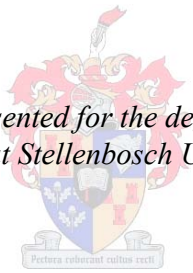
The investigation of genotypic antiretroviral drug resistance in the context of the South African national antiretroviral roll-out programme

by
Gert Uves van Zyl



Dissertation presented for the degree of Doctor of Pathology
at Stellenbosch University

Promoter: Professor Wolfgang Preiser
Division of Medical Virology, Department Pathology, Faculty of Health Sciences
Co-promotor: Professor Jean Nachega
Department of Medicine, Faculty of Health Sciences

March 2012

Declaration

I declare that the work contained herein is my own, that I am the sole author of the abstract, introduction and summative comment sections and first author of the 5 articles included in the thesis. The articles referred to are subjected to international copy right regulations.

Gert Uves van Zyl

March 2012

Abstract

Introduction: Since the South African public sector antiretroviral roll-out programme started in 2004, the success of antiretroviral combination therapy (cART) has been experienced in terms of survival, prevention of mother-to-child transmission (PMTCT) and quality of life. However, as the programme matures, viral resistance to the constituent drugs will increase. Monitoring antiretroviral drug resistance (ARVDR) should therefore be a priority in the public health approach to HIV treatment.

Methods: A cross-sectional investigation of genotypic antiretroviral drug resistance in:

a) HIV-infected mothers who were exposed to a PMTCT regimen of short course azidothymidine (AZT) with single dose nevirapine (NVP) during labour.
b) HIV-infected adults and children who were cART-naïve (transmitted or initial resistance).
c) HIV-infected adults and children who were failing cART (drug-induced or acquired resistance). In case of adults, this includes patients on a first-line, non-nucleoside reverse transcriptase (NNRTI)-based regimen, or on a second-line, protease inhibitor (PI)-based regimen, and in case of children, this includes patients on a first-line PI-based regimen.

Results: In mothers who received a PMTCT-regimen that combined AZT and NVP the prevalence of NNRTI resistance mutations was 17.1% (95% CI: 8.7-25.6%).

The prevalence of transmitted ARVDR in adults was low, as was initial ARVDR in young children (mostly PMTCT-exposed), except for NNRTI resistance in children who had received NVP as part of PMTCT.

Drug-induced resistance was found in adults failing first-line NNRTI-based cART, with 83% having resistance to ≥1 drug. In contrast, adult patients failing second-line PI-based cART had a low prevalence of PI resistance; the predominant reason for failure was poor drug exposure, as detected by measuring lopinavir concentrations in blood plasma and hair samples. In contrast, PI resistance in children was not rare, largely due to historic exposure to un-boosted PIs. This resulted in extensive resistance to PIs and reverse transcriptase inhibitors (RTI) in some children.

Conclusions: A combined regimen of short course AZT with intrapartum NVP for PMTCT may, in addition to reducing the risk of neonatal infection, also reduce the risk of NVP resistance in the mothers compared to a regimen of NVP only. In South Africa, the prevalence of transmitted ARVDR remains low relative to industrialised countries, probably as comparatively little time has elapsed since the scale-up of cART. Adults failing first-line cART are likely to respond to second-line cART, without failure due to resistance. However some children with PI and RTI resistance cannot be adequately treated with drugs currently available through the roll-out programme. This emphasizes the urgent need for a rational and science-based approach to managing cART-experienced children, including access to additional drugs to form a third-line paediatric cART regimen.

Opsomming (Afrikaans)

Inleiding: Sedert die begin van die Suid Afrikaanse publieke sektor antiretrovirale uitrol program in 2004 is die sukses van antiretrovirale kombinasie-behandeling (k-ARB) ervaar in terme van oorlewing, voorkoming van moeder na kind oordrag (VMKO) en lewenskwaliteit. Nietemin, sal weerstandigheid teen die middels wat in die antiretrovirale program gebruik word toeneem soos wat die program gevestig raak. Die monitoring van antiretrovirale middel-weerstandigheid is derhalwe ‘n prioriteit in gemeenskap-gesondheid benadering tot MIV behandeling.

Metodes: ‘n Deursnit ondersoek van genotipiese antiretrovirale middel-weerstandigheid in:

a) MIV-geïnfekteerde moeders wat blootgestel is aan VMKO regimen bestaande uit ‘n kort kursus AZT met ‘n enkeldosis nevirapien (NVP) tydens kraam.
b) MIV-geïnfekteerde volwassenes en kinders wat komibinasieterapie-naïef (oorgedraagde of inisiële weerstandigheid) is.
c) MIV-geïnfekteerde volwassenes en kinders wat k-ARB faal (middel-geïnduseerde weerstandigheid). In geval van volwassenes, sluit dit pasiënte op ‘n eerste-linie, non-nucleosied tru-transkriptase inhibitor (NNRTI)-regimen, en tweede-linie protease inhibitor (PI)-gebaseerde regimen, en in geval van kinders, sluit dit pasiënte in op ‘n eerste-linie PI-gebaseerde regimen.

Resultate: In moeders wat ‘n gekombineerde AZT en NVP VMKO-regimen ontvang het, was die voorkoms van NNRTI weerstandigheid 17.1% (95%-vertrouensinterval: 8.7-25.6%). Die voorkoms van oorgedraagde ARVMW in MIV-geïnfekteerde volwassenes en kinders wat kombinasieterapie-naïef is, was laag, so ook ARVMW in jong kinders (meestal VMKO-blootgestel), behalwe vir non-nukleosied tru-transkriptase inhibitor (NNRT) weerstandigheid in kinders wat NVP ontvang het deur VMKO. Middel-geïnduseerde weerstandigheid was gevind in volwassenes wat die eerste-linie NNRTI-gebaseerde k-ARB gefaal het, met 83% wat weerstandigheid teen ≥1 middel het. Volwassenes wat ‘n tweede-linie protease inhibitor (PI) –gebaseerde k-ARB gefaal het , het ‘n lae voorkoms van PI weerstandigheid, met die oorwegenede oorsaak, swak middel-bloostelling, soos bepaal deur van lopinavir-konsentrasies in bloed plasma en hare.

In teenstelling hiermee was PI weerstandigheid nie skaars in kinders nie, hoofsaaklik weens historiese blootstelling an ongeskraagde PI-behandeling. Dit het tot uitgebreide weerstandigheid tot PIs en tru-transkritptase inhibitors (RTI) in sommige kinders gelei.

Gevolgtrekkings: ‘n Gekombineerde regimen van ‘n kort kursus AZT met NVP tydens kraam vir VKMO, mag bykomend tot die vermindering die risiko van pasgebore infeksie, ook die kans vir weerstandigheid teen NVP in die moeders verlaag in vergelyking met ‘n regimen van NVP-alleen. Die voorkoms van oorgedraagde ARVMW is tans laag in vergelyking met geïndustrialiseerde lande, waarskynlik aangesien daar nog betreklik min tyd verloop het sedert k-ART wyd beskikbaar gemaak is. Volwassenes wat eerstelyn kombinasie terapie faal sal waarskynlik goed reageer op tweede-linie terapie, sonder terapie faling weens middelweerstandigheid. Daarenteen kan sommige kinders met protease inhibitor en tru-transkriptase weerstandigheid nie voldoende behandel word met die huidig-beskikbare middels in die uitrol program nie. Dit beklemtoon die dringende noodsaaklikheid van ‘n rasionele en wetenskaplike benadering tot k-ART in kinders, met ‘n lang terapie geskiedenis, wat toegang tot bykomende medikasie behels om `n derde-linie regimen saam te stel.

Acknowledgements

I would like to thank my supervisors, Prof. Wolfgang Preiser and Prof. Jean Nachega, for their valuable guidance and advice, prof Mark Cotton for valuable inputs with reference to career development and asking the right questions, clinical colleagues: Drs. Michele Zeier, Helena Rabie, Jantjie Taljaard, Hans Prozesky, Clair Edson, Prof. Gerhard Theron, and many others, whose enthusiasm and support for the projects made this possible. Without Prof. Susan Engelbrecht's technical advice and Ms. Mathilda Claassen's problem solving and analytical skills, none of this would have been possible. The fact that I could trust Drs. Leana Maree and Jean Maritz and Ms Tania Stander and the whole team of capable technologists to keep the ship afloat when I was pre-occupied with writing, made my life a lot easier. I appreciate the valuable statistical advice provided by Prof Lize van der Merwe. Sister Charmaine Abrahams did more than her part as research nurse. Last, but not least, I would like to thank my family in particular my wife Yvette, children Annika and Daniël, my father Danie and parents in law, Neels and Eileen, who had to put up with me through times.

Soli Deo gloria

Table of Contents

Introduction

1. South Africa and the antiretroviral roll-out programme

Today an estimated 33.3 million people worldwide are living with human immunodeficiency virus (HIV) infection. Sub-Saharan Africa is the region most severely affected, with about two thirds of the worldwide tally. Swaziland, Lesotho and Botswana are the countries with the highest prevalences, above 30% in antenatal clinic attendees, and South Africa is the single country in the world with the highest number of HIV-infected individuals (UNAIDS, 2008, UNAIDS, 2010). The adult prevalence, projected from the 2009 antenatal clinic survey, is 17.8% with 5.63 million adults and children infected and about 1.6 million adults in need of antiretroviral therapy (ART) (D.O.H., 2010a). South Africa launched a national ART roll-out programme in 2004, enabling HIV-infected individuals to regain or retain a reasonable quality of life. Adult patients with CD4+ T-cell counts below 200/µl or paediatric patients with CD4% below 15% or AIDS-defining conditions qualified for ART (D.O.H., 2004). These guidelines were revised in April 2010 with diagnosis of HIV infection in infancy an indication for ART, and a revised CD4 count cut-off of 350 cells/microliter, initially applying to pregnant women and patients with *Mycobacterium tuberculosis* co-infection only (D.O.H., 2010b), and recently extended to all patients with CD4 counts below 350 cells/microliter. For adults the first-line ART regimen was a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen containing stavudine (D4T), lamivudine (3TC) and either efavirenz (EFV) or nevirapine (NVP). Stavudine could be substituted with zidovudine (azidothymidine, AZT) for toxicity. The second-line regimen consisted of didanosine (DDI), AZT and lopinavir (LPV) boosted with RTV (LPV/r). Children below 3 years of age received a first-line regimen of LPV/r combined with 3TC and D4T, and older children D4T, 3TC and EFV. Second-line therapy was dependent on the first-line used, with NVP combined with AZT and DDI replacing a LPV/r-regimen and LPV/r, AZT and DDI replacing the first line NNRTI-based regimen. According to the updated 2010 guidelines (D.O.H., 2010b), adults initiated on ART now receive tenofovir (TDF), 3TC and either EFV or NVP and second-line comprises AZT, 3TC and LPV/r if they previously received TDF. For children under 3 months of age the new first-line regimen is abacavir (ABC), 3TC and LPV/r and for older children ABC, 3TC and EFV. Second-line therapy for children who failed ABC, 3TC and EFV consists of AZT, DDI and LPV/r, however children who fail a LPV/r-based regimen need to be referred for specialist opinion before a regimen switch is considered, on the premise that resistance is unlikely (D.O.H., 2010b) The limited availability of antiretroviral drugs necessitates an approach different from industrialised countries, where any sustained breakthrough viraemia triggers a switch of regimen. In the South African public sector which has used regular monitoring of HIV viral load from the beginning, the virological criterion for regimen switch in adult patients was two consecutive viral loads of 5000 copies per ml or more, the second after intensified adherence counselling; this threshold has recently been revised to 1000 copies per ml. In paediatric patients the approach was even more conservative and the criteria for virological failure not clearly defined. The conservative approach to regimen switch resulted in the retention of patients on non-suppressive ART regimens for prolonged periods of time. There are limited data on the effect of this on the accumulation of different genotypic resistance mutations in South African patients.

2. An introduction to antiretroviral drug resistance

2.1 The virus life cycle and antiretroviral drugs

Although viruses are absolutely dependent on living host cells to replicate, viral genomes contain unique genes that encode various structural proteins and non-structural viral proteins. Targeting these proteins and associated steps in the HIV life cycle confers specificity to antiretroviral drugs. Structural proteins consist of capsid, matrix and nucleocapsid proteins, encoded by the *gag* gene and "membrane-embedded" or envelope proteins, encoded by the *env* gene. Non-structural proteins include enzymes encoded by the *pol* gene, regulatory proteins encoded by the *tat* and *ref* genes and accessory proteins encoded by the *nef*, *vif*, *vpr* and *vpu* genes. The *pol*-encoded viral enzymes are protease (PR), reverse transcriptase (RT; this includes a polymerase and an RNAse H domain) and integrase. Most important antiretroviral targets are virally encoded enzymes which catalyse steps in the viral life cycle. Ideal antiretroviral agents inhibit stages in the viral life cycle with minimal inhibition of normal cellular function. Agents with high specificity and efficacy (as proven in clinical trials) have become part of the armamentarium of antiretroviral drugs.

In order to explain the mode of action of these drugs, a brief description of the life cycle of HIV-1 follows: The envelope protein of HIV-1, gp120, binds to CD4 receptors on host cells (predominantly CD4-positive T-lymphocytes, but also other CD4-positive cells such as macrophages and dendritic cells). Initial binding to CD4 is followed by binding to co-receptors, the most important being CCR5 and CXCR4. CCR5 is the predominant co-receptor in the early stages of infection whereas CXCR4-tropic viruses often emerge during late stages of infection (Clapham and McKnight, 2001). Following co-receptor binding, the trans-membrane portion of the other envelope protein, gp41, undergoes conformational changes which allow fusion of the viral membrane with the host cell membrane. Fusion is followed by release of the viral capsid into the cytoplasm where uncoating occurs, at which stage the major capsid protein p24 dissociates. The RT enzyme, RNAse H, vpr and the integrase enzyme remain complexed to the two copies of viral RNA in the "reverse transcription complex" (Gleenberg et al., 2007). Reverse transcription follows: First, RNA is transcribed to a RNA-DNA duplex, catalysed by RT; thereafter the RNA strand is digested by RNAse H followed by synthesis of double-stranded DNA through the DNA dependent DNA polymerase activity of RT. The now double-stranded DNA, still complexed to vpr and integrase, then moves across pores in the nuclear membrane into the nucleus. In the nucleus chromosomal DNA is cleaved by integrase and the viral DNA is inserted into the chromosomal DNA of the cell, now referred to as "proviral DNA". From this step onwards, the cellular machinery (RNA polymerase II) is used to transcribe viral genes into mRNA or full genomic viral RNA. Viral mRNAs are translated, using cellular ribosomes, to yield viral proteins. Viral capsid proteins self-assemble, incorporating viral RNAs and enzymes, and the viral particles or 'virions' then bud through the cell membrane, which has viral envelope proteins (gp120 and gp41) imbedded in it. During assembly of the viral particle, virus-encoded protease, an aspartyl protease, cleaves gag, gag-pol and nef proteins. The gag protein is cleaved into matrix, capsid, nucleocapsid and other proteins. In total twelve proteolytic reactions are needed to allow the viral particle to mature to full infectivity (de Oliveira et al., 2003). This protease cleavage occurs at particular sites in the precursor proteins. This process results in mature virions that can infect other cells resulting in another cycle of viral replication or a

"new viral generation". The inhibition of specific steps in this process results in a decreased yield of infectious virions. Important steps in HIV replication and the antiretroviral drugs currently used in clinical practice that inhibit these steps are shown in Table 1. An historic perspective on the development of these antiretroviral drugs is given in the section "A brief history of antiretroviral drug resistance".

Table 1: Important HIV replication steps for which antiretroviral drugs are available.

Viral replication step	**Antiviral agent**	**Clinical status**
Co-receptor binding	CCR5 antagonist maraviroc binds to CCR5	Maraviroc FDA approved in 2007
Fusion	Fusion inhibitor enfuvirtide binds to gp41 and prevents conformational change needed for fusion.	Injectable formulation, FDA approved in 2003.
Reverse transcription	Competitive inhibitors = nucleos(t)ide analogues: Nucleos(t)ide RT inhibitors (NRTIs) compete with the natural substrates and cause chain termination when incorporated. Allosteric inhibitors: Non-nucleoside RT inhibitors (NNRTIs) bind to pocket outside the active site.	NRTIs in current use are: Thymidine analogues: azidothymidine (AZT), stavudine (D4T) Cytosine analogues: lamivudine (3TC), emtricitabine (FTC). Guanosine analogue: abacavir (ABC) Adenosine/Adenine analogues: didanosine (DDI), tenofovir (TDF) (TDF is a nucleotide analogue as it is mono-phosphorylated) NNRTIs: first generation: nevirapine (NVP), efavirenz (EFV), delavirdine (DLV); second generation: etravirine (ETV), rilpivirine (RPV)
Integrase	Integrase inhibitor raltegravir inhibits chromosomal DNA cleavage and DNA strand transfer of the pre-integration complex	Raltegravir received accelerated FDA approval in 2007 followed by standard approval in 2009.
Protease	Protease inhibitors (PIs) inhibit cleavage of precursor proteins through binding to the catalytically active site (cleft) of the protease enzyme . Maturation inhibitor bevirimat binds to the protease target, the gag protein, and prevents cleavage by the protease.	Nine protease inhibitors currently in use: atazanavir (ATV), darunavir (DRV), fosamprenavir (FPV), indinavir (IDV), lopinavir/ritonavir (LPV/r), nelfinavir (NFV), ritonavir (RTV), saquinavir (SQV), tripanavir (TPV). RTV is used in low dose in combination with the other PIs (except NFV) e.g. DRV/r, SQV/r as it results in

		higher plasma concentrations through inhibition of their metabolism by cytochrome peroxidise enzymes. Bevirimat is not yet licensed.

2.2 Mechanisms leading to induced antiretroviral drug resistance

Untreated HIV-infected individuals produce at least 10^{10} viruses per day. The half-life of a virion is typically around 30 minutes and the average half-life of a virus-producing CD4 lymphocyte is 0.7 days. The generation time of HIV (the time it takes for a virus to be released by one cell, infect another cell and be released again) is about 2 days which means that there may be up to 180 generations of HIV per year (Markowitz et al., 2003). Due to the extremely high replication rate of HIV and since the viral RT has a high error rate and lacks proof-reading ability (Roberts et al., 1988), one extensive viral diversity (so-called quasi-species) can be found in any chronically infected individual (Saag et al., 1988). Therefore numerous viruses, harbouring a particular mutation, may be produced per day; estimates are 10^4 to 10^5 (Coffin, 1995). When HIV replicates in the presence of a particular antiretroviral drug, viral variants that by chance harbour mutations that enable these viruses to better replicate in the presence of the drug, experience a selective advantage over the rest of the viral population that lacks such mutations and may over time become the predominant subpopulation or quasi-species (Ho et al., 1995, Wei et al., 1995).

2.3 The importance of combination therapy in averting resistance

The success of antiretroviral therapy can largely be ascribed to the use of drug combinations. It has recently been shown that although viral reservoirs may continue to release viruses at levels below the detection limit of standard viral load assays, appropriate triple combination therapy can probably completely prevent new rounds of replication, and therefore viral evolution (Dinoso et al., 2009). This is due to the highly potent inhibition of different steps in the viral replication cycle. Therefore the first reason for the success of triple combination therapy is the hugely increased inhibitory effect on viral replication relative to mono or dual therapy. Furthermore the prevention of new rounds of viral replication does not allow for ongoing viral evolution and thus averts the selection of resistant viruses. An associated reason for the success of combination therapy is stochastic (Prosperi et al., 2009): In order to achieve viral replication that would result in virological failure (e.g. a viral load of > 1000 copies/ml) in the presence of three antiretroviral drugs, the particular viral strain would require more than one resistance-associated mutation (RAM). From epidemiological studies it is clear that at the time when failure is detected patients usually have resistance to at least two of the components of triple therapy – which in case of acquired resistance is the result of an evolutionary process: If one regards the generation of a particular nucleoside substitution a random event with a particular probability, which is determined by the replication rate and RT error rate, the probability of spontaneous generation of a variant harbouring multiple resistance mutations would be the product of the individual probabilities. Therefore the random generation of variants with several independent mutations occurring in cis on the same viral lineage resulting in multiply resistant virus would be an extremely rare event in a previously sensitive viral population. Selection for resistance mutations,

however, can occur when therapy interruption or poor adherence result in periods when the patient effectively receives monotherapy, due to different half-lives of the different drugs. The acquisition of a first mutation often results in the loss of activity of one of the three regimen components. Subsequently, should conditions again favour viral replication, the likelihood of acquiring additional mutations would be increased – in the first place through allowing a higher level of viral replication and therefore more rapid evolution and in the second place through the principle of conditional probability (when one mutation has appeared the likelihood of having two mutations increases). This could set the viral lineage on an evolutionary trajectory of resistance mutation accumulation. The same happens when a patient is infected with a strain that already harbours mutations, called transmitted resistance. Additional mutations are henceforth acquired through sequential mutation of the particular viral lineage or through recombination with other lineages that bear different mutations (Althaus and Bonhoeffer, 2005, Carvajal-Rodriguez et al., 2007). The result of this evolutionary process is multiple resistance and viral escape from therapy as evident from a persistently high viral load (Nachega et al., 2011).

2.4 The virus reservoir and archived resistance

Although actively virus-producing CD4 cells have a short half-life, resting CD4 cells and other cell types act as HIV reservoirs (Chun et al., 1995, Chun et al., 1997, Finzi et al., 1997, Finzi et al., 1999, Persaud et al., 2000). When therapy is discontinued, resistant viruses in the circulation may be rapidly displaced by wild-type viruses which are fitter (i.e. more replication competent) in the absence of drug pressure (Birk et al., 2001). However, resistant viruses survive as integrated proviral DNA sequences in peripheral blood mononuclear cells (Verhofstede et al., 2004) and lymphoid tissues (Chinnadurai et al., 2007, Lafeuillade et al., 2001) from where they can re-emerge when therapy with the same or similar drugs is initiated (Masquelier et al., 2001).

2.5 Mutation nomenclature

Non-synonymous HIV mutations (those that result in amino acid changes) are named according to the position of the amino acid (codon) in the particular protein (e.g. protease, RT or integrase). The number (position in sequence) of the amino acid is preceded by the letter indicating the native or ”wild-type” amino acid and followed by the amino acid change. Example: In the RT a nucleotide substitution from **A**TG to **G**TG at codon position 184 results in an amino acid change from methionine (M) to valine (V) and is denoted as “**M184V**”.

2.6 Resistance to specific drug classes

2.6.1 Protease inhibitors:

The protease gene encodes a 99 amino acid protein, which assembles into homodimers in order to be catalytically active. Mutations in the substrate cleft result in a reduction in binding affinity between the protease inhibitor and the enzyme, whereas mutations in the protease flap area, or other areas of the enzyme, may influence enzymatic processing.

Substrate cleft mutations such as V82A/T/F/S and I84V cause cross-resistance to most PIs whereas D30N confers resistance to NFV and I50L to atazanavir (Weinheimer et al., 2005). Protease flap mutations such as I47V, I54V and M46I/L confer cross-resistance to most PIs. L90M, although not at

the cleavage site, is in close association with the catalytic site on a secondary protein structure level and confers cross-resistance to most PIs.

“Primary” or “major” PI mutations confer resistance by directly decreasing the protease enzyme's susceptibility to being inhibited by the drug, often for the price of decreased replication efficiency or “fitness”. “Secondary” or “minor” mutations enable such resistant viruses to regain fitness – that is, these mutations enable viruses to replicate efficiently despite the presence of the primary or major resistance mutations by compensating for a loss in fitness. With the acquisition of primary PI resistance mutations, compensatory mutations may occur in the gag cleavage site which increase the substrate affinity of the modified protease. Recently gag cleavage site mutations which confer resistance in the absence of major PI mutations have been identified, and the presence of single nucleotide polymorphisms at positions 128 and 449, before therapy initiation, has been associated with PI therapy failure (Larrouy et al., 2010).

2.6.2 RT inhibitors:

HIV RT is a heterodimer made up of two sub-units: a large catalytically active p66, which contains all 560 amino acids of RT and RNAse H, and the p51, which has a scaffolding function and shares the first 440 amino acids of p66 (Abbondanzieri et al., 2008). The ultrastructural appearance of the polymerase domain of p66 reminds of a human hand with a palm, fingers and a thumb. The catalytically active area is the palm region. Antiretroviral drugs that target RT are either nucleos(t)ide reverse transcriptase inhibitors (N(t)RIs) that compete with the natural substrates and cause chain termination, or non-nucleoside reverse transcriptase inhibitors (NNRTIs) that are allosteric inhibitors of the enzyme.

NNRTIs do not bind to the RT's active substrate binding site, but to the NNRTI binding pocket, a hydrophobic area 10 Å away from the active site that includes Leu-100, Lys-101, Lys-103, Val-106, Thr-107, Val-108, Val-179, Tyr-181, Tyr-188, Val-189, Gly-190, Phe-227, Trp-229, Leu-234, and Tyr-318 (Shen et al., 2003). Binding of the drug to this pocket results in allosteric inhibition of the enzyme. Amino acid changes in the NNRTI binding pocket do not directly affect the active site and the enzyme can therefore easily tolerate them without loss of activity, i.e. without compromising the virus's “fitness”. When viral replication occurs in the presence of non-suppressive NNRTI levels, NNRTI mutations are rapidly selected, with the consequent rapid emergence of resistance. These drugs therefore have a low genetic barrier to resistance (De Clercq, 2004). Various mutations are associated with differential resistance to first-generation NNRTIs (NVP, EFV and delavirdine), and the mutation pathways may depend on the HIV-1 subtype, with some mutations conferring cross resistance to second-generation NNRTIs such as etravirine (Vingerhoets et al., 2010, Lai et al., 2010). As NNRTI resistance evolves and mutations accumulate on ART primary and secondary NNRTI resistance mutations have been described (Shafer and Schapiro, 2008).

NRTIs are triphosphorylated by cellular kinases, yielding nucleoside-analogue triphosphates as the active compounds whereas nucleotide reverse transcriptase inhibitors (NtRTIs), such as tenofovir, already have a phosphonated adenine base and only require diphosphorylation by cellular kinases. The active metabolites, triphosphates, of NRTIs and N(t)RTIs compete with the natural substrates, nucleoside triphosphates (NTPs), and are incorporated into the nucleic acid strand being extended. However since these compounds do not have 3’-hydroxyl groups, the next NTP cannot be incorporated and the result is chain termination (Mitsuya et al., 1990).

HIV RT can become resistant to N(t)RTIs through different pathways. Mutations that alter the deoxynucleotide triphosphate (dNTP) binding site and thus enable the enzyme to discriminate between natural and synthetic NTPs are called “discriminatory mutations”. Discriminatory mutations, such as M184V, K65R and L74V, change the processivity of RT and therefore come at a fitness price (Wainberg, 2004). Other mutations occur at the “finger” sub-domain (Matamoros et al., 2004) and allow the ATP-mediated excision of incorporated synthetic NTPs (Arion et al., 1998), a process referred to as primer rescue. These mutations are selected by thymidine analogues such as AZT or D4T and are called thymidine analogue mutations (TAMs) (Miller and Larder, 2001, Pellegrin et al., 1999, Picard et al., 2001, Mouroux et al., 2001, Kuritzkes, 2002). Multiple TAMs also confer cross-resistance to other N(t)RTIs. Two alternative TAM pathways have been described. The type-I TAM pathway includes the M41L, L210W and T215Y and the type-II TAM pathway the D67N, K70R, T215F, K219Q/E/N mutations. The type-I pathway is associated with the highest degree of cross-resistance to NRTIs (Marcelin et al., 2004). However in HIV-1 subtype C a non-classic combination of TAMs, D67N, K70R and T215Y has been observed (Novitsky et al., 2007).
M184V, which confers 3TC resistance, increases the enzyme's susceptibility to AZT, D4T and TDF. A possible explanation is that that it reduces primer unblocking and thereby potentiates the action of the chain terminating nucleosides. Furthermore the acquisition of other NRTI mutations in addition to M184V could further decrease enzyme processivity which could limit the fitness of these multiply resistant variants and therefore decrease the rate of evolution (Miller et al., 2002, Petrella and Wainberg, 2002).and can potentially protect against resistance to these drugs (Larder et al., 1995), K65R causes resistance to all N(t)RTIs except AZT to which it increases susceptibility. Some other discriminatory mutations such as Q151M or a nucleotide insertion at position 69 result in multiple NRTI resistance. These mutations could co-occur with other “associated mutations”, which on their own have little effect on resistance but when occurring in association with important resistance mutations may contribute to resistance.

3. Antiretroviral drug resistance testing

3.1 Phenotypic resistance testing

Antiretroviral drug resistance (ARVDR) can be detected in viral culture as a reduction in susceptibility of an isolate or molecular clone to a particular antiviral agent in comparison to a reference or standard viral isolate or molecular clone. This is referred to as phenotypic resistance. These phenotypic changes are conferred by mutations (non-synonymous nucleoside substitutions or rarely insertions or deletions). Therefore genotypic testing of viral strains can be used to infer phenotypic resistance. Genotypic associations, however, do not only rely on *in vitro* data, but the association of a particular viral genotype with clinical response to ART is also taken into account. To diagnose ARVDR, phenotypic testing (PT) was the first to be developed. An HIV isolate was grown in a CD4-expressing HeLa cell line and the reduction of syncytial foci in the presence of AZT was associated with the degree of AZT susceptibility (Larder, Darby et al. 1989; Larder, Chesebro et al. 1990). However this assay can only be used for syncytium-inducing isolates which are usually found late in disease. An assay based on the co-culture of infected cells with peripheral blood mononuclear cells (PMBCs) from negative donors which could also detect non-syncytium-inducing isolates was subsequently developed

(Japour, Mayers et al. 1993). Other developments that improved phenotypic testing were the determination of HIV p24 antigen levels by enzyme immunoassay or RT activity in the cell culture supernatant which relates to the concentration of virus and thus replication activity. Using these assays the concentration of drug that inhibits virus production by 50%, the IC_{50}, can be calculated and phenotypic resistance measured as the "fold change" in IC_{50} , i.e. the ratio of the IC_{50} of the test strain compared to the IC_{50} of a susceptible or "wild-type" reference strain. For each antiretroviral drug a significant "cut-off" in fold-change is derived from *in vitro* data and clinical trials. At least two cut-offs are necessary to interpret phenotypic testing for each drug – the first indicating some degree of reduced susceptibility and the second complete resistance to the drug.
Viral culture and co-culture require biosafety level 3 facilities in order to protect laboratory workers. These special safety measures were no longer necessary following the development of reporter cell lines transfected with non-infectious plasmid vectors with inserts from the patient-derived RT and/ or protease genes and expressing a fluorescent or chemiluminescent reporter protein (Gervaix et al., 1997, Miyake et al., 2003, Hachiya et al., 2001, Spenlehauer et al., 2001, Chiba-Mizutani et al., 2007). The use of vector systems also normalises for differences in growth between different viral strains and allows for the commercialisation of phenotypic testing.

3.2 Genotypic resistance testing

Most genotypic antiretroviral drug resistance testing (GART) methods are based on polymerase chain reaction (PCR) amplification of the target gene followed by sequencing by automated capillary electrophoresis. For the purpose of this dissertation, unless otherwise specified, genotypic resistance refers to resistance detected by PCR and sequencing. GART was also used at an early stage of antiretroviral development: Mutations associated with AZT resistance in culture, namely D67N, K70R, T215Y/F and K219Q, were identified using PCR and sequencing or selective PCR which paved the way for genotypic resistance testing (Larder and Kemp, 1989, Larder et al., 1991). Many more mutations were subsequently discovered that are associated with resistance to particular drugs or drug classes. Several clinical interpretation systems have been developed for genotypic drug resistance interpretation, such as the Stanford University HIV Drug Resistance Database (Shafer et al., 2000a, Shafer et al., 2000b), the RegaV7.1.1 algorithm for HIV drug resistance testing from the Rega Institute in Leuven, Belgium, (Vercauteren and Vandamme, 2006) and the French ANRS rules (Vercauteren and Vandamme, 2006).These interpretation systems are based on phenotypic resistance and clinical outcomes associated with these mutations. They are frequently updated. Some commercial assays have their own resistance interpretations systems but these are not always updated with the most recent mutations.
The use of commercial GART assays in resource-limited settings is limited by high cost and technical complexity as well as the fact that these tests have primarily been designed for HIV-1 subtype B although they are reported to perform well with other subtypes (Pandit et al., 2008).

3.3 Comparing genotypic and phenotypic testing

Both GART and PT have advantages and limitations. GART is more affordable (although still expensive) and has shorter turn-around time. GART can also detect revertant mutations which indicate that resistance has been present but has started to revert to wild-type but GART cannot

interpret complex interactions between different mutations or new mutations. PT is especially valuable in establishing resistance and the associated mutations in the case of new drugs or in validating genotypic algorithms for non-subtype B viruses and together with clinical data provide the data from which genotypic interpretation systems are derived. PT has the advantage of being able to study the net effect of mutation interactions. The fact that some antiretroviral drugs, for example D4T or TDF, are subject to extensive intracellular metabolism (which is dependent on the cell cycle and metabolic activity of the host cell) results in big differences between the *in vitro* and the *in vivo* metabolism of these drugs. This together with the biological variation between HIV strains and technical variation make the selection of clinical cut-offs difficult and limit the clinical value of PT. Furthermore, PT is limited by the high cost and long turn-around time.

In various clinical studies GART and / or PT have been compared to standard of care (SOC). GART generally showed benefit. In these studiesthere was a lack of evidence that PT is beneficial in the clinical management of patients (Meynard et al., 2002, Durant et al., 1999, Baxter et al., 2000, Cohen et al., 2002) although the combined use of GART and PT is often preferred in cases with complex resistance.

Neither GART nor PT can detect minority quasi-species (viral populations comprising less than 20% - 30% of the total viral population) that may be of clinical and prognostic relevance (Shafer and Schapiro 2005; Sen, Tripathy et al. 2006) nor will they detect resistant viral strains surviving as as proviral DNA or “archived” resistant variants able to re-emerge when the same therapy is re-introduced.

3.4 New assays to detect minority viral populations

Various assays have been developed employing different technologies to quantify minority resistant viral species. Allele-specific real-time PCR uses the PCR methodology designed to either detect the resistant allele, the wild-type allele, or both. This enables the relative or absolute quantification of the resistant sub-populations. Different methods to selectively amplify and detect one allelic variant (e.g. the resistant mutant or wild-type allele) have been used (Metzner, 2006). Allele-specific PCR can only evaluate the presence of one particular mutation with a particular primer or probe set (Paredes et al., 2007, Metzner, 2006, Detsika et al., 2007, Bergroth et al., 2005). Parallel allele-specific sequencing is an alternative to allele-specific PCR. This method uses acrydited reverse primers immobilised in an agarose gel and diffusible forward primers to focally amplify individual viral species. It then differentiates the wild-type from the mutant type by extending sequencing primers with fluorescent dideoxynucleotides within the gel. This assay allows the detection of minority species with prevalences as low as 0.01% and the study of mutations that occur in cis in the same viral lineage (Cai et al., 2007).

Assays based on hybridisation are the heteroduplex tracking assay (HTA) and line probe assays. HTA, which is less expensive but less sensitive than allele-specific PCR, is based on differential electrophoresis of native or mutated templates hybridised to probes. HTA has recently been modified to allow sequencing of the particular variant (Schnell et al., 2008). Line probe assays require limited equipment and are not expensive but they are insensitive to the quantification of minorities (Puchhammer-Stockl et al., 1999). Single-genome sequencing (Palmer et al., 2005) provides a method which could be regarded as the benchmark in terms of accuracy, but it requires dilution and multiple sequencing reactions and is therefore very expensive and time-consuming.

A further challenge in the development of allele-specific PCR and any assays based on hybridisation is the difficulty of designing oligonucleotides that can discriminate between particular mutations in the context of a high background variability which is due to the high natural diversity in HIV sequences and the presence of other drug-induced mutations in close proximity to the target mutation. This is further complicated by the degeneracy of the genetic code which means that for every functional amino acid, silent mutations exist that could influence the binding of a primer or probe. Different adaptations have been attempted in order to improve the discriminative ability of these assays: Mismatches have been introduced adjacent to the target mutation site, which would make a particular nucleotide substitution lethal (Bergroth et al., 2005, Paredes et al., 2007). Also a competitive blocking primer to prevent non-specific probe binding to the alternative allele has been used with success (Detsika et al., 2007). Another limitation is that any allele-specific PCR assay can only differentiate between a particular wild type and a mutated variant. Therefore, in order to detect more mutations, multiple reactions are needed in parallel, or assays need to be multiplexed.
Recently, pyrosequencing, on the Roche 454 system, has gained popularity. This allows massive parallel sequencing (simultaneous sequencing) of multiple minor variants (O'Meara et al., 2001, Hoffmann et al., 2007, Mitsuya et al., 2008).and is a valuable and robust method to detect low abundance variants in various clinical contexts (Varghese et al., 2009, Lataillade et al., Le et al., 2009). It is however costly, and requires specialised equipment, but cost can be reduced by optimal pooling (Ji et al., 2010).

4. A brief history of antiretroviral drug resistance

4.1 Resistance as the cause of limited success of antiretroviral monotherapy

Acquired Immunodeficiency Syndrome (AIDS) was first described in 1981 in men who had sex with men (Siegal et al., 1981, Gottlieb et al., 1981), and the Human Immunonodeficiency Virus (HIV) identified as the cause of AIDS in 1983 when it was cultured from the blood of AIDS patients (Barré-Sinoussi et al., 1983). Subsequently HIV was found to cause AIDS also in other risk such as patients with haemophilia, following heterosexual exposure and children born to HIV-infected mothers. Initially management of AIDS patients was limited to the treatment of opportunistic infections or malignancies, until the first antiretroviral drug, the NRTI AZT, was licensed by FDA on 20 March 1987 (Kolata, 1987, 1987) following a randomised controlled trial where it had been shown to have clinical benefit, at least in the short run (Fischl et al., 1987). Although AZT therapy initially slowed progression to AIDS, it did however not prevent it or improve survival (Mulder et al., 1994, Hamilton et al., 1992). Prolonged AZT treatment failed to maintain the initial improvement (Bach, 1989) and the duration of AZT benefit was limited (Volberding et al., 1995, Volberding et al., 1994). However, investigators only later realised that this limited duration of benefit was due the development of antiretroviral drug resistance.
Viral resistance to AZT was first described in 1989 (Marx, 1989, Jeffries, 1989, Larder et al., 1989); at that stage it was not known if drug resistance correlated with clinical failure although it was associated with advanced disease (Richman, 1991). Only in 1992, when AZT resistance was shown to correlate with poor clinical outcome in children (Tudor-Williams et al., 1992), and in 1995, from the analysis of the findings from the ACTG protocol 116B/11, which studied the relationship of phenotypic AZT resistance and disease progression, was resistance established as the reason for failure on prolonged AZT monotherapy (D'Aquila et al., 1995). As more antiretroviral drugs became registered, the era of

AZT monotherapy was followed by investigations of other drugs and of combination therapies (McLeod and Hammer, 1992). The second antiretroviral drug to be FDA licensed was DDI in 1991 for use in patients with AZT toxicity (1991); subsequently DDI was used to replace AZT in patients where AZT no longer had benefit (Spruance et al., 1994). Dual therapy with AZT and DDI was also shown to be effective in children (Husson et al., 1994) and superior to AZT monotherapy (Hammer et al., 1996).

4.2 The development and improvement of highly active antiretroviral therapy

Whereas antiretroviral monotherapy had a short-term benefit, the sustained success of current ART can largely be ascribed to the principle of combination therapy. Highly active antiretroviral *(combination)* therapy (HAART) is effective since, when used appropriately, it suppresses HIV replication and the combined effect of a low replication rate and the low likelihood of spontaneous generation of viruses that are simultaneously resistant to all three drugs in a regimen, dramatically reduces the risk of resistance.

Already in 1995 J. Lange postulated, based on evidence from other infections, that combination therapy with three drugs for HIV will be more effective than monotherapy since the combined effect will suppress viral replication more effectively and prevent the development of resistance (Lange, 1995). Antiretroviral combination therapy was made possible by the development of additional drug classes or new drugs within existing classes that select for alternative resistance mutation patterns. There was an early concern that combination of different classes of drugs would lead to multiple resistance (Larder et al., 1993) and initially combination therapy did not appear to reduce the risk of resistance (Richman, 1994). However, perceptions changed when multiple studies, presented at the Eleventh International Conference on AIDS in Vancouver, Canada, in July 1996, provided evidence for the long-term suppressive effect of various combination therapies: either including one protease inhibitor (PI) plus two NRTIs or one non-nucleoside reverse transcriptase inhibitor (NNRTI) plus two NRTIs (Williams and De Cock, 1996, Cohn, 1997). These breakthroughs led to the birth of a new concept: “Highly Active Antiretroviral Therapy” (HAART).

Many different investigations and findings contributed to the eventual paradigm shift towards HAART: Patients who received PIs from the outset as part of combination therapy were less likely to develop resistance than patients treated with monotherapy or who have had prior exposure to PI monotherapy (Condra, 1998). It was also shown that simultaneous initiation was more effective than the sequential initiation of indinavir, AZT and 3TC – the emergence of 3TC resistance in the sequential arm was strongly associated with failure to suppress the viral load (Gulick et al., 1998)

The first PI to be tested in clinical trials was saquinavir (SQV) (Johnson et al., 1992). Combination therapy of SQV with AZT was shown to be better than either drug alone (Vella, 1994). Furthermore, the combination of AZT and SQV delayed the appearance of resistance to either drug (Vella et al., 1996). Mutations conferring resistance towards SQV, the first approved PI (Baker, 1995), namely the L90M and I54V mutations were characterised in *in vitro* experiments before approval of SQV (Eberle et al., 1995). In *vivo,* the L90M mutation was associated with resistance and resistance was more likely to develop after 8-12 months of therapy in patients who received SQV alone, versus those who received combination therapy with ddC and AZT (Jacobsen et al., 1996b).

Furthermore SQV improved survival when combined with ddC (1996). A combination of SQV with AZT and ddC (ACTG229) was also shown to be better than either dual therapy combinations of SQV and

AZT or AZT and ddC (Collier et al., 1996) and a sustained response was associated with absence of SQV resistance (Jacobsen et al., 1996a). Approval of other protease inhibitors, RTV and indinavir, followed in 1996.

The approval of the NRTI, 3TC, in 1995, for use in combination with AZT (James, 1995), rapidly advanced the field of antiretroviral combination therapy as this combination showed promise in terms of CD4 response and because the M184V mutation, selected by 3TC (1995), conferred increased susceptibility to AZT. 3TC and AZT in combination were shown to be superior, in terms of immunological benefit and adverse effects, to the combination of AZT with zalcitabine (ddC) (Bartlett, Benoit et al. 1996). Also, 3TC combined with AZT was superior to AZT monotherapy, in suppressing viral load in AZT-naive (Katlama et al., 1996) and AZT-experienced patients, respectively (Staszewski et al., 1996). Stavudine (d4T) in combination with 3TC was shown to be at least as effective as AZT and 3TC in dual regimens. However, 3TC resistance, mediated by the M184V mutation, always developed, when it was used as part of such a dual regimen (Foudraine et al., 1998).

NVP was the first non-nucleoside reverse transcriptase inhibitor (NNRTI) to be approved in 1996 (Bowersox, 1996). Another NNRTI, efavirenz (EFV), approved in 1998 (James, 1998a), was shown to be at least as effective as PIs in initial combination ART (James, 1998b).

The development of these drugs and the initial trials of combination therapy laid the groundwork for triple combination therapy. The combination of AZT, 3TC and indinavir was shown to result in effective and sustained suppression of viral load in patients that were previously exposed to AZT (Gulick et al., 1997) and a decreased risk of progression to AIDS or death compared to patients on dual AZT and 3TC (Hammer et al., 1997). A combination of AZT, DDI and NVP was also shown to be effective in achieving sustained viral suppression and was thought to be able to prevent resistance (Montaner et al., 1998), prolong life and decrease the rate of progression to AIDS (Henry et al., 1998)

After the results of HAART trials had become available, there was an initial euphoria and optimism that potent combination therapy could potentially cure HIV; however this hope was thwarted when it was discovered that even after suppression on combination therapy for up to two years, HIV rapidly re-emerged soon after therapy had been discontinued, due to the long half-life of resting CD4 memory cells which harbour HIV (Wong et al., 1997).

Despite the success of PI- or NNRTI-based HAART some patients still developed multiple drug resistance and cross-class resistance, which necessitated the development of new drugs with different targets. The next successfully developed target was the fusion step of the HIV particle with the cell membrane, with enfuvirtide the first fusion inhibitor to be FDA approved (2003). Unfortunately, this drug is expensive and needs administration by subcutaneous injection, with frequent local reactions being reported, which does not make it suitable to large scale roll-out. This was followed by targeting the CCR5 co-receptor, which unlike other antiretroviral therapies does not target a part of the virus but blocks a host cell receptor. The first CCR5 inhibitor to be approved was maraviroc in 2007 (2007). It is generally well-tolerated and has high potency. However, patients must be pre-screened with a phenotypic receptor tropism assay to determine whether they have HIV strains with tropism for the CCR5 or for the CXCR4 co-receptor. At the stage of primary HIV infection, viruses are CCR5-tropic, but during later stages a tropism switch can occur to using CXCR4 receptors, making these viruses not susceptible to inhibition by a CCR5 antagonist. Unfortunately, these tropism assays are prohibitively expensive, which limits the use of maraviroc in developing countries. The development of

alternative cost-effective tropism screening assays may expand the usefulness of for CCR5 antagonists and is currently a research priority.

Another recent drug target is the HIV integrase enzyme. Raltegravir, an HIV-1 integrase inhibitor, was FDA approved in 2007 (Summa et al., 2008). This drug is well tolerated, highly potent and administered orally and thus a welcome addition to the antiretroviral repertoire. Subsequently new integrase inhibitors and additional drug classes have been developed, increasing the repertoire of therapies available to evaluate in clinical trials, and if shown to be effective, for clinical therapy.

The new generation ritonavir-boosted PIs, darunavir (DRV/r) and tripanavir (TPV/r), regularly form the backbone of ART in therapy experienced patients (Youle, 2007), and due to their high genetic barrier, are frequently used in these patients together with Raltegravir and ETV, a new generation NNRTI. Another NNRTI, rilpivirine (RPV), was licensed in 2011 as part of a combination tablet with emtricitabine and tenofovir, but it is targeted for first-line use (2011). However due to the high cost there is a lack of access to new drug classes in resource-limited countries, where therapy remains based on NRTIs, either combined with NNRTIs or PIs.

5. Antiretroviral drug resistance in a clinical context

5.1 Resistance in the context of prevention of mother-to-child transmission (PMTCT)

In the absence of any intervention the rate of HIV transmission, from mother to child, varies between 15-30% in non-breastfeeding populations and between 25-40% in breastfed children (De Cock et al., 2000). HIV transmission can occur *in utero*, during labour or delivery (perinatally), or after birth through breastfeeding. In non-breastfeeding mothers 30% of transmissions occur *in utero* and 70% perinatally (De Cock et al., 2000). With long-term AZT therapy (from 14 to 34 weeks of gestation), according to the Pediatric AIDS Clinical Trials Group (PACTG) 076 Guidelines, the risk of infection is only 4-8% in a non-breastfeeding population (Connor et al., 1994). Shorter AZT PMTCT courses were evaluated in breastfeeding (Dabis et al., 1999) and non-breastfeeding populations (Lallemant et al., 2000). These regimens reduced the transmission rate, but not as markedly as the PACTG 076 study. A very affordable and attractive strategy for resource-limited settings is single-dose NVP to the mother and the infant. This has been evaluated in Uganda (HIVNET 012) and in the South African Intra Partum NVP Trial (SAINT). These trials showed that the use of NVP could have equal benefit to shorter course AZT but at decreased cost (Mofenson and McIntyre, 2000, Moodley et al., 2003). The combination of AZT from 28 weeks of pregnancy with intra-partum NVP and single-dose NVP to the neonate with one week of AZT has been shown to be a highly effective PMTCT strategy, having a transmission rate of only 1.9% (Lallemant et al., 2004). This regimen has until recently been in use in the Western Cape, South Africa (Eley, 2006). Elective caesarean section was found to reduce the risk of transmission from 10.5% to 1.8% in the “European Mode of Delivery Collaboration” (1999). However this is not feasible to implement in resource limited settings with a high HIV prevalence. Although more costly than single or dual PMTCT regimens, the most effective strategy appears to be the use of HAART throughout pregnancy and given to the neonate which could reduce the risk of transmission to 1-2% (Cooper et al., 2002).

PMTCT regimens, employing a single drug or dual therapy, often result in antiretroviral resistance in the mother and / or baby; single-dose NVP in particular is associated with frequent and rapid selection for resistance mutations. In the HIVNET 012116 and HIVNET 006117 studies the detected prevalence

of resistance mutations in mothers were 19% and 20%, respectively, using PCR and sequencing (Jackson et al., 2000, Eshleman et al., 2001). However, in a study using sensitive real-time PCR tests at least 65% of South African women who received single-dose NVP were found to develop resistance (Johnson et al., 2005a). The prevalence of resistance in infected newborns, born to mothers receiving a single-dose NVP regimen, was also high (Eshleman et al., 2005). Despite these high levels of resistance, shortly after receiving NVP, clinical resistance may fade, as resistant viral populations would decay and would be replaced by wild-type, leading to a good clinical response, in PMTCT exposed patients compared to unexposed patients, when initiated on a HAART regimen that includes NVP, more than 6 months (Lockman et al., 2007) or 18 months (Coovadia et al., 2009) after the PMTCT intervention. Although AZT monotherapy in the PACTG 076 study was also associated with resistance, the incidence of the K70R mutation that confers low level AZT resistance was only 2.6% (Eastman et al., 1998). Even though HAART regimens used during pregnancy and interrupted after labour have been shown to have a low risk of resistance compared to other PMTCT regimens the risk cannot be ignored (Perez et al., 2008, Andreotti et al., 2007, Duran et al., 2007).
In this dissertation, the prevalence of genotypic drug resistance in mothers who received the Western Cape PMTCT regimen consisting of AZT from 34 weeks plus single-dose NVP intra-partum, followed by single-dose NVP and 7 days of AZT to the neonate, will be described.

5.2. Transmitted antiretroviral drug resistance

When antiretroviral drug resistance emerges in patients on therapy, these patients can transmit resistant viral strains to others who then acquire primary infections with these resistant strains, and which could be further transmitted in transmission chains. This is called “primary” or “transmitted” antiretroviral drug resistance (TDR). Surveillance for TDR is most commonly done using PCR and ‘bulk’ sequencing, therefore unless stated otherwise; resistance prevalence refers to the prevalence as detected using this method.
There is a lack of standardization in reporting TDR. Patient selection is subject to bias – such as selection of high risk groups which may over-estimate transmitted resistance; whereas testing patients that are not recently infected may lead to underestimation since the proportion of resistant viruses within the circulating viral population could have dropped below the level of detection (20%-25%), as they could have been replaced by wild-type virus which may be fitter. Furthermore there has until recently been no consensus about which mutations to include in resistance surveillance. In order to address this, the WHO published an updated surveillance mutation list in 2008 (Shafer et al., 2008). The World Health Organisation (WHO) also proposed guidelines for resistance surveillance using binomial sequential sampling for detecting a threshold of transmitted HIV drug resistance: at most 47 recently infected patients are sequentially tested and prevalence is classified as either low (< 5%), intermediate (between 5 and 15%) or high (> 15%) (Bertagnolio and Sutherland, 2005). Bennett et al. recently published an update to these guidelines (Bennett et al., 2008) for countries where antiretroviral therapy is being scaled up.
In industrialised countries, such as Europe and North America, 5-20% of newly HIV-infected individuals are infected with a strain with some degree of antiretroviral drug resistance (Pillay, 2004). In certain high-risk settings such as men-who-have-sex-with-men (MSM) in New York City, the prevalence can be above 20% (Shet et al., 2006). In developing countries with high levels of

antiretroviral exposure, such as Brazil (Rodrigues et al., 2006) and Argentina (Petroni et al., 2006), low to intermediate levels of transmitted resistance are found (Petroni et al., 2006, Rodrigues et al., 2006). Using mathematical models with Monte Carlo sampling techniques it is estimated that due to the low treatment coverage, it will probably take more than 10 years for transmitted resistance in many sub-Sahara African countries to reach the WHO surveillance threshold, with the exception of urban centres with a high treatment coverage (Blower et al., 2005). It is not known when TDR will reach levels that could compromise first-line therapy in areas with high treatment coverage, such as the Western Cape, South Africa. We therefore investigated TDR, based on WHO surveillance principles, in adults and infants. Since in adults the date of infection is often unknown, obtaining sufficient numbers of recently infected adults for accurate surveillance may be problematic.

Except for PMTCT cohorts there are very limited data on TDR in infants. In infants where infection is predominantly perinatal it is easier to establish the time of infection than in adults. Furthermore it is clinically beneficial to start infants on ART at an early age (Violari et al., 2008). Therefore, we have done TDR surveillance in infants who were being prepared for ART. Since the mothers of these infants had been receiving PMTCT regimens or may have been exposed to ART, the detection of TDR in these children may be an early warning-sign of an increase in TDR in the general population and simultaneously provide valuable information to clinicians treating these infants.

TDR surveillance may also be pivotal in selecting appropriate regimens for the new approach that attempts to reduce HIV transmission by universal access to treatment (Granich et al., 2009).

5.3 Antiretroviral resistance evolution and barrier to resistance

The genetic barrier to resistance differs among different antiretroviral drugs. The number of mutations needed to confer high-level resistance is the most important factor in determining the genetic barrier for a particular drug. With reference to antiretrovirals with a high genetic barrier, any single non-synonymous drug resistance mutation could result in an increase in the IC_{50}, but this would remain far below the trough level of the particular drug during a normal dosing schedule, until a sufficient number of mutations, each contributing to resistance, have accumulated to allow escape of viral replication. In the case of the NNRTIs, one mutation, such as K103N, confers high level resistance (Maga et al., 1997, Miller et al., 1998, Clotet, 1999), whereas in the case of PIs, multiple mutations need to accumulate in order to cause resistance (Condra, 1998). However when some single PIs are used, accumulation may be much more rapid, since low trough drug levels allow viral replication and also select for mutations that confer viral survival benefit; whereas when RTV-boosted PIs are used, this results in much higher sustained plasma drug concentrations that require many more mutations before viruses would have a survival benefit. This is due to the fact that RTV inhibits the metabolism of other PIs, excluding NFV, and when used in combination with a PI such as LPV, ATV or SQV increases the trough level of the active PI component (Kuritzkes, 2003). In the case of 3TC, one mutation (M184V or M184I) also confers high-level resistance; however, this mutation occurs at a fitness cost (Feng and Anderson, 1999), i.e. it reduces the rate of viral replication. 3TC resistance also predominantly occurs at one locus only, whereas in the case of NNRTIs, resistance mutations, each on their own conferring high level resistance, can occur at many loci, further increasing the probability of the resistance evolving. Both 3TC and NNRTIs are therefore low-resistance barrier drugs, with the expected barrier to 3TC being slightly higher than NNRTIs. Since thymidine analogues (AZT and D4T) and unboosted

PIs require the accumulation of three or more mutations for high-level resistance (Kellam et al., 1994) these drugs can be regarded as having intermediate resistance barriers, whereas RTV-boosted PIs have a high resistance barrier. This is due to their high plasma trough levels and the requirement for a number of non-synonymous mutations, each that would increase the IC50, having to occur, for the viral strain to escape therapy.

Observations concur with this: When patients fail first-line therapy containing 3TC and an NNRTI, failure is usually due to NNRTI and 3TC resistance without resistance to a thymidine analogue or PIs (Johnson et al., 2005b). Resistance evolution in a patient can thus be viewed as a stepwise process, where the appearance of resistance to a low-threshold drug, as part of triple therapy, effectively results in dual therapy with increased viral replication, which would enable resistance mutations to a second drug to arise. The genetic barriers of individual drugs and resistance interactions and cross-resistance explain the differences in drug resistance barrier associated with different therapy combinations. Some resistance mutations may protect against the development of other mutations. Mutations such as M184V, K65R and L74V as well as NNRTI-associated mutations Y181C and L100I may reverse or reduce the effect of TAMs on AZT susceptibility (Goldschmidt and Marquet, 2004) and may delay the appearance of subsequent mutations (Larder, Kemp et al. 1995).

Resistance against AZT was shown to be a stepwise progressive process (Richman, 1990). The population of AZT-resistant viruses was shown to decrease in the absence of drug pressure (Wainberg et al., 1992). This is driven by competition between wild-type and resistant virus, as the fitter wild type virus, released from the viral reservoir, would replicate at a higher rate than the less-fit mutant and outcompete it over time (McLean and Nowak, 1992). NVP resistance was shown to arise both after monotherapy and after combination therapy with AZT but with different respective mutation patterns (Richman et al., 1994). Combination therapy with AZT and DDI was shown to select for different mutation patterns than AZT alone (Shafer, Kozal et al. 1994). Prolonged AZT therapy was also shown to lead to cross-resistance to dideoxynucleosides (Mayers et al., 1994). The use of NRTI regimens such as tenofovir (TDF) combined with 3TC or emtricitabine (FTC) or abacavir in a primary regimen rather than AZT or D4T may prevent the selection of TAMs and thus prevent the emergence of mutations conferring cross-resistance to other NRTIs. Such a strategy would retain future therapy options by limiting cross-resistance in case of regimen failure (Martinez-Cajas and Wainberg, 2008). A pattern of genotypic drug resistance (GDR) evolution that differs from patients with HIV-1 subtype B has been observed in patients from developing countries where HIV-1 subtype C is prevalent: Regarding NRTI mutations, K65R and K70E may be found more often than TAMs (Doualla-Bell et al., 2006). In case of K65R this has been shown to be template dependent, with the reverse transcriptase pausing at the amino acid 64 and 65 position due to the unique homopolymeric region. This results in an increased mutation rate at the locus, which explains the high prevalence of K65R in HIV-1 subtype C (Coutsinos et al., 2009). However when TAMs do occur, a non-classic combination can be observed (Novitsky et al., 2007). With reference to NNRTIs, V106M is more common in subtype C than in subtype B where V106A is the most common resistant variant at the 106 position. This is due to the difference in template codon usage: in case of subtype B, valine is encoded by GTA, which requires two base changes to ATG (methionine) whereas in case of subtype C, only one base substitution is required, as the codon usage for valine at position 106 is GTG. This has implications for phenotypic resistance as V106A remains susceptible to EFV whereas V106M shows 10 fold resistance (Lai et al.,

2010). Also with regards to PIs, D30N occurs at a lower frequency in subtype C patients from Botswana and Ethiopia treated with NFV than observed in subtype B patients from other regions who received the same treatment (Martinez-Cajas et al., 2009). Limited data from South Africa are available: a study from KwaZulu Natal concurs with other studies in that a high prevalence of V106M was observed despite a low prevalence of K65R (Marconi et al., 2008). The observed pattern of resistance can influence the choice of second-line therapy and therefore additional data from South Africa are needed.

5.4. Monitoring of patients on antiretroviral therapy and prevention of resistance

The prevention of resistance in patients on ART is dependent on maintaining adequate drug levels of antiretroviral drugs that have activity against the particular virus. The importance of adequate drug levels is two-fold. Firstly, adequate levels of combination antiretroviral drugs prevent viral replication and therefore slow down viral evolution; secondly, adequate drug levels ensure that any mutations that could confer a marginal advantage, with regard to one of the drugs, would not present the virus with an overall evolutionary advantage, since it would still not be able to effectively replicate in the presence of adequate concentrations of the other drugs.

Therefore, apart from the genetic barrier of a particular drug or regimen, any factor that reduces drug levels could allow viral evolution and emergence of so-called “acquired resistance”. Adherence to therapy (compliance) is the most important factor (Meya et al., 2009, Goldman et al., 2008) but pharmacokinetic factors are also important (Boulle et al., 2008, Cohen et al., 2008). A concomitantly taken drug such as rifampicin that lowers the levels of many antiretroviral drugs can increase the risk of resistance on particular regimens (Boulle et al., 2008). There is a complex relationship between resistance and adherence. With PI therapy the risk of resistance follows a bell-shaped curve with the highest risk at intermediate levels of adherence (Bangsberg, Porco et al. 2004); however with non-nucleoside reverse transcriptase inhibitor (NNRTI) therapy, there is a linear relationship with a continuous decrease in risk of resistance as adherence increases(Bangsberg et al., 2006, Nachega et al., 2007).

Detecting patients that are failing antiretroviral therapy early is important for the prevention of resistance. The detection of an increase in HIV-1 RNA load, i.e. virological failure, is the best early warning sign of treatment failure. It allows for intensified adherence counselling to avoid the development of resistance. However, in many African countries therapy programmes rely entirely on clinical monitoring, with or without CD4 count testing, but with no access to viral load testing. This delays the detection of virological failure and allows for the emergence of resistance or the accumulation of additional resistance mutations.

5.5 Salvaging patients with multiple antiretroviral drug resistance

Patients who have failed more than two different antiretroviral regimens are difficult to treat. However, high-barrier drugs, such as LPV/r, can often be used despite the presence of resistance mutations: When the mutation score, derived from the number of relevant PI resistance mutations, is less than 5, the use of a boosted PI regimen such as LPV/r can usually achieve virological suppression and clinical benefit (Marcelin et al., 2005, Maillard et al., 2007) and may prevent the progressive accumulation of resistance despite the presence of resistance to the other drugs in the regimen. In these cases

therapeutic drug monitoring and the use of higher doses of LPV/r may be necessitated – the combined use of GDR and therapeutic drug monitoring (TDM) in calculating the genotypic inhibitory quotient, that is the ratio of the trough drug level over the expected IC_{50} of the resistant viral strain, has been shown to be of clinical benefit (Marcelin et al. 2005; Maillard et al. 2007). The new generation PIs, DRV/r and TPV/r, are even more useful in this context as their genetic barriers are higher than that of LPV (Youle, 2007). In addition to the new generation PIs, the new class of integrase inhibitors and a new generation NNRTI, etravirine (Lunzen, 2007), are valuable in salvage, but unlike boosted PIs they require full virologic suppression to prevent resistance, due to their low genetic barriers. Although new generation PIs, NNRTIs and new drug classes are valuable, their availability is very limited in resource-constrained settings. Where possible, for effective salvage one needs at least two, ideally three fully active drugs in the regimen. Therefore the simultaneous introduction of drugs with full susceptibility is the best strategy in salvage as long as the patient is adherent to this salvage regimen. Resistance testing is valuable in establishing the optimal background regimen or best salvage drugs but resistance information should be interpreted together with a complete ART history. Since wild-type virus may again become predominant after discontinuation of a particular drug and because commercial resistance tests can only reliably detect resistance when at least 20% of the viral population harbour that particular mutation, resistance testing often cannot detect resistance to prior regimens. However when these or similar drugs are reintroduced in a subsequent regimen, resistance will re-emerge from archived resistant populations.

The use of new assays, such as ultradeep pyrosequencing, which could possibly enable the detection of these minority or archived viruses may thus improve the value of resistance testing in patients needing salvage regimens and other patients with prior antiretroviral exposure needing ART (Codoner et al., 2011).

6. Research questions

Despite the huge amount of existing research on antiretroviral drug resistance, many questions with relevance to the sub-Saharan region, and South African setting, in particular, remain. The specific questions addressed in this dissertation are: The Western Cape was the first South African setting to replace a regimen of single dose NVP-only for PMTCT with a combined regimen of AZT and NVP, based on data of its improved efficacy (in other parts of the world). Nevertheless there were limited data on the effect of this regimen on the prevalence of genotypic antiretroviral resistance. In the first enclosed article the prevalence of NVP-associated resistance will be investigated in these patients in comparison to other sites where NVP-only was used.

As discussed above, surveillance of the prevalence of transmitted resistance is necessary to know whether baseline genotypic resistance testing is required before prescribing a first-line antiretroviral regimen. Infants and young children born to HIV-infected mothers represent true recent infections, and as they acquire their viruses from sexually active, and often relatively healthy, mothers, their viruses may reflect currently circulating strains in the heterosexual population. However, single dose NVP to the mother and baby is associated with a high prevalence of NVP-induced resistance in infants who become infected regardless. Due to the long half-life of NVP and the fact that most transmissions occur perinatally, resistance is more likely “acquired” than “transmitted”. As first-line therapy in these children, once initiated on therapy, would consist of a regimen that includes a RTV-boosted PI and two

NRTIs, investigating the prevalence of PI and NRTI resistance is important for the choice of first-line therapy. Furthermore the presence of transmitted PI and NRTI mutations in these children would signify the presence of such mutations in the heterosexually active adult population. In order to study the prevalence of initial (the combination of NVP PMTCT induced and transmitted resistance) we investigated the prevalence of antiretroviral resistance in children under 18 months of age, using the WHO surveillance strategy modified for young children and the WHO standardised surveillance mutation list. Similarly, the prevalence of transmitted resistance in recently infected, antiretroviral naïve adults, classified as recently infected through the use of the proxies: young age (15-20 years), high CD4 count and the absence of HIV-associated disease, will be studied. This will be included as an addendum to the dissertation, in the form of the abstract of a poster presented at the 6th IAS Conference on HIV Pathogenesis, Treatment and Prevention, Rome, Italy, 17-20 July, 2011.

The study of the prevalence of acquired antiretroviral drug resistance and its clinical associations e.g. with prescribing practices, response to failure, concurrent CD4 count and viral load would provide important information for program management and public health officials. As the epidemic in South Africa is predominantly HIV-1 subtype C, and the selection for particular mutations is template dependent, information about resistance patterns in this region is informative. For this purpose the prevalence of antiretroviral drug resistance (most likely “acquired”) at the time of virological failure in children receiving a first-line PI-based regimen and in adults receiving a first-line NNRTI-based or a second-line PI-based regimen were studied.

Until recently, paediatric patients under 6 months of age or those also taking rifampicin received the unboosted PI RTV instead of LPV/r, since the appropriate dose of LPV/r was not known. In our setting patients are sometimes retained on a failing regimen despite detectable viral loads, resulting in a chance for resistance accumulation. Data on the prevalence of drug resistance in these patients, and its association with particular therapies and other clinical factors, especially in a population with predominant HIV-1 subtype C infection, would be valuable. Knowing the prevalences of different mutations and the factors contributing to cross-resistance to second-line therapies, after first-line failure, could inform the roll-out programme.

In addition, as adults who receive a second-line LPV/r-based PI regimen, are unlikely to have PI resistance, due to the high genetic barrier of the drug combination, but are likely to fail due to inadequate adherence (since the regimen consisting of DDI, AZT and LPV/r has poor tolerability), lopinavir concentrations in blood plasma and hair will be determined as indicators of intermediate- and recent-term drug exposure, in an attempt to elucidate the cause of failure in these patients.

These studies are included as articles that form part of this dissertation. To summarize, the investigation of transmitted resistance in children and adults, and of acquired resistance in adults failing a first-line NNRTI regimen or a second-line PI regimen, and children failing a first-line PI regimen, will not only shed light on the prevalence of resistance in the South African region but also on the factors that are associated with an increased risk of having particular mutations or combinations of mutations.

Bibliography

(1987) Zidovudine approved by FDA for treatment of AIDS. *Clin Pharm,* 6**,** 431,435.
(1991) Second AIDS drug approved for those who can't tolerate AZT. *Oncology (Williston Park),* 5**,** 103.
(1996) Saquinavir plus ddC reduces deaths by more than two-thirds. *J Int Assoc Physicians AIDS Care,* 2**,** 51.
(1999) Elective caesarean-section versus vaginal delivery in prevention of vertical HIV-1 transmission: a randomised clinical trial. The European Mode of Delivery Collaboration. *Lancet,* 353**,** 1035-9.
(2003) FDA notifications. FDA approves Fuzeon, the first fusion inhibitor. *AIDS Alert,* 18**,** 78-9.
(2007) FDA notifications. Maraviroc approved as a CCR5 co-receptor antagonist. *AIDS Alert,* 22**,** 103.
(2011) FDA notifications. Complera approved. *AIDS Alert,* 26**,** 130-1.
ABBONDANZIERI, E. A., BOKINSKY, G., RAUSCH, J. W., ZHANG, J. X., LE GRICE, S. F. & ZHUANG, X. (2008) Dynamic binding orientations direct activity of HIV reverse transcriptase. *Nature,* 453**,** 184-9.
ALTHAUS, C. L. & BONHOEFFER, S. (2005) Stochastic interplay between mutation and recombination during the acquisition of drug resistance mutations in human immunodeficiency virus type 1. *J Virol,* 79**,** 13572-8.
ANDREOTTI, M., GUIDOTTI, G., GALLUZZO, C. M., MANCINELLI, S., GERMANO, P., PIRILLO, M. F., MARAZZI, M. C., VELLA, S., PALOMBI, L. & GIULIANO, M. (2007) Resistance mutation patterns in plasma and breast milk of HIV-infected women receiving highly-active antiretroviral therapy for mother-to-child transmission prevention. *AIDS,* 21**,** 2360-2.
ARION, D., KAUSHIK, N., MCCORMICK, S., BORKOW, G. & PARNIAK, M. A. (1998) Phenotypic mechanism of HIV-1 resistance to 3'-azido-3'-deoxythymidine (AZT): increased polymerization processivity and enhanced sensitivity to pyrophosphate of the mutant viral reverse transcriptase. *Biochemistry,* 37**,** 15908-17.
BACH, M. C. (1989) Failure of zidovudine to maintain remission in patients with AIDS. *N Engl J Med,* 320**,** 594-5.
BAKER, R. (1995) FDA approves 3TC and saquinavir. Food and Drug Administration. *BETA***,** 5, 9.
BANGSBERG, D. R., ACOSTA, E. P., GUPTA, R., GUZMAN, D., RILEY, E. D., HARRIGAN, P. R., PARKIN, N. & DEEKS, S. G. (2006) Adherence-resistance relationships for protease and non-nucleoside reverse transcriptase inhibitors explained by virological fitness. *AIDS,* 20**,** 223-31.
BARRÉ-SINOUSSI, F., CHERMANN, J. C., REY, F., NUGEYRE, M. T., CHAMARET, S., GRUEST, J., DAUGUET, C., AXLER-BLIN, C., VEZINET-BRUN, F., ROUZIOUX, C., ROZENBAUM, W. & MONTAGNIER, L. (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science,* 220**,** 868-71.
BAXTER, J. D., MAYERS, D. L., WENTWORTH, D. N., NEATON, J. D., HOOVER, M. L., WINTERS, M. A., MANNHEIMER, S. B., THOMPSON, M. A., ABRAMS, D. I., BRIZZ, B. J., IOANNIDIS, J. P. & MERIGAN, T. C. (2000) A randomized study of antiretroviral management based on plasma genotypic antiretroviral resistance testing in patients failing therapy. CPCRA 046 Study Team for the Terry Beirn Community Programs for Clinical Research on AIDS. *AIDS,* 14**,** F83-93.
BENNETT, D. E., MYATT, M., BERTAGNOLIO, S., SUTHERLAND, D. & GILKS, C. F. (2008) Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. *Antivir Ther,* 13 Suppl 2**,** 25-36.
BERGROTH, T., SONNERBORG, A. & YUN, Z. (2005) Discrimination of lamivudine resistant minor HIV-1 variants by selective real-time PCR. *J Virol Methods,* 127**,** 100-7.
BERTAGNOLIO, S. & SUTHERLAND, D. (2005) WHO approach to track HIV drug resistance emergence and transmission in countries scaling up HIV treatment. *AIDS,* 19**,** 1329-30.
BIRK, M., SVEDHEM, V. & SONNERBORG, A. (2001) Kinetics of HIV-1 RNA and resistance-associated mutations after cessation of antiretroviral combination therapy. *AIDS,* 15**,** 1359-68.
BLOWER, S., BODINE, E., KAHN, J. & MCFARLAND, W. (2005) The antiretroviral rollout and drug-resistant HIV in Africa: insights from empirical data and theoretical models. *AIDS,* 19**,** 1-14.
BOULLE, A., VAN CUTSEM, G., COHEN, K., HILDERBRAND, K., MATHEE, S., ABRAHAMS, M., GOEMAERE, E., COETZEE, D. & MAARTENS, G. (2008) Outcomes of nevirapine- and efavirenz-based antiretroviral therapy when coadministered with rifampicin-based antitubercular therapy. *JAMA,* 300**,** 530-9.
BOWERSOX, J. (1996) Nevirapine approved by FDA. Food and Drug Administration. *NIAID AIDS Agenda***,** 10.
CAI, F., CHEN, H., HICKS, C. B., BARTLETT, J. A., ZHU, J. & GAO, F. (2007) Detection of minor drug-resistant populations by parallel allele-specific sequencing. *Nat Methods,* 4**,** 123-5.

CARVAJAL-RODRIGUEZ, A., CRANDALL, K. A. & POSADA, D. (2007) Recombination favors the evolution of drug resistance in HIV-1 during antiretroviral therapy. *Infect Genet Evol,* 7**,** 476-83.

CHIBA-MIZUTANI, T., MIURA, H., MATSUDA, M., MATSUDA, Z., YOKOMAKU, Y., MIYAUCHI, K., NISHIZAWA, M., YAMAMOTO, N. & SUGIURA, W. (2007) Use of new T-cell-based cell lines expressing two luciferase reporters for accurately evaluating susceptibility to anti-human immunodeficiency virus type 1 drugs. *J Clin Microbiol,* 45**,** 477-87.

CHINNADURAI, R., RAJAN, D., MUNCH, J. & KIRCHHOFF, F. (2007) Human immunodeficiency virus type 1 variants resistant to first- and second-version fusion inhibitors and cytopathic in ex vivo human lymphoid tissue. *J Virol,* 81**,** 6563-72.

CHUN, T. W., FINZI, D., MARGOLICK, J., CHADWICK, K., SCHWARTZ, D. & SILICIANO, R. F. (1995) In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. *Nat Med,* 1**,** 1284-90.

CHUN, T. W., STUYVER, L., MIZELL, S. B., EHLER, L. A., MICAN, J. A., BASELER, M., LLOYD, A. L., NOWAK, M. A. & FAUCI, A. S. (1997) Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci U S A,* 94**,** 13193-7.

CLAPHAM, P. R. & MCKNIGHT, A. (2001) HIV-1 receptors and cell tropism. *Br Med Bull,* 58**,** 43-59.

CLOTET, B. (1999) Efavirenz: resistance and cross-resistance. *Int J Clin Pract Suppl,* 103**,** 21-5.

CODONER, F. M., POU, C., THIELEN, A., GARCIA, F., DELGADO, R., DALMAU, D., ALVAREZ-TEJADO, M., RUIZ, L., CLOTET, B. & PAREDES, R. (2011) Added value of deep sequencing relative to population sequencing in heavily pre-treated HIV-1-infected subjects. *PLoS One,* 6**,** e19461.

COFFIN, J. M. (1995) HIV population dynamics in vivo: implications for genetic variation, pathogenesis, and therapy. *Science,* 267**,** 483-9.

COHEN, C. J., HUNT, S., SENSION, M., FARTHING, C., CONANT, M., JACOBSON, S., NADLER, J., VERBIEST, W., HERTOGS, K., AMES, M., RINEHART, A. R. & GRAHAM, N. M. (2002) A randomized trial assessing the impact of phenotypic resistance testing on antiretroviral therapy. *AIDS,* 16**,** 579-88.

COHEN, K., VAN CUTSEM, G., BOULLE, A., MCILLERON, H., GOEMAERE, E., SMITH, P. J. & MAARTENS, G. (2008) Effect of rifampicin-based antitubercular therapy on nevirapine plasma concentrations in South African adults with HIV-associated tuberculosis. *J Antimicrob Chemother,* 61**,** 389-93.

COHN, J. A. (1997) Recent advances. HIV infection--I. *BMJ,* 314**,** 487-91.

COLLIER, A. C., COOMBS, R. W., SCHOENFELD, D. A., BASSETT, R., BARUCH, A. & COREY, L. (1996) Combination therapy with zidovudine, didanosine and saquinavir. *Antiviral Res,* 29**,** 99.

CONDRA, J. H. (1998) Resistance to HIV protease inhibitors. *Haemophilia,* 4**,** 610-5.

CONNOR, E. M., SPERLING, R. S., GELBER, R., KISELEV, P., SCOTT, G., O'SULLIVAN, M. J., VANDYKE, R., BEY, M., SHEARER, W., JACOBSON, R. L. & ET AL. (1994) Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. *N Engl J Med,* 331**,** 1173-80.

COOPER, E. R., CHARURAT, M., MOFENSON, L., HANSON, I. C., PITT, J., DIAZ, C., HAYANI, K., HANDELSMAN, E., SMERIGLIO, V., HOFF, R. & BLATTNER, W. (2002) Combination antiretroviral strategies for the treatment of pregnant HIV-1-infected women and prevention of perinatal HIV-1 transmission. *J Acquir Immune Defic Syndr,* 29**,** 484-94.

COOVADIA, A., HUNT, G., ABRAMS, E. J., SHERMAN, G., MEYERS, T., BARRY, G., MALAN, E., MARAIS, B., STEHLAU, R., LEDWABA, J., HAMMER, S. M., MORRIS, L. & KUHN, L. (2009) Persistent Minority K103N Mutations among Women Exposed to Single-Dose Nevirapine and Virologic Response to Nonnucleoside Reverse-Transcriptase Inhibitor-Based Therapy. *Clin Infect Dis*.

COUTSINOS, D., INVERNIZZI, C. F., XU, H., MOISI, D., OLIVEIRA, M., BRENNER, B. G. & WAINBERG, M. A. (2009) Template usage is responsible for the preferential acquisition of the K65R reverse transcriptase mutation in subtype C variants of human immunodeficiency virus type 1. *J Virol,* 83**,** 2029-33.

D'AQUILA, R. T., JOHNSON, V. A., WELLES, S. L., JAPOUR, A. J., KURITZKES, D. R., DEGRUTTOLA, V., REICHELDERFER, P. S., COOMBS, R. W., CRUMPACKER, C. S., KAHN, J. O. & RICHMAN, D. D. (1995) Zidovudine resistance and HIV-1 disease progression during antiretroviral therapy. AIDS Clinical Trials Group Protocol 116B/117 Team and the Virology Committee Resistance Working Group. *Ann Intern Med,* 122**,** 401-8.

D.O.H. (2004) National Antiretroviral Treatment Guidelines. South Africa. Available from: http://www.doh.gov.za/search/index.html. .

D.O.H. (2010a) Department of Health, 2010. National Antenatal Sentinel HIV and Syphilis Prevalence Survey in South Africa, 2009. Available from:

http://www.uj.ac.za/EN/CorporateServices/ioha/Documentation/Documents/2009%20HIV%20Antenatal%20Survey.pdf. Accessed on 31 December 2010.

D.O.H. (2010b) National Department of Health, South Africa. Clinical Guidelines for the Management of HIV&AIDS in Adults and Adolescents, 2010. Available at http://www.sanac.org.za/documents/2010 Adult ART Guidelines.pdf. Accessed 7 September 2010.

DABIS, F., MSELLATI, P., MEDA, N., WELFFENS-EKRA, C., YOU, B., MANIGART, O., LEROY, V., SIMONON, A., CARTOUX, M., COMBE, P., OUANGRE, A., RAMON, R., KY-ZERBO, O., MONTCHO, C., SALAMON, R., ROUZIOUX, C., VAN DE PERRE, P. & MANDELBROT, L. (1999) 6-month efficacy, tolerance, and acceptability of a short regimen of oral zidovudine to reduce vertical transmission of HIV in breastfed children in Cote d'Ivoire and Burkina Faso: a double-blind placebo-controlled multicentre trial. DITRAME Study Group. DIminution de la Transmission Mere-Enfant. *Lancet,* 353**,** 786-92.

DE CLERCQ, E. (2004) Non-nucleoside reverse transcriptase inhibitors (NNRTIs): past, present, and future. *Chem Biodivers,* 1**,** 44-64.

DE COCK, K. M., FOWLER, M. G., MERCIER, E., DE VINCENZI, I., SABA, J., HOFF, E., ALNWICK, D. J., ROGERS, M. & SHAFFER, N. (2000) Prevention of mother-to-child HIV transmission in resource-poor countries: translating research into policy and practice. *JAMA,* 283**,** 1175-82.

DE OLIVEIRA, T., ENGELBRECHT, S., JANSE VAN RENSBURG, E., GORDON, M., BISHOP, K., ZUR MEGEDE, J., BARNETT, S. W. & CASSOL, S. (2003) Variability at human immunodeficiency virus type 1 subtype C protease cleavage sites: an indication of viral fitness? *J Virol,* 77**,** 9422-30.

DETSIKA, M. G., CHANDLER, B., KHOO, S. H., WINSTANLEY, C., CANE, P., BACK, D. J. & OWEN, A. (2007) Detection and quantification of minority HIV isolates harbouring the D30N mutation by real-time PCR amplification. *J Antimicrob Chemother,* 60**,** 881-4.

DINOSO, J. B., KIM, S. Y., WIEGAND, A. M., PALMER, S. E., GANGE, S. J., CRANMER, L., O'SHEA, A., CALLENDER, M., SPIVAK, A., BRENNAN, T., KEARNEY, M. F., PROSCHAN, M. A., MICAN, J. M., REHM, C. A., COFFIN, J. M., MELLORS, J. W., SILICIANO, R. F. & MALDARELLI, F. (2009) Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. *Proc Natl Acad Sci U S A,* 106**,** 9403-8.

DOUALLA-BELL, F., AVALOS, A., BRENNER, B., GAOLATHE, T., MINE, M., GASEITSIWE, S., OLIVEIRA, M., MOISI, D., NDWAPI, N., MOFFAT, H., ESSEX, M. & WAINBERG, M. A. (2006) High prevalence of the K65R mutation in human immunodeficiency virus type 1 subtype C isolates from infected patients in Botswana treated with didanosine-based regimens. *Antimicrob Agents Chemother,* 50**,** 4182-5.

DURAN, A. S., LOSSO, M. H., SALOMON, H., HARRIS, D. R., PAMPURO, S., SOTO-RAMIREZ, L. E., DUARTE, G., DE SOUZA, R. S. & READ, J. S. (2007) Drug resistance among HIV-infected pregnant women receiving antiretrovirals for prophylaxis. *AIDS,* 21**,** 199-205.

DURANT, J., CLEVENBERGH, P., HALFON, P., DELGIUDICE, P., PORSIN, S., SIMONET, P., MONTAGNE, N., BOUCHER, C. A., SCHAPIRO, J. M. & DELLAMONICA, P. (1999) Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial. *Lancet,* 353**,** 2195-9.

EASTMAN, P. S., SHAPIRO, D. E., COOMBS, R. W., FRENKEL, L. M., MCSHERRY, G. D., BRITTO, P., HERMAN, S. A. & SPERLING, R. S. (1998) Maternal viral genotypic zidovudine resistance and infrequent failure of zidovudine therapy to prevent perinatal transmission of human immunodeficiency virus type 1 in pediatric AIDS Clinical Trials Group Protocol 076. *J Infect Dis,* 177**,** 557-64.

EBERLE, J., BECHOWSKY, B., ROSE, D., HAUSER, U., VON DER HELM, K., GURTLER, L. & NITSCHKO, H. (1995) Resistance of HIV type 1 to proteinase inhibitor Ro 31-8959. *AIDS Res Hum Retroviruses,* 11**,** 671-6.

ELEY, B. (2006) Addressing the paediatric HIV epidemic: a perspective from the Western Cape Region of South Africa. *Trans R Soc Trop Med Hyg,* 100**,** 19-23.

ESHLEMAN, S. H., HOOVER, D. R., CHEN, S., HUDELSON, S. E., GUAY, L. A., MWATHA, A., FISCUS, S. A., MMIRO, F., MUSOKE, P., JACKSON, J. B., KUMWENDA, N. & TAHA, T. (2005) Resistance after single-dose nevirapine prophylaxis emerges in a high proportion of Malawian newborns. *AIDS,* 19**,** 2167-9.

ESHLEMAN, S. H., MRACNA, M., GUAY, L. A., DESEYVE, M., CUNNINGHAM, S., MIROCHNICK, M., MUSOKE, P., FLEMING, T., GLENN FOWLER, M., MOFENSON, L. M., MMIRO, F. & JACKSON, J. B. (2001) Selection and fading of resistance mutations in women and infants receiving nevirapine to prevent HIV-1 vertical transmission (HIVNET 012). *AIDS,* 15**,** 1951-7.

FENG, J. Y. & ANDERSON, K. S. (1999) Mechanistic studies examining the efficiency and fidelity of DNA synthesis by the 3TC-resistant mutant (184V) of HIV-1 reverse transcriptase. *Biochemistry,* 38**,** 9440-8.

FINZI, D., BLANKSON, J., SILICIANO, J. D., MARGOLICK, J. B., CHADWICK, K., PIERSON, T., SMITH, K., LISZIEWICZ, J., LORI, F., FLEXNER, C., QUINN, T. C., CHAISSON, R. E., ROSENBERG, E., WALKER, B., GANGE, S., GALLANT, J. & SILICIANO, R. F. (1999) Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med,* 5**,** 512-7.

FINZI, D., HERMANKOVA, M., PIERSON, T., CARRUTH, L. M., BUCK, C., CHAISSON, R. E., QUINN, T. C., CHADWICK, K., MARGOLICK, J., BROOKMEYER, R., GALLANT, J., MARKOWITZ, M., HO, D. D., RICHMAN, D. D. & SILICIANO, R. F. (1997) Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science,* 278**,** 1295-300.

FISCHL, M. A., RICHMAN, D. D., GRIECO, M. H., GOTTLIEB, M. S., VOLBERDING, P. A., LASKIN, O. L., LEEDOM, J. M., GROOPMAN, J. E., MILDVAN, D., SCHOOLEY, R. T. & ET AL. (1987) The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. *N Engl J Med,* 317**,** 185-91.

FOUDRAINE, N. A., DE JONG, J. J., JAN WEVERLING, G., VAN BENTHEM, B. H., MAAS, J., KEET, I. P., JURRIAANS, S., ROOS, M. T., VANDERMEULEN, K., DE WOLF, F. & LANGE, J. M. (1998) An open randomized controlled trial of zidovudine plus lamivudine versus stavudine plus lamivudine. *AIDS,* 12**,** 1513-9.

GERVAIX, A., WEST, D., LEONI, L. M., RICHMAN, D. D., WONG-STAAL, F. & CORBEIL, J. (1997) A new reporter cell line to monitor HIV infection and drug susceptibility in vitro. *Proc Natl Acad Sci U S A,* 94**,** 4653-8.

GLEENBERG, I. O., HERSCHHORN, A. & HIZI, A. (2007) Inhibition of the activities of reverse transcriptase and integrase of human immunodeficiency virus type-1 by peptides derived from the homologous viral protein R (Vpr). *J Mol Biol,* 369**,** 1230-43.

GOLDMAN, J. D., CANTRELL, R. A., MULENGA, L. B., TAMBATAMBA, B. C., REID, S. E., LEVY, J. W., LIMBADA, M., TAYLOR, A., SAAG, M. S., VERMUND, S. H., STRINGER, J. S. & CHI, B. H. (2008) Simple adherence assessments to predict virologic failure among HIV-infected adults with discordant immunologic and clinical responses to antiretroviral therapy. *AIDS Res Hum Retroviruses,* 24**,** 1031-5.

GOLDSCHMIDT, V. & MARQUET, R. (2004) Primer unblocking by HIV-1 reverse transcriptase and resistance to nucleoside RT inhibitors (NRTIs). *Int J Biochem Cell Biol,* 36**,** 1687-705.

GOTTLIEB, M. S., SCHROFF, R., SCHANKER, H. M., WEISMAN, J. D., FAN, P. T., WOLF, R. A. & SAXON, A. (1981) Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med,* 305**,** 1425-31.

GRANICH, R. M., GILKS, C. F., DYE, C., DE COCK, K. M. & WILLIAMS, B. G. (2009) Universal voluntary HIV testing with immediate antiretroviral therapy as a strategy for elimination of HIV transmission: a mathematical model. *Lancet,* 373**,** 48-57.

GULICK, R. M., MELLORS, J. W., HAVLIR, D., ERON, J. J., GONZALEZ, C., MCMAHON, D., JONAS, L., MEIBOHM, A., HOLDER, D., SCHLEIF, W. A., CONDRA, J. H., EMINI, E. A., ISAACS, R., CHODAKEWITZ, J. A. & RICHMAN, D. D. (1998) Simultaneous vs sequential initiation of therapy with indinavir, zidovudine, and lamivudine for HIV-1 infection: 100-week follow-up. *JAMA,* 280**,** 35-41.

GULICK, R. M., MELLORS, J. W., HAVLIR, D., ERON, J. J., GONZALEZ, C., MCMAHON, D., RICHMAN, D. D., VALENTINE, F. T., JONAS, L., MEIBOHM, A., EMINI, E. A. & CHODAKEWITZ, J. A. (1997) Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. *N Engl J Med,* 337**,** 734-9.

HACHIYA, A., AIZAWA-MATSUOKA, S., TANAKA, M., TAKAHASHI, Y., IDA, S., GATANAGA, H., HIRABAYASHI, Y., KOJIMA, A., TATSUMI, M. & OKA, S. (2001) Rapid and simple phenotypic assay for drug susceptibility of human immunodeficiency virus type 1 using CCR5-expressing HeLa/CD4(+) cell clone 1-10 (MAGIC-5). *Antimicrob Agents Chemother,* 45**,** 495-501.

HAMILTON, J. D., HARTIGAN, P. M., SIMBERKOFF, M. S., DAY, P. L., DIAMOND, G. R., DICKINSON, G. M., DRUSANO, G. L., EGORIN, M. J., GEORGE, W. L., GORDIN, F. M. & ET AL. (1992) A controlled trial of early versus late treatment with zidovudine in symptomatic human immunodeficiency virus infection. Results of the Veterans Affairs Cooperative Study. *N Engl J Med,* 326**,** 437-43.

HAMMER, S. M., KATZENSTEIN, D. A., HUGHES, M. D., GUNDACKER, H., SCHOOLEY, R. T., HAUBRICH, R. H., HENRY, W. K., LEDERMAN, M. M., PHAIR, J. P., NIU, M., HIRSCH, M. S. & MERIGAN, T. C. (1996) A trial comparing nucleoside monotherapy with combination therapy in HIV-infected adults with CD4 cell counts from 200 to 500 per cubic millimeter. AIDS Clinical Trials Group Study 175 Study Team. *N Engl J Med,* 335**,** 1081-90.

HAMMER, S. M., SQUIRES, K. E., HUGHES, M. D., GRIMES, J. M., DEMETER, L. M., CURRIER, J. S., ERON, J. J., JR., FEINBERG, J. E., BALFOUR, H. H., JR., DEYTON, L. R.,

CHODAKEWITZ, J. A. & FISCHL, M. A. (1997) A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. *N Engl J Med,* 337**,** 725-33.

HENRY, K., ERICE, A., TIERNEY, C., BALFOUR, H. H., JR., FISCHL, M. A., KMACK, A., LIOU, S. H., KENTON, A., HIRSCH, M. S., PHAIR, J., MARTINEZ, A. & KAHN, J. O. (1998) A randomized, controlled, double-blind study comparing the survival benefit of four different reverse transcriptase inhibitor therapies (three-drug, two-drug, and alternating drug) for the treatment of advanced AIDS. AIDS Clinical Trial Group 193A Study Team. *J Acquir Immune Defic Syndr Hum Retrovirol,* 19**,** 339-49.

HO, D. D., NEUMANN, A. U., PERELSON, A. S., CHEN, W., LEONARD, J. M. & MARKOWITZ, M. (1995) Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature,* 373**,** 123-6.

HOFFMANN, C., MINKAH, N., LEIPZIG, J., WANG, G., ARENS, M. Q., TEBAS, P. & BUSHMAN, F. D. (2007) DNA bar coding and pyrosequencing to identify rare HIV drug resistance mutations. *Nucleic Acids Res,* 35**,** e91.

HUSSON, R. N., MUELLER, B. U., FARLEY, M., WOODS, L., KOVACS, A., GOLDSMITH, J. C., ONO, J., LEWIS, L. L., BALIS, F. M., BROUWERS, P. & ET AL. (1994) Zidovudine and didanosine combination therapy in children with human immunodeficiency virus infection. *Pediatrics,* 93**,** 316-22.

JACKSON, J. B., BECKER-PERGOLA, G., GUAY, L. A., MUSOKE, P., MRACNA, M., FOWLER, M. G., MOFENSON, L. M., MIROCHNICK, M., MMIRO, F. & ESHLEMAN, S. H. (2000) Identification of the K103N resistance mutation in Ugandan women receiving nevirapine to prevent HIV-1 vertical transmission. *AIDS,* 14**,** F111-5.

JACOBSEN, H., HAENGGI, M., OTT, M., DUNCAN, I. B., ANDREONI, M., VELLA, S. & MOUS, J. (1996a) Reduced sensitivity to saquinavir: an update on genotyping from phase I/II trials. *Antiviral Res,* 29**,** 95-7.

JACOBSEN, H., HANGGI, M., OTT, M., DUNCAN, I. B., OWEN, S., ANDREONI, M., VELLA, S. & MOUS, J. (1996b) In vivo resistance to a human immunodeficiency virus type 1 proteinase inhibitor: mutations, kinetics, and frequencies. *J Infect Dis,* 173**,** 1379-87.

JAMES, J. S. (1995) Lamivudine (3TC) approved for combination use with AZT. *AIDS Treat News***,** 1-5.

JAMES, J. S. (1998a) Efavirenz (SUSTIVA) approved. *AIDS Treat News***,** 1-4.

JAMES, J. S. (1998b) Efavirenz (Sustiva) may equal or exceed protease inhibitor in initial antiretroviral combination. *AIDS Treat News***,** 1-3.

JEFFRIES, D. J. (1989) Zidovudine resistant HIV. *BMJ,* 298**,** 1132-3.

JI, H., MASSE, N., TYLER, S., LIANG, B., LI, Y., MERKS, H., GRAHAM, M., SANDSTROM, P. & BROOKS, J. (2010) HIV drug resistance surveillance using pooled pyrosequencing. *PLoS One,* 5**,** e9263.

JOHNSON, J. A., LI, J. F., MORRIS, L., MARTINSON, N., GRAY, G., MCINTYRE, J. & HENEINE, W. (2005a) Emergence of drug-resistant HIV-1 after intrapartum administration of single-dose nevirapine is substantially underestimated. *J Infect Dis,* 192**,** 16-23.

JOHNSON, V. A., BRUN-VEZINET, F., CLOTET, B., CONWAY, B., KURITZKES, D. R., PILLAY, D., SCHAPIRO, J., TELENTI, A. & RICHMAN, D. (2005b) Update of the Drug Resistance Mutations in HIV-1: 2005. *Top HIV Med,* 13**,** 51-7.

JOHNSON, V. A., MERRILL, D. P., CHOU, T. C. & HIRSCH, M. S. (1992) Human immunodeficiency virus type 1 (HIV-1) inhibitory interactions between protease inhibitor Ro 31-8959 and zidovudine, 2',3'-dideoxycytidine, or recombinant interferon-alpha A against zidovudine-sensitive or -resistant HIV-1 in vitro. *J Infect Dis,* 166**,** 1143-6.

KATLAMA, C., INGRAND, D., LOVEDAY, C., CLUMECK, N., MALLOLAS, J., STASZEWSKI, S., JOHNSON, M., HILL, A. M., PEARCE, G. & MCDADE, H. (1996) Safety and efficacy of lamivudine-zidovudine combination therapy in antiretroviral-naive patients. A randomized controlled comparison with zidovudine monotherapy. Lamivudine European HIV Working Group. *JAMA,* 276**,** 118-25.

KELLAM, P., BOUCHER, C. A., TIJNAGEL, J. M. & LARDER, B. A. (1994) Zidovudine treatment results in the selection of human immunodeficiency virus type 1 variants whose genotypes confer increasing levels of drug resistance. *J Gen Virol,* 75 (Pt 2)**,** 341-51.

KOLATA, G. (1987) FDA approves AZT. *Science,* 235**,** 1570.

KURITZKES, D. R. (2003) Preventing and managing resistance in the clinical setting. *J Acquir Immune Defic Syndr,* 34 Suppl 2**,** S103-10.

KURITZKES, D. R., R.L. BASSETT, R.K. YOUNG, H. BARRETT, J.L. KOEL, J.D. HAZELWOOD, V.A. JOHNSON (2002) Rate of emergence of thymidine analogue resistance mutations in

HIV-1 reverse transcriptase selected by stavudine or zidovudine-based regimens in treatment naive patients. *XI International HIV Drug Resistance Workshop.* Seville, Spain.

LAFEUILLADE, A., POGGI, C., HITTINGER, G. & CHADAPAUD, S. (2001) Phenotypic and genotypic resistance to nucleoside reverse transcriptase inhibitors in HIV-1 clinical isolates. *HIV Med,* 2**,** 231-5.

LAI, M. T., LU, M., FELOCK, P. J., HRIN, R. C., WANG, Y. J., YAN, Y., MUNSHI, S., MCGAUGHEY, G. B., TYNEBOR, R. M., TUCKER, T. J., WILLIAMS, T. M., GROBLER, J. A., HAZUDA, D. J., MCKENNA, P. M. & MILLER, M. D. (2010) Distinct mutation pathways of non-subtype B HIV-1 during in vitro resistance selection with nonnucleoside reverse transcriptase inhibitors. *Antimicrob Agents Chemother,* 54**,** 4812-24.

LALLEMANT, M., JOURDAIN, G., LE COEUR, S., KIM, S., KOETSAWANG, S., COMEAU, A. M., PHOOLCHAROEN, W., ESSEX, M., MCINTOSH, K. & VITHAYASAI, V. (2000) A trial of shortened zidovudine regimens to prevent mother-to-child transmission of human immunodeficiency virus type 1. Perinatal HIV Prevention Trial (Thailand) Investigators. *N Engl J Med,* 343**,** 982-91.

LALLEMANT, M., JOURDAIN, G., LE COEUR, S., MARY, J. Y., NGO-GIANG-HUONG, N., KOETSAWANG, S., KANSHANA, S., MCINTOSH, K. & THAINEUA, V. (2004) Single-dose perinatal nevirapine plus standard zidovudine to prevent mother-to-child transmission of HIV-1 in Thailand. *N Engl J Med,* 351**,** 217-28.

LANGE, J. M. (1995) Triple combinations: present and future. *J Acquir Immune Defic Syndr Hum Retrovirol,* 10 Suppl 1**,** S77-82.

LARDER, B. A., DARBY, G. & RICHMAN, D. D. (1989) HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science,* 243**,** 1731-4.

LARDER, B. A., KELLAM, P. & KEMP, S. D. (1991) Zidovudine resistance predicted by direct detection of mutations in DNA from HIV-infected lymphocytes. *AIDS,* 5**,** 137-44.

LARDER, B. A., KELLAM, P. & KEMP, S. D. (1993) Convergent combination therapy can select viable multidrug-resistant HIV-1 in vitro. *Nature,* 365**,** 451-3.

LARDER, B. A. & KEMP, S. D. (1989) Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). *Science,* 246**,** 1155-8.

LARDER, B. A., KEMP, S. D. & HARRIGAN, P. R. (1995) Potential mechanism for sustained antiretroviral efficacy of AZT-3TC combination therapy. *Science,* 269**,** 696-9.

LARROUY, L., CHAZALLON, C., LANDMAN, R., CAPITANT, C., PEYTAVIN, G., COLLIN, G., CHARPENTIER, C., STORTO, A., PIALOUX, G., KATLAMA, C., GIRARD, P. M., YENI, P., ABOULKER, J. P., BRUN-VEZINET, F. & DESCAMPS, D. (2010) Gag mutations can impact virological response to dual-boosted protease inhibitor combinations in antiretroviral-naive HIV-infected patients. *Antimicrob Agents Chemother,* 54**,** 2910-9.

LATAILLADE, M., CHIARELLA, J., YANG, R., SCHNITTMAN, S., WIRTZ, V., UY, J., SEEKINS, D., KRYSTAL, M., MANCINI, M., MCGRATH, D., SIMEN, B., EGHOLM, M. & KOZAL, M. Prevalence and clinical significance of HIV drug resistance mutations by ultra-deep sequencing in antiretroviral-naive subjects in the CASTLE study. *PLoS One,* 5**,** e10952.

LE, T., CHIARELLA, J., SIMEN, B. B., HANCZARUK, B., EGHOLM, M., LANDRY, M. L., DIECKHAUS, K., ROSEN, M. I. & KOZAL, M. J. (2009) Low-abundance HIV drug-resistant viral variants in treatment-experienced persons correlate with historical antiretroviral use. *PLoS One,* 4**,** e6079.

LOCKMAN, S., SHAPIRO, R. L., SMEATON, L. M., WESTER, C., THIOR, I., STEVENS, L., CHAND, F., MAKHEMA, J., MOFFAT, C., ASMELASH, A., NDASE, P., ARIMI, P., VAN WIDENFELT, E., MAZHANI, L., NOVITSKY, V., LAGAKOS, S. & ESSEX, M. (2007) Response to antiretroviral therapy after a single, peripartum dose of nevirapine. *N Engl J Med,* 356**,** 135-47.

LUNZEN, J. (2007) Treatment of heavily antiretroviral-experienced HIV-infected patients. *AIDS Rev,* 9**,** 246-53.

MAGA, G., AMACKER, M., RUEL, N., HUBSCHER, U. & SPADARI, S. (1997) Resistance to nevirapine of HIV-1 reverse transcriptase mutants: loss of stabilizing interactions and thermodynamic or steric barriers are induced by different single amino acid substitutions. *J Mol Biol,* 274**,** 738-47.

MAILLARD, A., CHAPPLAIN, J. M., TRIBUT, O., BENTUE-FERRER, D., TATTEVIN, P., ARVIEUX, C., MICHELET, C. & RUFFAULT, A. (2007) The use of drug resistance algorithms and genotypic inhibitory quotient in prediction of lopinavir-ritonavir treatment response in human immunodeficiency virus type 1 protease inhibitor-experienced patients. *J Clin Virol,* 38**,** 131-8.

MARCELIN, A. G., COHEN-CODAR, I., KING, M. S., COLSON, P., GUILLEVIC, E., DESCAMPS, D., LAMOTTE, C., SCHNEIDER, V., RITTER, J., SEGONDY, M., PEIGUE-LAFEUILLE, H., MORAND-JOUBERT, L., SCHMUCK, A., RUFFAULT, A., PALMER, P., CHAIX, M. L., MACKIEWICZ, V., BRODARD, V., IZOPET, J., COTTALORDA, J., KOHLI, E., CHAUVIN, J. P., KEMPF, D. J., PEYTAVIN, G. & CALVEZ, V. (2005) Virological and pharmacological

parameters predicting the response to lopinavir-ritonavir in heavily protease inhibitor-experienced patients. *Antimicrob Agents Chemother,* 49**,** 1720-6.

MARCELIN, A. G., DELAUGERRE, C., WIRDEN, M., VIEGAS, P., SIMON, A., KATLAMA, C. & CALVEZ, V. (2004) Thymidine analogue reverse transcriptase inhibitors resistance mutations profiles and association to other nucleoside reverse transcriptase inhibitors resistance mutations observed in the context of virological failure. *J Med Virol,* 72**,** 162-5.

MARCONI, V. C., SUNPATH, H., LU, Z., GORDON, M., KORANTENG-APEAGYEI, K., HAMPTON, J., CARPENTER, S., GIDDY, J., ROSS, D., HOLST, H., LOSINA, E., WALKER, B. D. & KURITZKES, D. R. (2008) Prevalence of HIV-1 drug resistance after failure of a first highly active antiretroviral therapy regimen in KwaZulu Natal, South Africa. *Clin Infect Dis,* 46**,** 1589-97.

MARKOWITZ, M., LOUIE, M., HURLEY, A., SUN, E., DI MASCIO, M., PERELSON, A. S. & HO, D. D. (2003) A novel antiviral intervention results in more accurate assessment of human immunodeficiency virus type 1 replication dynamics and T-cell decay in vivo. *J Virol,* 77**,** 5037-8.

MARTINEZ-CAJAS, J. L., PAI, N. P., KLEIN, M. B. & WAINBERG, M. A. (2009) Differences in resistance mutations among HIV-1 non-subtype B infections: a systematic review of evidence (1996-2008). *J Int AIDS Soc,* 12**,** 11.

MARTINEZ-CAJAS, J. L. & WAINBERG, M. A. (2008) Antiretroviral therapy : optimal sequencing of therapy to avoid resistance. *Drugs,* 68**,** 43-72.

MARX, J. L. (1989) Drug-resistant strains of AIDS virus found. *Science,* 243**,** 1551-2.

MASQUELIER, B., NEAU, D., CHENE, G., LARBERE, J., BIRAC, V., RAGNAUD, J. M. & FLEURY, H. J. (2001) Mechanism of virologic failure after substitution of a protease inhibitor by nevirapine in patients with suppressed plasma HIV-1 RNA. *J Acquir Immune Defic Syndr,* 28**,** 309-12.

MATAMOROS, T., FRANCO, S., VAZQUEZ-ALVAREZ, B. M., MAS, A., MARTINEZ, M. A. & MENENDEZ-ARIAS, L. (2004) Molecular determinants of multi-nucleoside analogue resistance in HIV-1 reverse transcriptases containing a dipeptide insertion in the fingers subdomain: effect of mutations D67N and T215Y on removal of thymidine nucleotide analogues from blocked DNA primers. *J Biol Chem,* 279**,** 24569-77.

MAYERS, D. L., JAPOUR, A. J., ARDUINO, J. M., HAMMER, S. M., REICHMAN, R., WAGNER, K. F., CHUNG, R., LANE, J., CRUMPACKER, C. S., MCLEOD, G. X. & ET AL. (1994) Dideoxynucleoside resistance emerges with prolonged zidovudine monotherapy. The RV43 Study Group. *Antimicrob Agents Chemother,* 38**,** 307-14.

MCLEAN, A. R. & NOWAK, M. A. (1992) Competition between zidovudine-sensitive and zidovudine-resistant strains of HIV. *AIDS,* 6**,** 71-9.

MCLEOD, G. X. & HAMMER, S. M. (1992) Zidovudine: five years later. *Ann Intern Med,* 117**,** 487-501.

METZNER, K. J. (2006) Detection and significance of minority quasispecies of drug-resistant HIV-1. *J HIV Ther,* 11**,** 74-81.

MEYA, D., SPACEK, L. A., TIBENDERANA, H., JOHN, L., NAMUGGA, I., MAGERO, S., DEWAR, R., QUINN, T. C., COLEBUNDERS, R., KAMBUGU, A. & REYNOLDS, S. J. (2009) Development and evaluation of a clinical algorithm to monitor patients on antiretrovirals in resource-limited settings using adherence, clinical and CD4 cell count criteria. *J Int AIDS Soc,* 12**,** 3.

MEYNARD, J. L., VRAY, M., MORAND-JOUBERT, L., RACE, E., DESCAMPS, D., PEYTAVIN, G., MATHERON, S., LAMOTTE, C., GUIRAMAND, S., COSTAGLIOLA, D., BRUN-VEZINET, F., CLAVEL, F. & GIRARD, P. M. (2002) Phenotypic or genotypic resistance testing for choosing antiretroviral therapy after treatment failure: a randomized trial. *AIDS,* 16**,** 727-36.

MILLER, V., DE BETHUNE, M. P., KOBER, A., STURMER, M., HERTOGS, K., PAUWELS, R., STOFFELS, P. & STASZEWSKI, S. (1998) Patterns of resistance and cross-resistance to human immunodeficiency virus type 1 reverse transcriptase inhibitors in patients treated with the nonnucleoside reverse transcriptase inhibitor loviride. *Antimicrob Agents Chemother,* 42**,** 3123-9.

MILLER, V. & LARDER, B. A. (2001) Mutational patterns in the HIV genome and cross-resistance following nucleoside and nucleotide analogue drug exposure. *Antivir Ther,* 6 Suppl 3**,** 25-44.

MILLER, V., STARK, T., LOELIGER, A. E. & LANGE, J. M. (2002) The impact of the M184V substitution in HIV-1 reverse transcriptase on treatment response. *HIV Med,* 3**,** 135-45.

MITSUYA, H., YARCHOAN, R. & BRODER, S. (1990) Molecular targets for AIDS therapy. *Science,* 249**,** 1533-44.

MITSUYA, Y., VARGHESE, V., WANG, C., LIU, T. F., HOLMES, S. P., JAYAKUMAR, P., GHARIZADEH, B., RONAGHI, M., KLEIN, D., FESSEL, W. J. & SHAFER, R. W. (2008) Minority human immunodeficiency virus type 1 variants in antiretroviral-naive persons with reverse transcriptase codon 215 revertant mutations. *J Virol,* 82**,** 10747-55.

MIYAKE, H., IIZAWA, Y. & BABA, M. (2003) Novel reporter T-cell line highly susceptible to both CCR5- and CXCR4-using human immunodeficiency virus type 1 and its application to drug susceptibility tests. *J Clin Microbiol,* 41**,** 2515-21.

MOFENSON, L. M. & MCINTYRE, J. A. (2000) Advances and research directions in the prevention of mother-to-child HIV-1 transmission. *Lancet,* 355**,** 2237-44.

MONTANER, J. S., REISS, P., COOPER, D., VELLA, S., HARRIS, M., CONWAY, B., WAINBERG, M. A., SMITH, D., ROBINSON, P., HALL, D., MYERS, M. & LANGE, J. M. (1998) A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS Trial. Italy, The Netherlands, Canada and Australia Study. *JAMA,* 279**,** 930-7.

MOODLEY, D., MOODLEY, J., COOVADIA, H., GRAY, G., MCINTYRE, J., HOFMYER, J., NIKODEM, C., HALL, D., GIGLIOTTI, M., ROBINSON, P., BOSHOFF, L. & SULLIVAN, J. L. (2003) A multicenter randomized controlled trial of nevirapine versus a combination of zidovudine and lamivudine to reduce intrapartum and early postpartum mother-to-child transmission of human immunodeficiency virus type 1. *J Infect Dis,* 187**,** 725-35.

MOUROUX, M., DESCAMPS, D., IZOPET, J., YVON, A., DELAUGERRE, C., MATHERON, S., COUTELLIER, A., VALANTIN, M. A., BONMARCHAND, M., AGUT, H., MASSIP, P., COSTAGLIOLA, D., KATLAMA, C., BRUN-VEZINET, F. & CALVEZ, V. (2001) Low-rate emergence of thymidine analogue mutations and multi-drug resistance mutations in the HIV-1 reverse transcriptase gene in therapy-naive patients receiving stavudine plus lamivudine combination therapy. *Antivir Ther,* 6**,** 179-83.

MULDER, J. W., COOPER, D. A., MATHIESEN, L., SANDSTROM, E., CLUMECK, N., GATELL, J. M., FRENCH, M., DONOVAN, B., GRAY, F., YEO, J. M. & ET AL. (1994) Zidovudine twice daily in asymptomatic subjects with HIV infection and a high risk of progression to AIDS: a randomized, double-blind placebo-controlled study. The European-Australian Collaborative Group (Study 017). *AIDS,* 8**,** 313-21.

NACHEGA, J. B., HISLOP, M., DOWDY, D. W., CHAISSON, R. E., REGENSBERG, L. & MAARTENS, G. (2007) Adherence to nonnucleoside reverse transcriptase inhibitor-based HIV therapy and virologic outcomes. *Ann Intern Med,* 146**,** 564-73.

NACHEGA, J. B., MARCONI, V. C., VAN ZYL, G. U., GARDNER, E. M., PREISER, W., HONG, S. Y., MILLS, E. J. & GROSS, R. (2011) HIV Treatment Adherence, Drug Resistance, Virologic Failure: Evolving Concepts. *Infect Disord Drug Targets*.

NOVITSKY, V., WESTER, C. W., DEGRUTTOLA, V., BUSSMANN, H., GASEITSIWE, S., THOMAS, A., MOYO, S., MUSONDA, R., VAN WIDENFELT, E., MARLINK, R. G. & ESSEX, M. (2007) The reverse transcriptase 67N 70R 215Y genotype is the predominant TAM pathway associated with virologic failure among HIV type 1C-infected adults treated with ZDV/ddI-containing HAART in southern Africa. *AIDS Res Hum Retroviruses,* 23**,** 868-78.

O'MEARA, D., WILBE, K., LEITNER, T., HEJDEMAN, B., ALBERT, J. & LUNDEBERG, J. (2001) Monitoring resistance to human immunodeficiency virus type 1 protease inhibitors by pyrosequencing. *J Clin Microbiol,* 39**,** 464-73.

PALMER, S., KEARNEY, M., MALDARELLI, F., HALVAS, E. K., BIXBY, C. J., BAZMI, H., ROCK, D., FALLOON, J., DAVEY, R. T., JR., DEWAR, R. L., METCALF, J. A., HAMMER, S., MELLORS, J. W. & COFFIN, J. M. (2005) Multiple, linked human immunodeficiency virus type 1 drug resistance mutations in treatment-experienced patients are missed by standard genotype analysis. *J Clin Microbiol,* 43**,** 406-13.

PANDIT, A., MACKAY, W. G., STEEL, C., VAN LOON, A. M. & SCHUURMAN, R. (2008) HIV-1 drug resistance genotyping quality assessment: results of the ENVA7 Genotyping Proficiency Programme. *J Clin Virol,* 43**,** 401-6.

PAREDES, R., MARCONI, V. C., CAMPBELL, T. B. & KURITZKES, D. R. (2007) Systematic evaluation of allele-specific real-time PCR for the detection of minor HIV-1 variants with pol and env resistance mutations. *J Virol Methods,* 146**,** 136-46.

PELLEGRIN, I., IZOPET, J., REYNES, J., DENAYROLLES, M., MONTES, B., PELLEGRIN, J. L., MASSIP, P., PUEL, J., FLEURY, H. & SEGONDY, M. (1999) Emergence of zidovudine and multidrug-resistance mutations in the HIV-1 reverse transcriptase gene in therapy-naive patients receiving stavudine plus didanosine combination therapy. STADI Group. *AIDS,* 13**,** 1705-9.

PEREZ, H., VIGNOLES, M., LAUFER, N., GOMEZ, A., COLL, P., LOTTNER, J., ROLON, M., SALOMON, H. & CAHN, P. (2008) Low rate of emergence of nevirapine and lamivudine resistance after post-partum interruption of a triple-drug regimen. *Antivir Ther,* 13**,** 135-9.

PERSAUD, D., PIERSON, T., RUFF, C., FINZI, D., CHADWICK, K. R., MARGOLICK, J. B., RUFF, A., HUTTON, N., RAY, S. & SILICIANO, R. F. (2000) A stable latent reservoir for HIV-1 in resting CD4(+) T lymphocytes in infected children. *J Clin Invest,* 105**,** 995-1003.

PETRELLA, M. & WAINBERG, M. A. (2002) Might the M184V substitution in HIV-1 RT confer clinical benefit? *AIDS Rev,* 4**,** 224-32.

PETRONI, A., DELUCHI, G., PRYLUKA, D., ROTRYNG, F., BORTOLOZZI, R., LOPARDO, G., BOUZAS, M. B., ZAPIOLA, I., GARONE, D., RODRIGUEZ, C., CHIOCCONI, E., LAZARO, M. E., MURANO, F., MARANZANA, A., OLIVA, S. M., APARICIO, M., BELTRAN, M. & BENETUCCI, J. A. (2006) Update on primary HIV-1 resistance in Argentina: emergence of mutations conferring high-level resistance to nonnucleoside reverse transcriptase inhibitors in drug-naive patients. *J Acquir Immune Defic Syndr,* 42**,** 506-10.

PICARD, V., ANGELINI, E., MAILLARD, A., RACE, E., CLAVEL, F., CHENE, G., FERCHAL, F. & MOLINA, J. M. (2001) Comparison of genotypic and phenotypic resistance patterns of human immunodeficiency virus type 1 isolates from patients treated with stavudine and didanosine or zidovudine and lamivudine. *J Infect Dis,* 184**,** 781-4.

PILLAY, D. (2004) Current patterns in the epidemiology of primary HIV drug resistance in North America and Europe. *Antivir Ther,* 9**,** 695-702.

PROSPERI, M. C., D'AUTILIA, R., INCARDONA, F., DE LUCA, A., ZAZZI, M. & ULIVI, G. (2009) Stochastic modelling of genotypic drug-resistance for human immunodeficiency virus towards long-term combination therapy optimization. *Bioinformatics,* 25**,** 1040-7.

PUCHHAMMER-STOCKL, E., SCHMIED, B., MANDL, C. W., VETTER, N. & HEINZ, F. X. (1999) Comparison of line probe assay (LIPA) and sequence analysis for detection of HIV-1 drug resistance. *J Med Virol,* 57**,** 283-9.

RICHMAN, D. D. (1990) Zidovudine resistance of human immunodeficiency virus. *Rev Infect Dis,* 12 Suppl 5**,** S507-10; discussion S510-2.

RICHMAN, D. D. (1991) AZT resistance in isolates of HIV. *Immunodefic Rev,* 2**,** 315-8.

RICHMAN, D. D. (1994) Resistance, drug failure, and disease progression. *AIDS Res Hum Retroviruses,* 10**,** 901-5.

RICHMAN, D. D., HAVLIR, D., CORBEIL, J., LOONEY, D., IGNACIO, C., SPECTOR, S. A., SULLIVAN, J., CHEESEMAN, S., BARRINGER, K., PAULETTI, D. & ET AL. (1994) Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. *J Virol,* 68**,** 1660-6.

ROBERTS, J. D., BEBENEK, K. & KUNKEL, T. A. (1988) The accuracy of reverse transcriptase from HIV-1. *Science,* 242**,** 1171-3.

RODRIGUES, R., SCHERER, L. C., OLIVEIRA, C. M., FRANCO, H. M., SPERHACKE, R. D., FERREIRA, J. L., CASTRO, S. M., STELLA, I. M. & BRIGIDO, L. F. (2006) Low prevalence of primary antiretroviral resistance mutations and predominance of HIV-1 clade C at polymerase gene in newly diagnosed individuals from south Brazil. *Virus Res,* 116**,** 201-7.

SAAG, M. S., HAHN, B. H., GIBBONS, J., LI, Y., PARKS, E. S., PARKS, W. P. & SHAW, G. M. (1988) Extensive variation of human immunodeficiency virus type-1 in vivo. *Nature,* 334**,** 440-4.

SCHNELL, G., INCE, W. L. & SWANSTROM, R. (2008) Identification and recovery of minor HIV-1 variants using the heteroduplex tracking assay and biotinylated probes. *Nucleic Acids Res,* 36**,** e146.

SHAFER, R. W., JUNG, D. R. & BETTS, B. J. (2000a) Human immunodeficiency virus type 1 reverse transcriptase and protease mutation search engine for queries. *Nature Medicine,* 6**,** 1290-1292.

SHAFER, R. W., JUNG, D. R., BETTS, B. J., XI, Y. & GONZALES, M. J. (2000b) Human immunodeficiency virus reverse transcriptase and protease sequence database. *Nucleic Acids Res,* 28**,** 346-8.

SHAFER, R. W., RHEE, S. Y. & BENNETT, D. E. (2008) Consensus drug resistance mutations for epidemiological surveillance: basic principles and potential controversies. *Antivir Ther,* 13 Suppl 2**,** 59-68.

SHAFER, R. W. & SCHAPIRO, J. M. (2008) HIV-1 drug resistance mutations: an updated framework for the second decade of HAART. *AIDS Rev,* 10**,** 67-84.

SHEN, L., SHEN, J., LUO, X., CHENG, F., XU, Y., CHEN, K., ARNOLD, E., DING, J. & JIANG, H. (2003) Steered molecular dynamics simulation on the binding of NNRTI to HIV-1 RT. *Biophys J,* 84**,** 3547-63.

SHET, A., BERRY, L., MOHRI, H., MEHANDRU, S., CHUNG, C., KIM, A., JEAN-PIERRE, P., HOGAN, C., SIMON, V., BODEN, D. & MARKOWITZ, M. (2006) Tracking the prevalence of transmitted antiretroviral drug-resistant HIV-1: a decade of experience. *J Acquir Immune Defic Syndr,* 41**,** 439-46.

SIEGAL, F. P., LOPEZ, C., HAMMER, G. S., BROWN, A. E., KORNFELD, S. J., GOLD, J., HASSETT, J., HIRSCHMAN, S. Z., CUNNINGHAM-RUNDLES, C., ADELSBERG, B. R. & ET AL. (1981) Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions. *N Engl J Med,* 305**,** 1439-44.

SPENLEHAUER, C., GORDON, C. A., TRKOLA, A. & MOORE, J. P. (2001) A luciferase-reporter gene-expressing T-cell line facilitates neutralization and drug-sensitivity assays that use either R5 or X4 strains of human immunodeficiency virus type 1. *Virology,* 280**,** 292-300.

SPRUANCE, S. L., PAVIA, A. T., PETERSON, D., BERRY, A., POLLARD, R., PATTERSON, T. F., FRANK, I., REMICK, S. C., THOMPSON, M., MACARTHUR, R. D., MOREY, G. E., RAMIREZ-RONDA, C. H., BERNSTEIN, B. M., SWEET, D. E., CRANE, L., PETERSON, E. A., PACHUCKI, C. T., GREEN, S. L., BRAND, J., RIOS, A., DUNKLE, L. M., CROSS, A., BROWN, M. J., INGRAHAM, P., GUGLIOTTI, R., SCHINDZIELORZ, A. H. & SMALDONE, L. (1994) Didanosine compared with continuation of zidovudine in HIV-infected patients with signs of clinical deterioration while receiving zidovudine. A randomized, double-blind clinical trial. The Bristol-Myers Squibb AI454-010 Study Group. *Ann Intern Med,* 120**,** 360-8.

STASZEWSKI, S., LOVEDAY, C., PICAZO, J. J., DELLARNONICA, P., SKINHOJ, P., JOHNSON, M. A., DANNER, S. A., HARRIGAN, P. R., HILL, A. M., VERITY, L. & MCDADE, H. (1996) Safety and efficacy of lamivudine-zidovudine combination therapy in zidovudine-experienced patients. A randomized controlled comparison with zidovudine monotherapy. Lamivudine European HIV Working Group. *JAMA,* 276**,** 111-7.

SUMMA, V., PETROCCHI, A., BONELLI, F., CRESCENZI, B., DONGHI, M., FERRARA, M., FIORE, F., GARDELLI, C., GONZALEZ PAZ, O., HAZUDA, D. J., JONES, P., KINZEL, O., LAUFER, R., MONTEAGUDO, E., MURAGLIA, E., NIZI, E., ORVIETO, F., PACE, P., PESCATORE, G., SCARPELLI, R., STILLMOCK, K., WITMER, M. V. & ROWLEY, M. (2008) Discovery of raltegravir, a potent, selective orally bioavailable HIV-integrase inhibitor for the treatment of HIV-AIDS infection. *J Med Chem,* 51**,** 5843-55.

TUDOR-WILLIAMS, G., ST CLAIR, M. H., MCKINNEY, R. E., MAHA, M., WALTER, E., SANTACROCE, S., MINTZ, M., O'DONNELL, K., RUDOLL, T., VAVRO, C. L. & ET AL. (1992) HIV-1 sensitivity to zidovudine and clinical outcome in children. *Lancet,* 339**,** 15-9.

UNAIDS (2008) Report on the global HIV/AIDS epidemic 2008. UNAIDS/08.25E / JC1510E. Available from: http://www.unaids.org/en/KnowledgeCentre/HIVData/GlobalReport/2008/2008_Global_report.asp.

.

UNAIDS (2010) Global Report: UNAIDS Report on the global AIDS epidemic. Geneva, 2010. Available from: http://www.unaids.org/globalreport/documents/20101123_GlobalReport_full_en.pdf. Accessed on 16 August 2011. *UNAIDS*.

VARGHESE, V., SHAHRIAR, R., RHEE, S. Y., LIU, T., SIMEN, B. B., EGHOLM, M., HANCZARUK, B., BLAKE, L. A., GHARIZADEH, B., BABRZADEH, F., BACHMANN, M. H., FESSEL, W. J. & SHAFER, R. W. (2009) Minority variants associated with transmitted and acquired HIV-1 nonnucleoside reverse transcriptase inhibitor resistance: implications for the use of second-generation nonnucleoside reverse transcriptase inhibitors. *J Acquir Immune Defic Syndr,* 52**,** 309-15.

VELLA, S. (1994) HIV therapy advances. Update on a proteinase inhibitor. *AIDS,* 8 Suppl 3**,** S25-9.

VELLA, S., GALLUZZO, C., GIANNINI, G., PIRILLO, M. F., DUNCAN, I., JACOBSEN, H., ANDREONI, M., SARMATI, L. & ERCOLI, L. (1996) Saquinavir/zidovudine combination in patients with advanced HIV infection and no prior antiretroviral therapy: CD4+ lymphocyte/plasma RNA changes, and emergence of HIV strains with reduced phenotypic sensitivity. *Antiviral Res,* 29**,** 91-3.

VERCAUTEREN, J. & VANDAMME, A. M. (2006) Algorithms for the interpretation of HIV-1 genotypic drug resistance information. *Antiviral Res,* 71**,** 335-42.

VERHOFSTEDE, C., NOE, A., DEMECHELEER, E., DE CABOOTER, N., VAN WANZEELE, F., VAN DER GUCHT, B., VOGELAERS, D. & PLUM, J. (2004) Drug-resistant variants that evolve during nonsuppressive therapy persist in HIV-1-infected peripheral blood mononuclear cells after long-term highly active antiretroviral therapy. *J Acquir Immune Defic Syndr,* 35**,** 473-83.

VINGERHOETS, J., TAMBUYZER, L., AZIJN, H., HOOGSTOEL, A., NIJS, S., PEETERS, M., DE BETHUNE, M. P., DE SMEDT, G., WOODFALL, B. & PICCHIO, G. (2010) Resistance profile of etravirine: combined analysis of baseline genotypic and phenotypic data from the randomized, controlled Phase III clinical studies. *AIDS,* 24**,** 503-14.

VIOLARI, A., COTTON, M. F., GIBB, D. M., BABIKER, A. G., STEYN, J., MADHI, S. A., JEAN-PHILIPPE, P. & MCINTYRE, J. A. (2008) Early Antiretroviral Therapy and Mortality among HIV-Infected Infants. *N Engl J Med,* 359**,** 2233-2244.

VOLBERDING, P. A., LAGAKOS, S. W., GRIMES, J. M., STEIN, D. S., BALFOUR, H. H., JR., REICHMAN, R. C., BARTLETT, J. A., HIRSCH, M. S., PHAIR, J. P., MITSUYASU, R. T. & ET

AL. (1994) The duration of zidovudine benefit in persons with asymptomatic HIV infection. Prolonged evaluation of protocol 019 of the AIDS Clinical Trials Group. *JAMA,* 272**,** 437-42.

VOLBERDING, P. A., LAGAKOS, S. W., GRIMES, J. M., STEIN, D. S., ROONEY, J., MENG, T. C., FISCHL, M. A., COLLIER, A. C., PHAIR, J. P., HIRSCH, M. S. & ET AL. (1995) A comparison of immediate with deferred zidovudine therapy for asymptomatic HIV-infected adults with CD4 cell counts of 500 or more per cubic millimeter. AIDS Clinical Trials Group. *N Engl J Med,* 333**,** 401-7.

WAINBERG, M. A. (2004) The impact of the M184V substitution on drug resistance and viral fitness. *Expert Rev Anti Infect Ther,* 2**,** 147-51.

WAINBERG, M. A., TREMBLAY, M., ROOKE, R., FANNING, M., TSOUKAS, C., MONTANER, J. S., O'SHAUGHNESSY, M. & RUEDY, J. (1992) Characterization of zidovudine resistant variants of HIV-1 isolated from patients on prolonged therapy. *Drugs Exp Clin Res,* 18**,** 283-90.

WEI, X., GHOSH, S. K., TAYLOR, M. E., JOHNSON, V. A., EMINI, E. A., DEUTSCH, P., LIFSON, J. D., BONHOEFFER, S., NOWAK, M. A., HAHN, B. H. & ET AL. (1995) Viral dynamics in human immunodeficiency virus type 1 infection. *Nature,* 373**,** 117-22.

WEINHEIMER, S., DISCOTTO, L., FRIBORG, J., YANG, H. & COLONNO, R. (2005) Atazanavir signature I50L resistance substitution accounts for unique phenotype of increased susceptibility to other protease inhibitors in a variety of human immunodeficiency virus type 1 genetic backbones. *Antimicrob Agents Chemother,* 49**,** 3816-24.

WILLIAMS, I. G. & DE COCK, K. M. (1996) The XI international conference on AIDS. Vancouver 7-12 July 1996. A review of Clinical Science Track B. *Genitourin Med,* 72**,** 365-9.

WONG, J. K., HEZAREH, M., GUNTHARD, H. F., HAVLIR, D. V., IGNACIO, C. C., SPINA, C. A. & RICHMAN, D. D. (1997) Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science,* 278**,** 1291-5.

YOULE, M. (2007) Overview of boosted protease inhibitors in treatment-experienced HIV-infected patients. *J Antimicrob Chemother,* 60**,** 1195-205.

van Zyl, G. U., M. Claassen, S. Engelbrecht, J. D. Laten, M. F. Cotton, G. B. Theron, and W. Preiser. 2008. *Zidovudine with nevirapine for the prevention of HIV mother-to-child transmission reduces nevirapine resistance in mothers from the Western Cape, South Africa*. J Med Virol **80:**942-6.

About this article:

Background:

Simplified regimens for the prevention of mother-to-child transmission of HIV (PMTCT), being less costly than those developed in the industrialised world, have nevertheless been able to reduce transmission and thereby significantly reduce the burden of infected infants. The simplest of these regimens was a single dose of nevirapine (NVP) given to the mother during labour and to the newborn within 72 hours after birth. However due to the drug's long half-life and low genetic barrier , NVP resistance is commonly selected in mothers receiving this regimen and in children who become infected despite prophylaxis. Adding a short course of AZT given to the mother during the latter part of gestation (from 34 or 36 weeks), and to the baby during the first week of life, has been shown to further reduce the rate of transmission. However, little data were available on the impact of this intervention on resistance in the mothers. The objective of this study was to investigate the prevalence of NVP and AZT resistance in mothers after having received this combined regimen. For this purpose specimens were collected at baseline and after PMTCT exposure and genotypic resistance detected by PCR and sequencing by capillary electrophoresis (bulk sequencing).

Main findings:

In specimens obtained within 60 days after delivery, acquired NVP resistance was detected in 17.1% (8.7-25.6%).

Limitations:

Timing of testing for NVP resistance is important as the prevalence of NVP resistance as found by bulk sequencing wanes over time. The optimal period for testing seems to be 6 weeks after exposure. In this study 82% of specimens were collected between 4 and 8 weeks after exposure. Variable follow-up was a result of logistical problems.

The use of bulk sequencing, although it allows the detection of all NVP-associated mutations, is insensitive to minor resistant variants (making up less than 20-30% of the total viral population), which have been shown to be detectable in the large majority of patients after single dose NVP with the use of allele-specific PCR. However a panel of many different PCRs will be necessary to detect all minor variant mutations and another alternative such as deep sequencing is costly and requires access to specialised technology.

Conclusions:

A lower prevalence of NVP-associated mutations was observed in these patients who received the dual regimen compared to settings where NVP-only had been used. This probably indicates a reduction in the risk of resistance probably due to AZT-use resulting in a lower viral load, thereby limiting replication, viral evolution and resistance emergence.

Journal of Medical Virology 80:942–946 (2008)

Zidovudine With Nevirapine for the Prevention of HIV Mother-to-Child Transmission Reduces Nevirapine Resistance in Mothers From the Western Cape, South Africa

G.U. van Zyl,[1]* M. Claassen,[1] S. Engelbrecht,[1] J.D. Laten,[1] M.F. Cotton,[2] G.B. Theron,[3] and W. Preiser[1]

[1]Faculty of Health Sciences and National Health Laboratory Service (NHLS) Tygerberg, Division of Medical Virology, Department Pathology and Centre for Infectious Diseases, Stellenbosch University, Cape Town, South Africa
[2]Department of Paediatrics and Child Health and Centre for Infectious Diseases, Faculty of Health Sciences, Stellenbosch University and Tygerberg Hospital, Cape Town, South Africa
[3]Department of Obstetrics and Gynaecology, Faculty of Health Sciences, Stellenbosch University and Tygerberg Hospital, Cape Town, South Africa

van Zyl, G. U., M. F. Cotton, M. Claassen, C. Abrahams, and W. Preiser. 2010. *Surveillance of transmitted resistance to antiretroviral drug classes among young children in the Western Cape Province of South Africa.* Pediatr Infect Dis J **29:**370-1.

About this article:

Background:

Except for non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance, which is common in infants in sub Saharan Africa due to nevirapine (NVP)-containing prevention of mother-to-child transmission of HIV (PMTCT) regimens, little is known about resistance in young South African children who are combination antiretroviral therapy-naïve. Data on resistance to nucleoside reverse transcriptase inhibitors (NRTI) and protease inhibitors (PI) would be valuable especially as South African children younger than 3 years of age are initiated on a first-line regimen of the PI lopinavir boosted with low-dose ritonavir (LPV/r) combined with two NRTI, due to the high risk of NNRTI resistance. If such NRTI or PI resistance is transmitted this would likely occur at the time when most transmission occurs, that is around birth. Therefore in young children the timing of transmission can be more accurately estimated than in adults, with an unknown date of infection, for whom indirect measures such as young age, high CD4 count and absence of clinical disease are used as proxies of recent infection. The prevalence of resistance to PIs and NRTIs in very young untreated children therefore reflects the prevalence of these mutations in viruses currently circulating in the sexually active maternal population, and an increase in mutation prevalence in infants may be an early warning sign of an increase in transmitted resistance in a predominantly heterosexual epidemic.

At the stage of this investigation no guidelines for the study of initial (a combination of transmitted and PMTC-induced) resistance in young children had been formulated. Therefore children < 18 months of age, who were antiretroviral combination therapy-naive, were included and the most recent surveillance drug resistance mutation (SDRM) list was used to identify initial resistance. Furthermore we used the WHO sequential testing strategy, which includes a stopping rule and does not aim to calculate an exact prevalence but to classify resistance to respective drug classes as either high (>15%), intermediate (5-15%), or low (<15%).

Main findings:

No SDRM mutations conferring resistance to PIs or NRTIs were detected in 49 children (49 reverse transcriptase and 48 protease sequences).). Three patients harboured NNRTI resistance (2 with K103N and 1 with V90I), indicating NNRTI resistance at intermediate level (5-15%).

Limitations:

True transmitted resistance mutations would have likely persisted through the first 18 months of life (in adults a cut-off of 3 years is used) because, in most instances, a single variant is transmitted which takes some time for reversion to wild-type. Nevertheless PMTCT-induced resistance could have waned rapidly and could have become undetectable by bulk sequencing at the time of testing, whereas an alternative method such as allele-specific PCR might have detected NNRTI resistance in a larger proportion of individuals. As 35 of the 49 patients had been exposed to NVP via (unsuccessful) PMTCT the prevalence of NNRTI resistance was likely underestimated by bulk sequencing.

Conclusions

The prevalence of transmitted resistance to PIs and NRTIs in children < 18 months of age is can be classified as low (<5%), which suggests a probable low prevalence of antiretroviral resistance against the same drug classes in the parental population. NNRTI resistance, probably due to NVP PMTCT, was nevertheless detected in three of these children.

The Pediatric Infectious Disease Journal • Volume 29, Number 3, March 2010

SURVEILLANCE OF TRANSMITTED RESISTANCE TO ANTIRETROVIRAL DRUG CLASSES AMONG YOUNG CHILDREN IN THE WESTERN CAPE PROVINCE OF SOUTH AFRICA

Gert U. van Zyl, MD, Mark F. Cotton, MD, PhD,† Mathilda Claassen, BSc(Hons),* Charmaine Abrahams, RN,* and Wolfgang Preiser, MD, PhD**

van Zyl, G. U., L. van der Merwe, M. Claassen, M. F. Cotton, H. Rabie, H. W. Prozesky, and W. Preiser. 2009. *Protease inhibitor resistance in South African children with virologic failure*. Pediatr Infect Dis J **28:**1125-7.

About this article:

Background:

First-line antiretroviral therapy for children less than 3 years of age is protease inhibitor (PI)-based, due to an expected high prevalence of nevirapine (NVP) resistance resulting from (unsuccessful) exposure to prevention of mother-to-child transmission of HIV (PMTCT). The PI of choice is lopinavir combined with low-dose ritonavir (LPV/r) for pharmacodynamic purposes (s-called "boosting"). However lack of pharmacokinetic data on the use of LPV/r in infants or when co-administered with rifampicin resulted in some children in South Africa receiving an alternative regimen of ritonavir (RTV) as single PI (sPI).

In order to investigate associations with PI resistance, therapy history and laboratory information were recorded for children who had virological failure on a PI-based regimen. As multiple resistance tests were available for some patients, mixed effect linear and logistic regression models were used to study associations with resistance outcomes.

Main findings:

Major PI resistance mutations were detected in 12 of 17 patients who had historic or current use of RTV sPI versus in only 1 of 13 patients without such exposure (i.e. who had received LPV/r throughout). The presence of major PI resistance mutations was also associated with a prolonged failing period.

Not specifically highlighted in the manuscript is the problem of multiple resistance: PI resistance was combined with the M184V mutation and thymidine analogue mutations (TAMs) in some children. These children cannot be adequately treated with available drugs in the South African treatment programme and would require third-line options. This issue will be more fully addressed in a future publication.

Limitations:

As this study is an observational study confounding can not be totally eliminated. Factors such as the longer therapy history or the use of rifampicin, although not significantly associated with resistance in our study, may contribute to a larger number of patients with RTV sPI exposure having resistance.

Conclusions

Due to a lack of pharmacokinetic data on LPV/r, in infants, or when co-administered with rifampicin, RTV as sPI was used instead of LPV/r, which resulted in a high prevalence of PI resistance in children, who had virological failure, on this regimen.

The Pediatric Infectious Disease Journal • Volume 28, Number 12, December 2009

PROTEASE INHIBITOR RESISTANCE IN SOUTH AFRICAN CHILDREN WITH VIROLOGIC FAILURE

Gert U. van Zyl, MD, Lize van der Merwe, PhD,†‡ Mathilda Claassen, B.Sc.Hons,* Mark F. Cotton, MD, PhD,§ Helena Rabie, MD,§ Hans W. Prozesky, MD,¶ and Wolfgang Preiser, MD, PhD**

van Zyl, G. U., L. van der Merwe, M. Claassen, M. Zeier, and W. Preiser. *Antiretroviral resistance patterns and factors associated with resistance in adult patients failing NNRTI-based regimens in the Western Cape, South Africa.* Epublished 13 August 2011. J Med Virol **83:**1764-9.

About this article:

Background:

The prevalence of antiretroviral resistance mutations in adult patients on non-nucleoside reverse transcriptase inhibitor (NNRTI) based first-line regimens is informative to clinicians and policy makers. Although randomised controlled trials are ideal to study the effects of particular regimens on mutation prevalence, follow-up is often limited and therefore not able to detect long-term effects. Furthermore in resource-limited settings patients are often retained for prolonged periods on failing regimens as monitoring for virologic failure may be infrequent and the response to failure conservative. The choice of therapeutic regimen is also governed by clinical considerations, and it is therefore in some instances difficult to randomise the use of two drugs. Lastly HIV-1 subtypes influence the prevalence of particular polymorphisms and resistance-associated mutations. The effect of these factors on resistance outcomes can therefore best be studied in an observational or cross-sectional study. Although the prevalence of resistance in many settings, including South Africa, has been presented, few investigations attempted to study associations with particular resistance outcomes, which could provide guidance to clinicians. In this study we investigated the following resistance outcomes: the presence of NNRTI resistance (and susceptibility to etravirine (ETV) in particular); of M184V/I which confers resistance to lamivudine; of K65R, a mutation usually associated with TDF, DDI or ABC use, but which may be selected by stavudine in HIV-1 subtype C; and of thymidine analogue mutations (TAM) which are selected by AZT or D4T but, when they accumulate, can confer resistance to most NRTIs. Possible associations with these outcomes, investigated, were: therapy choice, duration of failure and laboratory data (HIV-1 viral load and CD4 count results).

Main findings:

Of 167 patients with virologic failure on a first-line NNRTI-based regimen, 17% had no resistance mutations, 82% NNRTI resistance, 60% M184V/I, 12% TAMs and 4% K65R. A prolonged failure period predicted having ≥ 3 TAMs. Nevirapine (NVP) rather than efavirenz use and prolonged failure on NVP were associated with resistance to ETV.

Limitations:

This cross-sectional study suffers from the limitations of any observational study, as not all confounding factors can be excluded. Accurate baseline data were not always available and therefore concurrent laboratory data were included in models. Since we did not have exact dates of failure onset, survival models for the presence or absence of particular mutations could not have been used; therefore we opted to use generalised linear models including estimated duration of failure, which we associated with resistance outcomes. Further details of the limitations are given in the discussion section of the article.

Conclusions

In patients failing an NNRTI-based regimen, NNRTI mutations were most often detected, followed by the M184V mutation that confers resistance to lamivudine. The prevalence of K65R and TAMs were low, with TAMs more often observed in patients with prolonged failure.

Journal of Medical Virology 83:1764–1769 (2011)

Antiretroviral Resistance Patterns and Factors Associated With Resistance in Adult Patients Failing NNRTI-Based Regimens in the Western Cape, South Africa

Gert U. van Zyl,[1,2]* Lize van der Merwe,[3,4] Mathilda Claassen,[1,2] Michele Zeier,[5,6] and Wolfgang Preiser[1,2]

[1]*Division of Medical Virology, Stellenbosch University, Tygerberg, Cape Town, South Africa*
[2]*National Health Laboratory Service, Tygerberg, Cape Town, South Africa*
[3]*Biostatistics Unit, Medical Research Council, Tygerberg, Cape Town, South Africa*
[4]*Department of Statistics, University of Western Cape, Bellville, Cape Town, South Africa*
[5]*Department of Medicine, Stellenbosch University, Tygerberg, Cape Town, South Africa*
[6]*Tygerberg Academic Hospital, Tygerberg, Cape Town, South Africa*

van Zyl, G. U., T. E. van Mens, H. McIlleron, M. Zeier, J. B. Nachega, E. Decloedt, C. Malavazzi, P. Smith, Y. Huang, L. van der Merwe, M. Gandhi, and G. Maartens. 2011. *Low lopinavir plasma or hair concentrations explain second-line protease inhibitor failures in a resource-limited setting*. J Acquir Immune Defic Syndr **56:**333-9.

About this article:

Background:

This study was conducted at the stage when second-line therapy for adults consisted of LPV/r, DDI and AZT. This regimen was poorly tolerable, and a large proportion of these patients who had previously failed an NNRTI-based regimen had detectable or even high viral loads. Similar to findings from other study sites, we were aware of very high rates of failure on second-line therapy for our adult patients both at the Tygerberg Family Clinic and the Ubunthu Clinic in Khayelitsha. However, pill-counts, which were the standard for monitoring adherence in the Tygerberg Clinic, were too insensitive to detect poor adherence. This study was motivated by our attempts to characterise the cause of failure in these patients.

Main findings:

Only 2 of 93 patients (of which 40% were failing) had major protease inhibitor resistance mutations. In a nested case-control study of 50 patients, low plasma and low hair lopinavir concentrations were highly predictive of failure and could together explain failure in all patients with viral loads above 1000 copies/ml that did not have resistance mutations.

Limitations:

The high prevalence of failure of a second-line regimen, largely due to poor adherence, as found in this setting, may not be representative of drug adherence in other settings. The cost-effectiveness of screening for drug exposure before conducting resistance testing would be dependent on the pre-test probability of failing due to poor drug exposure. Furthermore, in other settings unlike South Africa where the first PI used was LPV/r, patients with prior exposure to un-boosted protease inhibitors such as nelfinavir may be at increased risk of PI resistance. Patients with poor adherence generally have low CD4 counts and an increased risk of death. Therefore since the second-line regimen is currently the last resort, and as over time the duration on PI therapy of a ‘natural cohort’ increases, the proportion of patients with relatively good adherence (which is probably the proportion harbouring resistance) may increase relative to those with poor adherence. Other limitations such as the cross-sectional nature of the study, unavailability of LPV trough levels, small sample size and out-referral of patients are mentioned in the manuscript.

Conclusions

In a setting where poor adherence is a major cause of failure on a boosted PI regimen, the use of LPV plasma concentrations (as indicative of recent drug exposure) and LPV hair concentrations (as indicative of exposure over the last few weeks) could be valuable to exclude patients with poor adherence from unnecessary and costly genotypic drug resistance testing.

J Acquir Immune Defic Syndr • Volume 56, Number 4, April 1, 2011

CLINICAL SCIENCE

Low Lopinavir Plasma or Hair Concentrations Explain Second-Line Protease Inhibitor Failures in a Resource-Limited Setting

Gert Uves van Zyl, MBChB, FCPath(SA) Virol, Thijs E. van Mens,† Helen McIlleron, MBChB, PhD,‡ Michele Zeier, MBChB,§ Jean B. Nachega, MD, PhD,§‖ Eric Decloedt, MBChB,‡ Carolina Malavazzi, MD,¶ Peter Smith, PhD,‡ Yong Huang, PhD,# Lize van der Merwe, PhD,**†† Monica Gandhi, MD, MPH,‡‡ and Gary Maartens, MBChB, FCP, DTM&H‡*

Summative comment

In the publications included in this thesis, the investigator used cross-sectional data to investigate antiretroviral drug resistance trends in the Western Cape province of South Africa in adults and children.

The following were investigated:

1) The prevalence of antiretroviral resistance mutations in patients who received the Western Cape regimen for the prevention of mother-to-child transmission of HIV (PMTCT).
2) Intitial resistance in children, i.e. before being initiated on combination antiretroviral therapy.
3) Acquired resistance in children exposed to a first-line protease inhibitor (PI)-based treatment regimen.
4) Acquired resistance in adults failing a first-line non nucleoside reverse transcriptase inhibitor(NNRTI)-based treatment regimen.
5) Acquired resistance in adults failing a second-line PI-based regimen.
6) Transmitted resistance in adults: an abstract is included as an addendum.

These investigations were conducted in order to define priorities for further studies.

Resistance in PMTCT-exposed mothers:

Simplified regimens for the PMTCT, being less costly than the strategies used in the industrialised world, have nevertheless been able to reduce transmission and thereby significantly reduce the burden of infected infants. The simplest of these regimens is a single dose of the NNRTI nevirapine (NVP) given to the mother during labour and to the newborn within 72 hours after birth. However, due to the long half-life and low genetic barrier of NVP, resistance is commonly selected in mothers receiving this regimen and in children who become infected despite prophylaxis. This is relevant as it limits treatment options. Adding a short course of azidothymidine (AZT) to the mother during the latter part of gestation (starting at 34 to 36 weeks), and to the baby in the first week of life, was shown to further reduce the rate of transmission. However little data were available regarding the impact of this intervention on resistance in the mothers. The objective of this study was to investigate the prevalence of NVP and AZT resistance in mothers after having received this combined regimen. Specimens were collected at baseline and after PMTCT exposure and genotypic resistance detected by PCR and sequencing using capillary electrophoresis. The prevalence of NNRTI resistance was 13 out of 76 (17%; 95% CI: 8.7-25.6%) and thus lower than recorded for cohorts where a NVP-only regimen was used. Only one patient had the K70R mutation, which confers low level resistance to AZT – this concurred with a low prevalence of AZT resistance when used for PMTCT interventions. This was the first publication of antiretroviral resistance data in this population. The relative lower prevalence of NVP resistance compared to settings where a NVP-only regimen was used, is encouraging and suggests that the use of AZT with NVP could protect against NVP resistance. The possible mechanism is that AZT reduces the viral load and thereby limits viral evolution and the development of resistance. A potential bias towards under-estimation of resistance in this study was that the patients had relative high CD4 counts and corresponding low viral loads, as patients who had CD4 counts below 200 per microliter qualified for HAART and were therefore excluded from receiving the AZT and NVP regimen. On the other hand a possible counteracting bias (towards an over-estimation of

resistance) could be due to a higher reported risk of NVP resistance in subtype C virus compared to subtypes A and D.
Implications for the national antiretroviral roll-out programme: At the stage of this investigation NVP was used as single agent for PMTCT in the rest of South Africa. Following Western Cape data on the relatively lower prevalence of transmitted infections in patients receiving the dual regimen, and possibly the presented data on the lower risk of resistance, the national guideline adopted the use of a dual regimen that includes AZT during gestation, single-dose NVP and 3-hourly AZT during labour with the recent addition (in 2010) of a single post-partum TDF and FTC combination tablet, with the purpose to further reduce the risk of resistance in the mother.

Initial resistance in children:
Except for NNRTI resistance, which is common in sub-Saharan African infants due to NVP-containing PMTCT regimens, little is known about resistance in young South African children, who are combination antiretroviral therapy-naïve. Data on resistance to nucleoside reverse transcriptase inhibitors (NRTI) and protease inhibitors (PI) would be valuable especially as South African children, < 3 years of age, are initiated on a first-line regimen of the PI lopinavir boosted with low-dose ritonavir (LPV/r) combined with two NRTIs, due to the high prevalence of NNRTI resistance. If such NRTI or PI resistance is transmitted this would likely occur at the time when most transmission occurs, that is around birth. Therefore in young children the event of transmission can be more accurately estimated than in adults, who often have an unknown date of infection and for whom indirect measures such as young age, high CD4 count and absence of clinical disease are used as proxies of recent infection. The prevalence of resistance to PIs and NRTIs in young children would therefore reflect the prevalence of mutations in HIV strains currently circulating in the sexually active maternal population, and an increase in mutation prevalence in infants may be an early warning sign of an increase in transmitted resistance in a predominantly heterosexual epidemic.
At the stage of this investigation no guidelines for the study of initial (which denotes a combination of transmitted and PMTC-induced) resistance in young children had been formulated. Therefore children < 18 months of age, who were antiretroviral-combination-therapy-naive, were included and the most recent surveillance drug resistance mutation (SDRM) list was used to identify initial resistance. Furthermore we used the WHO sequential testing strategy, which includes a stopping rule and which does not aim to calculate an exact prevalence but aims to classify resistance to respective drug classes as either high (>15%), intermediate (5-15%) or low (<15%).
Main findings: No SDRM mutations conferring resistance to PIs or NRTIs were detected in 49 children (49 RT and 48 PI sequences). Therefore the prevalence of resistance to PIs and NRTIs can be classified as low (<5%). Three patients harboured NNRTI resistance (2 with K103N and 1 with V90I), classifying NNRTI resistance as intermediate level (5-15%).
Implications for the national antiretroviral roll-out programme: An estimated low prevalence of pre-existing NRTI and PI resistance in children initiated on combination antiretroviral therapy (including 2 NRTIs and a boosted PI) would suggest a good response to therapy, as long as patients are adherent and dosing is adequate.
Limitations of this investigation: True transmitted resistance mutations would have likely persisted through the first 18 months of life (in adults a cut-off of 3 years is used) because, in most instances, a

single variant is transmitted and as it takes some time for reversion to wild-type. Nevertheless PMTCT-induced resistance could have waned rapidly and become undetectable by bulk sequencing at the time of testing, whereas an alternative method such as allele specific PCR might have detected NNRTI resistance in a larger proportion of individuals. Furthermore, as 35 of the 49 patients had been exposed to unsuccessful PMTCT using NVP, the prevalence of NNRTI resistance was likely underestimated by bulk sequencing.

Transmitted resistance in adults

Our investigation of transmitted resistance in adults, presented at the 6th IAS Conference on HIV Pathogenesis, Treatment and Prevention, Rome, Italy, 17-20 July 2011, shows that the prevalence of transmitted resistance to NNRTIs, NRTI and PIs is currently low in adults, too, probably as a result of the only relatively recent scale-up of antiretroviral therapy in our region.

Implications for the national antiretroviral roll-out programme: The low estimated prevalence of baseline resistance concurs with the current data from other investigations in South Africa, and therefore, at this stage, baseline screening for antiretroviral resistance is not necessary before initiation combination antiretroviral therapy. However, as the treatment cohort in the South African public sector 'matures' and thus the average duration of treatment increases, a larger proportion of new infections may be acquired from patients failing therapy. Therefore surveillance must be repeated regularly to monitor trends in transmitted resistance prevalence. This will determine whether resistance testing would be required before therapy initiation.

Acquired resistance in children on a first-line PI regimen:

Studying acquired antiretroviral drug resistance allows one to study the effect of therapy choice, prolonged failure, CD4 count and viral load on the emergence and detection of resistance mutations. This provides valuable information on the antiretroviral roll-out programme and can provide evidence on choosing the best therapy and monitoring approach in order to limit resistance after first-line failure, and guide the choice of an appropriate second-line regimen with a probable high response rate.

In the investigation of children treated with a PI-based regimen we found that 12 of 17 patients with virologic failure who had been treated with an un-boosted PI regimen, RTV as single PI, had one or more major PI resistance mutations, compared to only one of 13 patients who exclusively received a boosted PI-based regimen, LPV/r. Furthermore patients with PI resistance had a longer exposure time to RTV as single unboosted PI indicating a 'dose response effect' which further supports the notion that the use of RTV is probably a true contributor to PI resistance. As this study was observational confounding can not be totally eliminated. Factors such as the longer therapy history or the use of rifampicin, although not significantly associated with resistance in our study, could have contributed to a larger number of patients with RTV sPI exposure having resistance.

The use of RTV as single PI was necessitated by the absence of pharmacokinetic data on LPV/r in children under 6 months of age or when LPV/r was co-administered with rifampicin. This resulted in the use of this suboptimal regimen which has a low genetic barrier and was associated with a high prevalence of PI resistance in patients with virologic failure.

Implications for the national antiretroviral roll-out programme: Since data on the appropriate dosage of LPV/r in these populations had become available in 2007 and 2008 respectively, RTV was replaced

with LPV/r. Nevertheless the extensive PI resistance, often combined with NRTI resistance (M184V with or without TAMs), in the RTV sPI-exposed cohort made the choice of an appropriate regimen in many of these patients very difficult.

Acquired resistance in adults on a first-line NNRTI-based regimen

The prevalence of antiretroviral resistance mutations in adult patients on NNRTI-based first-line regimens is informative to clinicians and policy makers. Although randomised controlled trials are ideal to study the effects of particular regimens on mutation prevalence, follow-up is often limited, and therefore not able to detect long-term effects. Furthermore in resource-limited settings patients are often retained for prolonged periods on failing regimens as monitoring for virologic failure may be infrequent and response to failure suboptimal or on purpose conservative. The choice of therapy regimen is also governed by clinical considerations, and it is therefore in some instances difficult to properly randomise the use of different drugs. Lastly HIV-1 subtypes influence the prevalence of particular polymorphisms and resistance associated mutations. The effect of these factors on resistance outcomes can therefore best be studied in an observational or cross-sectional study. Although the prevalence of resistance in many settings, including South Africa, has been well-published, few investigations attempted to study associations with particular resistance outcomes, which could provide guidance to clinicians. In this study we investigated the following resistance outcomes: the presence of NNRTI resistance mutations (and susceptibility to etravirine (ETV) in particular), of M184V/I which confers resistance to lamivudine, of K65R, a mutation usually associated with TDF, DDI or ABC use, but which may be selected by stavudine in HIV-1 subtype C; and of thymidine analogue mutations (TAMs), which are selected by AZT or D4T, but when they accumulate, could confer resistance to most NRTIs. Possible associations with these outcomes which we investigated were: therapy choice, duration of failure and laboratory data (HIV-1 viral load and CD4 count).

Main findings: Of 167 patients with virologic failure on a first-line NNRTI-based regimen, 17% had no resistance, 82% NNRTI resistance, 60% M184V/I, 12% TAMs and 4% K65R. M184V/I was associated with having a low viral load and high CD4 count. A prolonged failure period predicted having ≥ 3 TAMs. NVP use rather than EFV use and prolonged failure on NVP were associated with resistance to ETV.

The limitations of this study were: As a cross-sectional study it suffers from the limitations of any observational study, as not all confounding factors can be excluded. Accurate baseline data were not always available and therefore concurrent laboratory data were included in models. Since we did not have exact dates of failure onset, survival models for the presence or absence of particular mutations could not be used; therefore we opted to use generalised linear models including estimated duration of failure, which we associated with resistance outcomes.

The association of low viral load and high CD4 count with the presence of the M184V mutation is an observation with several possible explanations: This mutation sensitises the viral strain to AZT, D4T and TDF and reduces viral fitness; furthermore patients harbouring M184V are more likely ‘true’ regimen failures (failing despite adequate adherence) than patients who harbour NNRTI mutations only, which usually have little effect on viral fitness and could remain detectable long after self-interruption of therapy or in patients with very poor or intermittent adherence. The relatively low

prevalence of TAMs (12%) compared to settings that do not use viral load monitoring is probably attributable to the early recognition of virological failure in a setting with regular virologic monitoring. This was further supported by the association of having at least 3 TAMs with prolonged failure in our study. Although the prevalence of K65R was associated with the use of D4T in HIV-1 subtype C, only 6 (4%) of the 137 patients who received a regimen that contained D4T had K65R. This corresponds to other studies from South Africa, and although we did not find an association of K65R with prolonged failure, access to frequent viral load monitoring and subsequent response to failure (adherence intensification or regimen switch) may limit the selection of K65R. Data from our study also concurred with other investigations in a non-subtype B settings that associated the use of NVP, rather than EFV, with cross-resistance to etravirine (ETV), with an adjusted odds ratio of 5.17% (95% CI: 2.02-14.85). This is important as patients who had failed NVP, especially those with prolonged failure, may be less likely to respond to ETV when later used in salvage or third-line regimens.
Implications for the national antiretroviral roll-out programme: This investigation highlighted the importance of early detection of failure on the prevention of accumulative resistance, as having at least 3 TAMs and having ETV resistance were associated with prolonged failure. This study was conducted when most patients received D4T therapy as part of their first-line regimen. Since then, TDF has replaced D4T as first choice, and surveillance for the prevalence of acquired resistance in patients receiving the new regimen, especially being a cohort in a predominantly sub-type C region, would be necessary.

Antiretroviral therapy failure in adults treated with a second-line PI-based regimen:
In the investigation of adults who were treated with a second-line LPV/r-based regimen, we found a high prevalence of virological treatment failure (37/93, 40%) but a low prevalence of PI resistance (2/33, 6%) in those with genotypic resistance testing). Furthermore, lopinavir plasma and hair concentrations were helpful to elicit the cause of failure in these patients. The negative predictive values of having a high plasma or hair concentration were 92% and 96% respectively, when using 1000 viral RNA copies/ml as a cut-off for failure. The only one patient who failed despite having high plasma and hair lopinavir concentrations had PI resistance. As lopinavir concentration determination is less costly than genotypic resistance testing, screening patients who fail a high-barrier regimen (such as a boosted PI-based one) for medium term (hair concentration) and recent (plasma level) drug exposure could be cost-saving, as only those who fail despite adequate concentrations would require resistance testing, whereas adherence would have to be addressed first in the patients with low lopinavir concentrations.
Implications for the national antiretroviral roll-out programme: As no third-line options are available in the state sector, second-line PI-based therapy is the last line for adults. An improved understanding of the reasons for the frequently observed failure in these patients is therefore urgently necessary. In this investigation we identified inadequate drug exposure, most likely due to poor adherence, as the most important contributor to failure. Identifying poor adherence first, prior to attempting resistance testing, could be cost-saving in these patients. Nevertheless 6% of the patients with virological failure, and who had resistance testing, had PI resistance and therefore could not be adequately treated with the regimens available in the national programme. Although the prevalence was lower than in children

who had exposure to RTV as single PI, the number of adults who would require third-line options is likely to grow as more patients are switched to second-line and over time fail this ‘last-line’.

Overall limitations of the work:
The publications were all based on cross-sectional studies and thus suffer from the potential confounding effects or biases typical for this design. Randomised controlled studies of resistance outcomes of various therapy regimens would not suffer from these biases but would be more expensive and, for most of our observations, unachievable. For example a study of the impact of ritonavir as single PI versus the use of lopinavir boosted with ritonavir on resistance would not be ethically justifiable, as a boosted PI was the preferred regimen component, based on the higher known genetic barrier in other populations (adult patients), and ritonavir as single PI was only used in cases where the correct dosing for LPV/r was not known, due to lack of pharmacokinetic data. Nevertheless as real-life settings were studied, factors such as delayed response to failure and therefore increased time on a failing regimen could be studied. Another limitation arising from using cross-sectional data was that we could not accurately model time-trends and used an estimated failure time and concurrent patient characteristics as determinants of mutation risk rather than using survival models (which one could have done with a more expensive cohort study). Likewise, for comparing the risk of NVP resistance in mothers that received either NVP-only or a combination of AZT and NVP for PMTCT, a head-to-head comparison in a randomised controlled trial would have been more statistically sound. However, at the time of this study, available evidence of the better efficacy of the combined regimen in preventing transmission would have made such a trial for the purpose of studying a reduction in resistance risk unethical.

Conclusion:
Despite the limitations of cross-sectional data, these investigations were helpful in studying real-life associations of therapy choice on resistance emergence and prevalence. Surveillance of transmitted resistance was also informative and should be repeated on a regular basis. The investigations also highlighted the considerable differences in resistance patterns between paediatric and adult patients, probably not only as a result of the different regimens used, but also due to other factors such as care-giver adherence, poorly palatable liquid formulations and the different viral replication kinetics in children, who have much higher baseline viral loads, putting them at an increased risk of resistance evolution.

Therapy choice could also influence the resistance profile: Use of nevirapine (NVP) in combination antiretroviral regimens is associated with a higher risk of cross-resistance to etravirine than use of efavirenz. When used in PMTCT regimens, the addition of AZT to NVP reduced the risk of NVP resistance. In children previous exposure to suboptimal regimens is a major contributor to resistance. These investigations also confirmed that clinician behaviour is an important factor in determining resistance mutation prevalence. The accumulation of thymidine analogue mutations and NNRTI mutations when a patient is retained on a failing regimen could limit future therapy choices, emphasising the importance of ongoing virological monitoring and appropriate response to treatment failure. According to the current South African guidelines, the frequency of viral load monitoring should

be 6-monthy in the first year and thereafter only annually. This is a change from the previous guideline that allowed for 6-montly testing. In many patients annual testing is probably a best case scenario as phlebotomy is often omitted in very busy clinics. Infrequent monitoring, combined with a delayed response to failure which is a feature of overextended clinical services, could therefore result in resistance accumulation in patients. Lastly, the investigation of adults who failed a second-line PI-based regimen also explored the use of a new innovative way to monitor lopinavir drug exposure (and indirectly adherence), through hair concentration determination, a method that can be employed to select patients with adequate drug exposure; these patients are more likely to be failing due to resistance and should receive genotypic resistance testing, while adherence should be addressed in the others.

In conclusion, antiretroviral resistance testing, for individual patient management and for programmatic surveillance, is proving to be invaluable and is likely to become more so with the maturing HIV epidemic in South Africa. Nevertheless the roll-out of antiretroviral therapy in the region faces huge challenges, especially in sustaining funding for patient management and laboratory testing. Furthermore there is no provision for antiretroviral resistance testing in the roll-out budget and no guideline for the therapy of patients who cannot be adequately treated with either of two regimens, such as patients with triple class resistance. Access to third-line or salvage regimens is therefore likely to become a priority in the near future. Based on the findings presented in this dissertation and the necessity to rationalise antiretroviral resistance testing, I would suggest the following the flow diagram for resistance surveillance in a resource-limited setting (Figure 1).

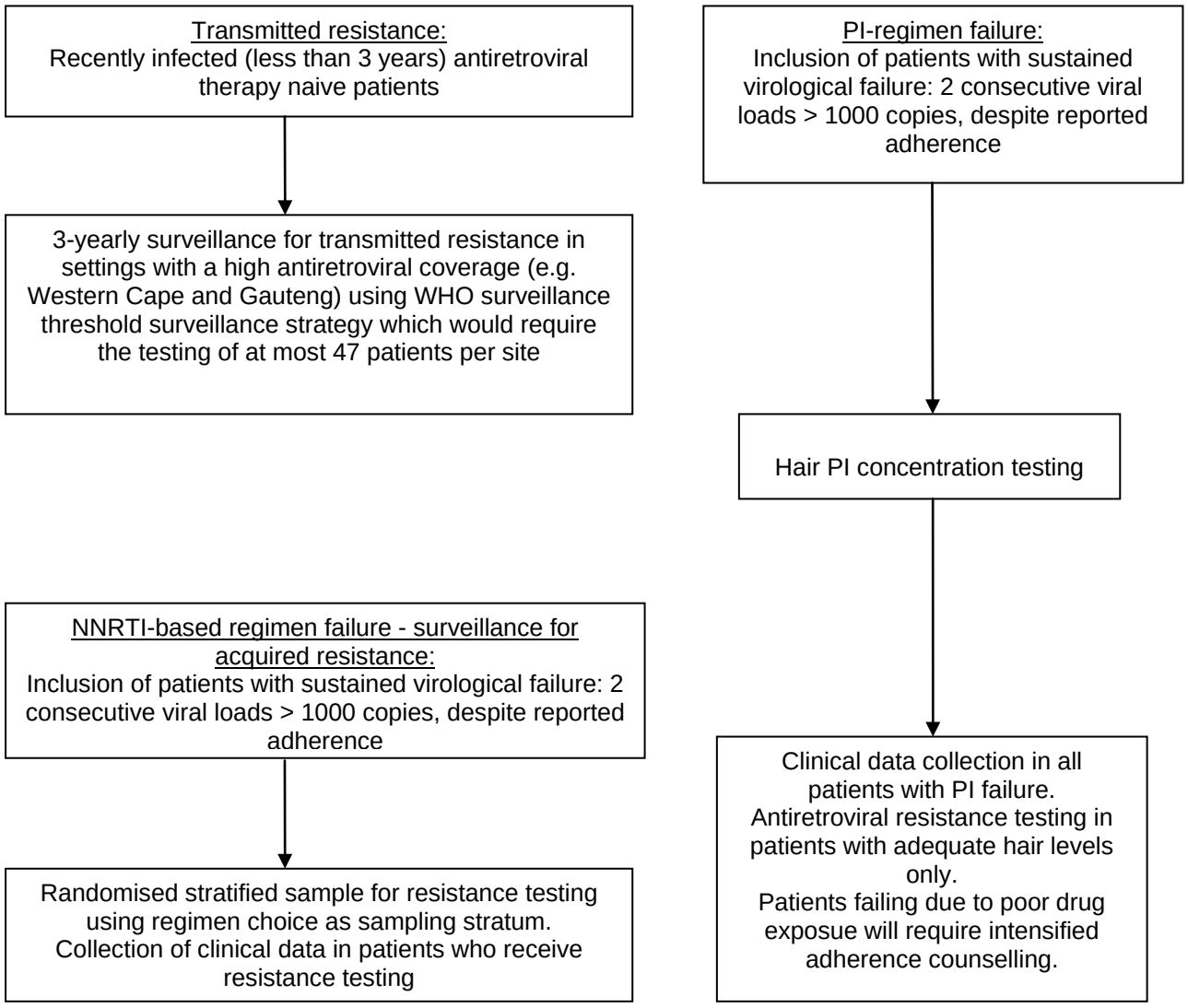


Figure 1: Suggested flow diagram for antiretroviral resistance testing for a resource-limited setting

The flow diagram (Figure 1) suggests a targeted approach to antiretroviral resistance surveillance for resource-limited settings. For transmitted resistance surveillance it suggests the WHO surveillance strategy. For patients, who had failure of a low-barrier NNRTI-based regimen, especially when used as first-line therapy, response to a PI-based regimen is likely, and resistance testing at the time of failure is therefore not absolutely necessary for clinical management. However surveillance is needed to compare the resistance outcomes of different regimens (e.g. different NRTI or NNRTI components) in order to describe common patterns of resistance after NNRT-based regimen failure. In case of PI-based regimen failure (which is the second and last line in adults or older children) it suggests screening for low PI concentrations in hair to exclude patients who are non-adherent from resistance testing, with only those patients with high PI levels and persisting failure qualifying for resistance testing. The overall purpose of this strategy is to monitor the roll-out program for the prevalence of

resistance mutations and the clinical associations with having particular resistance patterns. This has the potential to identify particular regimens or monitoring approaches which could result in a high likelihood of resistance, and to influence the choice of therapy regimens. One needs to emphasize the importance of regular viral load monitoring and rapid response to virologic failure.
To conclude, antiretroviral drug resistance is an important therapy outcome and the prevention of resistance should be considered in the formulation of guidelines and therapy policy.

Future research:
Since these publications appeared, the NRTI component, stavudine, has been replaced in adults by TDF and in children by ABC. As limited data on the prevalence of resistance in patients on these 'new' regimens are available, continued surveillance for acquired resistance in these patients is necessary. Similarly as the roll-out programme matures, continued surveillance for transmitted resistance in adults and children will be required. With reference to resistance testing methods, research in this field is ongoing to develop and evaluate new methods to detect resistance mutations. One promising method is the use of an allele-specific assays employing loop-mediated isothermal amplification (LAMP). We are also studying the use of deep sequencing methods for pooled antiretroviral resistance testing. Deep sequencing is currently expensive but, with optimal pooling and increased automation, it may in the near future become an affordable alternative to standard genotyping.

Abbreviations used

Abbreviation	Full term
ABC	Abacavir
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
ARVDR	Antiretroviral drug resistance
AZT	Azido thymidine/ Zidovudine
cART	Combination antiretroviral therapy
CI	Confidence interval
DDC	Zalcitabine
DDI	Didanosine
DR	Drug resistance
D4T	Stavudine
DNA	Deoxiribonucleic acid
EFV	Efavirenz
EFT	Estimated failure time
ETV	Etravirine
GART	Genotypic antiretroviral resistance testing
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
LPV	Lopinavir
LPV/r	Lopinavir boosted with low dose ritonavir
MPIRM	Major protease inhibitor resistance mutations
MSM	Men-who-have-sex-with-men
NHLS	National Health Laboratory Service (South Africa)
NVP	Nevirapine
NNRTI	Non nucleoside reverse transcriptase inhibitor
NRTI	Nucleos(t)ide reverse transcriptase inhibitor
NA	Not available
NS	Not significant
NTP	Nucleos(t)ide triphosphates
OR	Odds ratio
PCR	Polymerase chain reaction
PEPFAR	President's emergency plan for AIDS Relief
PI	Protease inhibitor
PMTCT	Prevention of mother to child transmission of HIV
PT	Phenotypic testing
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
RTV	Ritonavir
RTV sPI	Ritonavir as single protease inhibitor
RTI	Reverse transcriptase inhibitor
SDRM	Surveillance drug resistance mutations
TAM	Thymidine associated mutation
TAMs	Thymidine associated mutations
TB	Tuberculosis (mycobacterium tuberculosis infection)
TDF	Tenofovir
TDR	Transmitted drug resistance
VL	Viral load (with reference to HIV-1 RNA load)
WHO	World Health Organisation
3TC	Lamivudine

Addendum: Abstract

van Zyl, G., E. Braaf, and W. Preiser. 2011. Surveillance of transmitted drug resistance in HIV-infected adults in the Western Cape province, South Africa, 6th IAS Conference on HIV Pathogenesis, Treatment and Prevention, Rome, Italy, 17-20 July 2011.

About this abstract:

Background:

Surveillance for transmitted drug resistance is important for national therapy guidelines and testing policies, as an increase in the prevalence of transmitted resistance would signify the need to do baseline resistance testing at the time of starting antiretroviral combination therapy (or more ideally at first presentation of an HIV-positive individual to care). With transmitted surveillance one attempts to include patients in the first 3 years after infection, as in these recently infected patients one is most likely to detect resistant variants which may later wane due to reversion to wild-type virus, due to back-mutations and increased fitness of wild-type virus. For this purpose the WHO developed criteria that act as proxies for recent infection. In this study we used the following proxies derived from the WHO criteria: young age 15-20 years, high CD4 count (above 400) and absence of disease. We also employed the sequential binomial testing strategy originally developed for 'lot quality assurance'. This strategy does not aim to determine an exact point prevalence but aims to categorise prevalence as either low (<5%), intermediate (5-15%) or high (>15%). An advantage of this strategy is the cost-effectiveness of sequential testing allowing the use of stopping rules which enable one to stop testing as soon as a threshold number of negative or positive tests (harbouring or not harbouring resistance) is reached. In order not to include polymorphic loci, which may be HIV-1 subtype associated, we made use of the WHO surveillance drug resistance mutations list (SDRM).

Limitations of this investigation:

In an ideal setting, with frequent voluntary testing of patients, accurate estimates of the date of infection would be possible, obviating the need to use proxies for recent infection. Furthermore although we attempted to test patients during early infection, some resistant variants already may have waned and become undetectable by bulk sequencing.

Main findings and conclusion:

Although we detected T74S, an HIV-1 subtype C polymorphism associated with resistance to nelfinavir, we did not detect any SDRM mutations in 38 specimens tested (which are more than the stopping criterion of 34 tests). Therefore antiretroviral resistance prevalence can be classified as low (<5%). However as the HIV epidemic and the South African antiretroviral treatment programme mature, ongoing vigilance is required and there is a need to regularly repeat transmitted drug resistance surveillance.

6th IAS Conference on HIV Pathogenesis, Category: B10 Viral resistance testing in clinical trials and practice

van Zyl, G., E. Braaf, and W. Preiser. 2011. Surveillance of transmitted drug resistance in HIV-infected adults in the Western Cape province, South Africa, 6th IAS Conference on HIV Pathogenesis, Treatment and Prevention, Rome, Italy, 17-20 July 2011.

Introduction: Transmitted HIV drug resistance (TDR) can compromise initial antiretroviral therapy (ART), resulting in early therapy failure despite good adherence. In a population with a high prevalence of TDR, early or baseline resistance testing may be necessary to determine an optimal, individual ART regimen, which is not feasible in resource-constrained settings. We evaluated the prevalence of TDR in recently HIV-infected adults in the Western Cape province, South Africa, using the World Health Organization's threshold surveillance method.
Methods: We included specimens sent consecutively for CD4 counts to the Tygerberg laboratory if they fulfilled the following criteria: patient 15 - 20 years old; CD4 count >500/µl; not on ART according to request form. After anonymisation, population sequencing was performed. Sequences were interpreted using the calibrated population resistance (CPR) tool of the Stanford University database according to the updated WHO surveillance drug resistance mutation (SDRM) list.
Results: Specimens from 49 females and 1 male, median age 19 (range 15-20) years, median CD4 count 655/µl (range 505 - 2569) were included, of which 38 (76%) were successfully amplified and sequenced. The survey was discontinued thereafter based on the absence of SDRM list mutations; using the WHO threshold analysis classification, this result predicts a low (< 5%) prevalence of TDR to all three drug classes in this population. The T74S resistance associated polymorphism was detected in two samples. This polymorphism is frequently observed in HIV-1 subtype C and together with other primary resistance mutations causes resistance to certain protease inhibitors.
Conclusion: According to the WHO SDRM list no TDR was detected in this survey, suggesting a low prevalence (< 5%) of TDR in the Western Cape Province four years after the public-service ART roll-out programme was started. While this is encouraging, ongoing vigilance is required to ensure the continued success of the programme.