- Age between 18 and 65 years
- BMI 18-35 kg/m2. Women of child-bearing potential were required to use a suitable contraceptive method during the study and for one month after study completion.

Subjects were excluded if any of the following applied:

- Any significant acute or chronic medical illness
- Abnormal physical examination, electrocardiogram (ECG) or clinical laboratory determinations
- Positive screen HIV, hepatitis B or hepatitis C serology
- Current or recent (within 3 months) gastrointestinal disease
- Clinically relevant alcohol of drug use that the investigator felt would adversely affect compliance with trial procedures
- Exposure to any investigational drug or placebo within 4 weeks of the first dose of study drug
- Consumption of grapefruit or Seville oranges or any grapefruit- or Seville orange-containing product during the study
- Use of any other drugs, including over-the-counter medications and herbal preparations, within 2 weeks before the first dose of study drug
- Previous allergy to any of the constituents of the pharmaceuticals administered during the trial.

3.2.1.2 Study methodology

This was a 57-day (excluding screening and follow-up visits), open-label, randomised, prospective, two arm, pharmacokinetic study conducted at the Pharmacokinetic Unit of the St Stephen's Centre, Chelsea and Westminster Hospital, London. The study protocol was reviewed and approved by the Riverside Research Ethics Committee, London. All subjects

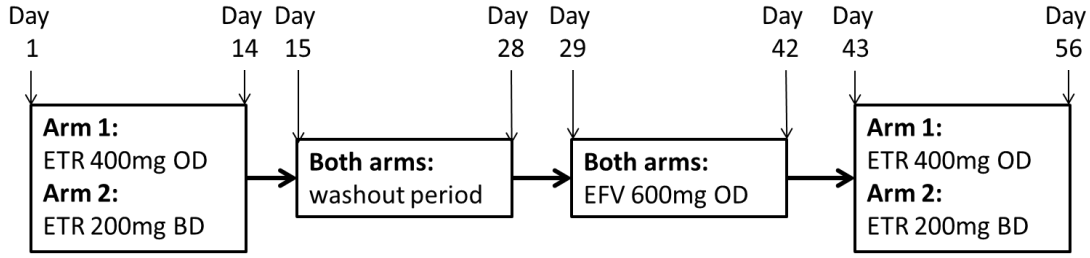
provided written informed consent, and the trial was conducted in accordance with Good Clinical Practice, the Declaration of Helsinki, and applicable regulatory requirements.

After provision of written, informed consent, subjects underwent screening with clinical assessment and routine laboratory investigations. Demographic details and full medical, drug and social history were recorded followed by a complete physical examination (including weight, height, and vital signs). Volunteers then underwent ECG assessment, non-fasted blood testing (drug screen, HIV antibody, hepatitis C antibody, hepatitis B surface antigen, haematology [full blood count & differential, and clotting] and biochemistry [creatinine, urea, potassium, sodium, bicarbonate, total cholesterol and triglycerides, calcium, phosphate, liver function tests (alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT)), albumin, glucose, amylase]) and urine testing (drug screen, macro analysis and, for females of childbearing potential, pregnancy testing).

After successful screening, eligible subjects underwent an enhanced ECG assessment one day prior to day 1 (baseline visit) with hourly ECG assessment over a three hour period in order to attain detailed baseline data. Similar ECG assessments were also performed on days 14, 28 and 42 in order to gain detailed information on the impact, if any, of ETR or EFV on ECG activity, particularly QT interval.

Prior to baseline, subjects were randomised 1:1 to arm 1 or arm 2. Subjects in arm 1 were assigned to receive ETR (4 x 100mg tablets) once daily (unlicensed ETR dose) whereas arm 2 received ETR 200mg (2 x 100mg tablets) twice daily (licensed ETR dose) throughout the study. For both arms, subjects received their respective doses of ETR with food daily for 14 days (1-14) followed by 14 days of washout period (15-28). They then commenced EFV 600mg once daily (on an empty stomach) intake and continued for the next 14 days (29-42). ETR was then administered again, at the same dose as in the first phase of the study, for an additional 14 days (43-56). The dosing schedule is summarised in figure 3.1 below.

Figure 3.1: Flowchart summary of drug dosing for EFV to ETR pharmacokinetic study



At each visit subjects underwent the following assessments: review of adverse event and concomitant medications, adherence assessment, vital signs, and blood draw for haematology and fasting biochemistry tests. Additionally urine testing (as per screening visit) was performed on each of the intensive PK days (see below).

After a standardised breakfast, all subjects had serial blood samples for estimation of ETR concentration collected on **days 1, 14, 43, and 56** (intensive PK days 1, 2, 3 and 4, respectively) at the following times:

- Pre-dose
- 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 hours after administration of ETR.
- 24 hour post-dose ETR trough concentration (C_{trough}) in arm 1 only

Samples for the determination of EFV concentrations were collected on:

- **Days 42 and 43**, 12 hours after EFV dosing
- **Daily** after stopping EFV, at the same time each day until **day 56**.

Finally, a follow-up visit was performed 7 to 14 days after the final study assessment (day 56) in order to assess vital signs, adverse events, concomitant medication and haematology/biochemistry.

The safety and tolerability of study medications were evaluated throughout the study using the NIAID Division of AIDS table for grading the severity of adult and paediatric adverse events to characterise abnormal findings (published December 2004). Causality was determined by the principal or a co-investigator (relatedness options: unrelated, unlikely, possible, probable or definite).

3.2.1.3 Pharmacokinetic analysis

Plasma ETR concentrations were measured by the team at The University of Liverpool using a validated high-performance liquid chromatography (HPLC)-tandem mass spectrometry method. In brief, ETR was extracted from heat-inactivated plasma (100mcl; 58^{o}C, 40 minutes) using acetonitrile (500mcl) after the addition of an internal standard (6,7-dimethyl-2,3-di-(2-pyridyl)-quinoxaline; Sigma Chemical Co). After centrifugation, the organic layer was separated and 200mcl water plus 0.05% formic acid added to each tube. Analytes were separated on a Ascentis C18 column (3mcm; 100mm x 2.1mm; Supelco). The HPLC system consisted of a Surveyor AS autosampler, a PDA detector, and LC pump interfaced with an ion trap LCQ Deca XP Plus mass spectrometer with an electrospray ionisation source (Thermo Electron Corporation, Hemel Hempstead, United Kingdom). Recovery of ETR was >90%; the lower limit of quantification was 2497 ng/ml. Intra-assay and inter-assay coefficient of variation at the low, medium and high quality controls were favourable (<11%).

Concentrations of EFV were determined using a validated HPLC method [Almond *et al*. 2005] with a lower limit of 110 ng/ml.

3.2.1.4 Data analysis

ETR maximum concentration C_{max}, time to C_{max} (T_{max}), and trough concentration C_{trough} were derived. C_{max} is considered the pharmacokinetic parameter most correlated with risk of drug toxicity. Area under the curve from 0 to 24 hours (AUC_{0-24}) for once-daily ETR and from 0 to 12 hours (AUC_{0-12}) for twice-daily ETR were calculated using WinNonLin version 5.2 (Mountain View, CA), by non-compartmental linear-linear trapezoidal method. Inter-individual variability in plasma concentrations during drug intake and after drug cessation were assessed by measuring the coefficient of variation (CV = standard deviation/mean x 100). Within-subject changes for ETR concentrations (before and after the 14-day EFV intake period) were assessed by calculating geometric mean ratios (GMRs) and 90% confidence intervals (CIs). The CIs were first determined using logarithms of the individual geometric mean plasma concentration values and then expressed linearly. The changes in pharmacokinetic parameters were considered significant when the CI for the GMR did not cross the value of 1.

Relationships between the weight and BMI and EFV daily concentrations or ETR plasma exposure (expressed as the ratio between AUC before and after 14-day efavirenz intake

period) were assessed by Pearson correlation (the groups were separated into halves by median weight and BMI). Gender differences in drug exposure were calculated using analysis of variance. P values of ≤0.05 were considered statistically significant. Statistical analysis was performed using SPSS (version 16.0, SPSS Inc Headquarters, Chicago, IL).

Adverse events were collated and described in a qualitative manner.

3.2.2 HIV-positive patient study

3.2.2.1 Subject selection & screening

HIV-1-infected adults who had received at least 12 weeks of EFV plus 2 NRTI with ongoing CNS symptoms were recruited from three sites (see section 2.2.2). Patients were required to have an undetectable plasma viral load (less than 50 copies/ml) and CD4 cell count greater than 50 cells/mm^3 at screening. Exclusion criteria included previous exposure to ETR or RPV, a serious medical or psychiatric condition (including hypnotic and anti-depressant use), acute viral hepatitis, an active AIDS-defining illness, significant laboratory abnormality, disallowed concomitant medication (as per the summary of product characteristics for EFV and ETR), resolution of CNS toxicity between screening and baseline, and patients who were pregnant or breast-feeding. All subjects provided written, informed consent as previously described.

Other inclusion and exclusion criteria are listed below:

Inclusion criteria:

1. Receiving stable ART, with two NRTI + EFV, for at least 12 weeks
2. Symptomatic toxicity associated with the EFV after at least 12 weeks
3. If female and of childbearing potential, the subject must be using effective birth control methods during and for least 30 days after the end of the trial:
    a. Double barrier method
    b. Hormonal method plus a barrier contraceptive
    c. Intrauterine device (IUD) plus a barrier contraceptive **or**
    d. No heterosexual sex, or vasectomised partner with confirmed sterility
4. If heterosexually active male, the subject must use effective birth control during and for at least 30 days after the end of the trial

Exclusion criteria:

1. Primary HIV-1 infection
2. HIV-2 infection
3. Life expectancy <6 months according to the investigator's judgement
4. Chronic hepatitis B and/or C with AST and/or ALT > 5 x upper limit of normal
5. Any investigational drug within 30 days prior to the trial drug administration
6. Clinically significant allergy or hypersensitivity to any of the excipients of the investigational medications
7. Any grade 3 or grade 4 toxicity except for:
    a. Grade 3 glucose elevation
    b. Asymptomatic grade 3 pancreatic amylase elevation
    c. Asymptomatic grade 3 cholesterol or grade 4 triglyceride elevation
8. Clinical or laboratory evidence of significantly decreased hepatic function
9. Prior exposure to ETR, TMC120, or rilpivirine

3.2.2.2 Study methodology

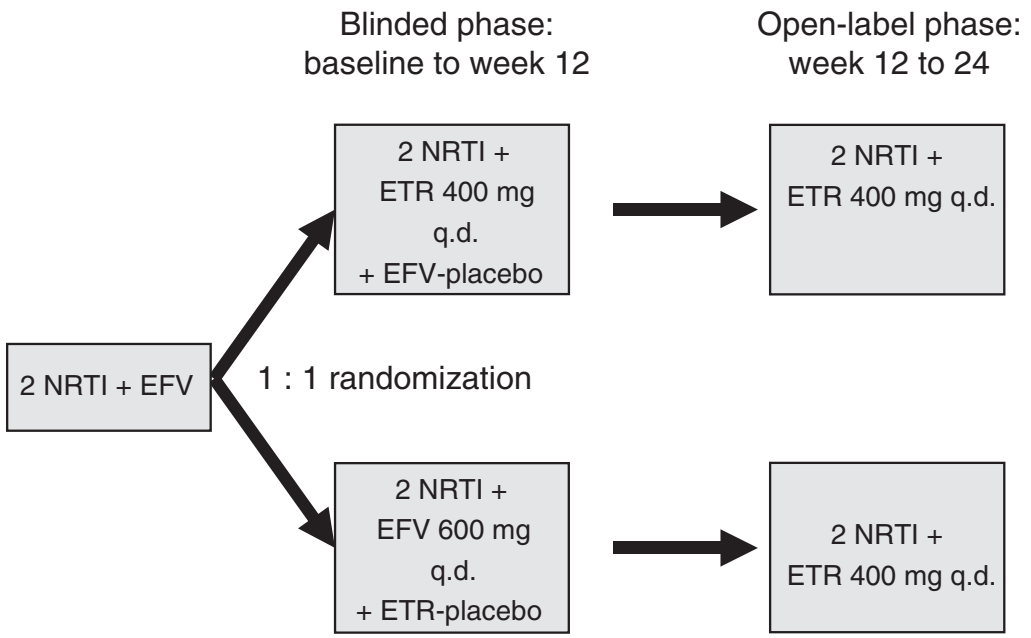
The primary endpoint of this study was change in the proportion of patients experiencing grade 2–4 CNS toxicity from baseline to week 12. Secondary endpoints were as follows:

1. Change in CNS score
    a. At week 12 and week 24
    b. Combined (immediate switch and delayed switch) after 12 weeks on ETR
2. Median number of grade 2 – 4 CNS adverse events
    a. At week 12 and week 24
    b. Combined (immediate switch and delayed switch) after 12 weeks on ETR
3. The rate of neuropsychiatric and CNS toxicity after 12, 16 and 24 weeks as measured by the change from baseline in the HADS
4. Change from baseline in adherence at weeks 12, 16 and 24 as measured by the adherence questionnaire: MMASRI
5. Viral suppression at week 12 and week 24
6. CD4 change at week 12 and week 24
7. Fasting lipids
8. Safety and non-CNS tolerability

The study comprised of two phases as outlined in figure 2. Eligible patients were randomized at baseline to one of two arms:

1. Immediate switch: 2 NRTI + ETR 400mg QD + EFV-placebo
2. Delayed switch: 2 NRTI + EFV 600 mg QD + ETR- placebo

Figure 3.2: EFV to ETR switch study design



At each study visit patients underwent:

- Assessment of vital signs
- Urine testing:
    - Macro- analysis
- Blood testing:
    - Plasma HIV-RNA (Chiron bDNA)
    - CD4/8 cell count and percentage
    - Biochemistry (hepatic, renal and bone profiles)
    - Haematology (full blood count and differential)
    - Fasting lipids (total, HDL and LDL cholesterol, total: HDL cholesterol ratio and triglycerides) and glucose
- Assessment of concomitant medications, symptoms, adverse events and symptom-directed physical examination
- Questionnaires:
    - CNS symptoms
    - HADS

- M-MASRI

Adverse events were based on the summary of product characteristics and local clinical experience; these were documented at each visit and graded in a manner based on the to the ACTG DAIDS scale (2004) – see section 2.5.

The following CNS adverse events were investigated:

1. Dizziness
2. Depression
3. Insomnia
4. Anxiety
5. Impaired concentration
6. Headache
7. Somnolence
8. Fatigue
9. Abnormal dreams
10. Nervousness
11. Hallucinations

Study participants were specifically questioned by a co-investigator about each of the adverse events listed above. Adverse events were then graded on a four-point scale as described in section 2.5; essentially 0 = absent, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe.

CNS adverse events were described as the proportion of patients with any grade 2–4 adverse events and individual grade 2–4 adverse events; the median number of grade 2–4 CNS adverse event was also calculated. A 'CNS score' was calculated based on the sum total of all grades of CNS adverse events; for example, a patient with grade 1 depression, grade 3 anxiety, grade 2 insomnia and no other CNS adverse events would have a CNS score of 6.

3.2.2.3 Data analysis

All data analyses were performed in SAS V9 (SAS Institute Inc., Cary, North Carolina, USA). Quantitative data with hypergeometric distribution was presented as medians with interquartile ranges (IQRs); qualitative data have been presented as proportions. Between-

group quantitative comparisons were analysed with Mann– Whitney U test, between-groups qualitative comparisons with chi-squared testing (and Yates' correction for small numbers). Within-participant changes over time were analysed using: Wilcoxon signed-rank test (quantitative data) or McNemar's chi-squared test (qualitative data). Due to the relatively small sample size, P values are presented as Yate's corrected; all P values are two-tailed.

3.3 Results

3.3.1 Healthy volunteers

Twenty-five subjects (12 in arm 1 and 13 in arm 2) completed the study. Median (range) age, weight and BMI were 45 (19-61) years, 73 (54-116) kg, and 25 (19-32) kg/m^2. Nine of the 25 subjects (36%) were female and on terms of ethnicity, 18 were white, 4 were of Asian origin and 3 were black.

3.3.1.1 Pharmacokinetics

ETR pharmacokinetic parameters before and after the EFV intake period at initiation (day 14 and day 56) are shown in table 3.1.

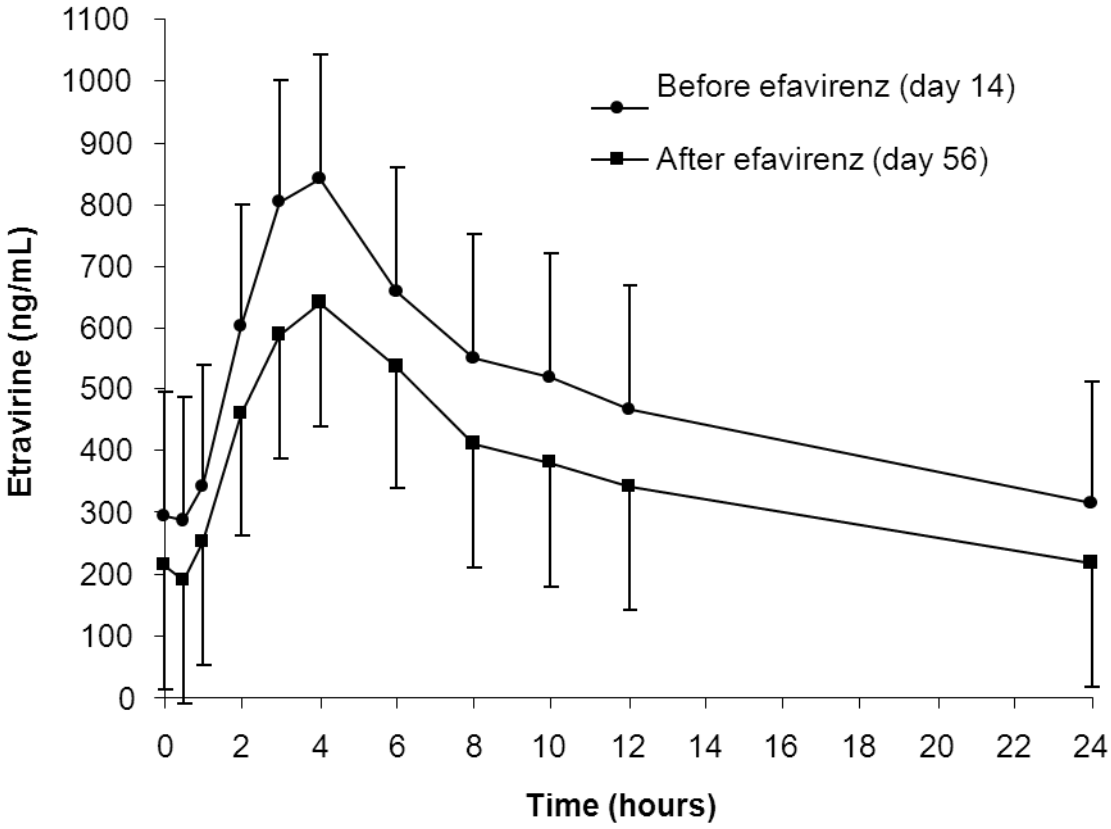
Table 3.1: ETR pharmacokinetic parameters measured before (days 1 and 14) and after (days 43 and 56) the EFV intake period

	Etravirine PK parameter	Before EFV GM (90% CI)	After EFV GM (90% CI)	GMR (90% CI)	Before EFV GM (90% CI)	After EFV GM (90% CI)	GMR (90% CI)
		Day 1	*Day 43*	*Day 1/Day 43*	*Day 14*	*Day 56*	*Day 14/Day 56*
Arm 1400 mg OD	AUC_{0-24h} (ng.h/mL)	2996 (2626-4070)	2245 (1899-3171)	**0.74** (0.66-0.83)	11064 (9741-14077)	7677 (6373-11134)	**0.71** (0.62-0.81)
	C_{max} (ng/mL)	385 (345-476)	293 (246-405)	**0.78** (0.76-0.90)	863 (796-972)	676 (597-832)	**0.78** (0.70-0.86)
	C_{trough} (ng/mL)	41 (36-67)	31 (26-55)	**0.71** (0.59-0.85)	270 (243-389)	175 (142-296)	**0.67** (0.49-0.91)
	CL/F_{0-24h} (L/h)	132 (116 – 177)	172 (155 – 221)	**1.32** (1.17 – 1.48)	36 (32 – 43)	52 (47 – 67)	**1.44** (1.28 – 1.62)
		Day 1	*Day 43*	*Day 1/Day 43*	*Day 14*	*Day 56*	*Day 14/Day 56*
Arm 2 200 mg BID	AUC_{0-12h} (ng.h/mL)	1399 (1259-1777)	1090 (930-1452)	**0.78** (0.66-0.92)	8756 (7755-10848)	6481 (5881-7615)	**0.72** (0.63-0.81)
	C_{max} (ng/mL)	286 (250-372)	201 (168-277)	**0.70** (0.57-0.86)	1001 (892-1219)	816 (744-949)	**0.79** (0.70-0.90)
	C_{trough} (ng/mL)	52 (48-74)	34 (30-51)	**0.66** (0.51-0.85)	596 (528-769)	395 (354-486)	**0.63** (0.54-0.73)
	CL/F_{0-12h} (L/h)	140 (121 – 187)	180 (163 – 221)	**1.28** (1.09 – 1.51)	23 (21 – 28)	31 (28 – 37)	**1.39** (1.23 – 1.58)

Arm 1 and arm 2 steady-state ETR concentrations before and after EFV intake period are illustrated in Figure 3.3. EFV concentrations after drug cessation are shown in figure 3.4.

Figure 3.3: Mean steady-state ETR plasma concentrations before (day 14) and after (day 56) EFV intake period. Bars indicate standard deviation (SD).

A – Arm 1, ETR 400mg once daily (n=12)



B – Arm 2, ETR 200mg twice daily (n=13)

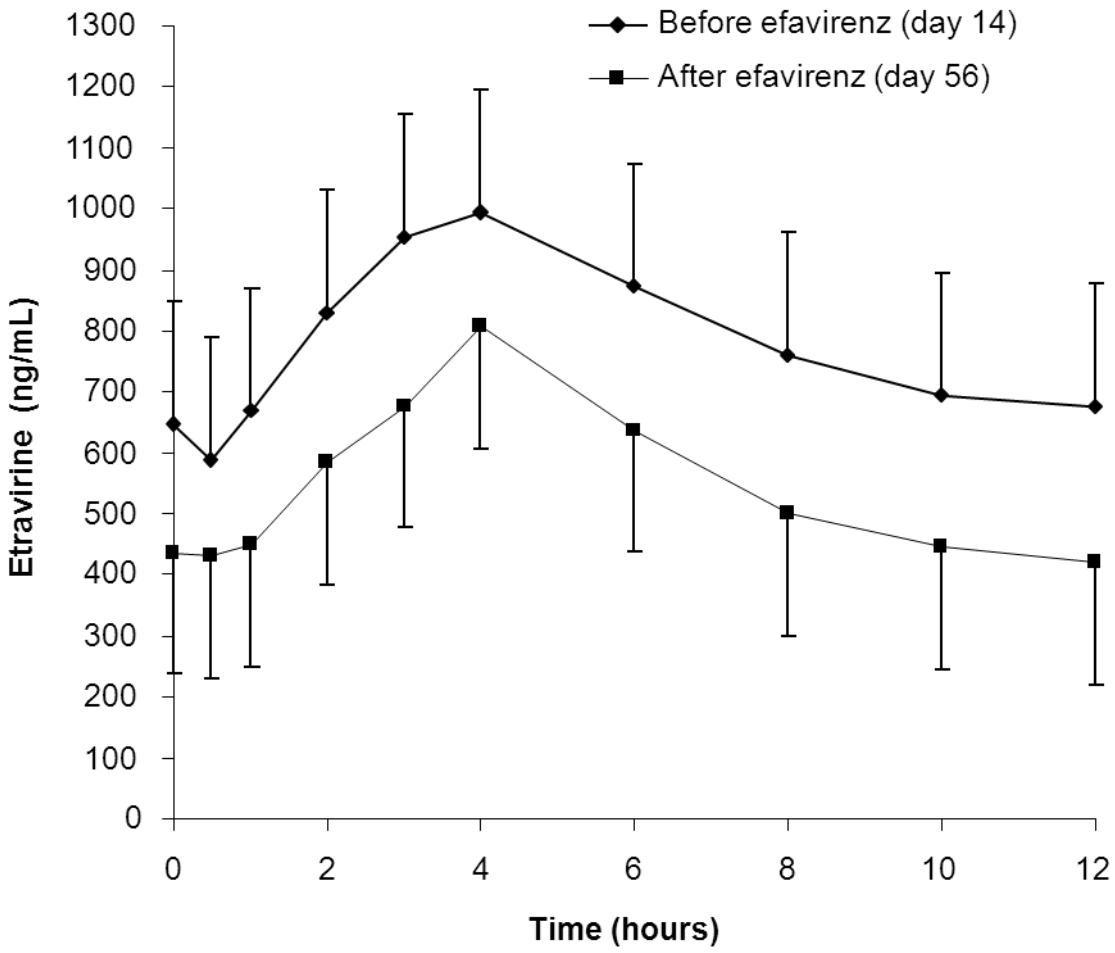
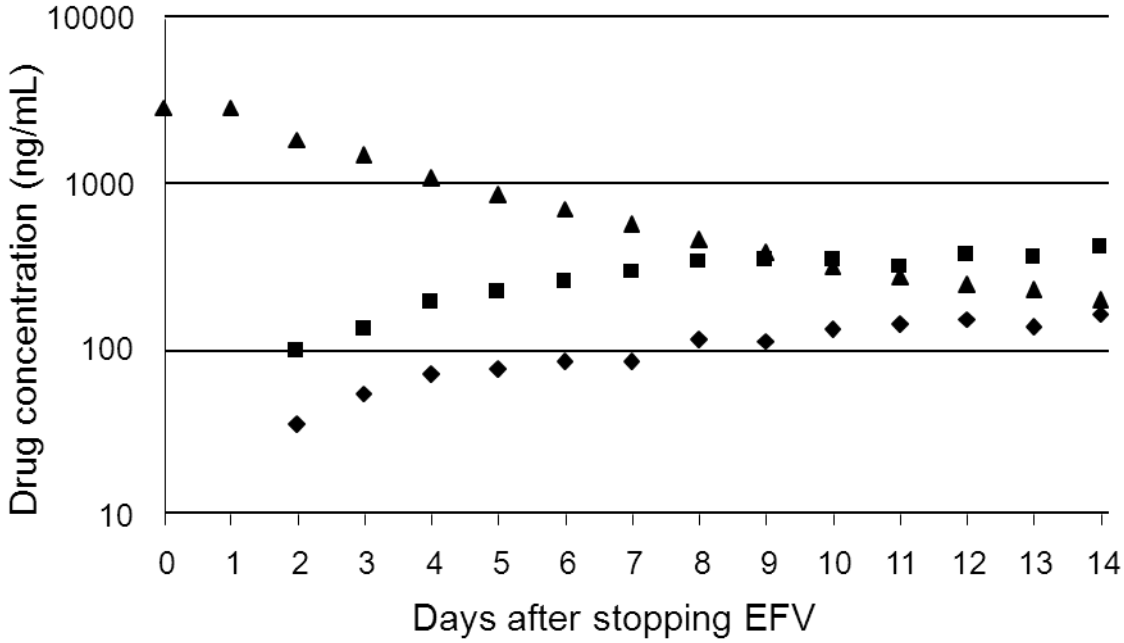


Figure 3.4: ETR (squares: twice daily; diamonds: once daily) trough concentrations and EFV (triangles) daily concentrations after stopping EFV intake and restarting etravirine



Although weight and BMI did not correlate with the ratio (before:after EFV) of ETR AUC, C_{trough}, or C_{max}, a significant effect of gender on the ratio of ETR AUC and C_{trough} was observed at steady state (day 14:day 56). A decrease in AUC of 26.5% in males versus 7.5% in females (P=0.050) and 35% decrease in C_{trough} in males versus 2.4% in females (P=0.017) was apparent.

ETR 400mg Once Daily (Arm 1)

Steady-state (GM, 90% CI) C_{max}, C_{trough} and AUC_{0-24} were 863 (796 to 972) ng/mL, 270 (243 to 389) ng/mL and 11,064 (9741 to 14,077) ng/h/mL before EFV intake and 676 (597 to 832) ng/mL (22% lower), 175 (142 to 296) ng/mL (33% lower) and 7677 (6373 to 11,134) ng/h/mL (29% lower) after the EFV intake period.

Steady-state ETR CV for C_{max}, C_{trough} and AUC_{0-24} were 23%, 53% and 41% before EFV intake and 37%, 80% and 62% after the EFV intake period.

ETR 200mg Twice Daily (Arm 2)

Steady-state (GM, 90% CI) C_{max}, C_{trough} and AUC_{0-12} were 1001 (892 to 1219) ng/mL, 596 (528 to 769) ng/mL, and 8756 (7755 to 10,848) ng/h/mL before EFV intake and 816 (744 to 949)

ng/mL (21% lower), 395 (354 to 486) ng/mL (37% lower), and 6481 (5881 to 7615) ng/h/mL (28% lower) after the EFV intake period.

Steady-state ETR CV for C_{max}, C_{trough} and AUC_{0-12} were 35%, 42%, and 38% before EFV intake and 27%, 36%, and 29% after the EFV intake period.

EFV Pharmacokinetics

EFV intake was stopped on day 42 after a 14-day treatment period. Terminal half-life (median, range) for subjects enrolled in arm 1 was 83 (45-183) hours and 64 (30-185) hours for subjects in arm 2. All subjects had detectable EFV concentrations 7 days after stopping intake (day 50). In 5 subjects (3 in arm 1), concentrations measured above 1000ng/ml (the lower limit plasma concentration of the therapeutic range for EFV) on day 50. Of these 5 participants with therapeutic EFV concentrations at day 50:

- Two (1 in each arm) weighed less than 60kg
- Three were female
- Three were of white origin and 2 were Asian

EFV concentrations after EFV cessation (ie during the FEV decay period) are described, by gender, in table 3.2.

Table 3.2: Median (range) EFV concentrations 3, 7, 10 and 13 days after stopping EFV intake (study days 46, 50, 53 and 56) in male and female subjects

Days after EFV cessation	Males (n=16)	Females (n=9)
3	672 (398-4736) ng/ml)	1349 (403-6580) ng/ml
7	339 (110-3020) ng/ml	454 (131-3857) ng/ml
10	166 (110-1982) ng/ml	292 (110-3411) ng/ml
13	120 (110-1605) ng/ml	171 (110-2241) ng/ml

After adjusting for age, BMI, height, and treatment arm, there was no significant effect of gender on EFV concentrations.

Inter-individual variability of EFV concentrations after drug withholding was very wide with a CV of 97% on day 46 (3 days post EFV cessation), 127% on day 50 (7 days post EFV cessation), and 159% on day 53 (10 days post EFV cessation).

EFV concentrations were negatively correlated with ETR AUC and C_{trough} ratios on corresponding days after and before the efavirenz intake period. Correlation coefficients (P values) for EFV concentrations on days 50 and 56 and ETR AUC ratio at steady state (AUC day 56/AUC day 14) were -0.48 (P=0.032) and -0.45 (P=0.049), respectively.

3.3.1.2 Safety and tolerability

The study drugs were well tolerated, and no grade 2-4 adverse events or laboratory abnormalities were reported.

3.3.2 HIV-positive patients

Thirty-eight men were enrolled with a median age was 43 years (range 26 – 64) and a median duration of EFV exposure of 21.4 months (range 3.5 – 117.5 months). Median baseline CD4 cell count was 510 cells/mm^3 (IQR 365 – 611 cells/mm^3) and plasma viral load was less than 50 copies/ml in all patients at both the screening and baseline visits. Twenty patients were randomized to the immediate switch arm and 18 to delayed switch.

NRTI backbones at baseline were:

- Truvada (TDF/FTC fixed-dose combination)
    - 60% immediate switch arm (50% as Atripla)
    - 61% delayed switch arm (50% as Atripla)
- Kivexa (ABC/3TC fixed-dose combination)
    - 35% immediate switch arm
    - 22% delayed switch arm
- ABC/TDF
    - 5% immediate switch arm
    - 11% delayed switch arm
- TDF/3TC
    - 6% delayed switch arm

None of the differences in backbone use between the two study arms were statistically significant. Patient disposition is described in table 3.3.

Table 3.3: Patient disposition

	Immediate Switch	Delayed Switch
Baselined	20	18
Completed	19	13
Lost to Follow-up	1	2
Withdrawal of consent	0	2
Adverse Event	0	1
Virological Failure	0	0

3.3.2.1 Central nervous system symptoms

Rates of baseline CNS toxicity were similar in the two arms (table 4); 90% in the IS arm and 88.9% in the DS arm had at least one grade 2-4 CNS AE and the frequency of individual grade 2-4 CNS AE were similar with the exception of insomnia (75% in the IS arm compared with 39% in the DS arm; p=0.024). Baseline CNS score was 14 (IQR 6-17.5) in the IS arm and 10 (IQR 5.5-17.5) in the DS arm; this difference was not significant (p=0.534).

Table 3.4: Baseline CNS toxicity

Grade 2-4 CNS conditions at baseline	N=20 N (%;95% CI)	N=18 N(%;95% CI)	p-value
Proportion with any Grade 2-4 CNS AE	18 (90.0%; 68.3-98.8%)	16 (88.9%; 65.3-98.6%)	0.676
Dizziness	2 (10.0%)	3 (16.7%)	0.544
Depression	7 (35.0%)	6 (33.3%)	0.914
Insomnia	15 (75.0%)	7 (38.9%)	0.024
Anxiety	7 (35.0%)	5 (27.8%)	0.633
Pain	2 (10.0%)	1 (5.6%)	0.612
Impaired concentration	7 (35.0%)	4 (22.2%)	0.386
Headache	2 (10.0%)	4 (22.2%)	0.302
Somnolence	8 (40.0%)	6 (33.3%)	0.671
Fatigue	9 (45.0%)	8 (44.4%)	0.973
Abnormal dreams	10 (50.0%)	12 (66.7%)	0.299
Nervousness	5 (25.0%)	4 (22.2%)	0.841
Hallucinations	1 (5.0%)	1 (5.6%)	0.939

At week 12 (table 3.5) the proportion of subjects with grade 2-4 CNS AE (primary endpoint) had declined to 60% in the IS arm and remained unchanged (81.3%) in the DS arm (p=0.041 and p=0.999 compared with baseline, respectively). In terms of individual grade 2-4 CNS AE there were no significant changes in the DS arm; the proportion experiencing abnormal dreams declined significantly in the IS arm (from 50% at baseline to 20% at week 12;

p=0.041) and was significantly less than in the DS arm (62.5%; p=0.009). The median number of grade 2-4 CNS AE at baseline was 4 in the IS arm and 3 in the DS arm and at week 12 this had changed to 1.5 in the IS arm (p=0.003) and was unchanged in the DS arm. Finally, CNS score declined from 14 at baseline to 6 at week 12 in the IS arm (p=0.001) and from 10 to 7.5 in the DS arm (p=0.192).

Table 3.5: Rates of Grade 3-4 CNS events at week 12 and week 24 by study arm (p-values refer so difference at each time point compared with event rates at baseline)

	Week 12		Week 24	
	IS (n=20) n(%)	DS (N=16) n(%)	IS (n=20) n(%)	DS (n=15) n(%)
Overall CNS events	12 (60%) 95% CI 36-81%	13 (81.3%) 95% CI 54-96%	14 (70%) 95% CI 46-88%	9 (60%) 95% CI 32-84%
Dizziness	3 (15%) p=0.999	3 (18.8%) p=0.618	3 (15%) p=0.999	0 p=0.248
Depression	4 (20%) p=0.371	3 (18.8%) p=0.372	5 (25%) p=0.724	5 (33%) p=0.683
Insomnia	10 (50%) p=0.074	7 (43.8%) p=0.999	7 (35%) p=0.013	3 (20%) p=0.371
Anxiety	5 (25%) p=0.617	7 (43.8%) p=0.617	5 (25%) p=0.683	4 (26.7%) p=0.999
Impaired concentration	6 (30%) p=0.999	5 (31.3%) p=0.999	7 (35%) p=0.617	4 (26.7%) p=0.999
Headache	1 (5%) p=0.999	4 (25%) p=0.617	3 (15%) p=0.999	4 (26.7%) p=0.999
Somnolence	6 (30%) p=0.683	5 (31.3%) p=0.999	4 (20%) p=0.134	6 (40%) p=0.999
Fatigue	7 (35%) p=0.480	7 (43.8%) p=0.999	5 (25%) p=0.134	6 (40%) p=0.999
Abnormal dreams	4 (20%) p=0.041	10 (62.5%) p=0.724	2 (10%) p=0.027	3 (20%) p=0.077
Nervousness	2 (10%) p=0.371	4 (25%) p=0.617	3 (15%) p=0.617	1 (6.7%) p=0.371
Hallucinations (auditory)	0 p=0.999	1 (6.7%) p=0.480	0 p=0.999	0 p=0.999

In the IS arm there was no further significant change in overall or individual grade 2-4 CNS AE from week 12 to week 24 (p-values for week 12 vs week 24 not presented). In the DS arm there was a numerical reduction in proportion of subjects with any grade 2-4 CNS AE in the DS arm from week 12 to week 24 although this was not statistically significant; there was,

however, a significant reduction in abnormal dreams in DS arm from week 12 to week 24 (p=0.023). In terms of median number of grade 2-4 CNS AE at week 24 there was a further small reduction compared with week 12 from 1.5 to 1 in the IS arm (p=0.085) and a decline from 3 to 1 in the DS arm (p=0.003).

Combined analyses were performed to calculate the change in CNS AE after 12 weeks of ETR; changes from baseline to week 12 in the IS arm and from week 12 to week 24 in the DS arm were combined for statistical analysis. After 12 weeks of ETR there were significant reductions in the proportion of subjects reporting any grade 2-4 CNS AE (89% to 60%; p=0.009), grade 2-4 insomnia (63% to 37%, p=0.016), abnormal dreams (57% to 20%, p=0.001) and nervousness (29% to 9%, p=0.046) compared with baseline. Combined change in other CNS events was not significant. Median number of grade 2-4 CNS AS declined from 3 at baseline to 1 at 12 weeks (p<0.001) and CNS score from 10 to 7 (p=0.001).

3.3.2.2 Hospital anxiety & depression scale (HADS)

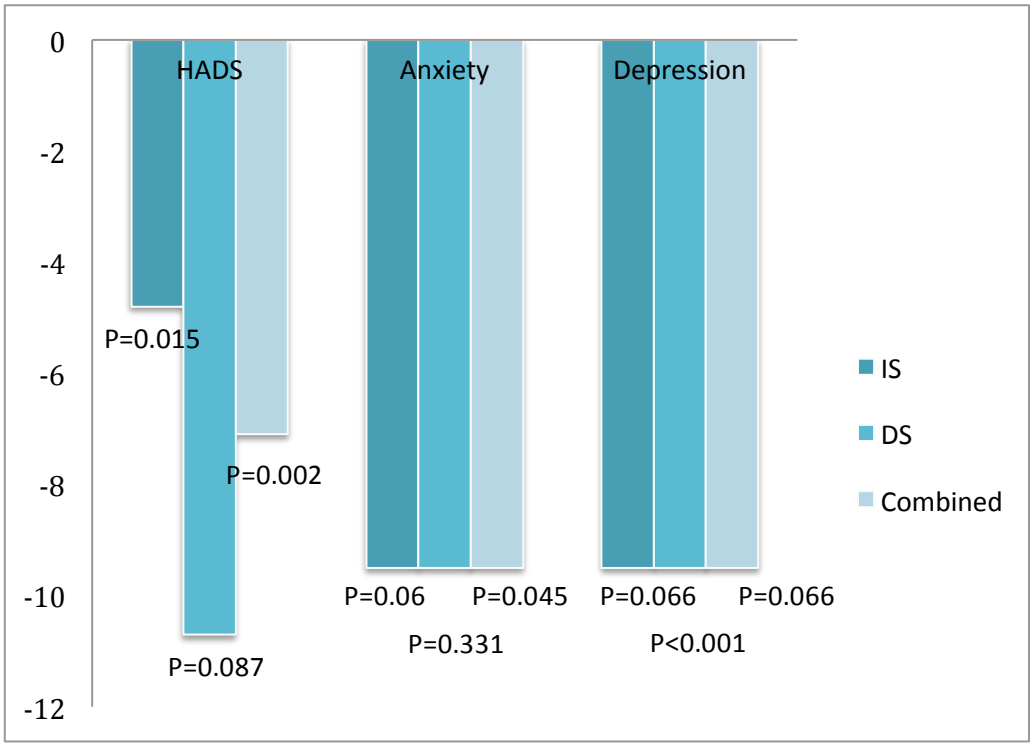
HADS score (total and as separate anxiety and depression scores) was similar prior to switch to ETR in both arms (table 3.6).

Table 3.6: Baseline HADS score in IS and DS arms

	IS Arm; Median (IQR)	DS Arm; Median (IQR)	*p*-value
Total HADS score	31.0 (19.0-50.0)	39.3 (15.5-47.6)	0.791
Anxiety score	35.7 (23.8-52.4)	28.6 (14.3-52.4)	0.525
Depression score	31.0 (9.5-42.9)	38.1 (9.5-47.6)	0.799

Switching EFV to ETR led to improvements in HADS, for total and individual anxiety/depression scores. These are illustrated for each arm (change in score from baseline to week 12 of ETR) and for both arms combined. Improvements were statistically significant for overall HADS score in the IS arm and for overall HADS score, anxiety score and depression score in the combined analysis (figure 3.5).

Figure 3.5: Graph summarising changes in HADS score (total, anxiety & depression) after 12 weeks of ETR therapy.



3.3.2.3 Safety and tolerability

There were two new grade 3/4 adverse events: one patient in the DS arm developed grade 3 flatulence (deemed probably drug-related) during the blinded phase (ie on EFV with ETR placebo) and one patient in the IS arm was hospitalised during the blinded phase with a viral upper respiratory tract infection (grade 4 [serious adverse event]; not drug-related. Patient subsequently experienced full recovery).

All other adverse events were grade 2 or less. Grade 2 adverse events deemed at least probably study drug related are outlined in table 3.7.

*Table 3.7: Grade 2 adverse events (probably/definitely drug related) by study arm (*occurring between baseline and week 12; ** occurring between week 12 and week 24).*

	IS (n=20) n (%)	DS (n=18) n (%)	*p*-value
Fatigue*	4(20.0)	4(20.0)	0.999
Headache**	1(0.0)	3(16.7)	0.097
Reduced libido*	1(5.0)	0(0.0)	0.998

There were no changes in liver function tests, no grade 2 or greater nausea and no cases of rash at any time point in the study.

Lipid changes are illustrated table 4. Analysing the two arms of the study individually, there was a significant reduction in total cholesterol in each arm after 12 weeks of ETR (-0.64 mmol/l in the IS arm and -0.63 mmol/l in the DS arm; p=0.017 and p=0.024, respectively). In addition there was a significant reduction in LDL-cholesterol in the IS arm after 12 weeks of ETR (-0.74mmol/l; p=0.043). There were no significant changes in HDL-cholesterol or triglycerides.

When the changes after 12 weeks of ETR in each arm were combined (ie the change from baseline to week 12 in the IS arm and from week 12 to week 24 in the DS arm) reductions in total cholesterol and LDL-cholesterol were significant at -0.64mmol/l ($p<0.001$) and -0.58mmol/l ($p=0.021$), respectively.

In terms of lipid-lowering agents, at baseline 3 participants in the IS arm were on a statin. In the DS arm 2 patients were receiving a statin at baseline and 1 was taking fish oil supplements. Lipid-lowering drug use remained stable during the study and no new agents were started.

Table 3.8: Lipid changes (mmol/l) after 12 weeks of ETR-based HAART (from baseline to week 12 in IS arm and from week 12 to week 24 in DS arm); note not all patients underwent triglyceride and LDL-cholesterol measurement (see numbers).

		IS Arm	DS Arm	Total
No.	Baseline	20	18	38
	Week 12	20	16	36
	Week 24	20	15	35
Total Cholesterol (mmol/l)	Mean	5.33	5.26	
	Change at W12 (SD)	-0.64 (1.09)	-0.63 (0.97)	-0.64 (1.02)
	p-value	**p=0.017**	**p=0.024**	**p<0.001**
HDL-cholesterol (mmol/l)	Mean	1.34	1.09	
	Change at W12 (SD)	-0.05 (0.24)	-0.04 (0.17)	-0.04 (0.21)
	p-value	**p=0.342**	**p=0.433**	**p=0.212**
LDL-cholesterol (mmol/l)	Number	13	9	22
	Mean	3.58	3.41	
	Change at W12 (SD)	-0.74 (1.18)	-0.34 (0.95)	-0.58 (1.09)
	p-value	**p=0.043**	**p=0.311**	**p=0.021**
Triglycerides (mmol/l)	Number	17	15	32
	Mean	1.45	1.73	
	Change at W12 (SD)	-0.10 (0.59)	-0.30 (0.64)	-0.19 (0.62)
	p-value	**p=0.517**	**p=0.096**	**p=0.092**

3.3.2.4 Immunological and virological efficacy

All subjects on study maintained viral suppression (less than 50 copies/ml) at all visits. By on-treatment analysis viral suppression rates were 100% at weeks 12 and 24 for both treatment arms.

3.3.2.5 Adherence

Using M-MASRI, adherence (proportion of prescribed doses taken in last 30 days) was high at all time points (median 100%) and there were no significant differences between or within arms at any visit.

Week 12 CD4 rise from baseline was +40 cells/mm3 in the IS arm and +6 cells/mm3 in the DS arm; at week 24 CD4 rises from baseline were +68 cells/mm3 and + 60 cells/mm3, respectively. In the combined analysis the median CD4 rise after 12 weeks of ETR was +43 cells/mm3 (p=0.027 compared with baseline).

3.4 Discussion

3.4.2 Healthy volunteers

This study investigated the pharmacokinetics of ETR, administered 400mg once daily or 200mg twice daily, before and after a 14-day EFV intake period. This is the first data describing the pharmacokinetics of ETR after a switch from EFV. However, Winston *et al* [2004] investigated the effect of a direct switch from EFV to NVP on the pharmacokinetics of NVP initiated at either 200mg once daily or 200mg twice daily in HIV-positive adults. In that study, NVP concentrations were transiently sub-therapeutic when initiated at the once daily dose (the usual recommended lead-in dose when initiating NVP). Therefore, the authors recommended starting NVP at the full dose of 200mg twice daily when switching from EFV.

In our study, the decrease in ETR pharmacokinetic parameters after EFV were modest (28-29% reduction in AUC). These changes are unlikely to be clinically significant as all subjects had ETR concentrations well above the median protein binding adjusted EC50 of 4ng/ml (concentration required to inhibit 50% of viral replication in vitro) [Vingerhoets *et al*. 2010] at all measurements. Our group previously described high rates of efficacy when ETR 200mg twice daily and DRV/r were co-administered in patients with extensive resistance, despite a 30% decrease in ETR pharmacokinetic parameters (compared with historical controls) [Winston *et al*. 2007]. In addition, in large randomised trials (DUET 1 and DUET 2) twice-daily ETR provided an additional and significant antiviral effect versus placebo despite concomitant administration of a boosted protease inhibitor [Katlama *et al*. 2009]. Furthermore, in a pooled analysis of the DUET studies, no association between ETR pharmacokinetics and 48-week efficacy (viral load < 50 copies/ml) was found [Kakuda *et al*. 2010]. In summary we observed reductions in ETR concentrations of a similar magnitude to those observed when ETR is administered with a boosted PI; high rates of efficacy have been observed when ETR and a PI/r are given together. In addition, previous studies have not shown a correlation between ETR concentrations and virological response and all subjects in our study had therapeutic ETR concentrations at all measurements leading us to conclude that the impact of EFV on ETR concentrations is not clinically significant.

In the present study, EFV persisted for several days after stopping it and showed highly variable pharmacokinetics. Single nucleotide polymorphisms influencing EFV pharmacokinetics have been shown to be associated with excessive EFV exposure after treatment discontinuation in some individuals [Ribaudo *et al*. 2006]; these polymorphisms may, at least partly, explain the variability we observed.

In terms of the relationship between EFV and ETR pharmacokinetics, subjects with higher EFV concentrations persisting after the switch had greater proportional decrease in ETR concentrations. A possible explanation is that the degree of induction of the enzyme responsible for the metabolism of ETR (CYP3A4) is dependent on systemic EFV exposure. In an earlier study in healthy volunteers, increasing doses of EFV resulted in greater induction of hepatic CYP3A4 [Mouly *et al*. 2002]. This suggests that individuals with extremely high EFV concentrations for prolonged periods after stopping drug intake (ie. slow EFV metabolisers) may be at greater risk of suboptimal ETR exposure. Identification of patients with very high EFV concentrations may be of clinical value but is not undertaken routinely.

Switching from EFV to ETR is feasible, well tolerated and no clinically significant changes in ETR concentrations were observed. We conclude that ETR dose adjustment is not necessary when switching directly from EFV to ETR, whether ETR is used once- or twice-daily. This is likely to be particularly safe when switching virologically suppressed patients; less 'potent' drug options such as PI/r monotherapy and NRTI-only regimens that are suboptimal for initial therapy yield much higher efficacy rates as switch options during viral suppression [Ghosn *et al*. 2010; Nunes *et al*. 2009]. This study supports the investigation of ETR as a switch option for individuals experiencing EFV-related toxicity.

3.4.3 HIV-positive individuals

This study demonstrated an improvement in several measures of CNS toxicity when switching from EFV to ETR in patients stable on an EFV-based regimen. All subjects were required to have been on EFV for at least 12 weeks but the median EFV exposure at study entry was much longer (21.4 months). This data further confirms the persistence of CNS toxicity in some EFV-treated patients as demonstrated in other studies and cohorts.

Although the study was open to male and female patients, by chance all recruited subjects were male; this was consistent with the patient demographics across the three sites. The study population reported high rates of CNS toxicity at baseline; majority of individuals had at least one grade 2-4 CNS AE and a median of three grade 2-4 CNS AE in total. By definition our study selected patients with persistent CNS toxicity and it is possible that the time commitment required of participants, and the higher pill burden (6 pills or more during the blinded phase), further selected individuals with more severe toxicity (ie those patients who had a greater reason to switch to a higher pill burden regimen). The prevalence of CNS toxicity cannot therefore be applied to the general clinic population.

Switching to ETR most improved sleep-related CNS AE (abnormal dreams and insomnia), as well as nervousness, but yielded no significant improvement in other CNS AE. The study was not powered to detect significant differences for each of the individual AE. It may be that individuals with other possible EFV-related CNS AE, such as depression, had already switched away to alternative regimens or were less likely to enter the study. Alternatively, some CNS symptoms on stable EFV regimens may be more likely secondary to other factors and not drug-related. Although the difference in baseline CNS score between the two arms was not statistically significant it was numerically higher in the IS arm; we cannot exclude that improvement in the CNS score at week 12 in the IS arm was secondary to regression to the mean rather than an effect of treatment switch.

This study also provides limited data supporting the use of ETR as a QD agent. Once daily dosing is unlicensed but supported by the pharmacokinetic profile of ETR [Scholler-Gyure, Kakuda, De Smedt *et al*. 2007; Boffito *et al*. 2009]. No virological failures occurred in the 19 and 15 subjects completing 24 and 12 weeks of once daily ETR-based HAART, respectively. However, the study population was small and all subjects were virologically suppressed, most for more than 48 weeks, prior to switching to ETR. These results cannot necessarily be extrapolated to patients with viraemia. Additionally all subjects had baseline resistance tests demonstrating full NNRTI susceptibility; switching to QD ETR may not be appropriate for subjects with any evidence of NNRTI resistance.

In addition we described an improvement in lipids with significant reductions in total and LDL-cholesterol after 12 weeks of ETR. EFV is known to be associated with lipid changes [Haubrich *et al*. 2009]; ETR, like other new agents including maraviroc [DeJesus *et al*. 2008]

and raltegravir [Lennox *et al*. 2009; Eron *et al*. 2010], is associated with better lipid parameters and may be a switch option for metabolic reasons. However, we did not collect data on cholesterol ratio ad as such were unable to estimate the impact of these lipid changes on estimated cardiovascular risk. The clinical relevance of these changes, if any, is likely very small.

Finally, although most patients were on 1-2 tablets once daily at baseline, and switch to ETR increased pill burden to at least 5 tablets once daily (more during the blinded phase), adherence was very high and unchanged throughout the study.

Limitations of this study include the small sample size and the highly selected patient group. In particular the analysis of individual symptoms should be interpreted with caution as the study was not powered for these comparison. The results may not be generalisable to individuals with less marked or less persistent CNS toxicity. For some parameters (insomnia and nervousness), reductions in symptoms reached statistical significance only when the changes after 12 weeks of ETR in each arm were combined; as this was blinded for the immediate switch arm but not for the delayed switch this could limit the accuracy of our results. By limiting ourselves to detailed questions on 12 classic EFV side effects only we may have missed other changes (positive or negative) associated with switch to ETR. Although adherence remained very high during the trial the impact of switching to a higher pill burden regimen on longer-term adherence is not clear. Finally we did not collect data on quality if life or activities if daily living so do not know if the observed improvements in CNS toxicity were associated with functional improvements also.

Proactive switch away from EFV may yield significant reductions in CNS toxicity. Patients can be reassured that, in the absence of noticeable improvement, they can resume an EFV-containing regimen.

In conclusion, once daily etravirine offers an efficacious, tolerable and lipid-friendly alternative to efavirenz in patients with persistent CNS toxicity.

Chapter 4:

The evolution of co-receptor tropism during viral suppression

&

The pharmacokinetics, efficacy and safety of switching efavirenz to maraviroc in HIV-positive patients

4: The feasibility, pharmacokinetics, safety and efficacy of switching from efavirenz to maraviroc based antiretroviral therapy

4.1 Introduction

4.2 Methods

4.2.1 Tropism evolution study

4.2.1.1 Subject selection

4.2.1.2 Tropism methodology

4.2.1.3 Data analysis

4.2.2 Switch study

4.2.2.1 Subject selection & screening

4.2.2.2 Methodology & CNS assessment

4.2.2.3 Pharmacokinetic methods

4.2.2.4 Statistical methods

4.3 Results

4.3.1 Tropism evolution study

4.3.2 Switch study

4.3.2.1 Demographic & clinical characteristics

4.3.2.2 Immunological & virological efficacy

4.3.2.3 Safety and tolerability

4.3.2.4 Pharmacokinetics

4.4 Discussion

4.4.1 Healthy volunteers

4.4.2 HIV-positive patients

4.1 Introduction

As previously discussed, switching therapy to address issues of tolerability or toxicity is complicated by the potential for drug-drug interactions at the time of switch. Drug interactions may result in decreased concentrations of the new agent, which may result in reduced efficacy and the potential for development of drug resistance mutations. It is important to not risk virological efficacy when switching for tolerability or toxicity reasons.

MVC is a CCR5 antagonist that prevents the entry of R5-tropic virus into target cells by blocking the cell surface CCR5 co-receptor. Use of MVC is dependent on demonstration of R5-utilising virus by tropism assay (as opposed to dual, mixed or CXCR4-tropic virus; see chapter 1.5.2). MVC was non-inferior to EFV for initial therapy in a *post hoc* analysis [Sax *et al*. 2008], and is well-tolerated in treatment-naïve [MERIT] and treatment-experienced subjects [Gulick *et al*. 2008]. In particular, MVC is associated with a low risk of neuropsychiatric toxicity and a favourable lipid profile [DeJesus *et al*. 2008]. The MERIT study used a phenotypic assay to determine tropism; since then data has accumulated on the use of genotypic tropism assays. Genotypic testing allows tropism determination in the presence of an undetectable viral load as sequencing can be performed on RNA or cellular DNA (unlike phenotypic tests which require detectable RNA). Genotypic methods are now preferred by British guidelines [Asboe e*t al*. 2012] but at the time of our tropism evolution analysis, phenotypic testing using Trofile was in routine use locally and is still used at many centres. Despite several cross-sectional studies describing tropism distribution in different patient populations there is a real lack of longitudinal data describing how tropism evolves over time. Arbitrarily it is recommended that a tropism test remains valid for 90 days and after this time a tropism test should be repeated in case evolution had occurred in the interim [Geretti *et al*. 2012]. In particular there is a lack of data regarding tropism evolution during viral suppression – although it is unlikely there is a possibility that tropism could evolve during low-grade viral replication below the limit of detection of standard viral load assays. A previous study comparing tropism pre-treatment (ascertained by 2 genotypic algorithms, Geno2pheno and PSSM, on plasma and PBMC) and after 48 weeks of suppressive ART (genotypic algorithms on peripheral blood mononuclear cell (PBMC) samples only) showed that only 1 of 34 patients experienced tropism change (from R5 to dual/mixed) [Soulie *et al*. 2007]. At the time of our tropism evolution analysis there was no data regarding change, if any, of phenotypic tropism during viral suppression.

As outlined in chapter 1.5.2, as MVC is a CYP3A4 substrate dose adjustment is required when MVC is co-administered with certain ARVs. MVC dose reduction is required when it is co-administered with some PIs but when switching to MVC from a PI, as PIs are cleared rapidly, no interim dose adjustment would be required. An increase from the standard 300mg BID to a 600mg BID dose is required. When switching from EFV to MVC, it can be expected that EFV may affect MVC concentrations for a period of time after the switch. The length of time that the inducing effect of EFV will persist for after stopping the drug is unclear. Crucially, the inducing effect of EFV could result in initial sub-therapeutic MVC concentrations if MVC is started immediately after EFV and consequently, reduced MVC efficacy. Moreover, maintaining an elevated dose of MVC after the induction effect of EFV has worn off could result in adverse events such as postural hypotension [Celsentri SPC]. We developed a switch strategy if using the recommended MVC dose when co-administered with EFV (ie 600mg BID) for the first 14 days after EFV cessation followed by dose reduction to the 300mg BID. This was based on the fact that there is less data to support the efficacy of MVC when administered with drugs that lower its concentrations and that, unlike ETR, virological failure on MVC dose may correlate with MVC concentrations [Heera *et al*. 2008]. In conclusion, at present, whilst MVC dose adjustments required during co-administration with EFV are clear, the correct dose of MVC when switching from an EFV-containing regimen remains unknown; this information is crucial in order to guide safe switching from EFV to MVC in individuals needing to switch.

The primary objective of the tropism analysis was to investigate the frequency of tropism change during viral suppression as determined by phenotypic testing (Trofile). The primary objective of the switch study was to assess the PK, efficacy and safety of MVC administered at 600 mg twice-daily for two weeks to HIV-1 infected patients who had achieved viral suppression on EFV-based therapy followed by 22 weeks of MVC 300 mg twice-daily. The secondary objectives of the switch study were to assess virological and immunological efficacy of MVC at 24 weeks after switch from EFV. Based on a presumed risk of toxicity if a higher MVC dose was maintained after the induction effect of EFV had waned we decided to investigate a strategy of increasing MVC dose for the first 2 weeks after switch from EFV followed by standard MVC dosing.

4.2 Methods

4.2.1 Tropism evolution study

4.2.1.1 Subject selection

At our centre data including demographics, disease characteristics, treatment history and surrogate markers (including CD4 cell count and HIV-RNA) are entered on a prospectively collected database. We used this database to identify patients who achieved viral suppression on ART (defined as continuous therapy for at least 12 weeks) and then had an interruption of therapy for reasons other than virological failure. All patients were required to have stored plasma samples available from within 3 months of initiating their regimen and within 3 months of stopping their drugs.

4.2.1.2 Tropism methodology

Tropism testing was performed on stored frozen samples by the Trofile® ES assay, and tropism before and after suppressive therapy was compared. Trofile is a phenotypic assay; although others are available, Trofile was the assay used to screen patients for inclusion in trials of CCR5 antagonists and as such became the 'gold standard' tropism test [Geretti *et al.* 2009]. The original Trofile assay had a sensitivity for detecting X4-tropic virus of 10%; a new enhanced sensitivity (ES) version became available in 2008 with a reported lower-limit sensitivity of 0.3% [Reeves *et al*. 2009]. As outlined in chapter 1.5.1, re-analysing the MERIT study with Trofile ES showed that 15% of patients who entered the study in fact had non-R5 tropic virus and when the analysis was restricted to indviduals with 'true' R5-tropism MVC was non-inferior to EFV [Sax *et al*. 2008]. This suggests that Trofile ES is better at predicting response to MVC. Trofile testing requires detectable HIV-RNA (said to be 1000 copies/ml or more for reliable amplification [Geretti *et al*. 2009]). As it is only available in a limited number of laboratories, frozen plasma samples must be shipped to the test provider, Monogram Biosciences Inc, San Francisco [Trofile methodology]. Trofile is a complex, cell-based assay and as such has a relatively slow turnaround time 2-3 weeks, longer than alternative, genotypic methods [Geretti *et al*. 2009].

4.2.1.3 Data analysis

The following information was collected: age, gender, year of diagnosis, treatment history (including line of therapy, history of prior virological failure [VF], and history of mono/dual NRTI therapy) viral load before, during and after interruption of ART. Tropism before and

after switch was summarised and described. Viral loads in samples yielding successful and failed Trofile® results were compared using Mann-Whitney non-parametric testing.

4.2.2 Switch study

4.2.2.1 Subject selection & screening

HIV-infected adults who had been receiving two NRTI + EFV for at least 12 weeks were invited to participate. After provision of written, informed consent, subjects underwent screening with clinical assessment and routine laboratory investigations. Demographic details and full medical, drug and social history were recorded followed by a complete physical examination (including weight, height, and vital signs). Volunteers then underwent ECG assessment, non-fasted blood testing (drug screen, HIV antibody, hepatitis C antibody, hepatitis B surface antigen, haematology [full blood count & differential, and clotting] and biochemistry [creatinine, urea, potassium, sodium, bicarbonate, total cholesterol and triglycerides, calcium, phosphate, liver function tests (ALT, AST, GGT), albumin, glucose, amylase]) and urine testing (drug screen, macro analysis and, for females of childbearing potential, pregnancy testing).

Patients were required to meet all eligibility criteria at screening. These included being between 18 to 65 years old, with documented HIV-1 infection for at least six months, CD4 count > 50 cells/mm^3, and HIV-RNA <50 copies/mL for at least 12 weeks on two NRTIs and EFV without any prior history of virological failure. Participants were required to have R5-tropic virus at study screening, as determined by genotypic tropism assay (geno2pheno^{TM}) performed on cellular DNA. Subjects were excluded if they had non-R5-tropic virus, hepatitis B or C co-infection, hepatic or renal impairment, any prior use of CCR5 antagonists or any genotypic resistance to NRTI or NNRTI.

Additional inclusion and exclusion criteria are listed below:

<u>Inclusion criteria:</u>

1. Males or non-pregnant, non-lactating females.
2. Documented HIV-1 infection of at least 6 months duration.
3. Women of childbearing potential (WOCBP) must be using an adequate method of contraception to avoid pregnancy throughout the study.
4. Viral load <50 copies/ml at and for at least 12 weeks prior to screening visit

5. No medical, psychiatric or substance misuse disorders felt by the investigator to impact on the subject's ability to participate in the study including a positive drugs of abuse test. (positive test for cannabinoids allowed).

Exclusion criteria:

1. Dual, mixed or X4-tropic virus on geno2pheno tropism sample
2. HIV-2 co-infection
3. Disallowed concomitant medication as per the SPC for MVC and NRTI
4. ALT or AST elevation greater than five times the upper limit of normal
5. Estimated GFR (Modification of Diet in Renal Disease (MDRD) equation) less than 50ml/min

4.2.2.2 Methodology

This was a 24-week, single arm, PK study conducted at the PK Unit of the St. Stephen's Centre, Chelsea and Westminster Hospital, London. The study protocol was reviewed and approved by the Riverside Research Ethics Committee, London. All subjects provided written informed consent and the trial was conducted in accordance with Good Clinical Practice, the Declaration of Helsinki, and applicable regulatory requirements.

The primary endpoint of this study was steady state plasma concentrations of MVC dosed at 600mg BID for 2 weeks followed by 300mg BID thereafter following cessation of EFV 600mg QD.

Secondary endpoints were:

1. Viral suppression rates (<50 copies/ml) 4 and 24 weeks after switch from EFV to MVC
2. CD4 rise at 24 weeks following switch from EFV to MVC
3. To assess the impact of switching from EFV to MVC on grade 2-4 adverse events and laboratory abnormalities

At the day 1, day 14, day 28, week 12 and week 24 visits, participants underwent the following assessments: vital signs, urine testing (macroanalysis, drugs of abuse screen, pregnancy test for WOCBP), bloods (biochemistry, haematology, CD4/8 count and

percentage and HIV-RNA), adverse event review, concomitant medication review and symptom-directed physical examination.

At the day 3, 6, 10, 17, 20 and 24 visits (single PK visits as described below) only adverse event review, concomitant medication review and vital signs were performed.

The safety and tolerability of study medications were evaluated throughout the study using the DAIDS grading system to describe the severity of adverse events and causality was determined by the principal or a co-investigator (relatedness options: unrelated, unlikely, possible, probable or definite). Adherence was assessed by direct questioning at every study visit and by pill count at drug dispensation visits (weeks 2, 4, 12 and 24).

There were three intensive PK days on visit 1 (day 1), visit 2 (day 14) and visit 3 (day 28). Patients were admitted fasting to the PK unit on day 1 (when EFV was switched to MVC 600 mg twice-daily), on day 14 (when patients had achieved steady-state on MVC 600 mg twice-daily and were then switched to MVC 300 mg twice-daily from day 15), and day 28 (when patients had achieved steady state MVC 300 mg twice-daily) for MVC concentration sampling (pre-dose, 1, 2, 4, 6, 8, 12 hours post-dose) with a standardized breakfast beforehand and witnessed drug taking.

Furthermore, patients attended the PK unit for MVC C_{trough} measurements 3, 6, and 10 days following regime switches. EFV concentrations were measured on days 1, 3, 6, 10, 14, 17, 20, 24 and 28, following the switch. HIV viral load and CD4 cell count were measured on days 1, 14, 28, 84 and 168.

4.2.2.3 Pharmacokinetic methods

All blood samples were collected into beadless, heparinised tubes. Plasma concentrations of MVC and EFV were measured using a validated HPLC-tandem mass spectrometry method [11]. Recovery of MVC and EFV was 113% and 83%, respectively. . The lower limits of quantification for MVC and EFV was 1.0 ng/mL and 8.5 ng/mL, respectively. Intra-assay and inter-assay accuracy and precision did not exceed 10%.

4.2.2.4 Statistical methods

No formal sample size calculation was performed for this pilot study. Twelve subjects completing the study was considered sufficient to allow for relevant conclusions to be drawn. The PK parameters calculated for plasma MVC concentrations on the intensive PK days were as follows: C_{trough}, defined as the plasma concentration at hour 12 after the observed drug dose, the maximum observed plasma concentration (C_{max}), and total drug exposure, expressed as the area under the plasma concentration–time curve from 0-12 hours after dosing (AUC). The PK parameter calculated for EFV was the projected Ctrough based on the EFV plasma concentrations measured pre-dose on day 1 and EFV elimination half-life ($t_{1/2}$) during the drug decay phase (days 3, 6, 10, 17, 20 and 24).

All pharmacokinetic parameters were calculated using non-compartmental modelling techniques (WinNonlin Phoenix (version 6.1; Pharsight Corp, Mountain View, CA, USA).

Within-subject changes in the assessed PK parameters when comparing visit 1, visit 2, and MVC Ct_{rough} on days 3, 6, and 10 with visit 3 (reference) were evaluated by calculating geometric mean ratios (GMRs) and 90 % confidence intervals (90% CI). The 90% CIs were determined using logarithms of the individual geometric mean values; which were then expressed as linear values. The changes in PK parameters were considered significant when the 90% CI did not cross the value 1.

Inter-individual variability in drug PK parameters was expressed as a coefficient of variation [(standard deviation/mean) x 100].

4.3 Results

4.3.1 Tropism evolution study

Thirty seven patients (33 male, 4 female) with stored plasma available from within 3 months of starting and 3 months of interrupting suppressive ART were identified. All had been on therapy for at least 12 weeks with a mean duration of 862 days (range 120 – 2708 days).

Of the 74 samples 19 did not amplify (26% assay failure rate) leaving 26 patients with paired amplifiable samples. Most sample volumes were less than the 3ml usually required for Trofile® testing (mean volume 1ml). There was no difference in storage time for the samples that did amplify and those that did not; the median time from sample collection for samples

was 82 months (range 4 – 133 months) and 68 months (range 3 – 123 months) for samples that did and did not amplify respectively. However, the difference between amplifiable and non-amplifiable samples in terms of viral load was significant at 69729 (4.84 log10) copies/ml compared with 12412 (4.09 log10) copies/ml respectively (p=0.001). In addition, all successful Trofile® assays were performed on samples with a viral load greater than 1000 copies/ml whereas 2/19 failed Trofile® tests were performed on samples with a viral load less than 1000 copies/ml (626 and 639 copies/ml respectively).

In terms of treatment line the median line of therapy at time of study was second line (range 1 – 11 lines of therapy) and median number of previous virological failures was 0.5 (range 0 – 6). Baseline characteristics of patients with R5-tropic or dual/mixed/X4-tropic (D/M/X4) at baseline test (before suppressive ART) are described in table 1.

Of the 26 individuals analysed, 24 retained the same viral tropism after treatment interruption: 17/18 with R5-tropic virus at baseline rebounded with R5-tropic virus and 7/8 with D/M/X4-tropic baseline HIV rebounded with the same strain. One patient with baseline R5-tropic virus and one with a baseline D/M/X4-tropic viral population rebounded with D/M/X4 and R5-tropic virus respectively. Both patients who experienced tropism change were highly treatment experienced (7th and 8th line of therapy and 2 and 4 prior virological failures respectively). Most patients maintained VL <50 during therapy preceding interruption (including the two patients with tropism switch); 5 individuals experienced blips (VL 50-500 preceded and followed by <50).

4.3.2 Switch study

4.3.2.1 Demographic and clinical characteristics

Twelve subjects completed the study. All were males, with a median (range) age of 42 (28-63) years, weight of 76 (62-103) kg, and BMI of 24 (19–34) kg/m2. Two subjects were Black Caribbean, and 9 were Caucasian. Median (range) baseline CD4 count was 548 (195-1238) cells/mm^3; all had an undetectable viral load and a CCR-5 tropic virus at screening.

4.3.2.2 Immunological and virological efficacy

All patients maintained an undetectable viral load throughout the 24 weeks of study follow up. There was an increase in CD4 cell count over follow-up (548, 555 and 593 cells/mm3 day

1, day 28 and week 24, respectively) although CD4% remained unchanged (30%, 30% and 31% at the same time points, respectively). CD8 count and % were unchanged.

4.3.2.3 Safety and tolerability

The study drugs were well tolerated and no grade 3/4 adverse events were reported. One grade 2 adverse event at least possibly related to study drug was reported: mood swings starting at day 16 and resolving without intervention at week 16. There was no change in fasting total cholesterol (4.95, 4.3 and 4.7 mmol/l at day 1, day 28 and week 24, respectively) and a small improvement in triglycerides (1.81, 1.38 and 1.08 mmol/l at day 1, day 28 and week 24, respectively). One subject experienced a transient grade 1 AST elevation during the 600 mg BID MVC phase (all other liver function parameters remained within normal limits). Median ALT, AST, GGT and alkaline phosphatase (ALP) were similar throughout follow-up.

4.3.2.4 Pharmacokinetics

Maraviroc pharmacokinetics

MVC pharmacokinetic parameters after the switch from EFV, on days 1, 14 and 28, and C_{trough} measurements at days 3, 6 and 10 are shown in Table 4.1. All day 3 and 6 measurements were greater than the IC_{90} for maraviroc inhibition of wild-type virus.

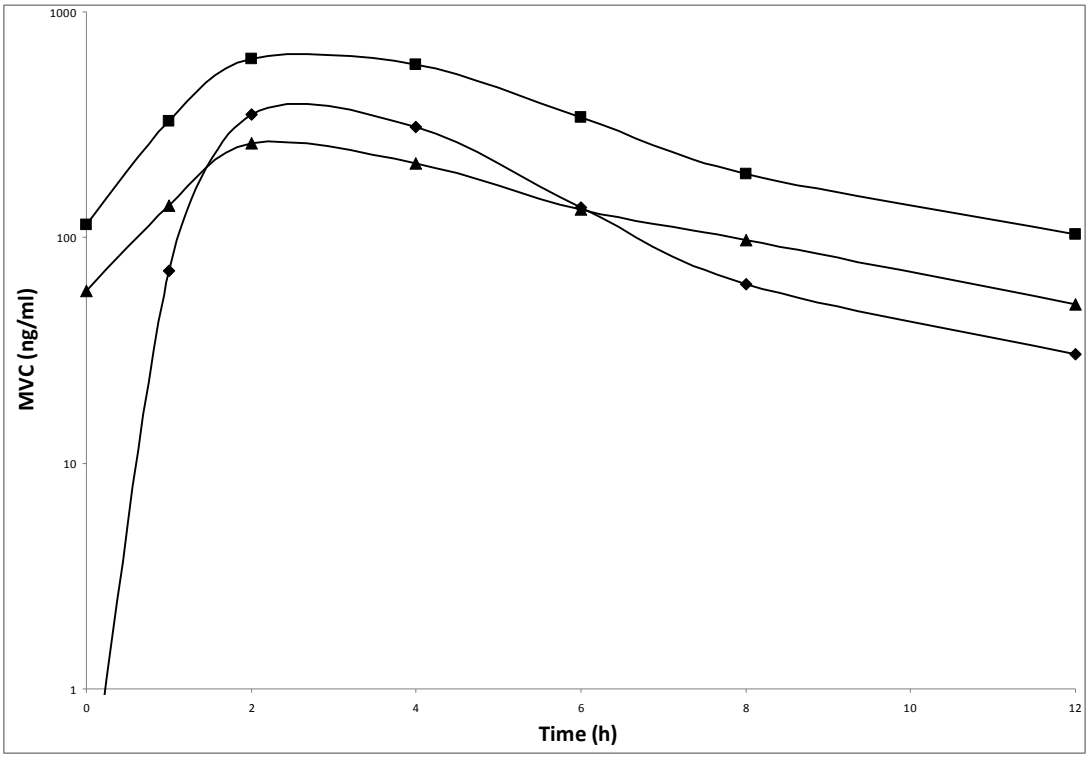
Table 4.1: MVC pharmacokinetic (PK) parameters measured throughout the study and their comparison to visit 3/day 28 (reference)

	MVC PK parameters		
	AUC (ng·h/mL)	C_{max} (ng/mL)	C_{trough} (ng/mL)
*Visit 1 (day 1) 600 mg BID 1st dose	2237 (1879-2594)	555 (398-711)	31 (23-38)
CV (%)	32	52	45
***Day 1 vs day 28*	*1.25 (1.02-1.52)*	*1.64 (1.22-2.18)*	*0.61 (0.48-0.77)*
*Visit 2 (day 14) 600 mg BID steady-state	4141 (3597-4685)	821 (672-970)	113 (85-142)
CV (%)	27	36	48
***Day 14 vs day 28*	*2.31 (1.91-2.80)*	*2.42 (1.95-3.00)*	*2.25 (1.81-2.80)*
*Visit 3 (day 28) 300 mg BID steady-state	1791 (1282-2300)	339 (233-445)	50 (36-65)
CV (%)	53	57	53
*Day 3			69 (45-93)
CV (%)			
***Day 3 vs day 28*			1.37 (1.03-1.81)
*Day 6			68 (47-89)
***Day 6 vs day 28*			1.34 (1.07-1.68)
*Day 10			101 (76-125)
***Day 10 vs day 28*			1.94 (1.60-2.37)

*Geometric mean (90% confidence intervals)
**Geometric mean ratio (90% confidence intervals)

MVC concentrations on days 1 (600 mg twice-daily), 14 (600 mg twice-daily) and 28 (300 mg twice-daily) are shown in Figure 4.1.

Figure 4.1: Geometric mean MVC plasma concentrations on days 1 (diamonds, 600 mg twice-daily first dose), 14 (squares, 600 mg twice-daily steady-state), and 28 (triangles, 300 mg twice-daily steady-state)



MVC (GM, 90% CI) C_{max}, C_{trough} and AUC_{0-12} were 555 (398-711) ng/mL (64% higher than reference), 31 (23-38) ng/mL, (39% lower than reference) and 2237 (1879-2594) ng.h/mL (25% higher than reference) on day 1; and 821 (672-970) ng/mL (142% higher than reference), 113 (85-142) ng/mL (125% higher than reference) and 4141 (3597-4685) ng.h/mL (131% higher than reference) on day 14 (steady-state).

When on 300 mg twice-daily (day 28; reference treatment), MVC (GM, 90% CI) C_{max}, C_{trough} and AUC_{0-12} were 339 (233-445) ng/mL, 50 (36-65) ng/mL, and 1791 (1282-2300) ng.h/mL.

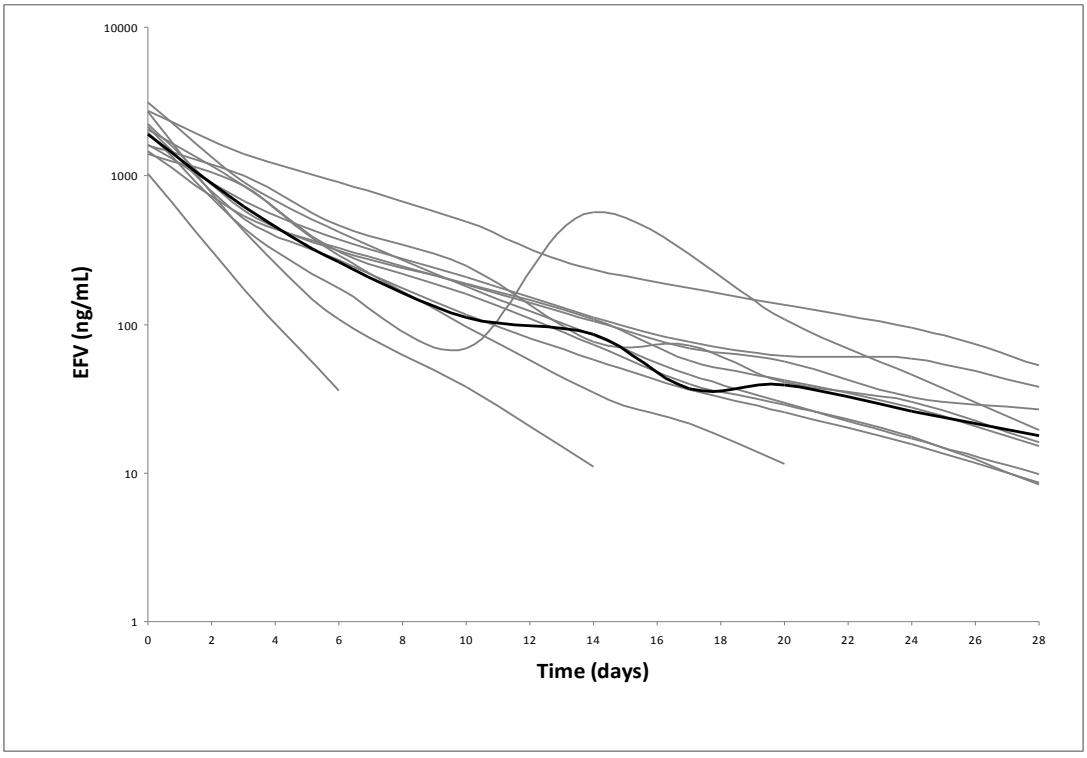
GM (90% CI) MVC C_{trough} on days 3, 6, and 10 was 69 (45-93), 68 (47-89), and 101 (76-125), ng/mL, respectively. MVC C_{trough} was higher than reference up to day 10 following the switch: 37% higher on day 3, 34% higher on day 6, and 95% higher on day 10. GM (90% CI) MVC

C_{trough} on days 17, 20 and 24 (on MVC 300mg BD) was 63 (46-79), 46 (31-60) and 59 (37-81) ng/mL, respectively; these values were 30% higher, 4% lower and 17% higher than reference C_{trough}, respectively. MVC inter-individual variability was wide (table 4.1).

Efavirenz pharmacokinetics

EFV concentrations decay measured up to day 28 after the switch is illustrated in Figure 4.2. Note that three subjects had EFV concentrations below the lower limit of quantification before day 28.

Figure 4.2: EFV plasma concentration decay up to 28 days following the drug switch in the 12 subjects studied. The black thick line indicates the geometric mean



Median EFV terminal half-life was 116 hours (range 59–148 hours). Nine/12 subjects had detectable EFV concentrations 28 days after stopping EFV intake. However, only one patient had concentrations measured above 1000 ng/mL up to three days after the switch and none had concentrations higher than 1000 ng/mL after this time point: median (range) EFV concentrations 3, 6 and 10 days after stopping EFV intake were 632 (173-1410) ng/mL, 310 (36-906) ng/mL and 180 (4-490) ng/mL, respectively. Inter-individual variability of EFV concentrations after drug cessation was wide. Of note many patients starting MVC where EFV levels fell below 1000 ng/ml had levels only just above this threshold at baseline.

4.4 Discussion

In an era where several choices of antiretroviral regimen exhibit similar virological efficacy, other factors such as short- and long-term toxicity and tolerability increasingly govern drug choice [BHIVA guidelines 2012]. Although highly efficacious, EFV-based cART may be associated with treatment-limiting toxicities, which could be addressed by switching to alternatives such as MVC. Crucially, MVC demonstrates a significant advantage over EFV in terms of estimated CV risk (Framingham equation). As long-term morbidities, including cardiovascular disease, are of ever greater importance in the modern era of HIV care; now that HIV-positive individuals can expect near normal life expectancy [Lewden *et al.* 2007; May *et al*. 2011] managing risk factors for these conditions is essential. However, due to the pharmacokinetic properties of EFV it is important to study the potential interactions when switching alternative drugs.

Our tropism evolution data shows that phenotypic tropism change is uncommon during periods of viral suppression. This is supported by an earlier study [Souile *et al*. 2007] and data published since our work was presented; a longitudinal analysis comparing genotypic tropism pre-ART (RNA and DNA) and after at least 2 years of suppressive ART (proviral DNA tropism only) using two genotypic algorithms [Seclen *et al*. 2010]. Seventy-eight individuals on suppressive ART for a median of 3.5 years were included; the rate of non-R5 tropism at baseline was 33% and 34% on RNA and DNA samples, respectively. At the second time point the proportion of non-R5 variants was 34% on proviral DNA sequencing and of the 8 patients with paired baseline and >2 year samples available, results were concordant in 82% of cases. When restricted to comparison of baseline and >2 year DNA samples tropism switch was observed in 12% [Seclen *et al*. 2010]. Since our study, phenotypic testing has been largly superseded by genotyoic methods which are preferred in UK [Geretti *et al*. 2009] guidelines; European guidelines recommend both methods but acknowledge that genotypic tests will likely be used more frequently because of "greater accessibility, lower cost and shorter turnaround time" [Vendekerckhove *et al*. 2011].

In our study, both patients who did experience a change in viral tropism after suppressive HAART were highly treatment experienced. We conclude that stored a pre-treatment tropism test or a tropism test performed on a stored plasma sample from prior to treatment initiation, in combination with clinical history, can reliably predict tropism in patients on

suppressive HAART. An alternative option to determine suitability for MVC in suppressed individuals is the use of genotypic testing on proviral DNA. Clinical validation data for HIV-DNA is relatively lacking but it is an option in UK guidelines [Geretti *et al*. 2009] and tropism results (geno2pheno) using HIV-DNA correlate with HIV-RNA more than 95% of the time [Verhofstede *et al*. 2011]. Further validation of DNA testing is required.

The switch study investigated the pharmacokinetics, safety and efficacy of switching EFV to MVC in patients on suppressive EFV-based ART. Firstly, from a PK perspective, all measured MVC concentrations exceeded the IC_{90} for wild-type virus. Increasing MVC to 600 mg BID, as recommended when MVC is co-administered with EFV, resulted in adequate plasma concentrations of MVC during week 1. However, the induction effect of EFV appeared to wane after week 1 and at day 14 MVC PK parameters were significantly higher than at day 28. At the trough measurements on day 3 and 6 MVC concentrations were around 35% higher than at day 28; by day 10 MVC C_{trough} was almost double that at day 28. This would suggest that a MVC dose increase for 1 week, rather than the 2 weeks selected in this study, would be appropriate when switching directly from EFV to MVC in clinical practice. Importantly, although higher doses of MVC were associated with postural hypotension in healthy volunteer studies [Celsentri SPC], no participants experienced symptoms of hypotension during the study suggesting that the supra-therapeutic levels of MVC observed from day 10 onwards were well-tolerated. However, in pooled phase 1 and 2a MVC studies, postural hypotension occurred only with a MVC C_{max} greater than 1000 ng/ml; all C_{max} concentrations in this study were below this threshold. Importantly all measured MVC concentrations exceeded the protein-adjusted IC_{90} for wild-type virus (2.0 ng/mL) [Dorr *et al*. 2005].

It is unclear why the induction effect of EFV on MVC persisted for a shorter period of time than that observed for ETR (chapter 3.3.2.1) and for rilpivirine (RPV) [Crauwels *et al*. 2012], both non-nucleoside reverse transcriptase inhibitors (NNRTI). This may be explained by the differences in the populations studied (ie healthy volunteers rather than HIV-positive individuals) since hepatic metabolism may be affected by HIV status [Jones *et al*. 2010]; however, a study switching HIV-positive subjects with a suppressed viral load on EFV to RPV also showed a prolonged induction effect relative to our study [Cohen *et al*. 2011]. Therefore, an alternative explanation may be that ETR and RPV somehow prolong the induction effect of EFV. ETR, for example, inhibits the 2C9 isoenzyme [140], which in turn

plays a small role in EFV metabolism [Ward *et al*. 2003]. The concentrations of EFV measured after cessation of EFV intake in this study were lower than those observed in the ETR switch study which may explain the shorter induction effect we observed. The degree of CYP3A4 induction is a function of EFV exposure; in a healthy volunteer study, increasing doses of EFV resulted in greater induction of hepatic CYP3A4 [Mouly *et al*. 2002]. However, whether the lower EFV concentrations we observed were due to differences between HIV-positive patients and healthy volunteers or due to some impact of ETR on EFV metabolism in the other study remains uncertain.

Limitations of this study include the small study population; although study numbers were typical, and sufficient, for pharmacokinetic analyses, they are not large enough to draw meaningful conclusions regarding efficacy and toxicity. We did not require subjects to be suffering from EFV side effects and did not collect detailed CNS symptom data. As a single arm study we did not compare different strategies and cannot conclude that switching directly from EFV to 'standard dose' MVC is not a reasonable option; ideally the study design would have incorporated a non-adjusted MVC arm for comparison. Finally, we have a relative lack of detailed pharmacokinetic data at some of the time points and our observation that the induction effect of EFV waned around day 7 is based only on MVC C_{trough}.

In summary we demonstrated that, when switching from EFV to MVC, increasing the dose of MVC from 300 mg BID to 600 mg BID for 1 week would be sufficient to compensate for the prolonged induction effect of EFV. Importantly switching from MVC to EFV was safe, well-tolerated at no point were sub-therapeutic MVC concentrations measured. Our study highlights the importance of studying different tail interactions to guide safe, effective antiretroviral switch strategies.

Chapter 5:

Switching from a failing 1st line, NNRTI-based regimen to a boosted protease inhibitor: how many nucleoside analogues are necessary?

Chapter 5: Switching from a failing 1st line, NNRTI-based regimen to a boosted protease inhibitor: how many nucleoside analogues are necessary?

5.1 Introduction

5.2 Methods

5.2.1 Subject selection

5.2.2 Definition of drug activity

5.2.3 Data analysis

5.3 Results

5.3.1 Virological response (overall)

5.3.2 Response by number of NRTI

5.3.3 Response by active NRTI

5.4 Discussion

5.1 Introduction

Despite marked improvements in antiretroviral therapy, treatment failures continue to occur. The commonest reason for therapy switch are for adverse events, but virological failures - defined as failure to achieve viral suppression or a rise in viral load above a defined threshold with or without resistance mutations - also occur [Boyle *et al*. 2012]. Recent clinical trials yield treatment success rates (HIV-RNA less than 50 copies/ml at week 48 or 96) of around 80-90% [Cohen *et al*. 2012; Ssax *et al*. 2011], and although many of the treatment failures are for other reasons (eg. toxicity, tolerability, loss to follow-up), a proportion are due to true virological failure (5-9%).

After failure on an NNRTI-based regimen, most 2nd line combinations are boosted protease inhibitor (PI/r)-based. Current UK guidelines advise the use of at least 2 active agents and it is common practice to construct a regimen of 3 active agents, based on results of resistance testing [BHIVA guidelines 2012]. World Health Organisation (WHO) guidelines recommend two NRTI, choice dependent on prior NRTI, with PI/r second line [WHO guidelines 2010]. However, the potency and high genetic barrier of PI/r is such that 2 additional fully active nucleoside drugs may not be necessary. PI/r such as lopinavir/ritonavir (LPV/r) and darunavir/ritonavir (DRV/r) have shown high rates of virologic efficacy as monotherapy [Arribas *et al*. 2009; Cameron *et al*. 2008; Arribas *et al*. 2010], although LPV/r performs better as an induction-maintenance or simplification strategy than as initial therapy [Nunes *et al*. 2009]; DRV/r has only been studied as a simplification option. Despite that, a systematic review of published protease inhibitor-monotherapy studies showed ritonavir-boosted protease inhibitor monotherapy is inferior to cART [Bierman *et al*. 2009].

If 2nd line PI/r cART efficacy is less dependent on NRTI activity this could have far-reaching implications for resource-rich and -limited settings in terms of toxicity, tolerability and cost. We aimed to generate more evidence on this issue by performing a retrospective analysis of a large UK cohort (UK CHIC). Our objectives were to describe the proportion of patients failing 2nd line PI/r-based cART (after failing 1st line NNRTI-based therapy), to investigate factors associated with failing 2nd line therapy and to investigate the impact of the number of new or fully active NRTI included with 2nd line therapy on virological success rates.

5.2 Methods

5.2.1 Subject selection

HIV-positive patients attending for care between 1st January 1996 and 31st December 2008 were identified using the UK CHIC cohort database. Eligible subjects had failed a 1st line NNRTI-based regimen then switched to a PI/r-based regimen 2nd line (see sections 2.2.3 and 2.3.5). Subjects with HIV-RNA less than 200 copies/ml by time of regimen change or less than 4 months follow-up in 2nd line therapy were excluded from the analysis as were those who failed a new NRTI between failing 1st line NNRTI and starting 2nd line PI/r. When analysing responses to 2nd line therapy, virological failure was defined as HIV-RNA greater than 200 copies/ml after 4 months of continuous use of a PI/r.

5.2.2 Definition of drug activity

We analysed the impact of backbone NRTI on virological response by considering the number of new NRTI (NRTI that a patient was naïve to) and the predicted activity of the NRTI backbone; for the latter a genotypic sensitivity score (GSS) was calculated based on a validated algorithm [Stanford]; FTC and 3TC were analysed as the same drug. For analysis purposes, GSS was considered in three categories (GSS ≤1, 1.25-1.75 and ≥2), and as a continuous variable; a higher GSS implies more NRTI activity with a GSS of 1 being equivalent to one fully active NRTI.

5.2.3 Data analysis

A Kaplan-Meier analysis was performed to describe time to failure of the 2nd line regimen and Cox Regression to identify factors associated with 2nd line virological failure. NRTI GSS categories in patients who did and did not fail 2nd line therapy were compared using chi-squared tests. Logistic regression was used to identify factors associated with having a resistance test in order to define any differences between individuals who did or did not undergo resistance testing prior to regimen switch.

5.3 Results

During the study period (Jan 1999 to Dec 2008) 10,583 subjects commenced NNRTI-based therapy first-line, and of these 1925 (18.2%) experienced virological failure according to our definition. Virological failure rates after 48 and 96 weeks of cART were 4% and 6.2%, respectively. Of these, 1348 started new drugs; 878 did not meet study criteria (table 5.1) leaving 470 individuals eligible for the analysis.

Table 5.1: Patient disposition (UK CHIC subjects starting a 1st line NNRTI regimen)

Patients starting HAART on NNRTI-regimen		10583
Failed 1st line NNRTI		1925 (18.2%)
Started new drugs		1348 (70.0%)
	2nd line non-PI/r	632
	Unboosted PI	*49*
	New NNRTI	*72*
	New NRTI only	*438*
	Other	*73*
	2nd line PI/r	716
Excluded due to VL<200 at 2nd line		135
<4 months follow up		62
Failed new NRTI between 1st line and 2nd line		49
Eligible for analysis		**470**

Baseline characteristics of the 470 patients are described in table 2. The median time between 1st and 2nd line regimen was 0 months (IQR 0-5.5 months) and the median [IQR] log VL at start of 2nd line was 4.4 [3.6, 5.0] $\log_{10}$ (compared with 5.1 [4.6, 5.5] $\log_{10}$ at start of 1st line). Most subjects initiated LPV/r (53.5%) or ATV/r (22.2%) based therapy 2nd line. In terms of new NRTI included in the 2nd line regimen, 19.6%, 34.5% and 46.0% started none,

one, or two or more, respectively. Median CD4 cell count at start of 2nd line therapy was 223 cells/mm3 (IQR 130-320 cells/mm3) and HIV-RNA 4.3 log10 (IQR 3.6-5.0 log10). A total of 246 (52.3%) of individuals underwent genotypic resistance testing after failing 1st line cART and before starting 2nd line cART.

Table 5.2: Baseline characteristics of subjects eligible for analysis

Patients starting 2nd line PI/r HAART		470
Time to failing 1st line HAART (months)	Median (IQR)	9.8 (5.4 – 20.9)
NRTIs taken at 1st line failure; no. (%)	AZT	238 (50.6)
	DDC	1 (0.2)
	DDI	94 (20.0)
	D4T	67 (14.2)
	3TC/FTC	391 (83.2)
	ABC	74 (15.7)
	TFV	91 (19.4)
	Unknown NRTI	3 (0.6)
NRTIs taken at 2nd line; no. (%)	AZT	156 (33.2)
	DDC	1 (0.2)
	DDI	132 (28.1)
	D4T	36 (7.7)
	3TC/FTC	230 (48.9)
	ABC	149 (31.7)
	TFV	307 (65.3)
	Unknown NRTI	8 (1.7)
New NRTI started at 2nd line[1]; no. (%)	0	92 (19.6)
	1	162 (34.5)
	≥2	216 (46.0)
Year of starting 2nd line; no. (%)	1999-2001	54 (11.5)

	2002-2004 2005-2008	184 (39.2) 232 (49.4)
Ethnicity; no. (%)	White Black Other	185 (39.4) 240 (51.1) 45 (9.6)
Risk group; no. (%)	MSM Heterosexual Other	174 (37.1) 247 (52.5) 49 (10.4)
VL <200 between 1st and 2nd line HAART; no. (%)		88 (18.7)
CD4 at start 1st line (cells/mm^3)	Median (IQR)	158 (66 – 232)
HIV-RNA at start 1st line (log_{10} copies/ml)	Median (IQR)	5.1 (4.5 – 5.5)
CD4 at start 2nd line (cells/mm^3)	Median (IQR)	223 (130– 320)
HIV-RNA at start 2nd line (log_{10} copies/ml)	Median (IQR)	4.3 (3.6 – 5.0)

5.3.1 Virological response (overall)

Of 470 patients starting 2nd line PI/r cART, 226 (48.1%) experienced virological failure overall; virological failure rates after 48 and 96 weeks of cART were 10.9% (n=51) and 13% (n=61), respectively. Factors associated with virological failure are presented in Table 5.3.

Table 5.3: Factors associated with virological failure of 2nd line HAART (n=470)

		Univariable		Multivariable	
		HR (95% CI)	P	HR (95% CI)	P
Age at start of 2nd line	Per 10 years older	0.99 (0.82, 1.19)	0.91	1.01 (0.83, 1.24)	0.89
Time between 1st line & 2nd line	Per month increase	1.01 (1.00, 1.01)	0.14	1.01 (1.00, 1.02)	0.03
No. new NRTI 2nd line	0	0.90 (0,62, 1.30)	0.56	1.02 (0.68, 1.52)	0.94
	1	1.04 (0.78, 1.40)	0.77	1.06 (0.78, 1.44)	0.73
	≥2	1	-	1	-
Ethnicity	White	1	-	1	-
	Black	1.58 (1.20, 2.09)	0.001	1.03 (0.67, 1.59)	0.88
	Other	0.55 (0.29, 1.02)	0.06	0.54 (0.27, 1.06)	0.07
Sex/Exposure	MSM	1	-	1	-
	Hetero male	2.08 (1.47, 2.92)	<0.0001	1.96 (1.23, 3.12)	0.004
	Hetero female	1.82 (1.30, 2.56)	0.001	2.07 (1.24, 3.43)	0.01
	Other	1.62 (1.01, 2.59)	0.04	1.48 (0.87, 2.50)	0.15
Year starting 2nd line	Per 1 year increase	0.95 (0.90, 1.02)	0.15	0.94 (0.88, 1.01)	0.11
HIV-RNA <200 between 1st line and 2nd line		1.04 (0.74, 1.45)	0.83	0.90 (0.60, 1.34)	0.59
CD4 at start 2nd line	Per 50 cells higher	0.90 (0.85, 0.94)	<0.001	0.92 (0.88, 0.97)	0.003
VL at start of 2nd line	Per 1 log increase	1.31 (1.16, 1.47)	<0.001	1.20 (1.05, 1.36)	0.01

In univariable analyses, patients of black ethnicity, heterosexual risk group and with higher VL at start of 2nd line cART were at an increased risk of virological failure. Conversely, patients with higher CD4 counts at start of 2nd line cART were less likely to experience virological failure. In multivariable analysis, factors independently associated with risk of virological failure were:

- Heterosexual risk group: increased risk
    - HR 1.96; 95% CI 1.23-3.12; p=0.004 and HR 2.07; 95% CI 1.24-3.43; p=0.01 for heterosexual males and females, respectively, vs MSM

- Higher HIV-RNA at start of 2nd line: increased risk
    - HR 1.20; 95% CI 1.05-1.36; p=0.01 per 1 $\log_{10}$ higher
- Higher CD4 cell count at start of 2nd line regimen: reduced risk
    - HR = 0.92; 95% CI 0.88-0.97; p=0.003 per 50 cells/mm^3 higher

There was a trend towards increased risk of virological failure of 2nd line therapy with increased time between virological failure of the first line regimen and the date of start of the second line regimen (HR 1.01; 95% CI 1.00-1.02; p=0.03 per month).

5.3.2 Response by number of NRTI

Number of new NRTI included in the 2nd line regimen was not associated with virological outcome; compared to individuals with 2 or more new NRTI, the hazard ratios for virological failure were 1.02 (95% CI 0.68-1.52; p=0.94) and 1.06 (95% CI 0.78-1.44; p=0.73) for those receiving no, or 1, new NRTI, respectively. When number of new NRTI was modelled as a continuous variable the HR for virological failure was 0.96 per new NRTI (95% CI 0.80-1.14; p=0.96).

5.3.3 Response by active NRTI

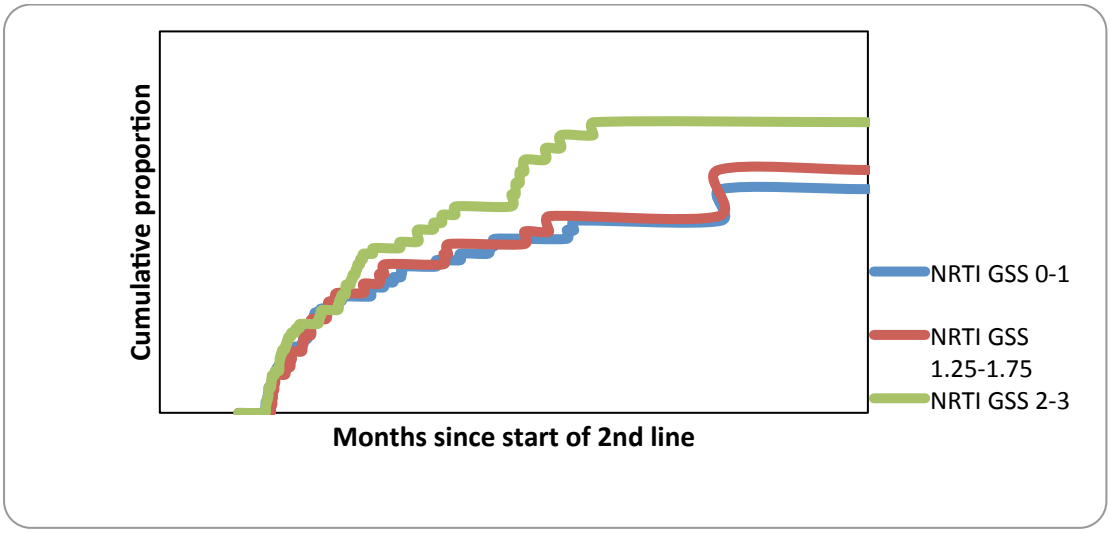
When comparing patients who did (n=246) and did not (n=224) undergo resistance testing prior to switch, patients who did not have a resistance test had significantly lower median CD4 cell count at virological failure (250 vs 287 cell/mm3; p=0.03) but other patient characteristics were similar (data not shown).

In an analysis based only on those with a resistance test available, there was no tendency observed for those with higher NRTI GSS (analysed as proportions with GSS ≤1, 1.25-1.75 and ≥2) to experience a lower risk of virological failure (table 5.4). In multivariable analysis, the HR for virological failure compared to subjects with GSS ≥2 was 0.62 (p=0.05) and 0.63 (p=0.11) for subjects with NRTI GSS of ≤1 and 1.25-1.75, respectively. When NRTI GSS was fitted as a continuous variable there was no significant association with increased risk of virological failure with increasing NRTI GSS (HR=1.22; (95% CI 0.90, 1.66) p=0.21). A Kaplan-Meier plot of time to virological failure by NRTI GSS is illustrated in figure 5.1.

Table 5.4: Factors associated with virological failure of 2nd line HAART amongst patients with resistance tests (N=246); 116 (47.2%) patients failed

		Univariable		Multivariable	
		HR (95% CI)	P-value	HR (95% CI)	P-value
NRTI GSS	≤1	0.69 (0.46, 1.03)	0.07	0.62 (0.39, 1.00)	0.05
	1.25-1.75	0.70 (0.43, 1.15)	0.16	0.63 (0.36, 1.10)	0.11
	≥2	1	-	1	-
Time from failing first line to starting 2nd line	Per 1 month increase	1.00 (0.99, 1.02)	0.51	1.00 (0.99, 1.02)	0.76
Age at start of 2nd line HAART (years)	Per 10 years older	0.95 (0.73, 1.25)	0.73	0.91 (0.67, 1.22)	0.56
Ethnicity	White	1	-	1	-
	Black	1.43 (0.98, 2.10)	0.07	0.84 (0.42, 1.64)	0.60
	Other	0.52 (0.22, 1.22)	0.13	0.38 (0.14, 1.01)	0.05
Sex/Exposure	MSM	1	-	1	-
	Heterosexual male	1.97 (1.19, 3.23)	0.01	2.07 (1.00, 4.31)	0.05
	Heterosexual female	1.85 (1.17, 2.93)	0.01	2.58 (1.23, 5.41)	0.01
	Other	1.59 (0.87, 2.91)	0.13	1.05 (0.47, 2.34)	0.90
Year of starting 2nd line HAART	Per 1 year increase	1.00 (0.91, 1.10)	0.94	1.04 (0.93, 1.15)	0.51
CD4 at start 2nd line	Per 50 cells higher	0.86 (0.79, 0.92)	<0.0001	0.90 (0.82, 0.99)	0.03
VL <500 before 2nd line	Yes	0.49 (0.32, 0.75)	0.001	0.45 (0.27, 0.73)	0.001
VL at start of 2nd line	Per 1 log increase	1.35 (1.07, 1.71)	0.01	1.07 (0.79, 1.46)	0.65

Figure 5.1: Kaplan-Meier plot of time to virological failure of 2nd regimen by NRTI GSS; log rank p-value = 0.14



GSS 0-1 N	104	73	47	32	26	10	6	2	1
GSS 1.25-2.75 N	50	35	24	20	11	9	4	1	0
GSS 2-3 N	92	64	35	19	9	5	3	3	1

5.4 Discussion

In UK practice, approaches differ between clinics with respect to NRTI choice when using a PI/r 2nd line. If two fully active NRTI are not required savings could be made in terms of both cost and toxicity.

The response rates to first and second line cART in this analysis are consistent with, or better than, those observed in other cohorts [Murphy *et al*. 2011] and clinical trials [Madrugs *et al*. 2007]. In our analysis there was a wide degree of variability in number of new and active NRTI started as part of second line PI/r cART. We found no association between GSS nor number of new NRTI, and 2nd line virological failure (although confidence intervals were wide). The risk of failing 2nd line therapy increased with time spent on failing 1st line treatment. It is established that longer time on failing cART may be associated with accumulation of resistance; it is possible that longer time on failing 1st line cART was associated with more low-level resistance (not analysable in our study) or adverse combinations of mutations.

Our findings regarding lack of impact of NRTI on 2nd line response are consistent with cohort data indicating that for individuals with M184V, therefore resistant to 3/FTC, outcomes on a PI/r were similar regardless of number of NRTI [Hull *et al*. 2009]. Possible explanations include the inherent potency of PI/r which could mask any small benefit of active NRTI. LPV/r monotherapy yields higher rates of viral suppression when used as simplification in virologically suppressed patients than as initial therapy. Most patients in our analysis switched directly from failing therapy and had lower viral load levels than patients starting first-line cART which could partly explain our results. In ACTG 5230, a single-arm study where individuals failing NNRTI-based therapy switched to lopinavir/ritonavir monotherapy high rates of viral suppression were achieved (87% less than 400 copies/ml at week 24) [Bartlett *et al*. 2012], consistent with our findings. However, in a recent Thai study although LPV/r monotherapy was similar to LPV/r + tenofovir (TDF) + 3TC after 1st line NNRTI failure by a <400 cut-off, it was inferior by <50 [Bunupuradah *et al*. 2012]. Similarly, a substudy of DART (a large trial investigating different monitoring and treatment strategies in Uganda and Zimbabwe) randomised individuals who had an undetectable viral load after 24 weeks of second line LPV/r-based therapy to continue that regimen or switch to LPV/r monotherapy. After a further 24 weeks the monotherapy group experienced more low-level viraemia though generally without any PI resistance [Gilks *et al*. 2012]. Less than 20% of the subjects in our study switched to PI/r monotherapy and it possible that our methods underestimated NRTI activity, over-estimating 'no NRTI' efficacy.

It is possible that individuals with poor adherence failed first-line cART with less resistance, therefore had more active NRTI, but continued to adhere poorly. This could explain the lack of benefit from additional NRTI, and the faster time to virological failure amongst those receiving more active NRTI, but cannot be explored in this study due to lack of adherence data. A study of 2nd line cART (LPV/r + NRTI backbone) in South Africa showed individuals with poor adherence to their first-line NNRTI regimen were at greater risk of lower adherence to second line therapy [Murphy *et al*. 2011]. Another reason for failure to demonstrate difference in second line response rates by number of NRTI was that the majority of patents in the cohort received zidovudine in their first line combination thereby leading to a resistance profile less likely to respond to tenofovir-based 2NRTI. As more modern regimens rarely include zidovudine, as such NRTI resistance at failure tends to be less extensive. Therefore, this finding may not be true using current first line NRTI where zidovudine will remain active.

Our results must be interpreted with caution. We had limited power and a strong possibility of confounding bias, meaning patients receiving more active NRTI differed in other important ways from those receiving fewer. A high proportion of individuals failing first-line NNRTI therapy were excluded from this analysis for reasons previously outlined; this is a clear source of potential confounding. Importantly almost half of the analysed subjects did not resistance testing prior to switch. This may limit the applicability of these results to both resource-rich settings (where a higher proportion of patient would be expected to undergo resistance testing) and resource-limited settings (where resistance testing may not be available routinely). Our cut-off for virological failure was 200 copies/ml; it was, and in many centres still is, common UK practice to request genotyping only on samples with HIV-RNA greater than 500-1000 copies/ml which may account for the high proportions with no resistance testing. Also, our cohort covers 1999 to 2008; during this decade standards of care evolved considerably and which may confound our observations and limit the applicability of our results to modern therapy. Due to the time period covered by our cohort, some of the early data would have been based on a less than 400 copies/ml rather than a less than 50 copies/ml HIV-RNA assay. This would have a minimal impact on results, if any, since by the time NNRTI were widely used first line, 400 cut-of assays were rarely used and, since an undetectable viral load is coded as zero in the database, the cut-off used should not affect the analysis. We selected a 200 cut-off to define viral suppression to allow for viral blips (detectable viraemia between 50 and 200 copies/ml, preceded and followed by an undetectable viral load); this cut-off is also used by the FDA for registrational antiretroviral trials. Of note, defining second line virological failure as failure to achieve a viral load less that 200 copies/ml by month 4 may have misclassified those individuals with slow viral decay as failures when they may have gone to achieve viral suppression.

Optimising 2nd line therapy is of global importance. With scarce 2nd line ARV options in resource-limited settings, minimising the number of agents required for 2nd line therapy could yield benefits in terms of toxicity, tolerability, adherence and cost. To definitively address this, a randomised trial is required to rule out confounding secondary to adherence or other factors. However, in areas without access to viral load monitoring the risk of resistance development on 1st line cART is greater and our findings may not be applicable to these settings.

Chapter 6:

General Conclusions

6: General conclusions

6.1 Conclusions

6.2 Hypothesis 1

6.3 Hypothesis 2

6.4 Hypothesis 3

6.5 Hypothesis 4

6.6 Hypothesis 5

6.7 Summary

6.1 Conclusions

Despite the advent of better-tolerated antiretrovirals, drug-related toxicity remains a common problem. Timely switch of antiretrovirals and, where feasible, simplifying therapy are important strategies for managing side effects and adherence difficulties.

This thesis sought to examine the following hypotheses:

- Switching directly from efavirenz will alter concentrations of newer antiretrovirals metabolised by the cytochrome P450 system and the induction effect of efavirenz will vary for different drugs.
- Switching from EFV to an alternative agent in the absence of prior drug resistance will not compromise virological efficacy.
- Switching from efavirenz to etravirine will improve CNS symptoms (in symptomatic patients) and improve lipids without compromising adherence.
- Increasing MVC dose for 2 weeks after switching to EFV will result in maintenance of therapeutic MVC concentrations throughout the early dosing period and can be guided by pre-cART tropism determination.
- Individuals starting a boosted protease inhibitor (PI/r) based regimen after first line treatment failure do not require two additional active drugs.

6.2 Hypothesis 1: Switching directly from efavirenz will alter concentrations of newer antiretrovirals metabolised by the cytochrome P450 system and the induction effect of efavirenz will vary for different drugs.

This hypothesis was tested in two studies: the EFV to ETR study in healthy volunteers (chapter 3) and the EFV to MVC study in HIV-positive patients (chapter 4). We demonstrated a significant reduction in plasma concentrations of antiretrovirals metabolised by the 3A4 cytochrome isoenzyme when started directly after stopping EFV.

In the first EFV to ETR switch study, healthy volunteers underwent intensive ETR PK sampling at day 1 (PK1) and after 14 days (steady-state; PK2) of ETR therapy. After a wash out period of 2 week, all commenced EFV for 2 weeks and then switched directly to ETR again for a further two weeks; intensive ETR pharmacokinetics were performed at day 1 (PK3) and day 14 (PK4) of the second ETR dosing period. By comparing drug concentrations primarily at PK2 and PK4 we were able to estimate the impact of the prolonged CYP induction effect of

EFV on steady-state (ie after 2 weeks) ETR plasma concentrations. Furthermore the participants received ETR at a dose of 200mg twice daily or at an unlicensed dose of 400mg once daily.

In the twice daily ETR group steady-state C_{max}, C_{trough} and AUC_{0-12} ETR concentrations were 21%, 37% and 28% lower directly after EV intake; corresponding figures for the once daily ETR arm were 22%, 33% and 28% lower, respectively. When comparing ETR concentrations before and after EFV on day 1 of ETR dosing (PK1 vs PK3) the magnitude of reduction in ETR concentrations was similar (although measured concentrations were expectedly lower). As ETR dosing ceased at day 14 of each intake period our data did not demonstrate how long the EFV induction effect persisted.

With regards to MVC we selected to investigate an adjusted dose strategy, based on the prediction that MVC would be subject to a significant EFV effect for the first 14 days. Unlike ETR, for MVC there is a lack of historical data to support the efficacy of MVC when combined with drugs that lower its plasma concentrations. In addition, also unlike ETR, the risk of virological failure on MVC does seem to correlate with MVC plasma concentrations [132]. With this in mind we felt it was safer to use MVC dose that is recommended when it is co-administered with EFV for the first 14 days. We actually observed a shorter period of 'post-EFV induction' for MVC than for ETR. On day 1 of switching from EFV, the higher dose of MVC produced plasma concentrations similar to reference (AUC_{0-12} was 25% higher). However, by 14 days after switch from EFV, MVC 600g BID yielded plasma concentrations greater than double the reference concentrations for all parameters. Looking at MVC trough concentrations on days 3, 6 and 10, MVC C_{trough} was higher than reference at each time point and by day 10 was almost double the reference concentration; by day 14, MVC C_{trough} was 125% higher than reference. This suggests that the induction effect of EFV on MVC was less than 2 weeks and we estimate that a significant impact of EFV on MVC concentrations persist for approximately 1 weeks after switch from EFV only.

The reasons for the apparent difference in terms of EFV induction effect is unclear but may be due to:

1) A difference in drug metabolism between HIV-negative and HIV-positive participants
2) An impact of ETR on EFV metabolism (which could account for (3))
3) A difference in the EFV concentrations after EFV cessation

Most importantly this highlights the importance of studying different tail interactions; the impact of EFV on one drug cannot be extrapolated from studies of different agents.

6.3 Hypothesis 2: Switching from EFV to an alternative agent in the absence of prior drug resistance will not compromise virological efficacy.

In both of the switch studies in HIV-positive patients, switching from EFV to an alternative agent was associated with maintained virological suppression (there were no virological failures in the 38 individuals who switched to ETR and the 12 who switched to MVC). It must be borne in mind however that these studies were designed to investigate the tolerability (ETR switch) and the pharmacokinetics (MVC switch) of switching from EFV, not virological or immunological efficacy. By definition all individuals were sufficiently adherent to have achieved and maintained viral suppression on their baseline regimen, supported by the very high adherence rates reported throughout both studies (only reported for ETR switch via M-MASRI questionnaire); it may not be possible to extrapolate our results to individuals with intermittent or persistent suboptimal adherence. In addition, we excluded any individuals with a history of detected or likely drug resistance (for example patients with periods of detectable viraemia on cART with no available resistance test form that time). As studies switching patients on suppressive PI/r- to raltegravir-based treatment have demonstrated, baseline resistance can contribute to inferior efficacy of the new regime [Eron *et al*. 2010; Vispo *et al*. 2010]. This was unlikely in our studies as individuals on EFV, with its low genetic barrier to resistance, are unlikely to maintain viral suppression in the face of significant drug resistance. Our studies contribute to the existing data that a component of successful cART can be switched without of loss of efficacy providing further reassurance to clinicians and patients alike. It also supports the earlier use of MVC and ETR; both agents have, anecdotally, tended to be reserved to later lines of therapy.

6.4 Hypothesis 3: Switching from efavirenz to etravirine will improve CNS symptoms (in symptomatic patients) and improve lipids without compromising adherence.

In many ways our results are not surprising in that switching an individual who is suffering from EFV-related side effects away from the EFV will yield symptomatic improvement. However, ours is the first study to switch from EFV in a blinded, placebo-controlled, randomised fashion thus minimising the risk of bias, particularly as EFV side effects are well understood; in addition, patients entering the study were all recruited because of possible

EFV toxicity and as such may have had an expectation that their symptoms would improve on switching away from that agent. During the blinded phase we observed significant improvements in overall CNS toxicity (as measured by the proportion of participants with any grade 2-4 CNS symptoms, the median number of CNS symptoms and the CNS score), a significant improvement in abnormal dreams and a trend towards improvement in insomnia amongst those patients who switched to EFV immediately; no improvement in any measured parameter was observed in the group who remained on EFV. Changes in CNS symptoms between baseline and week 12 in the immediate switch group and week 12 and 24 in the delayed switch group were also combined and confirmed significant improvement in all of the 'overall CNS toxicity' parameters and, individually, insomnia, abnormal dreams and nervousness. The fact that other CNS symptoms did not improve after switch from EFV suggests that, in the study population at least, these were unlikely to be EFV-related.

Although we observed no change in depression based on patient questionnaire we did see significant reductions in HADS score overall and the individual anxiety and depression scores. Our CNS symptoms questionnaire required a patient to identify themselves as suffering from depression whereas HADS provides a more objective and validated assessment of depressive symptoms. Side effects such as abnormal dreams may be clearer and simpler for patients to describe and identify whereas depression, arguably a more complex and subjective symptom, may be more difficult.

Furthermore we observed lipid improvements on switching from EFV to ETR with a significant reduction in total and LDL cholesterol and a trend to reduction in triglycerides. Reducing cardiovascular risk is an increasingly important art of HIV care and, although lifestyle changes are key, additive benefits ma be gained by switching antiretrovirals.

Our switch data supports the treatment-naïve and treatment-experienced studies that showed ETR to be a well-tolerated drug option and support its use in earlier lines of therapy as a switch option for patients with treatment-limiting EFV-related side effects.

6.5 Hypothesis 4: Increasing MVC dose for 2 weeks after switching to EFV will result in maintenance of therapeutic MVC concentrations throughout the early dosing period and can be guided by pre-cART tropism determination.

As previously discussed we decided the safest option for the EFV to MVC switch study would be to use the higher MVC dose for the first 2 weeks after EFV switch. In terms of maintaining therapeutic MVC throughout the switch period this strategy was successful with all subjects achieving MVC concentrations in excess of the IC_{50} at all time points. However, the induction effect of EFV seems to have waned sooner than has been observed when switching to NNRTI (ETR and RPV) and by day 10, MVC C_{trough} concentrations were almost double reference. It could therefore be extrapolated that an increased dose of MVC for the first week after switch only would also maintain high enough MVC concentrations. In the EFV to RPV switch work RV concentrations were initially low but as EFV concentrations were initially still in the therapeutic range the study authors used an additive score of EFV and RPV activity to show that, overall, the equivalent of one fully active drug was achieved. While this may be the case, since the increased dose of MVC was well tolerated, and post-cessation EFV concentrations were lower than observed in our EFV to ETR switch study, we conclude that a strategy of increasing MVC to 600mg BID for the first 1-2 weeks after switch from EFV is a safe strategy to employ.

6.6 Hypothesis 5: Individuals starting a boosted protease inhibitor (PI/r) based regimen after first line treatment failure do not require two additional active drugs.

NNRTI-based therapy remains the most common initial regimen in the UK. Although there are a variety of switch options for individuals who experience tolerability or toxicity problems, for those who develop virological failure, a PI/r based 2nd line regimen would usually be selected. This study was conceived following the observation that there was a lack of consensus amongst clinicians and between HIV treatment centres in terms of how many active NRTI should be used with a PI/r second line. The inherent potency of PI/r supports the hypothesis that two fully active NRTI are not required, and the desire to reduce toxicity, inconvenience and cost drives the need to simplify second line therapy as far as possible without compromising efficacy.

We demonstrated that the number of new or fully active NRTI does not impact on virological response rates to a second line PI/r-based regimen in individuals who have failed a first line NNRTI regimen. This supports the use of simpler regimens after failure of an NNRTI-based combination first line. Although potentially of particular importance in regions of the world with limited drug options, a major limitation is that individuals in the UK receive regular viral

load monitoring. In countries without access to routine viral load testing, individuals on failing therapy may accumulate more extensive NRTI resistance; these results therefore may not be generalisable. In addition the activity of NRTI was estimated based on available resistance tests and a large proportion of subjects analysed did not have resistance tests available making our study numbers relatively small. Our resistance interpretation was based on single time point sequences and we could not account for minority resistance species or mutations not listed at the time of a particular test. Despite this our data supports the practice of many HIV-treating clinicians which is to use a boosted PI with 1-2 active NRTI 2nd line and suggests further studies investigating different 2nd line strategies is warranted.

6.7 Summary

As cART regimens become increasingly inseparable in terms of virological and immunological efficacy, differences in toxicity, tolerability, drug-drug interactions and suitability for use in individuals with other co-morbidities drive prescribing decisions. Managing and, where necessary, changing cART on an individual basis is particularly important in an era when HIV-positive individuals will be treated for longer. Switching from one cART regimen to another may result in improvement in symptoms and toxicities but can be complicated by pharmacokinetic interactions. This thesis provides further evidence that the prolonged induction effect of EFV will alter concentrations of susceptible drug after EFV cessation. However, for the first time we showed that this induction effect may vary according to the switch studied consequently highlighting the importance of studying individual switch strategies. This thesis also presents the first randomised, blinded data confirming a significant improvement in CNS and metabolic toxicity when switching from EFV to an alternative drug; it also illustrated that self-report may mask objective improvement in some symptoms (for example, depression). The switch studies presented herein further support the advice that switching to a fully active regimen will not compromise antiretroviral efficacy.

Finally this thesis provides cohort data to show that potent drugs, such as boosted PI, may negate the need for two additional fully active agents. This supports the existing practice of many centres and could yield significant improvements in terms of the convenience, cost and toxicity profile of second line treatment.

Abstract presentations & publications

Listed below are data presented within this thesis that have been:

a) Published in peer-reviewed scientific journals
b) Presented as poster or oral abstracts at national and international scientific meetings

Publications:

A phase IV, double-blind, multicentre, randomized, placebo-controlled, pilot study to assess the feasibility of switching individuals receiving efavirenz with continuing central nervous system adverse events to etravirine.

AIDS 2011;25(1):65-71.

Waters L, Fisher M, Winston A, Higgs C, Hadley W, Garvey L, Mandalia S, Perry N, Mackie N, Nelson M.

The evolution of coreceptor tropism in HIV-infected patients interrupting suppressive antiretroviral therapy.

Clin Infect Dis 2011;52(5):671-3.

Waters LJ, Scourfield AT, Marcano M, Gazzard BG, Bower M, Nelson M, Stebbing J.

Pharmacokinetics and Safety of Etravirine Administered Once or Twice Daily After 2 Weeks Treatment With Efavirenz in Healthy Volunteers.

J Acquir Immune Defic Syndr 2009;52(2):222-7.

Boffito M, Jackson A, Lamorde M, Back D, Watson V, Taylor J, Waters L, Asboe D, Gazzard B, Pozniak A.

In press

Switching from a failing 1st line, NNRTI-based regimen to a boosted protease inhibitor: how many nucleoside analogues are necessary?

Antiviral Therapy (accepted for publication)

Waters L, Bansi L, Asboe D, Pozniak A, Smit W, Orkin C, Fearnhill E, Dunn D & Phillips A.

Submitted, decision awaited

Waters L, Jackson A, Else L, Rockwood N, Back D, Nelson M, Gazzard B & Boffito M.

Submitted to Lancet Infectious Diseases.

Switching safely: Pharmacokinetics, efficacy and safety of switching efavirenz to maraviroc twice-daily in patients on suppressive antiretroviral therapy.

Oral abstracts:

Pharmacokinetics (PK), efficacy and safety of switching from efavirenz (EFV) to maraviroc (MVC) twice-daily (BID) in patients suppressed on an EFV-containing regimen as initial therapy.

13th EACS Conference, Belgrade. October 2011. PS6/3.

Waters LJ, Newell S, Amara A, Hughes A, Cevik M, Rockwood N, Jackson A, Back D, Nelson M, Gazzard B & Boffito M.

A phase IV, double-blind, multi-centre, randomised, placebo-controlled, pilot study to assess the feasibility of switching individuals receiving efavirenz (EFV) with continuing central nervous system (CNS) adverse events (AE) to once daily etravirine (ETR).

2nd Joint Conference of BHIVA and BASHH, April 2010; Manchester. O7.

Waters L, Fisher M, Winston A, Higgs C, Hadley W, Garvey L, Mandalia S, Perry N, Mackie N & Nelson M.

Outcomes of second-line ritonavir-boosted protease inhibitor (PI/r)-based ART after failure of a first-line non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART.

2nd Joint Conference of BHIVA and BASHH, April 2010; Manchester. O8.

Waters L, Bansi L, Asboe D, Pozniak A, Orkin C, Smit E & Phillips A.

Poster abstracts:

Switching individuals on stable efavirenz (EFV)-based therapy with central nervous system (CNS) adverse events (AE) to etravirine (ETR): impact on modified medication adherence self-report inventory (M-MASRI) and hospital anxiety and depression scale (HADS).

13th EACS Conference, Belgrade. October 2011. PE9.8/2

Waters LJ, Perry N, Winston A, Higgs C, Fisher M, Garvey L, Mandalia S, Hadley W, Mackie N & Nelson M.

The evolution of co-receptor tropism in patients interrupting suppressive HAART.

16th CROI Conference 2009, San Francisco. Abstract 439a.

Waters LJ, Scourfield AT, Marcano M, Gazzard B & Nelson M.

References

Airoldi, M., Zaccarelli, M., Bisi, L. *et al*. One-pill once-a-day HAART: a simplification strategy that improves adherence and quality of life of HIV-infected subjects. *Patient Prefer Adherence* **4**: 115-25 (2010).

Alkhatib, G., Combadiere, C., Broder, C.C. *et al*. CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* **272**: 1955-8 (1996).

Almond, L.M., Hoggard, P.G., Edirisinghe, D. et al. Intracellular and plasma pharmacokinetics of efavirenz in HIV-infected individuals. *J Antimicrob Chemother* **56**: 738-744 (2005).

Applied Health Sciences 2011. Assessing the validity of the PHQ-9, HADS, BDI-II and QIDS-SR16 in measuring severity of depression in a UK sample of primary care patients with a diagnosis of depression. Available at healthcareimprovementscotland.org. Accessed on 30th April 2013.

Arribas, J.R., Delgado, R., Arranz, A. *et al*. Lopinavir-ritonavir monotherapy versus lopinavir-ritonavir and 2 nucleosides for maintenance therapy of HIV: 96-week analysis. *J Acquir Immune Defic Syndr* **51(2)**: 147-52 (2009).

Arribas, J.R., Horban, A., Gerstoft, J. *et al*. The MONET trial: darunavir/ritonavir with or without nucleoside analogues, for patients with HIV RNA below 50 copies/ml. *AIDS* **24(2)**: 223-30 (2010).

Asboe, D., Aitken, C., Boffito, M. et al. British HIV Association guidelines for the routine investigation and monitoring of adult HIV-1-infected individuals 2011. *HIV Medicine* **13**: 1-44 (2012).

Baede, P., Piscitelli, S., Graham, N., Van't Klooster, G. Drug interactions with TMC125, a potent next generation NNRTI [abstract A-1827]. *2nd Interscience Conference on Antimicrobial Agents and Chemotherapy*, September, San Diego (2002).

Bangalore, S., Kamalakkannan, G., Parkar, S., Messerli, F.H. Fixed-dose combinations improve medication compliance: a meta-analysis. *Am J Med* **120(8)**: 713-9 (2007).

Bartlett, J.A., Ribaudo, H.J., Wallis, C.L. *et al*. Lopinavir/ritonavir Monotherapy After Virologic Failure of First-line Antiretroviral Therapy in Resource-limited Settings'. *AIDS* **26(11)**:1345-1354 (2012).

Baum, P.D., Sullam, P.M., Stoddart, C.A., McCune, J.M. Abacavir increases platelet reactivity via competitive inhibition of soluble guanylyl cyclase. *AIDS* **25(18)**: 2243-8 (2011).

BHIVA guidelines for the treatment of HIV-1 positive adults with antiretroviral therapy (2012). Accessed at www.bhiva.org on 7th June 2012

Bierman, W.F.W. HIV monotherapy with ritonavir-boosted protease inhibitors: a systematic review. *AIDS* **23**: 279–291 (2009).

Bjelland, I., Dahl, A.A., Haug, T.T., Neckelmann, D. The validity of the Hospital Anxiety and Depression Scale. An updated literature review. *J Psychosom Res* **52(2)**: 69-77 (2002).

Boffito, M., Jackson, A., Lamorde, M. et al. Pharmacokinetics and safety of etravirine administered once or twice daily after 2 weeks treatment with efavirenz in healthy volunteers. *J Acquir Immune Defic Syndr* **52(2)**: 222-7 (2009).

Bommenel, T., Launay, O., Meynard, J.L. *et al*. Comparative effectiveness of continuing a Virologically effective first-line boosted protease inhibitor combination or of switching to a three-drug regimen containing either efavirenz, nevirapine or abacavir. *J Antimicrob Chemother* **66**: 1869–1877 (2011).

Boyle, A., Sonecha, S., Pacho, C. *et al*. An investigation into the frequency and reasons why patients switch antiretroviral therapy and which antiretrovirals are commonly implicated in toxicity. *HIV Medicine* **13(S1)**: O28 (2012).

Briggs, D.R., Tuttle, D.L., Sleasman, J.W., Goodenow, M.M. Envelope V3 amino acid sequence predicts HIV-1 phenotype (co-receptor usage and tropism for macrophages. *AIDS* **14**: 2937-9 (2000).

Bunupuradah, T., Chetchotisakd, P., Ananworanich, J. *et al*. A randomized comparison of second line lopinavir/ritonavir monotherapy vs tenofovir/lamivudine/lopinavir/ritonavir in patients failing NNRTI-regimens: the HIV STAR Study. *Antivir Ther* **17**:10.3851/IMP2222 (2012).

Cameron, D.W., da Silva, B.A., Arribas, J.R. *et al*. A 96-week comparison of lopinavir-ritonavir combination therapy followed by lopinavir-ritonavir monotherapy versus efavirenz combination therapy. *J Infect Dis* **198(2)**: 234-40 (2008).

Celsentri Summary of Product Characteristics. ViiV Healthcare UK Ltd; 22nd November 2011.

Clifford, D.B., Evans, S., Yang, Y. *et al*. Long-term impact of efavirenz on neuropsychological performance and symptoms in HIV-infected individuals (ACTG 5097s). *HIV Clin Trials* **10(6):** 343-55 (2009).

Cohen, C., Mills, A., DeJesus, E. *et al*. Switching from Efavirenz/Emtricitabine/Tenofovir Disoproxil Fumarate (EFV/FTC/TDF) Single Tablet Regimen (STR) to Emtricitabine/Rilpivirine/Tenofovir Disoproxil Fumarate (FTC/RPV/TDF) STR in Virologically Suppressed, HIV-1 Infected Subjects [abstract LBPS10/4]. *13th European AIDS Conference.* October 12-15, Belgrade (2011).

Cohen, C.J., Molina, J.M., Cahn, P. *et al*. Efficacy and safety of rilpivirine (TMC278) versus efavirenz at 48 weeks in treatment-naive HIV-1-infected patients: pooled results from the phase 3 double-blind randomized ECHO and THRIVE Trials. *J Acquir Immune Defic Syndr* **60(1)**: 33-42 (2012).

Collier, A.C., Coombs, R.W., Schoenfeld, D.A. *et al*. Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. AIDS Clinical Trials Group. *N Engl J Med* **334(16)**: 1011-7 (1996).

Combivir Summary of Product Characteristics. ViiV Healthcare Limited; 2nd May 2011.

Cooper, D.A., Heera, J., Goodrich, J. *et al*. Maraviroc versus efavirenz, both in combination with zidovudine-lamivudine, for the treatment of antiretroviral-naive subjects with CCR5-tropic HIV-1 infection. *J Infect Dis* **201(6)**: 803-13 (2010).

Cooper, V., Moyle, G.J., Fisher, M. *et al*. Beliefs about antiretroviral therapy, treatment adherence and quality of life in a 48-week randomised study of continuation of zidovudine/lamivudine or switch to tenofovir DF/emtricitabine, each with efavirenz. *AIDS Care* **23(6)**: 705-13 (2011).

Crauwels, H., Vingerhoets, J., Ryan, R., Witek, J., Anderson, D. Pharmacokinetic parameters of once-daily rilpivirine following administration of efavirenz in healthy subjects. *Antivir Ther* **17(3)**: 439-46 (2012).

Daar, E.S., Tierney, C., Fischl, M.A. *et al.* Atazanavir plus ritonavir or efavirenz as part of a 3-drug regimen for initial treatment of HIV-1. *Ann Intern Med* **154(7)**: 445-56 (2011).

DAD Study Group. The Data Collection on Adverse Events of Anti-HIV drugs (DAD) Study Group. Factors associated with specific causes of death amongst HIV-positive individuals in the DAD study. *AIDS* **24**: 1537–1548 (2010).

Dailly, E., Tribut, O., Tattevin, P. *et al*. Influence of tenofovir, nevirapine and efavirenz on ritonavir-boosted atazanavir pharmacokinetics in HIV-infected patients. *Eur J Clin Pharmacol* **62(7)**: 523-6 (2006).

DeJesus, E., Walmsley, S., Cohen, C. *et al*. Fasted lipid changes after administration of maraviroc or efavirenz in combination with zidovudine and lamivudine for 48 weeks to treatment-naive HIV-infected patients [abstract 791]. *15th Conference on Retroviruses and Opportunistic Infections*. February 3-6. Boston, MA (2008).

Dejesus, E., Young, B., Morales-Ramirez, J.O. *et al*. Simplification of antiretroviral therapy to a single-tablet regimen consisting of efavirenz, emtricitabine, and tenofovir disoproxil

fumarate versus unmodified antiretroviral therapy in virologically suppressed HIV-1-infected patients. *J Acquir Immune Defic Syndr* **51(2)**: 163-74 (2009).
DeJesus, E., Lalezari, J.P., Osiyemi, O.O. *et al*. Pharmacokinetics of once-daily etravirine without and with once-daily darunavir/ritonavir in antiretroviral-naive HIV type-1-infected adults. *Antivir Ther* **15(5)**: 711-20 (2010).
DHHS Guidelines. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services (2011). Available at http://www.aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf. Accessed 7th June 2012.
Dorr, P., Westby, M., Dobbs, S. *et al*. Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. *Antimicrob Agents Chemother* **49(11)**: 4721-32 (2005).
Duran, S., Spire, B., Raffi, F. *et al*. Self-reported symptoms after initiation of a protease inhibitor in HIV-infected patients and their impact on adherence to HAART. *HIV Clin Trials* **2(1)**: 38-45 (2001).
Durand, M., Sheehy, O., Baril, J.G., Lelorier, J., Tremblay, C.L. Association between HIV infection, antiretroviral therapy, and risk of acute myocardial infarction: a cohort and nested case-control study using Québec's public health insurance database. *J Acquir Immune Defic Syndr* **57(3)**: 245-53 (2011).
EACS guidelines v6. European AIDS Clinical Society Guidelines version 6 – October 2011. Accessed at http://www.europeanaidsclinicalsociety.org/images/stories/EACS-Pdf/EACSGuidelines-v6.0-English.pdf on 7th June 2012.
Elzi, L., Marzolini, C., Furrer, H. *et al*. Treatment modification in human immunodeficiency virus-infected individuals starting combination antiretroviral therapy between 2005 and 2008. *Arch Intern Med* **170(1)**: 57-65 (2010).
Ena, J., Leach, A., Nguyen, P. Switching from suppressive protease inhibitor-based regimens to nevirapine-based regimens: a meta-analysis of randomized controlled trials. *HIV Med* **9**: 747–756 (2008).
Eron, J.J., Young, B., Cooper, D.A. *et al*. Switch to a raltegravir-based regimen versus continuation of a lopinavir-ritonavir-based regimen in stable HIV-infected patients with suppressed viraemia (SWITCHMRK 1 and 2): two multicentre, double-blind, randomised controlled trials. *Lancet* **375**: 396–407 (2010).
Famularo, G., Di Toro, S., Moretti, S., De Simone, C. Symptomatic crystalluria associated with indinavir. *Ann Pharmacother* **34(12)**: 1414-8 (2000).
Fätkenheuer, G., Duvivier, C., Rieger, A. *et al*. Lipid profiles for etravirine versus efavirenz in treatment-naive patients in the randomized, double-blind SENSE trial. *J Antimicrob Chemother* **67(3)**: 685-90 (2012).
Fayet Mello, A., Buclin, T., Decosterd, L.A. *et al*. Successful efavirenz dose reduction guided by therapeutic drug monitoring. *Antivir Ther* **16(2)**: 189-97 (2011).
Fisher, M., Moyle, G.J., Shahmanesh, M. *et al*. A randomized comparative trial of continued zidovudine/lamivudine or replacement with tenofovir disoproxil fumarate/emtricitabine in efavirenz-treated HIV-1-infected individuals. *J Acquir Immune Defic Syndr* **51(5)**: 562-8 (2009).
Gatell, J., Salmon-Ceron. D., Lazzarin, A. *et al*. Efficacy and safety of atazanavir-based highly active antiretroviral therapy in patients with virologic suppression switched from a stable, boosted or unboosted protease inhibitor treatment regimen: the SWAN Study (AI424-097) 48-week results. *Clin Infect Dis* **44(11)**: 1484-92 (2007).
Gazzard, B.G., Anderson, J., Babiker, A. *et al*. British HIV Association Guidelines for the treatment of HIV-1-infected adults with antiretroviral therapy 2008. *HIV Med* **9(8)**: 563-608 (2008).

Gazzard, B., Duvivier, C., Zagler, C. *et al*. Phase 2 double-blind, randomized trial of etravirine versus efavirenz in treatment-naive patients: 48-week results. *AIDS* **25(18)**: 2249-58 (2011).
Geretti, A.M. & Mackie, N. Determining HIV-1 tropism in routine clinical practice (2009). Accessed at www.bhiva.org on 18th July 2012.
Ghosn, J., Carosi, G., Moreno, S. *et al*. Unboosted atazanavir-based therapy maintains control of HIV type-1 replication as effectively as a ritonavir-boosted regimen. *Antivir Ther* **15(7)**: 993-1002 (2010).
Gilks, C.F., Walker, A.S., Dunn, D.T. *et al*. Lopinavir/ritonavir monotherapy after 24 weeks of second-line anti-retroviral therapy in Africa: a randomised, controlled trial (SARA). *Antivir Ther* :10.3851/IMP2253 (2012).
Gilliam, B.L., Riedel, D.J., Redfield, R.R. *et al*. Clinical use of CCR5 inhibitors in HIV and beyond. *Journal of Translational Medicine* **9(Suppl 1)**:S9 (2010).
Grunfeld, C., Delaney, J.A., Wanke, C. *et al*. Preclinical atherosclerosis due to HIV infection: carotid intima-medial thickness measurements from the FRAM study. *AIDS* **23(14)**: 1841-9 (2009).
Gulick, R.M., Lalezari, J., Goodrich, J. *et al*. Maraviroc for previously treated patients with R5 HIV-1 infection. *N Engl J Med* **359(14)**: 1429-41 (2008).
Gutierrez, C., Diaz, L., Vallejo, A. *et al*. Intensification of Antiretroviral Therapy with a CCR5 Antagonist in Patients with Chronic HIV-1 Infection: Effect on T Cells Latently Infected. *PLoS ONE* 6(12): e27864 (2011).
Hariparsad, N., Nallani, S.C., Sane, R.S. *et al*. Induction of CYP3A4 by efavirenz in primary human hepatocytes: comparison with rifampin and phenobarbital. *J Clin Pharmacol* **44**: 1273-1281 (2004).
Haubrich, R.H., Riddler, S.A., DiRienzo, A.G. et al. Metabolic outcomes in a randomized trial of nucleoside, nonnucleoside and protease inhibitor-sparing regimens for initial HIV treatment. *AIDS* **23(9)**: 109-18 (2009).
Heera, J., Saag, M., Ive, P. et al. Virological correlates associated with treatment failure at week 48 in the phase 3 study of maraviroc in treatment-naïve patients. *15th Conference on Retroviruses and Opportunistic Infections*, Boston, MA; abstract 40LB (2008).
Herrmann, C. International experiences with the Hospital Anxiety and Depression Scale – a review of validation data and clinical results. *J Psychom Res* **42**: 17-41 (1997).
Hileman, C.O., Wohl, D.A., Tisch, D.J., Debanne, S.M., McComsey, G.A. Initiation of an Abacavir-Containing Regimen in HIV-Infected Adults Is Associated with a Smaller Decrease in Inflammation and Endothelial Activation Markers Compared to Non-Abacavir-Containing Regimens. *AIDS Res Hum Retroviruses* (2012).
HPA 2011 Report. Health Protection Agency HIV in the United Kingdom: 2011 Report. Accessed at http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317131685847 on 6th June 2012.
Hull, M., Moore, D., Harris, M. *et al*. A lamivudine (3TC)-based backbone in conjunction with a boosted protease inhibitor (PI) is sufficient to achieve virologic suppression in the presence of M184V mutations. *49th Interscience Conference on Antimicrobial Agents and Chemotherapy*, San Francisco, abstract H-916 (2009).
Intelence Summary of Product Characteristics. Janssen-Cilag International NV, March 2012; accessed 16th July 2012.
Islam, F., Wu, J., Jansson, J., Wilson. D. Relative risk of cardiovascular disease among people living with HIV: a systematic review and meta-analysis. *HIV Med* (2012).
Iwamoto, M., Wenning, L.A., Petry, A.S. *et al*. Minimal effects of ritonavir and efavirenz on the pharmacokinetics of raltegravir. *Antimicrob Agents Chemother* **52**: 4338–4343 (2008).
Jones, A.E., Brown, K.C., Werner, R.E. *et al*. Variability in drug metabolizing enzyme activity in HIV-infected patients. *Eur J Clin Pharmacol* **66(5)**: 475-85 (2010).

Kakuda, T.N., Wade, J.R., Snoeck, E. *et al*. Pharmacokinetics and pharmacodynamics of the non-nucleoside reverse-transcriptase inhibitor etravirine in treatment-experienced HIV-1-infected patients. *Clin Pharmacol Ther* **88(5)**: 695-703 (2010).
Kakuda, T.N., Van Solingen-Ristea, R., Aharchi, F. et al. Pharmacokinetics and Short-Term Safety of Etravirine in Combination With Fluconazole or Voriconazole in HIV-Negative Volunteers. *J Clin Pharmacol* (2012).
Katlama, C., Haubrich, R., Lalezari, J. *et al*. Efficacy and safety of etravirine in treatment-experienced, HIV-1 patients: pooled 48 week analysis of two randomized, controlled trials. *AIDS* 23(17): 2289-300 (2009).
Katlama, C., Clotet, B., Mills, A. *et al*. Efficacy and safety of etravirine at week 96 in treatment-experienced HIV type-1-infected patients in the DUET-1 and DUET-2 trials. *Antivir Ther* **15(7)**: 1045-52 (2010).
Kharasch, E.D., Whittington, D., Ensign, D. *et al*. Mechanism of efavirenz influence on methadone pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther* **91(4)**: 673-84 (2012).
Kilby, J.M., Sfakianos, G., Gizzi, N. *et al*. Safety and pharmacokinetics of once-daily regimens of soft-gel capsule saquinavir plus minidose ritonavir in human immunodeficiency virus-negative adults. *Antimicrob Agents Chemother* **44(10)**: 2672-8 (2000).
Lennox, J.L., DeJesus, E., Lazzarin, A. *et al*. Safety and efficacy of raltegravir-based versus efavirenz-based combination therapy in treatment-naive patients with HIV-1 infection: a multicentre, double-blind randomised controlled trial. *Lancet* **374(9692)**: 796-806 (2009).
Lewden, C., Chene, G., Morlat, P. *et al*. HIV-infected adults with a CD4 cell count greater than 500 cells/mm3 on long-term combination antiretroviral therapy reach same mortality rates as the general population. *J Acquir Immune Defic Syndr* **46(1)**: 72-7 (2007).
Madruga, J.V., Berger, D., McMurchie, M. *et al*. Efficacy and safety of darunavir-ritonavir compared with that of lopinavir-ritonavir at 48 weeks in treatment-experienced, HIV-infected patients in TITAN: a randomised controlled phase III trial. *Lancet* **370**: 49–58 (2007).
Maggiolo, F. Efavirenz: a decade of clinical experience in the treatment of HIV. *J Antimicrob Chemother* **64(5)**: 910-28 (2009).
Mallolas, J., Podzamczer, D., Milinkovic, A. *et al*. Efficacy and safety of switching from boosted lopinavir to boosted atazanavir in patients with virological suppression receiving a LPV/r-containing HAART: the ATAZIP study. *J Acquir Immune Defic Syndr* **51(1)**: 29-36 (2009).
Manfredi, R., Calza, L. HIV infection and the pancreas: risk factors and potential management guidelines. *Int J STD AIDS* **19(2)**: 99-105 (2008).
Mannheimer, S.B., Wold, N., Gardner, E.M. *et al*. Mild-to-moderate symptoms during the first year of antiretroviral therapy worsen quality of life in HIV-infected individuals. *Clin Infect Dis* **46(6)**: 941-5 (2008).
Martinez, E., Arnaiz, J.A., Podzamczer, D. *et al*. Substitution of nevirapine, efavirenz or abacavir for protease inhibitors in patients with human immunodeficiency virus infection. *N Engl J Med* **349**: 1036–1046 (2003).
Martínez, E., Larrousse, M., Llibre, J.M. *et al*. Substitution of raltegravir for ritonavir-boosted protease inhibitors in HIV-infected patients: the SPIRAL study. *AIDS* **24**: 1697–1707 (2010).
May, M., Gompels, M., Delpech, V. *et al*. Impact of late diagnosis and treatment on life expectancy in people with HIV-1: UK Collaborative HIV Cohort (UK CHIC) Study. *BMJ* **343**: d6016 (2011).
Mayers, D.L., Japour, A.J., Arduino, J.M. *et al*. Dideoxynucleoside resistance emerges with prolonged zidovudine monotherapy. *Antimicrob Agents Chemother* **38(2)**: 307-14 (1994).
McGovern, R., Dong, W., Zhong, X. *et al*. Population-based sequencing of the V3-loop is comparable to enhanced sensitivity Trofile assay in predicting virologic response to

maraviroc of treatment-naïve patients in the MERIT trial [abstract 92]. *17th Conference on Retroviruses and Opportunistic Infections.* February 16-19 San Francisco, CA (2010).
McGovern, R.A., Thielen, A., Mo, T. *et al*. Population-based V3 genotypic tropism assay: a retrospective analysis using screening samples from the A4001029 and MOTIVATE studies. *AIDS* **24(16)**: 2517-25 (2010).
Mills, A., Antinori, A., Clotet, B. *et al*. Neurological and psychiatric tolerability of rilpivirine (TMC278) vs. efavirenz in treatment-naïve, HIV-1-infected patients at 48 weeks. ***HIV Med***. doi: 10.1111/hiv.12012 (2013).
Mills, A., Cohen, C., Dejesus, E. *et al*. Switching from efavirenz/emtricitabine/tenofovir disoproxil fumarate (EFV/FTC/TDF) single tablet regimen (STR) to emtricitabine/rilpivirine/tenofovir disoproxil fumarate (FTC/RPV/TDF) STR in virologically suppressed, HIV-1 infected subjects [abstract H2-794c]. *51st Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC)*. September 17-20. Chicago (2011).
Molina JM, Andrade-Villanueva J, Echevarria J et al. Once-daily atazanavir/ritonavir versus twice-daily lopinavir/ritonavir, each in combination with tenofovir and emtricitabine, for management of antiretroviral-naive HIV-1-infected patients: 48 week efficacy and safety results of the CASTLE study. *Lancet* **372(9639)**: 646-55 (2008).
Montaner, J., Hill, A., Acosta, E. Practical implications for the interpretation of minimum plasma concentration/inhibitory concentration ratios. *Lancet* **357**: 1438-40 (2001).
Mouly, S., Lown, K.S., Kornhauser, D. et al. Hepatic but not intestinal CYP3A4 displays dose-dependent induction by efavirenz in humans. *Clin Pharmacol Ther* **72**: 1-9 (2002).
Moyle, G., Wildfire, A., Mandalia, S. *et al*. Epidemiology and Predictive Factors for Chemokine Receptor Use in HIV-1 Infection. *J Infect Dis* **191**: 866–72 (2005).
Moyle, G.J., Sabin, C.A., Cartledge, J. *et al*. A randomized comparative trial of tenofovir DF or abacavir as replacement for a thymidine analogue in persons with lipoatrophy. *AIDS* **20(16)**: 2043-50 (2006).
Moyle, G., Orkin, C., Fisher, M. *et al*. Switching to Atripla (EFV/FTC/TDF) from Kivexa (ABC/3TC) + efavirenz improves lipid levels towards NCEP recommendations: results of a 24-week randomized study [abstract THPE0133]. *Program and abstracts of the XVIII International AIDS Conference.* July 18-23; Vienna, Austria (2010).
Moyle, G.J., Andrade-Villanueva, J., Girard, P.M. *et al*. A randomized comparative 96-week trial of boosted atazanavir versus continued boosted protease inhibitor in HIV-1 patients with abdominal adiposity. *Antivir Ther* **17(4)**: 689-700 (2012).
Murphy, R., Sunpath, H., Castilla, C. *et al*. Adherence to Second-line ART and Long-term Virologic Outcomes in South Africa. *18th Conference on Retroviruses and Opportunistic Infections*, Boston. Abstract 582 (2011).
Nakagawa, F., Lodwick, R.K., Smith, C.J. *et al*. Projected life expectancy of people with HIV according to timing of diagnosis. *AIDS* **26(3)**: 335-43 (2012).
Nelson, M., Girard, P.M., Demasi, R. *et al*. Suboptimal adherence to darunavir/ritonavir has minimal effect on efficacy compared with lopinavir/ritonavir in treatment-naive, HIV-infected patients: 96 week ARTEMIS data. *J Antimicrob Chemother* **65(7)**: 1505-9 (2010).
Nunes, E.P., Santini de Oliveira, M. et al. Monotherapy with Lopinavir/Ritonavir as Maintenance After HIV-1 Viral Suppression: Results of a 96-Week Randomized, Controlled, Open-Label, Pilot Trial (KalMo Study). *HIV Clin Trials* **10(6)**: 368-74 (2009).
Nunes, V., Neilson, J., O'Flynn, N. *et al*. (2009). Clinical Guidelines and Evidence Review for Medicines Adherence: involving patients in decisions about prescribed medicines and supporting adherence. London: National Collaborating Centre for Primary Care and Royal College of General Practitioners.
O'Rourke S, MacHale S, Signorini D, Dennis M. Detecting psychiatric morbidity after stroke: comparison of the GHQ and the HAD Scale. *Stroke* **29(5)**: 980-5 (1998).
ONS Mortality Statistics 2010. Office for National Statistics: Mortality Statistics: Deaths

registered in England and Wales (Series DR), 2010. Accessed at http://www.ons.gov.uk/ons/publications/re-reference-tables.html?edition=tcm%3A77-230730 on 7th June 2012.

Ortiz, R., Dejesus, E., Khanlou, H. *et al.* Efficacy and safety of once-daily darunavir/ritonavir versus lopinavir/ritonavir in treatment-naive HIV-1-infected patients at week 48. *AIDS* **22(12)**: 1389-97 (2008).

Ovbiagele, B., Nath, A. Increasing incidence of ischemic stroke in patients with HIV infection. *Neurology* **76(5)**: 444-50 (2011).

Pahuja, M., Grobler, A., Glesby, M.J. *et al.* Effects of a reduced dose of stavudine on the incidence and severity of peripheral neuropathy in HIV-infected adults in South Africa. *Antivir Ther* **17(4)**: 737-43 (2012).

Parienti, J.J., Bangsberg, D.R., Verdon, R., Gardner, E.M. Better adherence with once-daily antiretroviral regimens: a meta-analysis. *Clin Infect Dis* **48(4)**: 484-8 (2009).

Pulido, F., Estrada, V., Baril, J.G. *et al*. Long-term efficacy and safety of fosamprenavir plus ritonavir versus lopinavir/ritonavir in combination with abacavir/lamivudine over 144 weeks. *HIV Clin Trials* **10(2)**: 76-87 (2009).

Rasmussen, T.A., Tolstrup, M., Melchjorsen, J. *et al*. Evaluation of cardiovascular biomarkers in HIV-infected patients switching to abacavir or tenofovir based therapy. *BMC Infect Dis* **11**: 267 (2011).

Reeves, J.D., Coakley, E., Petropoulos, C.J., Whitcomb, J.M. An enhanced sensitivity Trofile HIV coreceptor tropism assay for selecting patients for therapy with entry inhibitors targeting CCR5: A review of analytical and clinical studies. *J Viral Entry* **3**: 94-102 (2009).

Ribaudo, H.J., Haas, D.W., Tierney, C. et al. Pharmacogenetics of plasma efavirenz exposure after treatment discontinuation: an Adult AIDS Clinical Trials Group Study. *Clin Infect Dis* **42**: 401-407 (2006).

Rihs, T.A., Begley, K., Smith, D.E. *et al*. Efavirenz and chronic neuropsychiatric symptoms: a cross-sectional case control study. *HIV Med* **7(8)**: 544-8 (2006).

Saag, M., Ive, P., Heera, J. *et al*. A multicenter, randomized, double-blind, comparative trial of a novel CCR5 antagonist, maraviroc versus efavirenz, both in combination with Combivir (zidovudine [ZDV] / lamivudine [3TC]), for the treatment of antiretroviral naïve patients infected with R5 HIV-1: week 48 results of the MERIT study [abstract WESS104]. *Fourth International AIDS Society Conference on HIV Treatment and Pathogenesis*, Sydney (2007).

Satchell, C.S., O'Halloran, J.A., Cotter, A.G. *et al*. Increased platelet reactivity in HIV-1-infected patients receiving abacavir-containing antiretroviral therapy. *J Infect Dis* **204(8)**: 1202-10 (2011).

Savard, J., Laberge, B., Gauthier, J.G., Ivers, H., Bergeron, M.G. Evaluating anxiety and depression in HIV-infected patients. *J Pers Assess* **71**: 349-367 (1998).

Saves, M., Chene, G., Ducimetiere, P. *et al*. Risk factors for coronary heart disease in patients treated for human immunodeficiency virus infection compared with the general population. *Clin Infect Dis* **37**: 292 (2003).

Sax, P.E. Report from the 2008 joint ICAAC/IDSA meeting. Maraviroc vs. efavirenz: a reanalysis of MERIT. *AIDS Clin Care* **20(12)**: 98-104 (2008).

Sax, P.E., Tierney, C., Collier, A.C. *et al*. Abacavir/Lamivudine Versus Tenofovir DF/ Emtricitabine as Part of Combination Regimens for Initial Treatment of HIV: Final Results. *J Infect Dis* **204**: 1191–201 (2011).

Scholler-Gyure, M., Kakuda, T.N., De Smedt, G. et al. Pharmacokinetics of TMC125 in QD and BID regimens in HIV-1 negative volunteers [abstract A-1427]. *47th Interscience Conference on Antimicrobial Agents and Chemotherapy.* September 17-20; Chicago, IL (2007).

Scholler-Gyure, M., Kakuda, T.N., Sekar, V. et al. Pharmacokinetics of darunavir/ritonavir and TMC125 alone and coadministered in HIV-negative volunteers. *Antivir Ther* **12**:789-796 (2007).

Schöller-Gyüre, M., Kakuda, T.N., Raoof, A., De Smedt, G., Hoetelmans, R.M. Clinical pharmacokinetics and pharmacodynamics of etravirine. *Clin Pharmacokinet*; **48(9)**: 561-74 (2009).
Scourfield, A., Zhen, J., Chinthapalli, S., Waters, L., Martin, T., Mandalia, S. & Nelson, M. Discontinuation of Atripla as first-line therapy in HIV-1 infected individuals. *AIDS* **26(11)**: 1399-401 (2012).
Seclen, E., Del Mar Gonzalez, M., De Mendoza, C., Soriano, V., Poveda, E. Dynamics of HIV tropism under suppressive antiretroviral therapy: implications for tropism testing in subjects with undetectable viraemia. *J Antimicrob Chemother* **65(7)**: 1493-6 (2010).
Selzentry FDA. Accessed at http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/022128s002lbl.pdf on 25th May 2012.
Sevinsky, H., Eley, T., Persson, A. *et al*. The effect of efavirenz on the pharmacokinetics of an oral contraceptive containing ethinyl estradiol and norgestimate in healthy HIV-negative women. *Antivir Ther* **16(2)**: 149-56 (2011).
Shi, L., Liu, J., Fonseca, V. *et al*. Correlation between adherence rates measured by MEMS and self-reported questionnaires: a meta-analysis. *Health and Quality of Life Outcomes* **8**: 99 (2010).
Smith, P.F., DiCenzo, R., Morse, G.D. Clinical pharmacokinetics of non-nucleoside reverse transcriptase inhibitors. *Clin Pharmacokinet* **40**: 893-905 (2001).
Soulie, C., Marcelin, A.G., Ghosn, J. et al. HIV-1 X4/R5 co-receptor in viral reservoir during suppressive HAART. *AIDS* **21(16)**: 2243-5 (2007).
Sustiva Summary of Product Characteristics. Bristol-Myers Squibb Pharma EEIG. 13th July 2011.
Stanford algorithm. Accessed at http://hivdb.stanford.edu/pages/algs/HIVdb.html on 1st June 2009.
Svicher, V., Alteri, C., Montano, M. *et al*. Performance of genotypic tropism testing on proviral DNA in clinical practice: results from the DIVA Study Group. *New Microbiologica* **35**: 17-25 (2012).
Taylor, S., Boffito, M., Khoo, S. *et al.* Stopping antiretroviral therapy. *AIDS* **21**: 1673-1682 (2007).
The overvolunteering prevention system. Accessed at http://www.tops.org.uk/site/cms/contentChapterview.asp?chapter=1 on 16th July 2012.
Thielen, A., Lengauer, T. Geno2pheno[454]: a web server for the prediction of HIV-1 coreceptor usage from next-generation sequencing data. *Intervirology* **55(2)**: 102-7 (2012).**Thompson, M.A., Aberg, J.A., Hoy, J.F. *et al*.** Antiretroviral Treatment of Adult HIV Infection: 2012 Recommendations of the International Antiviral Society–USA Panel. *JAMA* 308(4): 387-402 (2012).
Triant, V.A., Lee, H., Hadigan, C., Grinspoon, S.K. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J Clin Endocrinol Metab* **92(7)**: 2506-12 (2007).
Trofile methodology. Accessed at http://www.trofileassay.com/pdf/MON-8459-TDNA-Only.pdf on 18th July 2012.
Tseng, Z.H., Secemsky, E.A., Dowdy, D. *et al*. Sudden cardiac death in patients with human immunodeficiency virus infection. *J Am Coll Cardiol* **59(21)**: 1891-6 (2012).
Vandekerckhove, L.P.R., Wensing, A.M.J., Kaiser, R. *et al*. European guidelines on the clinical management of HIV-1 tropism testing. *Lancet Infect Dis* **11(5)**: 394-407 (2011).
Verhofstede, C., Brudney, D., Reynaerts, J. *et al*. Concordance between HIV-1 genotypic coreceptor tropism predictions based on plasma RNA and proviral DNA. *HIV Med* **12(9)**: 544-52 (2011).

Vingerhoets, J., Tambuyzer, L., Azin, H. et al. Resistance profile of etravirine: combined analysis of baseline genotypic and phenotypic data from the randomized, controlled phase III clinical studies. *AIDS* **24(4)**: 503-14 (2010).
Vispo, E., Barreiro, P., Maida, I. *et al*. Simplification from protease inhibitors to once- or twice-daily raltegravir: the ODIS trial. *HIV Clin Trials* **11**: 197–204 (2010).
Viramune Summary of Product Characteristics. Boehringer Ingelheim International GmbH; 19th January 2012.
v**on Giesen, H.J., Köller, H., de Nocker, D., Haslinger, B.A., Arendt, G.** Long-term safety and efficacy of NNRTI within the central nervous system. *HIV Clin Trials* **4**: 382-90 (2003).
Walsh, J., Mandalia, S., Gazzard, B. Responses to a 1 month self-report on adherence to antiretroviral therapy are consistent with electronic data and virological treatment outcome. *AIDS* **16(2)**: 269-77 (2002).
Ward, B.A., Gorski, J.C., Jones, D.R., Hall, S.D., Flockhart, D.A., Desta, Z. The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. *J Pharmacol Exp Ther* **306(1)**: 287-300 (2003).
Ward, D.J., Curtin, J.M. Switch from efavirenz to nevirapine associated with resolution of efavirenz-related neuropsychiatric adverse events and improvement in lipid profiles. *AIDS Patient Care STDS* **20(8)**: 542-8 (2006).
Waters, L., Moyle, G. CCR5 antagonists in the clinic: a review. *J Viral Entry* **3(2)**: 42-53 (2008).
WHO guidelines 2010. Antiretroviral therapy for HIV infection in adults and adolescents Recommendations for a public health approach (2010); accessed at http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf on 27th May 2012.
Wilkin, T.J., Lalama, C.M., McKinnon, J. *et al.* A pilot trial of adding maraviroc to suppressive antiretroviral therapy for suboptimal CD4+ T-cell recovery despite sustained virologic suppression: ACTG A5256. *J Infect Dis* **206(4)**: 534-42 (2012).
Winston, A., Pozniak, A., Smith, N. *et al*. Dose escalation or immediate full dose when switching from efavirenz to nevirapine-based highly active antiretroviral therapy in HIV-1-infected individuals? *AIDS* **18(3)**: 572-4 (2004).
Winston, A., Jackson, A., Fletcher, C. et al. Pharmacokinetics and antiretroviral response to darunavir/ritonavir and etravirine combination in patients with high-level viral resistance. *AIDS* **21**: 1449-1455 (2007).
Ye, Y., Zeng, Y., Li, X. *et al*. HIV infection: an independent risk factor of peripheral arterial disease. *J Acquir Immune Defic Syndr* **53(2)**: 276-8 (2010).
Yeni, P., Mills, A., Peeters, M. *et al*. Etravirine in the treatment of HIV-1: a clinical overview for healthcare professionals. *Curr HIV Res* **8(7)**: 564-76 (2010).
Zhou, J., Watson, Z., Fisher, M. & Waters, L. Switch from Efavirenz to Etravirine improves neuropsychiatric side effects in a UK-HIV clinic cohort [P199]. *18th Annual BHIVA Conference*, 18-20 April, Birmingham (2012).
Zigmond, A.S., Snaith, R.P. The Hospital Anxiety and Depression Scale. *Acta Psychiatr Scand* **67**: 361-370 (1983).