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# "Descriptive study of HIV drug resistance genotype testing in a public sector paediatric population in Johannesburg"

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# “Descriptive study of HIV drug resistance genotype testing in a public sector paediatric population in Johannesburg”

**Phocas NGABIRE**

**397339**

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of

Master of Medicine in the field of Paediatrics.

Johannesburg, 2015

# DECLARATION

I, Phocas NGABIRE, declare that this dissertation is my own work. It is being submitted for the degree of Master of Medicine in the field of paediatric, University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.



Johannesburg

March 31st, 2015

# DEDICATION

To my family.

Despite the distance,

Your continuous support was unvaluable through this journey,

With much love and admiration.

# PRESENTATIONS ARISING FROM THIS STUDY

1. **Ngabire P.,** Technau K., Wallis L.C. HIV Drug resistance genotype testing in a Public sector paediatric population in Johannesburg. Poster presentation. Southern African HIV clinician society 2nd biennial conference, Cape town, South Africa, 24-27th September 2014.

# ABSTRACT

**Context**

The introduction of combined antiretroviral treatment (cART) reduced HIV related mortality more than 70% and the rate of new infection in children continue to decrease considerably. However this benefit is threatened by the emergence of drug resistant strains of HIV. Studies exploring the patterns of drug resistance in the paediatric population are crucial for policy makers and for individual patients' management. In sub-Saharan Africa where HIV-1 subtype C is more prevalent, there is a limited number of paediatric studies exploring the drug resistance patterns. To get more insight on this problem, we explored the drug resistance mutations (DRMs) patterns in a paediatric population attending a referral public paediatric HIV clinic.

**Methodology**

The study was a cross-sectional retrospective descriptive study. Convenience sampling method was used and all paediatric patients (0-14 years) who underwent genotypic HIV drug resistance testing at Empilweni Clinic between January 1st, 2004 and February 28th, 2012 were included. Demographic and clinical data were collected from the clinical electronic database and DRM frequencies related to treatment exposure were presented.

**Results**

During our study period, 63 patient samples were sent for HIV genotyping drug resistance testing. Eleven samples did not meet the inclusion criteria. Among the 52 patient samples retained, 44 patients (84.6%) had a successful HIV amplification and all were infected with HIV-1 subtype C. Ninety one percent (n=40) of the patients had at least one DRM isolated but in only 78% (n=34) did these mutations translate into genotypic drug resistance to at least one antiretroviral drug (ARV) used in South Africa. Nucleotide reverse transcriptase inhibitors (NRTI) mutations were the most commonly identified with M184V being the most prevalent (64.4%; n=29). This was associated with thymidine analogue mutations (TAMs) in 36.3% of the patients (n=16). TAMs were identified in 25% (n=11) of the patients. K65R and Q151M were rarely identified in our cohort. V106M and K103N were the most common non-nucleotide reverse transcriptase inhibitors (NNRTI) mutations and were both identified in 21.9% (n=7) of the patients exposed to NNRTI-based regimen. V82A was the most commonly identified protease gene (PR) mutation in 29.3% (n=12) of the cases.

Forty eight percent of the patients (n=21) had a dual class resistance and 11.4% (n=5) had resistance to ARVs from all the three classes. Over a quarter (27.2%, n=12) of the patients in our cohort were still sensitive to all ARVs used in South Africa. The development of drug resistance was not associated with any clinical characteristic in our cohort.

## Conclusion

The drug resistance mutations identified in paediatric patients failing cART show a complex pattern with some failing patients still sensitive to all ARVs while others harbour complex resistance mutations. Therefore, regular counselling to optimize adherence and regular viral load monitoring for early detection of failure may be important tools for continued cART success. Given the complexity of the DRMs patterns in paediatric patients, the HIV drug resistance test is warranted to guide the choice of appropriate cART regimens.

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# NOMENCLATURE

AIDS: Acquired Immune Deficiency Syndrome

ANRS: Agence Nationale de la Recherche sur le SIDA

ARV: Antiretroviral drug

cART: Combined Antiretroviral Therapy

CD4: Cluster of Differentiation 4

CEO: Chief Executive Officer

CMMT: Comprehensive Plan for the Care, Management and Treatment

CRF: Circulating Recombinant Form

DNA: Deoxyribonucleic Acid

DRM: Drug Resistance Mutation

HIV: Human Immune-deficiency Virus

HIVdb: HIV drug resistance database

IAS-USA: International AIDS society of United States of America

ID: Identification number

IQR: Interquartile Range

KZN: KwaZulu-Natal Province

µl: Microlitre

N: Number

NNRTI: Non-Nucleoside reverse transcriptase inhibitor

NRTI: Nucleoside reverse transcriptase inhibitor

PI: Protease Inhibitor

PMTCT: Prevention of Mother-to-Child Transmission

PR: Protease gene

RMMCH: Rahima Moosa Mother and Child Hospital

RNA: Ribonucleic Acid

TAM: Thymidine Analogue Mutation

TAM1: Thymidine Analogue Mutation pathway 1

TAM2: Thymidine Analogue Mutation pathway 2

TB: Tuberculosis

VL: Viral Load

WHO: World Health Organisation

# CHAPTER 1. INTRODUCTION

## 1.1. Background

Human immunodeficiency virus (HIV) infection is a major public health concern worldwide. It is one of the leading causes of child mortality in the world. In 2010, 4.0% of the total number of child deaths in Africa were due to HIV infection, a rate four times higher compared to Europe and North America (1). South Africa has the highest HIV burden in the world, with an estimated 14.0% of all infected children living with HIV in the world residing there (2). Furthermore, in 2010, it was estimated that HIV contributed around 40.0% of mortality among South African children aged between 1-59 months (2, 3).

Large-scale efforts have been made to stop this epidemic and it has been placed among the top world health priorities with target to virtually stop the epidemic spread by 2015. Nevertheless, South Africa still experience many new cases of HIV infection in children (4). One of the most successful interventions towards this goal was the initiation of antiretroviral drugs (ARVs) for prevention and treatment of HIV. The combinations of three ARVs, often referred to as combined antiretroviral therapy (cART), has drastically altered Acquired Immune Deficiency Syndrome (AIDS) from an almost uniformly fatal disease to a chronic manageable one (5).

To date, there are over 20 ARVs available to treat HIV infection. They fall into six categories inhibiting different viral enzymes and different steps in the viral lifecycle (6, 7).
Table 1 summarizes the main classes of ARVs, their mechanisms of action and the most commonly selected mutations associated with HIV drug resistance.

In South Africa, the Comprehensive Plan for the Care, Management and Treatment (CCMT) of HIV and AIDS, was initiated in April 2004 with the objective to progressively achieve universal antiretroviral treatment access (8).

As cART was initiated, it dramatically modified the course of HIV infection in children, reducing mortality five-fold or more and resulting in high survival rates into adulthood (9). cART have been shown to reduce HIV related mortality by more than 70% both in developed countries and in resource-limited settings (10, 11). Without treatment one third of HIV-infected children die in their first year of life and 50.0% before their second birthday (12). Furthermore, cART delays the emergence of drug resistant HIV strains, which accumulated rapidly in the pre-cART era when monotherapy was still in use, but challenges remain (13).

The roll-out of cART in resource-limited countries has been identified as a global public health priority. With the collaboration of various international organizations, governments of developing countries managed to scale-up cART access but have not yet reached universal access as the treatment coverage for children is only 34.0% (95% CI: 31-39%) (14).

However, increasing cART coverage in resource-limited settings also brings with it the risk of emerging HIV drug resistance potentially compromising future treatment options (15). Amongst the paediatric population with access to cART, the resistant virus can be acquired from inadequate adherence or from use of Prevention of Mother-to-Child Transmission (PMTCT) regimens (acquired HIV drug resistance) (16) or transmitted to newly infected individuals (transmitted HIV drug resistance) when a patient is infected by an HIV-1 strain already resistant to ARVs (17, 18). One of the consequences of cART scale-up is treatment failure that selects for drug resistant HIV-1 (15). This is more common in the paediatric population due to various adherence challenges (19, 20).

**Table 1: Major classes of ARVs and commonly selected mutations** (6, 7, 21).

| Drug class | Mode of action | Example | | Common mutations selected by the drug |
|---|---|---|---|---|
| | | Generic name | Abbre-viation | |
| Nucleoside reverse transcriptase inhibitor (NRTI) | Deoxyribonucleic acid (DNA) chain terminators and inhibit reverse transcription | Abacavir | ABC | K65R, L74V |
| | | Didanosine | ddI | K65R, L74V |
| | | Emtricitabine | FTC | M184V |
| | | Lamivudine | 3TC | M184V |
| | | Stavudine | d4T | TAMs (41L, 67N, 70R, 210W, 215Y/F, 219Q/E), K65R |
| | | Tenofovir | TDF | K65R, K70E |
| | | Zidovudine | AZT | TAMs (41L, 67N, 70R, 210W, 215Y/F, 219Q/E) |
| Non-nucleoside reverse transcriptase inhibitors (NNRTI) | Bind to the hydrophobic pocket inhibiting the reverse transcriptase enzyme | Efavirenz | EFV | K103N , V106M |
| | | Etravirine | ETR | L100I, K101E/P, Y181C |
| | | Nevirapine | NVP | Y181C , K103N, V106M |
| | | Rilpivirine | RPV | K101E/P, E138K, Y181C, Y188L, M230L |
| Protease inhibitors (PI) | Target the viral enzyme required for cleavage of viral precursors and final assembly of viral particles | Atazanavir/ Ritonavir | ATZ/r | I50L, I84V, N88S |
| | | Darunavir/ Ritonavir | DRV/r | I47V, I50V, I54M, L76V, I84V |
| | | Lopinavir/ Ritonavir | LPV/r | V32I, I47V, L76V, V82A/F/T/S |
| | | Indinavir/ Ritonavir | IDV/r | M46I, V82A/F/T/S, I84V |
| | | Nelfinavir/ Ritonavir | NFV/r | D30N, L90M |
| Integrase inhibitor (IN) | Inhibit the attachment of pro-viral DNA to host-cell genome | Raltegravir | RAL | G140S, Q148H, N155H |
| Fusion/Entry inhibitor (FI)* | Block the fusion of the viral particle to their target cells | Enfuvirtide | T-20 | G36S/V, 38M, Q40H |
| Co-receptor inhibitors (CRI) | Interfere with the entry of HIV in the target cell | Aplaviroc | APL | R305K, Q315R, T319K, P363S, A373T, N413T, S437P, and T467I. |
| | | Maraviroc | MVC | |

*Not yet registered in South Africa (22).

In developed countries, the rate of cART failure in children and adolescents is estimated to be twice that observed in adults, with risk increasing with duration of therapy and with entry into adolescence (23).

In developing countries it has been shown that the prevalence of transmitted drug resistance has increased progressively since the roll-out of cART (24). The overall prevalence of drug resistance was estimated to increase at 29.0% per year in east Africa where the cART roll out programme started earlier and 14.0% per year in southern Africa. This increase in prevalence was directly linked to cART coverage (14, 15).

Furthermore, it has been shown that children, highly vulnerable to HIV infection, are at higher risk of developing drug resistance and the rate is higher compared to adults (25). This is a major concern as this population is expected to be on lifelong cART from early infancy. Currently, in resource-limited settings, there are few treatment options while in developed countries it is possible to tailor cART regimens more individually and prescribe fully active cART even for multi-resistant HIV (26, 27).

In developed countries the prevalence of HIV drug resistance seems to be stabilizing over time mainly due to continuous and timely VL monitoring added to individualized cART from pre-treatment HIV drug resistance testing (28). Pre-treatment HIV drug resistance testing is not feasible in resource-limited settings at present due to the high cost. As expected, in developing countries, cases of drug resistance are more likely to increase progressively as the cART coverage and the duration on cART increases (15, 24).

HIV drug resistance has been extensively explored in adult populations in developed countries. However, data on paediatric HIV drug resistance is still scarce, especially in resource-limited settings like sub-Saharan Africa even though it is home to more than 90.0% of children living with HIV (14, 29). More studies from developing countries are needed to elucidate the problem.

## 1.2. Literature review

### 1.2.1. Development of HIV drug resistance

Due to the constantly changing HIV genome, the virus has the ability to change its genetic code, accumulating mutations which may confer a decreased susceptibility to the ARVs. However, all the mutations do not results in viable viruses. Some mutations arising in HIV genome are lethal to the virus itself and others are only polymorphisms which don't cause resistance to ARVs by themselves but may act in concert with other mutations to enhance resistance (30).

HIV is said to have developed 'drug resistance' as it progressively evades the effects of these medication on its replication abilities compared to wild type HIV. Drug resistance is a continuum and it varies from low level to high level drug resistance (30)

The development of drug resistance may be due to different factors, these include viral, drug and host related factors.

### 1.2.2. Factors contributing to HIV drug resistance

#### 1.2.2.1. HIV biology

HIV has been classified into two types: HIV-1 and HIV-2. HIV-1, which is the most common pathogenic strain, is divided into group M (major) and three minor groups N (non-M, Non-O), O (Outlier) and P (Pending identification of further human cases) which was recently discovered. Group P has so far been isolated from only one Cameroonian woman (31, 32). Due to substantial natural genetic variation, HIV-1 group M have been further sub-classified into nine subtypes (A through D, F through H,J and K) and numerous circulating recombinant forms (CRFs) (32).

Although subtype B is most prevalent in North America and Europe, non-B subtypes predominate elsewhere with subtype C infection dominant in Southern Africa (32). Various studies have shown that cART benefits patients irrespective of the viral subtype, but subtypes C and D may be more virulent (33, 34).

Different HIV subtypes are prone to encode different amino acids substitution due to differences in codon sequences at positions associated with drug-resistance mutations. This difference might affect not only the rate of emergence of resistance mutations but also the cross-resistance to ARV's within the same class, potentially affecting antiviral responsiveness and clinical outcomes (35, 36). For example, group O HIV-1 and HIV-2 viruses manifest high-level innate resistance to some NNRTIs as a result of mutations in the reverse transcriptase gene that are present as natural polymorphisms (37-39).

Furthermore, even though resistance mutations are comparable in different viral subtypes, it has been shown that different subtypes develop certain drug resistance mutations more readily than others and this might be through different mutation pathways. HIV-1 subtype C, which is more common in sub-Saharan Africa, is more prone to develop V106M and K65R mutations compared to subtype B common in Europe and North America (40, 41).

In addition, HIV has a short half-life. In order to keep the balance with its clearance it has a very high replication rate which is error prone as a result of the poor proof-reading capability of the HIV-1 reverse transcriptase enzyme during replication. This results in the introduction of either polymorphisms or mutations potentially reducing the efficacy of ARVs. The mutation rate averages $3 \times 10^5$ mutations/base/replication cycle (30, 42, 43).

This implies that circulating viruses are not represented by a unique virus genotype, but by numerous different genetically related viral variants referred to as "quasispecies" as initially designated by Eigen in 1993 (44). Quasispecies in a patient diversify progressively over time (45) which results in a progressive expansion of the complexity of HIV resistant variants in untreated patients (46). The mutation that develops can be either specific to one ARV or lead to reduced susceptibility to a number of ARVs (cross resistance) or increased susceptibility to certain ARVs (27). For example, the presence of K65R mutation confers resistance to TDF, as well as cross-resistance to ABC and ddI (Table 1). By contrast, the presence of M184V which causes resistance to 3TC (Table 1), enhances susceptibility to AZT, d4T and TDF (21).

### 1.2.2.2. Genetic barrier and HIV drug resistance

HIV can develop high level resistance to some ARVs with a single mutation while other drugs require accumulation of multiple mutations. Each ARV drug has a different genetic barrier, i.e. threshold number of mutations required to cause clinically significant drug resistance (47). For example NRTI (3TC and FTC) and NNRTI (NVP and EFV) require one resistance mutation to acquire high level resistance therefore are considered as having low genetic barrier. The PIs and other NRTIs, such as AZT require accumulation of multiple drug resistance mutations to achieve high level phenotypic resistance and they are considered therefore as having high genetic barrier (30, 47). However it is known that some mutations or combination of mutations can have greater effect than others (48), thus understanding the genetic barrier goes beyond the simple counting of DRMs and involve the determination of the effect of interaction of different mutations on HIV drug susceptibility (47).

### 1.2.2.3. Drug potency, Adherence and resistance

Adherence is defined as the extent to which the patients' health related behaviour corresponds with the medical advice (49). Nearly perfect adherence to cART is the key factor to gain the intended health benefit. Suboptimal adherence or partial adherence results in insufficient bioavailability of the ARV to ensure complete suppression of the virus and therefore result in the development of HIV drug resistance and virological failure in patients on cART (50). Some drug resistant variants are thought to exist at very low levels before drug initiation from natural viral recombination (51, 52). If the patient receives an inadequate drug regimen or is poorly adherent to a correct regimen, the resistant HIV variants emerge, continue replication, accumulate resistance mutations and eventually cause treatment failure (53).

In the absence of ongoing drug selection pressure, reversion to wild-type HIV which has higher replication capacity occurs. However, various studies confirmed the persistence of drug-resistant virus both in long-lived cellular reservoirs (54) and in blood plasma as minority species several months after treatment discontinuation (55, 56). Under drug selection pressure, this minority resistant virus can evolve later to cause significant pathogenicity (57). For this reason, the interruption of ineffective treatment as a strategy to permit the re-emergence of wild-type virus has been proven ineffective to address drug resistance issue (58).

Adherence is an even bigger challenge in children, a dynamic population at different developmental stages, and dependant on care givers for medications. Poor availability of adequate drug formulation for younger children may result in inadequate drug levels and HIV drug resistance (59, 60). Furthermore, due to the developing metabolic pathways and rapid growth, paediatric patients are more likely to develop drug resistance from under dosing. Frequent dose adjustment are required to address this issue which is not always done correctly by all health care providers. Other factors include the premature release of responsibility to older children before they are developmentally ready to take over their own medication administration and psychological factors like denial, anger and depression in adolescents (19). In addition, programmatic factors such as insufficient or inadequately trained health care personnel and weak supply systems impair adherence and therefore must be addressed to attain optimal adherence (61).

### 1.2.3. HIV drug resistance test and clinical significance

The immediate consequence of ARV resistance is reduction of treatment efficacy (27). The emergence of resistance to one ARV often leads to cross-resistance to other ARVs of the same class. This results from the similarities of the molecular structure within compounds of the same antiretroviral class and their interaction with similar target sites.

The reduction of therapeutic options which results from this cross-resistance lead to the prescription of more complex, expensive and often poorly tolerated regimens (27). This represents a major challenge especially in resource-limited settings where only few alternatives are available in the public sector and access to third-line agents may require significant time and resources.

The drug resistance mutations can have multiple effects on ARVs. Some drug resistance mutations confer increased susceptibility to ARVs (e.g: K65R increase susceptibility to AZT), can cause decreased viral replication abilities (e.g: M184V) (62) or are associated with a decreased incidence of other mutations like in case of M184V which is associated with low incidence of TAMs (63, 64). Due to the this decrease in viral fitness, drugs like 3TC and FTC which select for the M184V mutation continue to provide some level of virological benefit even in case of high level resistance (65).

### 1.2.4. HIV drug resistance testing

The testing of HIV drug resistance has become an integral part in HIV management in developed countries and is still not readily available in resource-limited countries (22, 66). Resistance assays can be grouped into two categories: Phenotypic and genotypic assays (67).

Phenotypic assays measure the *in vitro* ARV susceptibility of HIV strains in cell culture (68). Susceptibility to each ARV is reported as fold-changes in drug inhibiting concentrations as measured by comparing the mean inhibiting concentrations at which 50.0% replication of the testing HIV strain is suppressed (IC50) relative to that of a reference HXB2 HIV strain. This method, although considered the gold standard, has several limitations; it is time consuming, costly, only be performed in a laboratory with high safety level (P3) and requires highly skilled staff. It is therefore, not readily available in many (69).

Genotypic assays determine the presence of mutations that are known to confer decreased drug susceptibility. The viral RNA is extracted from plasma and reverse transcribed. The gene of interest, mainly the *pol* gene, is amplified and sequenced. The sequence is compared to the HXB2 sequence and differences are identified. These differences are analyzed for their level of resistance. It only uses the resistance mutations which are already known but the results have been shown to be comparable to those generated by the phenotypic resistance test. Genotypic assays are faster, require less skill, are superior at detecting evolving resistance and are more affordable. For this reason the genotypic test is the commonly used test in clinical settings (69).

Although the assay technology is improving, both types of HIV drug resistance tests still face some challenges for detection of clinically significant minority species particularly in patients who were previously heavily treated and who have changed therapy (52, 69).

### 1.2.5. Interpretation of genotypic drug resistance

Interpreting results of HIV-1 genotypic resistance tests is one of the most difficult tasks facing HIV clinicians. There are many different HIV-1 drug resistance mutations which may occur in complex patterns and may interact to cause varying levels of HIV-1 drug resistance to ARVs from each of the ARV classes.

The presence of some mutations induce decreases in drug susceptibility and are referred to as "primary drug resistance mutation" while other mutations impact the drug susceptibility by enhancing the viral fitness of the virus harboring a primary mutation. These are termed "secondary drug resistance mutations" and they do not cause a decreases in drug susceptibility by themselves. Multiple DRMs may occur in a single sample and their interaction may result in an antagonistic or synergistic effect on drug susceptibility (48, 69).

Given the complexity of these mutations, an interpretation system is necessary to help the clinicians. For this reason, several algorithms for the interpretation of HIV-1 genotypic drug resistance information have been designed (48).

Most clinicians ordering HIV drug resistance tests use the publicly available web-based academic interpretation systems. There are more than ten academic systems for genotypic interpretation but only three of them are commonly used by clinicians: the Agence Nationale de Recherche sur le SIDA (ANRS) available on www.hivfrenchresistance.org, the Rega institute system from the Catholic University of Leuven available on https://rega.kuleuven.be/cev/regadb and the HIV drug resistance data base (HIV db) system from Stanford University available on http://hivdb.stanford.edu (70).

All these interpretation systems are designed for clinical use and interpret HIV resistance using rules-based algorithms where each rule is conditioned upon the presence of certain mutations and assigns a level of inferred resistance to certain drugs. The rules used in these algorithms are frequently updated from HIV drug resistance literature and clinical data (70).

The ANRS and Rega Institute systems are rules-based systems that report three levels of resistance: susceptible, intermediate, and resistant. Both systems contain interpretations for all available antiretroviral drugs. They are frequently updated and widely accessible (70).

The HIVdb system, also using a rules-based algorithm, is the most widely used and freely available online allowing clinicians and laboratories to interpret the HIV-1 resistance mutation test. The HIVdb internet interface (http://hivdb.stanford.edu/DR/webservices) accepts either nucleic acid sequences or lists of mutations. The sequence analysis form allows users to paste one or more HIV-1 protease, RT and/or integrase sequences into a text box or to upload a text file containing the same. The mutation list form allows users to type in lists of RT, protease, and/or integrase mutations or to select ARV resistance mutations from a drop-down menu (71).

After uploading the sequence or the list of mutations, the output consists of:

- ✓ A list of penalty scores for each resistance mutation in a submitted sequence,
- ✓ Estimates of decreased susceptibility per ARVs class, and
- ✓ The comments about each resistance mutation in the submitted sequence.

The level of resistance are then determined based on the database algorithms. The HIVdb has 5 levels of resistances: susceptible, potential low level resistance, low level resistance, intermediate resistance and high level resistance (71).

### 1.2.6. Benefits of HIV genotypic susceptibility testing

It was demonstrated that the use of genotypic susceptibility testing in patients failing therapy leads to better viral suppression and improved survival compared to standard of care (72-74). In developed countries, HIV drug resistance testing has become an important clinical tool in the management of patients failing cART (66). In South Africa, drug resistance testing is not widely available in public settings. Other parameters such as viral load and CD4 cell count are monitored instead, to inform clinicians about patient compliance and treatment response.
One major consequence is that children failing first-line regimen may be kept on the failing regimen or switched blindly onto more costly regimens with high side effect profiles with no real evidence of resistance or enough knowledge as to what the optimal new regimen should consist of. This decreases the likelihood of favorable treatment response in paediatric patients and could potentially compromise patient care (74).

## 1.3. Study rationale

To date, although more than 90.0% of patients with HIV-1 infection worldwide have non-subtype B variants of HIV-1, there is limited knowledge of drug resistance mutations in non-B subtypes of HIV-1 and their clinical relevance (30).
Drug resistance mutation data is scarcer in children especially in resource-limited settings (29). To my knowledge few studies have been performed to explore HIV drug resistance patterns and consequences for future treatment regimens in the paediatric population in South Africa.

This study analyzed genotypic HIV drug resistance data from tests performed on selected patients at Empilweni Clinic, a paediatric HIV clinic in Johannesburg, with the aim of describing the HIV drug resistance profiles of patients who have had an HIV genotypic assay. Findings from this study will contribute to the availability of the HIV drug resistance data in resource-limited settings and will provide us with more insight on the HIV drug resistance patterns in paediatric patients.

# CHAPTER 2. OBJECTIVES OF THE STUDY

## 2.1. Aim of the study

To describe HIV-1 drug resistance patterns and the profile of patients who had genotypic HIV drug resistance tests at Empilweni Clinic between 1st January 2004 and 28th February 2012.

## 2.2. Specific objectives

1) To describe the profile of the patients who underwent a genotypic HIV drug resistance testing between 1st January 2004 and 28th February 2012.
2) To describe HIV drug resistance patterns obtained, including the:
    a. Success rate of genotypic HIV drug resistance amplification;
    b. Frequency of important HIV polymorphisms in the protease gene;
    c. Frequency of important HIV drug resistance mutations *[Appendix A]*.

# CHAPTER 3. MATERIALS AND METHODS

## 3.1. Study design

A descriptive cross-sectional retrospective study was conducted on HIV-positive children on cART followed up at Empilweni Clinic who underwent HIV genotypic drug resistance testing between January 1st, 2004 and February 28th, 2013. The study included all children who underwent HIV drug resistance testing and whose medical files and results were accessible at the clinic. Demographic profiles (age, gender, and referral status), HIV subtype, the virological mutations and the genotypic drug resistances were assessed.

## 3.2. Study setting

This study was conducted at Empilweni Clinic, a large public paediatric HIV clinic operating at the Rahima Moosa Mother and Child Hospital (RMMCH) in Johannesburg, South Africa. It has been functional since 1995 and has grown rapidly since the Comprehensive HIV and AIDS Care, Management and Treatment for South Africa began in April 2004 (8). The clinic acts as a referral centre for the public clinics from north western Johannesburg and occasionally private clinics from Johannesburg. The patients are treated according to the national paediatric cART guidelines (75).

During our study period the patients were treated according to national guideline. The following table summarizes the drug regimen used during our study period.

**Table 2: Drug regimen used during our study period (76)**

| Regimen | 0 months up to 3 years | Over 3years and >10 kg |
|---|---|---|
| First-line | Stavudine /Lamivudine<br>Lopinivir/ritonavir | Stavudine /Lamivudine<br>Efavirenz |
| Second-line | Zidovudine / Didanosine<br>Nevirapine/ Efavirenz* | Zidovudine/Didanosine<br>Lopinivir/ritonavir |

* Efavirenz if the child is over 3 years and Nevirapine if less than 3 years

As in many other public institutions, HIV drug resistance testing is not readily available. At Empilweni Clinic, the collaboration with external institutions (Department of Molecular Medicine and Haematology, University of the Witwatersrand and the National Health Laboratory Services) has made this test available but only for selected cases. The cases for genotypic HIV drug resistance testing were discussed in the clinical meetings at Empilweni Clinic and results were considered and reviewed on a dedicated clinic day.

HIV drug resistance testing was done following case discussion and at the clinicians' discretion in patients with virological failure whose viral load was above 1000 copies/ml for more than two visits done six months apart, whose adherence was judged to be adequate and where the child's treatment history caused concern as to whether standard second line therapy would be adequate. Thus not all patients failing first or second-line therapy have HIV drug resistance tested systematically.

The clinic has a database where all patient information is captured and updated on a daily basis. The patient's files are kept in the clinic and access to both electronic and paper records is controlled by a dedicated gate keeper.

## 3.3. Study population

Due to low frequency of genotypic HIV drug resistance testing in our setting, a convenience sampling method was used. All patients who underwent genotypic HIV drug resistance testing at the clinic during the study period were considered. Patients were excluded if their medical information was missing on the database or if their medical files could not be traced to retrieve missing information.

## 3.4. Ethics and institutional approval

This study was reviewed and approved by the scientific research committee *[Appendix B]*. Ethical clearance to use patient records was obtained through the human research ethic committee of the University of the Witwatersrand with Ethics clearance number M130222 *[Appendix C]*. Hospital approval was obtained from the chief executive officer of RMMCH *[Appendix D]*.

## 3.5. Data collection

The genotypic HIV drug resistance test was performed by the NHLS (national Health Laboratory Services). After the viral RNA was extracted from plasma and reverse transcribed, the gene of interest was amplified and sequenced. The sequence was then compared to HXB2 and differences from the sequence were identified. The level of resistance was determined using the HIVdb algorithm freely available on http://sierra2.stanford.edu/sierra/servlet/JSierra. The mutations were displayed on the results sheet together with the genotypic susceptibility of the common ARVs used in South Africa. We considered as relevant the drug resistance mutations from the IAS-USA March 2013 update *[Appendix A]*.

After obtaining the result sheets of the HIV drug resistance test, a research ID was allocated and relevant information including date of sample collection, HIV subtype, resistance mutations and polymorphism were captured on a Microsoft Excel database. For each patient whose genotypic HIV drug resistance results were available at the clinic, relevant medical files were extracted from the clinic archive.

They were used to review and update the clinic database for those specific patients. Further demographic and medical information was extracted from the clinic database after its review. Extracted information included gender, age, history of tuberculosis, PMTCT, treatment initiation CD4 cell count and viral load, regimen, date of initiation of cART and the date of HIV drug resistance genotype testing as well as whether the patient was transferred in on ART from another facility or not.

## 3.6. Statistical analysis

Data was analyzed descriptively using STATA (version 11). For categorical variables such as gender, race, clinical information and HIV drug resistance test results, frequencies and percentages were computed whereas, for continuous variables such as age, means and standard deviations were calculated for normally distributed data. For non-normally distributed data, medians and interquartile ranges (IQR) were computed. Resistance mutations were presented in bar graphs, and demographic and clinical characteristics were presented in frequency tables.

The $\chi^2$ (Chi-square) test was used for computation of different proportions between groups. The statistical significance was confirmed with $p<0.05$.

# CHAPTER 4. RESULTS

## 4.1. Source of the study population

The study overview is depicted in Figure 1. From the 1st January 2004 to 28th February 2012, 68 samples were sent for genotypic HIV drug resistance testing from the Empilweni Clinic. Five of these patients were excluded from our study as they had no information available on the clinic database and we could not trace the medical files.

Among the 63 patients whose samples were sent for genotypic HIV drug resistance testing and whose medical files were available for review, nine patients had a genotypic HIV drug resistance test repeated twice and one patient had the test repeated three times. The last time point on record for each patient was considered for genotypic HIV drug resistance analysis in these ten subjects.

Overall 52 patient samples were included in the analysis with an amplification rate of 84.6% (n=44). All the patients whose samples were successfully amplified were infected with HIV subtype C. The rest of the samples (16.4%, n=8) failed to amplify due to low viral load.
The following figure depicts an overview of the enrolment process in our study.

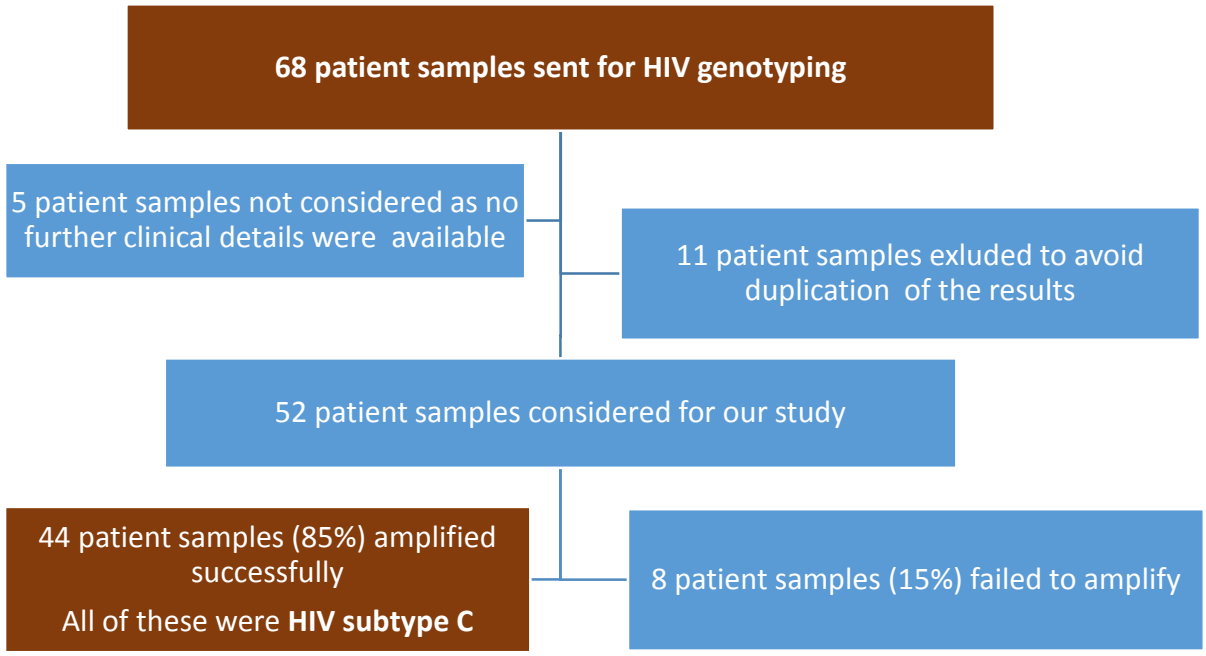



*Figure 1: Enrolment algorithm of the paediatric HIV drug resistance study at Empilweni Clinic*

## 4.2. Patient demographic characteristics

The main demographic characteristics of our study population are summarized in Table 1. Of the 52 children with genotypic HIV drug resistance results, the majority were males (55.8%; n=29). Most of the patients were initiated on cART at the clinic (N=39, 75%) as soon as possible after the first visit and 13 patients (25%) were referred to our centre already on cART. The median age at initial visit at our clinic was 28.5 months (IQR: 6-89.5). The mean age of initiation on cART was 25.5 months (IQR: 7-89.5) with 36.5% (n=15) initiating cART before one year of age and half of the patients (50.0%, n=26) having started cART before the age of two. At the time of genotypic HIV drug resistance testing, the median age was 59.5 months (IQR: 28-138.5) and the median duration on cART test was 26.5 months (IQR: 16-39.5).

***Table 3: Demographic characteristics of patients enrolled in our study***

| DEMOGRAPHIC CHARACTERISTICS | | | | |
|---|---|---|---|---|
| **Characteristic** | | **Designation** | **Number** | **Percentage (%)** |
| Sex | | Male | 29 | 55.8 |
| | | Female | 23 | 44.2 |
| Age (Months) | at initial visit | Median: 28.5 (IQR 6-89.5) | | |
| | at initiation of cART (median & IQR): 25.5 (IQR: 7-89.5) | < 12 | 17 | 32.7 |
| | | 13-24 | 9 | 17.3 |
| | | 25-60 | 6 | 11.5 |
| | | 61-120 | 15 | 28.9 |
| | | > 120 | 5 | 9.6 |
| | at genotypic HIV drug resistance test | Median: 59.5 ( IQR 11-205) | | |
| Transferred in on cART | | Yes | 13 | 25.0 |
| | | No | 39 | 75.0 |

## 4.2. Pre-treatment clinical characteristics

The main pre-treatment clinical characteristics are summarized in Table 2. Among 52 patients enrolled in this study, more than a third (N=19, 38%) presented early to the clinic at WHO stage I. The median VL at cART initiation was 5.2 logs (IQR: 3.1-6.6) with a median CD4 cell count of 447 (IQR: 214-796). The majority of the study population had no known history of PMTCT (N=28, 53%). Thirty patients (56.6%) had been treated for tuberculosis before HIV drug resistance testing was performed. The initial regimen which was commonly used in our population study consisted mainly of a PI-based regimen (53.9%) with LPV/r (Kaletra®) being the main PI used.

Full dose Ritonavir was used in only two patients (3.8%) as initial regimen, one of them was switched to Kaletra® after 6 months and the other patient switched after one year on Ritonavir. The switch was done more than a year before the resistance test.

All the patients who were on NNRTI-based regimens (46.1%) received EFV, except one patient who received NVP. D4T/3TC was the main NNRTI combination used in 73.5% (n=39) of the cases. Before the genotypic HIV drug resistance test many patients on NNRTI were already switched to PI-based therapy (40.4%; n=21) and 20 patients (38.5%) had only been on PI-based regimen; thus 78.9% of patients had PI exposure before the test.

***Table 4: Clinical characteristics of patients enrolled in our study***

| **CLINICAL CHARACTERISTICS** | | | |
|---|---|---|---|
| **Characteristic** | | **Count** | **Percentage (%)** |
| WHO stage at initial visit | I | 19 | 38.0 |
| | II | 9 | 18.0 |
| | III | 14 | 28.0 |
| | IV | 8 | 16.0 |
| | Not available | 2 | NA |
| CD4 cell count at initial visit<br>Median & range (cells/µl):<br>447 (214-796) | < 100 | 7 | 13.7 |
| | 100-500 | 20 | 39.2 |
| | 501-1000 | 13 | 25.5 |
| | > 1000 | 11 | 21.6 |
| | Not available | 1 | NA |
| Viral load in $log_{10}$ copies/ml<br>at initial visit<br>Median: 5.2 (IQR 3.1-6.6) | 3-5 | 21 | 42.0 |
| | >5 | 29 | 58.0 |
| | Not available | 2 | NA |

***Table 3: Clinical characteristics of patients enrolled in our study (continued)***

| Characteristic | | Count | Percentage (%) |
|---|---|---|---|
| Viral load before resistance test (in $log_{10}$ copies/ml) Median: 4.26 (IQR 3.6-4.5) | < 3 | 6 | 14.0 |
| | 3-5 | 28 | 65.0 |
| | >5 | 9 | 21.0 |
| PMTCT | Yes | 17 | 37.8 |
| | No | 28 | 62.2 |
| | Not available | 7 | NA |
| History of TB | Yes | 30 | 57.7 |
| | No | 22 | 42.3 |
| Drug exposure before HIV drug resistance testing | NNRTI backbone | 11 | 21.1 |
| | PI backbone | 20 | 38.5 |
| | Regimen switch | 21 | 40.4 |
| Duration on cART (months) before the HIV drug resistance test in months Median: 26.5 (IQR 16-39.5) | < 12 | 7 | 13.4 |
| | 12-24 | 16 | 30.8 |
| | > 24 | 29 | 55.8 |

## 4.3. Genotyping results

### 4.3.1. HIV subtype and overall resistance mutations

All the patients who had a successful amplification of the PR and RT coding regions (n=44) were infected with HIV-1 subtype C. At least one resistance mutation was identified in 90.9% (n=40) of the cases. Thirty five patients (79.5%) had at least one mutation isolated in the RT gene and 13 patients (29.5%) had two or more resistance mutations in the PR gene. Four patients (9.0%) had no known drug resistance mutations detected.

### 4.3.2. Nucleoside reverse transcriptase inhibitors (NRTI) mutations

The frequencies of the NRTI mutations are shown in Figure 2. The most commonly observed NRTI mutation was M184V (65.9%; n=29), it was the only mutation identified in 36.3% of the cases (n=16). This was followed by thymidine analogue mutations (TAMs) (25.0%, n=11) which resulted in a decreased susceptibility to AZT and d4T. Six patients (13.6%) had M184V associated with at least one TAM.

Of the 11 patients who had TAMs, 7 of them (77.8%) had TAM2 (D67N/K70R/T215F) and two patients (22.2%) had TAM1 mutations (M41L/L210W/T215F) identified. Two patients (4.5%) had two TAM1 mutations (K70R/T215F) accumulated and one patient had three TAM1 accumulated (M41L/L210W/T215Y). We did not identify both TAM pathways in the same viraemic sample. TAM2 mutations, the most commonly selected pathway, were identified as follows: D67N (8.9%, n=4), K70R (8.9%, n=4), and T215F (4.5%, n=2).

The K65R and L74V mutations, which result in decreased susceptibility to TDF/ddI were identified in only 2.2% (n=1) and 4.5% (n=2) cases, respectively. The Q151M complex and associated mutations and the T69N insertion were identified in only 2.2% (n=1) and 8.9% (n=4) patients, respectively.

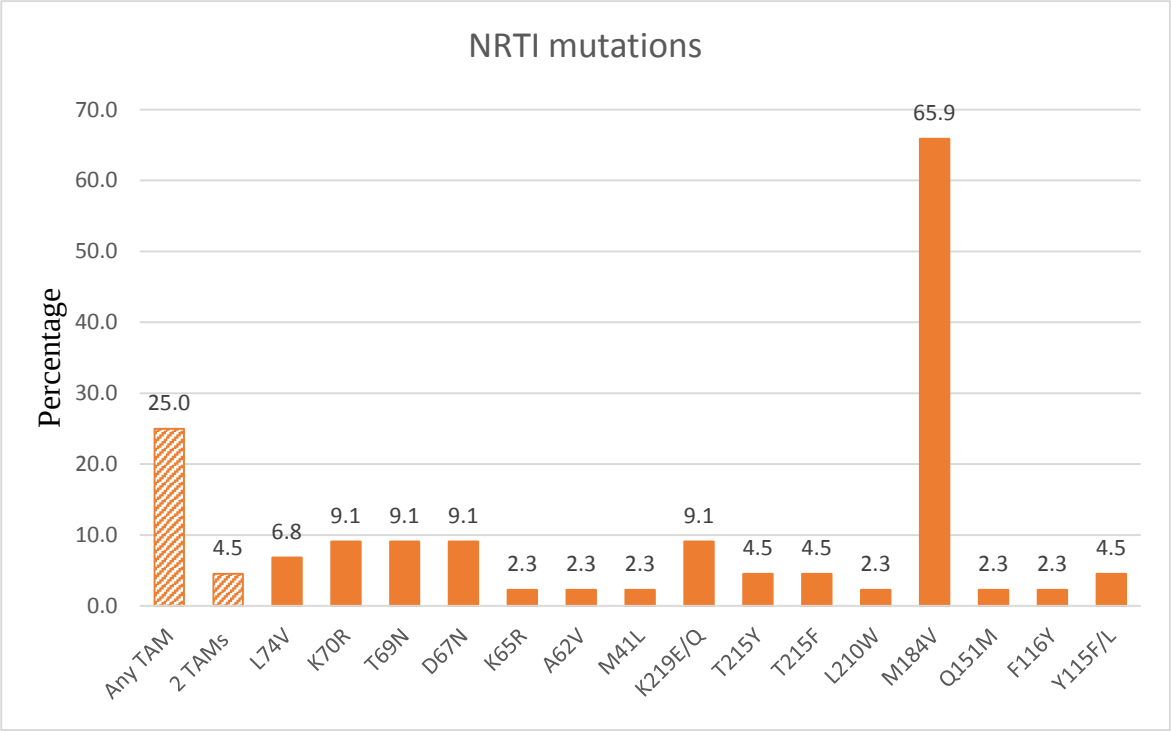



*Figure 2: Frequency of drug resistance mutations associated with resistance to Nucleotide Reverse Transcriptase Inhibitor (NRTI); the most frequent mutation observed was M184V (65.9%, n=29).*

### 4.3.3. Non-nucleoside reverse transcriptase inhibitors (NNRTI) mutations

The identified NNRTI mutations are summarized in Figure 3. Among the patients failing NNRTI-based therapy (n=32), 14 patients (43.8%) had at least one NNRTI mutation identified. The mutations V106M and K103N were the most identified and were equally observed (n=7, 21.9%). Both mutations were not observed in the same vireamic sample. Other NNRTI mutations identified were Y188H/L (15.6%), G190A/S/E (15.6%) and Y181C (6.3%). The minor NNRTI mutations identified were V179D/E (9.4%), K101P (6.3%), V108I (6.3%) and E138A (3.1%).

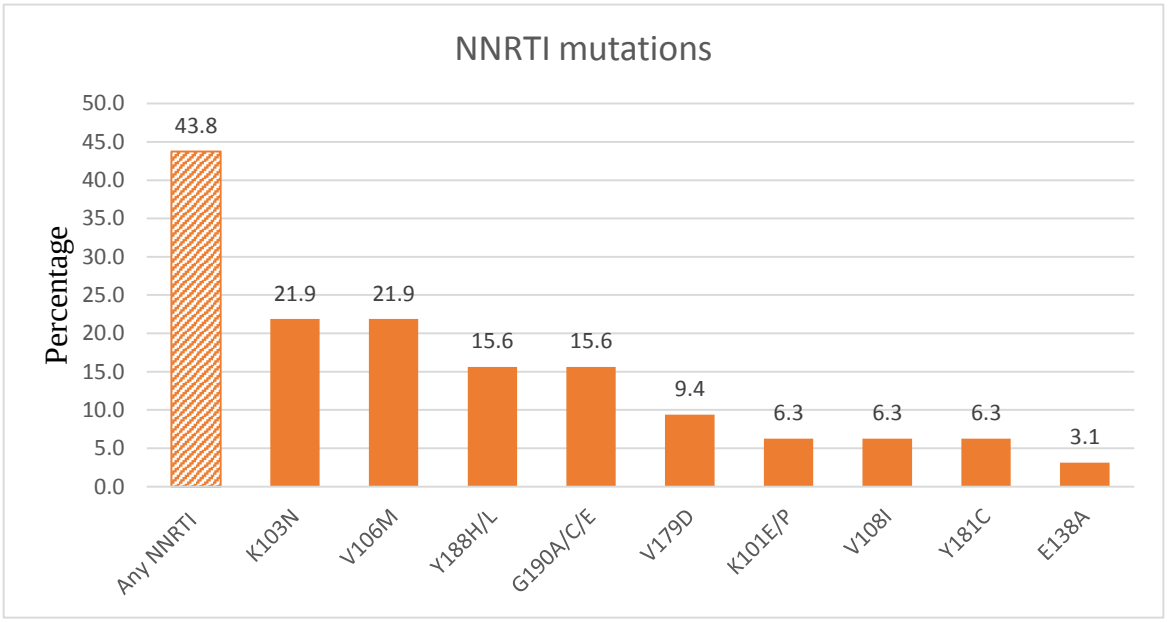



*Figure 3: Frequency of drug resistance mutations associated with resistance to Non-Nucleotide Reverse Transcriptase Inhibitor (NNRTI), the most frequent mutations observed were K103N (21.9%; n=7) and V106M (21.9%; n=7).*

### 4.3.4. Mutations associated with resistance to Protease inhibitors

Among the 41 patients who were exposed to PI based regimen, the PR gene mutations identified were V82A/F (29.3 %, n=12), T74S (24.4 %, n=10), I54V (22.0%, n=9), L76V (14.6%, n=6) and M46I (14.6%, n=6) as depicted in Figure 4.

Twelve of these patients (29.3%) had two or more PR gene mutations identified.

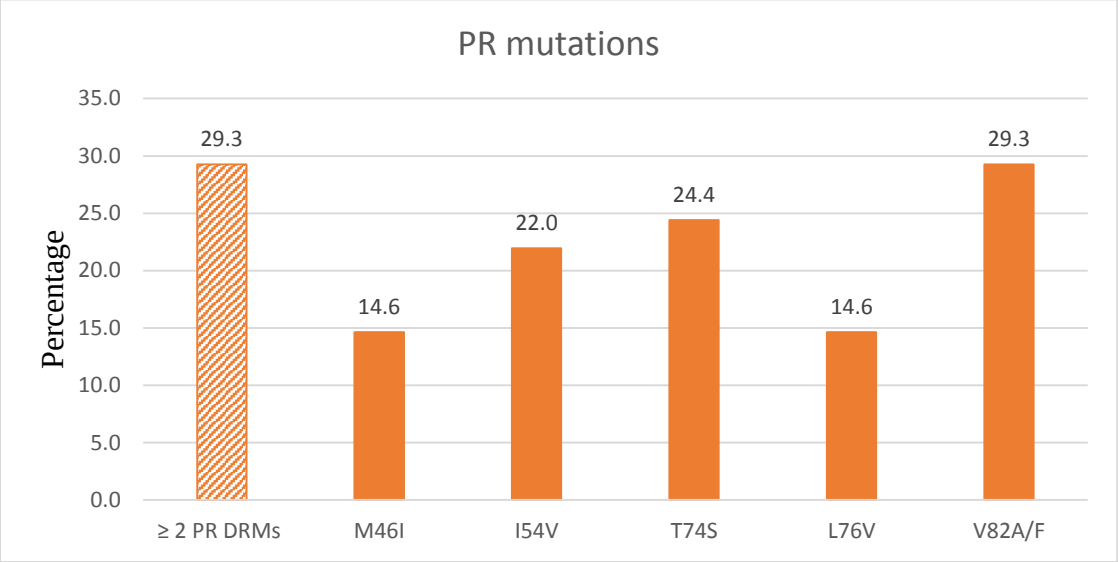



*Figure 4: Frequency of Protease (PR) mutations in the patients failing Protease Inhibitor (PI) based regimen, the most commonly identified mutation was V82A/F (29.3%, n=12)*

### 4.3.4. Protease gene polymorphisms

All the PR polymorphisms observed in patients exposed to PI based regimen (n=41) are summarized in Figure 5. The most common PI polymorphisms identified in our population were H69K (87.8%, n=36), M36I (75.6%, n=31), L89I/M (73.2%, n=30), L63P (63.4%, n=26) and R41K (56.1%, n=23).

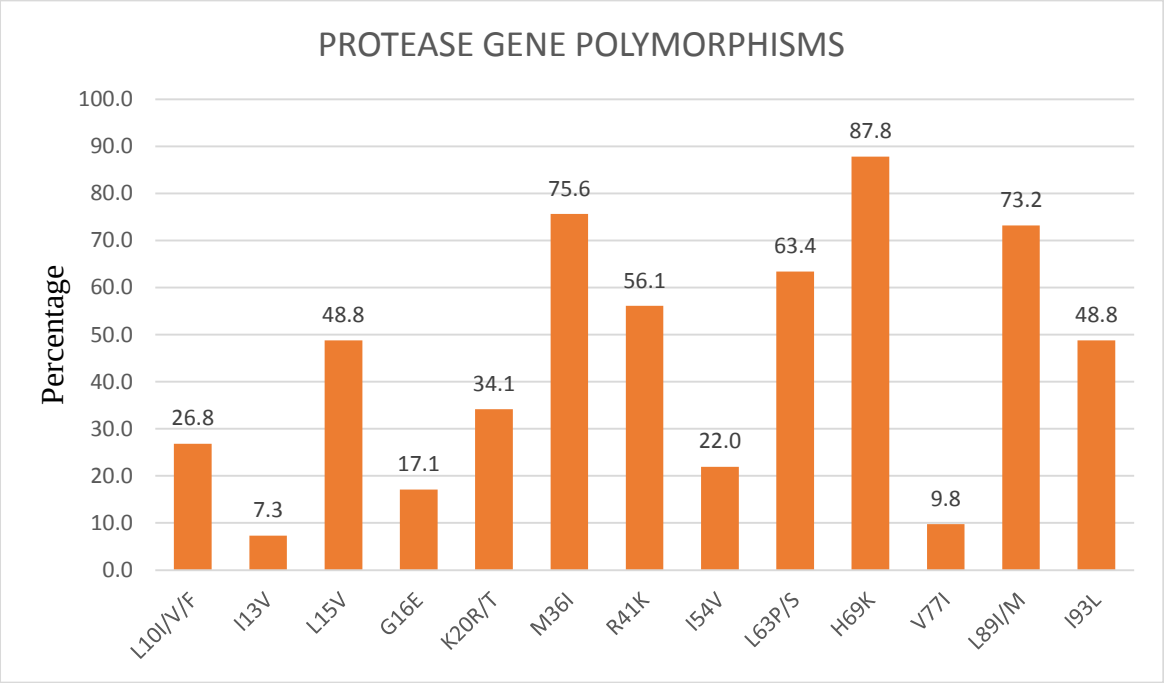



*Figure 5: Frequency of Protease (PR) polymorphisms in patients failing Protease Inhibitor (PI) based therapy.*

## 4.4. Genotypic susceptibility

Figure 6 shows the distribution of the patients according to the genotypic susceptibility. Of the patients who had successful amplification, almost a third (72.7%, n=32) had genotypic resistance to at least one ARV used in South Africa. All of these had a genotypic resistance to at least one NRTI with 50.0% of them (n=16) experiencing resistance to only 3TC and FTC. Twenty seven percent (n= 12) of these patients were still susceptible to all ARVs.

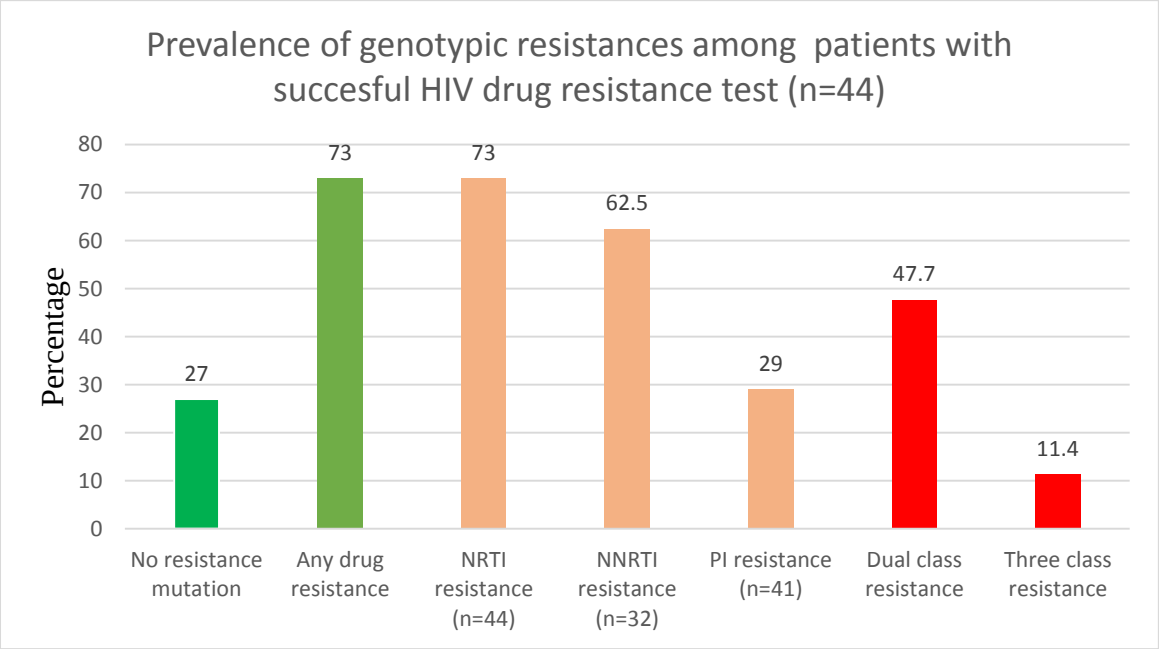



*Figure 6: Distribution of the patients according to genotypic susceptibility*

Two patients (4.5%) had genotypic drug resistance to all NRTI available in South Africa with intermediate susceptibility to TDF as they did not have K65R mutation. One had Q151M complex together with T69N insertion and the other one had accumulated 3TAMs amongst other mutations.

Among the patients exposed to NNRTI, sixty three percent (n=20) were resistant to at least one NNRTI. In our cohort, exposure to ART as part of PMTCT was not associated with having NNRTI genotypic resistance (p=0.393).

The majority of the patients exposed to PI backbone therapy before the genotypic HIV drug resistance test (n=41), were still sensitive to all the PI used in South Africa. Twelve patients (29.3%) had two or more PR gene mutation which was associated with genotypic drug resistance to at least one of the PI available in South Africa (p<0.001).

Among the patients who amplified successfully (n=44), 21 patients (47.7%) had a dual class resistance (NRTI and NNRTI) and 5 (11.4%) had genotypic resistance to ARVs from all the three classes (NRTI, NNRTI, and PI).

In a univariate logistic regression model *(table 4)*, no individual factor; including age, duration on cART before drug resistance test, history of TB treatment, viral load before the resistance test and PMTCT exposure, was associated with developing genotypic resistance to ARVs.

***Table 5: Univariate logistic regression analysis***

| **Independent variable** / **Dependent variable** | **Genotypic resistance to any ARV** |
|---|---|
| | **P value** |
| Age at initiation | 0.85 |
| Duration on cART before HIV drug resistance test | 0.93 |
| PMTCT exposure | 0.25 |
| Viral load at HIV drug resistance test | 0.90 |
| History of TB | 0.17 |

# CHAPTER 5. DISCUSSION

Although the HIV transmission rate is declining in South Africa (77), it still poses a serious public health problem with children being the most vulnerable group (25). This study focused on the mutations and genotypic susceptibility of the children who underwent genotypic HIV drug resistance testing from a public hospital after virological failure. The drug resistance mutations in our cohort has a complex pattern with almost half (47.7%) of the patients harbouring dual class drug resistance and 11% of the patients with genotypic resistance to all three classes while over a quarter of the patients (27%) still sensitive to all ARVs.

## 5.1. HIV clade and genotypic resistance profile

All the patients who had their vireamic samples successfully amplified were infected with HIV subtype C. Ninety one percent (91%) had at least one resistance mutation isolated. These results are similar to previously published studies from South Africa (78-80). The frequency of DRMs in our cohort is consistent with findings form a systematic review of resistance in children failing therapy from resource-limited settings which reported a pooled proportion of 90% of children failing cART with any DRM (25)

The majority of the patients (78%) were exposed to PI inhibitors before the genotypic HIV drug resistance test. As per current national paediatric ART protocol, the PI-based regimen was the commonly used initial cART in our population (53%) and a quarter of the patients (25%) had a regimen switch from NNRTI-based regimen to PI-based regimen.

We observed resistance mutations for all three major ARV drug classes used in South Africa. Almost two thirds of the patients (73%) had genotypic resistance to at least one ARV used in our settings, 47.7% had dual class resistance and 11% were resistant to drugs from three classes. TAMs were identified in 15.3% of the cases with 4.5% having two TAMs. This complexity of the mutation patterns in our population suggest a potential need for drug resistance test in paediatric population to determine appropriateness of regimen switch and to preserve first-line regimen where possible.

Over a quarter (27%) of the patients with virological failure had no genotypic drug resistance to any drug used in South Africa. The median RNA load at initiation was 5.1 logs (IQR: 3.1-6.6) and 4.2 logs (IQR: 3.6-4.5) at time of resistance testing. The most plausible reason for this treatment failure with no drug resistance identified is poor adherence even though the patients were reporting nearly perfect adherence. Previous studies have shown that self-report by paediatric patients overestimates adherence when compared to pharmacy records (81). This shows that clinicians may assess a patient as having good adherence and be suspicious of them having mutations but the results may show that this is not the case in up to a quarter of cases.

Furthermore, this underscores the importance of adherence counselling and various adherence support measures and the role of drug resistance testing in paediatric patients failing treatment to avoid unnecessary switching and prolonging first-line regimen, thereby prolonging the duration of ART success in resource-limited settings. Therefore, as the VL monitoring becomes increasingly available in resource-limited settings the adherence counselling and support should be the first focus before drug resistance testing. Adult studies from developing countries provided evidence that, in patients failing cART, targeted counselling can lead to viral re-suppression in some cases averting the need to switch therapy (82).

In our cohort, the genotypic drug resistance testing was selective and not every patient who required the test could get it. Only patients with relatively complex ARVs history had the test done. This could have contributed to the low percentage of patients with no genotypic drug resistance.

## 5.2. NRTI mutation patterns

The NRTI mutations were the most commonly observed DRMs in our series. The M184V conferring high level resistance to 3TC/FTC and TAMs conferring resistance to most NRTI were the most commonly identified in 64% and 15.6% of cases, respectively. Six patients (13.6%) had M184V with at least one TAM. Due to a small sample size, the comparison of the prevalence of TAMs in groups with and without M184V could not be performed, but it has been previously reported that the presence of M184V decrease the incidence of TAMs (83).

The most commonly identified TAM was D67N and K70R which was observed in 8.9% of cases. This suggest that in our series TAM2 pathway was the most favourably selected compared to TAM1 pathway which might be of some advantage since the TAM2 resistant virus is relatively less fit compared to TAM1 resistant virus (84, 85) 4.5% of our patients had accumulated two TAMs and only one patient had three TAMs.

The TAM patterns in this study are similar to those previously published studies done in South Africa and Kenya where the patients had routine follow up and VL monitoring (79, 86). However, the prevalence of TAMs and M184V observed in our cohort is lower compared to other studies done in developing countries where the monitoring was less intense.

A recently published study from Uganda by Musiime et al., reported the M184V mutation in 90.8%, TAMs in 43% and 10.9% of children failing first-line regimen had accumulated 3 or more TAMs. In the study, similarly to our findings, K65R was identified in only 2.8% (87).

This high prevalence of M184V mutation in both studies can be explained by the common use of 3TC or FTC in the first-line regimen. The high prevalence of TAMs in the Ugandan study might be explained by a prolonged duration on failing cART. This is consistent with Gupta et al.'s findings in his Zambian cohort (88) where a more rapid accumulation of TAM in children compared to the rate previously reported in adult studies was reported (89). While the average duration on cART in our study was only 26.5 months, it was 70.8 months in the Ugandan study (87).

Our results are different from those published by Almeida et al. from Brazil where the TAM T215Y/F was the most commonly identified mutations (69.6%) with M184V identified in only 56.5% of the cases. This difference might be explained by different drug regimens and the duration on cART. In their study the patients were mainly exposed to AZT/ddI containing regimen and the median time of exposure to cART was 60 months while our patients were mainly on d4T containing regimen with a median time of exposure of 26.5 months (90). Previous studies have suggested that AZT-containing regimen is more likely to select for TAMs compared to d4T containing regimen (25, 87).

Another possible explanation is the difference in pathways of developing DRMs among different HIV subtypes. In our cohort all the patients had HIV subtype C while in the study by Almeida et al. the patients were mainly infected by HIV subtype B (78.3%) and only 4.3% were infected with HIV-1 subtype C. In a published review article, Wainberg et al. suggested that HIV-1 subtype B have a higher propensity to acquire TAMs compared to HIV-1 subtype C, while subtype C have a differential selection of K65R instead (41).

The K65R resulting in decreased susceptibility to TDF and ddI was observed in only one patient (2.3%). Our rate is similar to the rates previously reported from studies done in similar settings. Van Zyl et al. who reported the K65R prevalence of 2.8% in South African patients failing d4T containing regimen and Wamalwa et al., in a Kenyan paediatric cohort, observed a rate of 2.9% (86, 91). However, our rate is lower than the rate reported by Puthanakit et al. in a cohort of Thai children failing NNRTI-based therapy where the K65R mutation was identified in 5% of the cases (92). The relatively high rate reported in the Thai study might have been a result of rare virological monitoring which was only done once treatment failure was suspected based on clinical and immunological criteria. A systematic review from adult studies showed that routine virological monitoring allows for on time identification of treatment failure and intervention thus limiting the occurrence of DRMs (92, 93).

The Q151M complex conferring multi-NRTI resistance except TDF, was defined as Q151M alone or in combination with one or more of the following mutations: A62V, V75I, F77L, and F116Y. It was identified in one patient (2.3%) and was not associated with K65R which results in multi-NRTI resistance including TDF. This patients was on failing regimen (d4T/3TC/EFV) since July 2005 and was never virally suppressed until switched to second-line PI based regimen in January 2008 after the genotyping resistance test.

These findings are consistent with the previously published adult study by Zaccarelli et al., suggesting that the detection of Q151M mutation increases with the duration on failing NRTI therapy (94). Our findings are also similar to a recently published study from South Africa by Van Zyl et al. who reported a Q151M prevalence of 1.9% among paediatric patients failing first-line regimen (91).

## 5.4. NNRTI mutation patterns

Almost half of the patients (43.8%, n=14) exposed to NNRTI were resistant to at least one NNRTI. The K103N and V106M mutation were the most common NNRTI mutations, identified in 29.1% of the patients exposed to NNRTI-based regimen before resistance test. These mutations confer high level resistance to most commonly used NNRTIs but they have no effect on the susceptibility ETR which is a recently introduced NNRTI.

The K101E and Y181C mutations were identified in only 6.3% of the patients exposed to NNRTI-based regimens. This rate is comparable to the rate reported in recently published study from KwaZulu-Natal Province (KZN) (79).

The patterns of the NNRTI mutations in our cohort suggest that ETR can be still an option after failing conventional NNRTI-based regimens in this age group.

The positive PMTCT history was not statistically associated with any NNRTI mutation or with genotypic resistance to NNRTI, consistent with an observation from a recently published study from South Africa noting that PMTCT history is not reliable in ruling out pre-treatment HIV drug resistance (95).

## 5.5. Protease mutation and polymorphism patterns

In the study population the PI based therapy was the main regimen used with 78% of the patients exposed to this regimen before resistance test. The median duration on treatment was 26.5 months (IQR 16-39.5). Despite this relatively long exposure, only 29% of these patients developed genotypic resistance to at least one PI commonly used in South Africa. This might be explained by the high genetic barrier of PI but also raise the possibility of poor and differential adherence to Kaletra® as it can be poorly tolerated especially in young children (96).

The V82A was the most commonly observed PR mutations and was observed in 29.3% of the patients exposed to PI. The double substitution V82A/I84V is known to confer resistance to all PR inhibitors was not identified in our study. In our study, the PR mutations rate was much higher than the rate recently published from KZN, where Pillay et al. identified V82A mutation as the only mutations in 6.3% of their rural area cohort (79). This difference might be explained by the different exposure to PI drugs. In our cohort 93.1% (n=41) of the patients had been exposed to a PI based regimen by the time of drug resistance testing while only 15.8% (n=16) had been on PI regimen in Pillay's cohort (79) . Two patients had received full dose Ritonavir but they were switched to Kaletra® more than a year before the genotypic resistance test.

This might have contributed to the increase of the rate of PR resistance mutations in our cohort. In Pillay's cohort, 17.8% of the cases were on PI based therapy with various adherence patterns by the time of genotyping (79). The relative high prevalence of PI resistance in our study is a cause of concern since PI are considered as the pillar of the paediatric HIV care.

The PR polymorphisms H69K, M36I and L89I/M were the most common and were identified in 87.8%, 75.6% and 73.2% of the patients exposed to PI based regimen, respectively. This high level of polymorphism is a cause of concern in our settings. Although they do not seem to cause drug resistance on their own but they may have compensatory roles such as enhancing viral replication or aid the virus in developing active site drug resistance mutations (97, 98). Velazquez-Campoy et al. proved that high level of polymorphisms in HIV-1 subtype C contribute on the reduction of the effectiveness of PR inhibitors binding onto substrate (99) and Clemente et al. suggested that HIV-1 subtype C harbouring M36I as a natural polymorphism could aid the virus to develop active site mutation while maintaining catalytic activity(100).

## 5.6. Limitations of the study

### 5.6.1. Sampling

In this study, a convenience sampling method was used. However, during the study period only a few resistance tests were performed due to the low incidence of the treatment failure and limited availability of the genotyping drug resistance test in the clinic. Therefore, due to the small size the mutation pattern observed might represent the reality of our clinic but not easy to generalize it to the rest of the population.

We could not identify any factor associated with developing resistance to ARVs in our study probably because of a small number of the patients who underwent genotyping in our study period.

### 5.6.2. Selection bias

The HIV drug resistance testing was performed following individual case discussion and at the clinicians' discretion in patients with virological failure whose HIV- RNA was above 1000 copies/ml for more than two visits done six months apart.

Thus not all patients failing therapy received systematic testing of HIV drug resistance; this may have caused selection of cases with complicated ARVs history and probably more complex mutation profile.

# CHAPTER 6. CONCLUSION AND RECOMMENDATIONS

The study findings suggest the existence of complex drug resistance mutations pattern in paediatric patients failing therapy in resource-limited settings.

Given this complex resistance mutation patterns in paediatric patients we recommend regular VL monitoring at least 6 monthly and genotypic HIV drug resistance testing in case of any virological failure.

The viral load monitoring will facilitate rapid identification of virological failure, and the genotypic HIV drug resistance test will prevent unnecessary switches, thereby preserve first-line regimens where appropriate and avoiding prolonged duration on a failing regimen where HIV drug resistance is identified. This will ensure the continued cART success in the paediatric patients.

Yet the complex patterns of resistance mutations in the paediatric population and the absence of resistance in some patients failing therapy as demonstrated in our study underscores the importance of adherence counselling and adherence support measures as a key factor for a durable ART success.

The frequency of drug resistance mutations are closely related to the drug regimen and duration of exposure in poorly compliant children.

Additional large studies on drug resistance mutation in paediatric population and their impact on clinical outcomes are warranted to further enlighten this clinical puzzle.

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# APPENDICES

## Appendix A: HIV drug resistance mutations updates, 2013

IAS–USA Topics in Antiviral Medicine

*Special Contribution*

Appendix A

# Update of the Drug Resistance Mutations in HIV-1: March 2013

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This March 2013 edition of the IAS–USA drug resistance mutations list updates the figures last published in November 2011.[1]

In this update, 2 integrase strand transfer inhibitors (InSTIs), elvitegravir and dolutegravir, have become available and were added to the figure. Elvitegravir was approved by the US Food and Drug Administration (FDA) in August 2012 for HIV-1 treatment-naive patients as part of a fixed-dose combination of elvitegravir/cobicistat/tenofovir/emtricitabine.[2,3] Dolutegravir is being evaluated in clinical trials for both initial HIV therapy and for use by treatment-experienced patients. It is available in an expanded access program and has been designated for priority review by the US FDA for treatment-experienced patients with detectable viral load who have documented HIV-1 resistance to raltegravir or elvitegravir. Relevant elvitegravir and dolutegravir mutations that have been identified to date are listed on the figure.

The following mutations have been added to existing classes or drugs: M230L has been added to the bars for the nonnucleoside analogue reverse transcriptase inhibitors (NNRTIs) efavirenz and nevirapine;[4,5] Y188L has been added to the NNRTI rilpivirine bar; the asterisk was removed from E138K (see revised user note).[6,7] L74M, T97A, E138A/K, and G140A/S have been added to the InSTI raltegravir bar; E92Q was unbolded.

## Methods

The IAS–USA Drug Resistance Mutations Group is an independent, volunteer panel of experts charged with delivering accurate, unbiased, and evidence-based information on these mutations to HIV clinical practitioners. As with all IAS–USA volunteer panels, members are rotated on a structured, planned basis. The group reviews new data on HIV drug resistance to maintain a current list of mutations associated with clinical resistance to HIV. This list includes mutations that may contribute to a reduced virologic response to a drug.

In addition, the group considers only data that have been published or have been presented at a scientific conference. Drugs that have been approved by the US FDA as well as any drugs available in expanded access programs are included (listed in alphabetical order by drug class). User notes provide additional information as necessary. Although the Drug Resistance Mutations Group works to maintain a complete and current list of these mutations, it cannot be assumed that the list presented here is exhaustive.

### Identification of Mutations

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

The development of more recently approved drugs that cannot be tested as monotherapy precludes assessment of the impact of resistance on antiretroviral activity that is not seriously confounded by activity of other drug components in the background regimen. Readers are encouraged to consult the literature and experts in the field for clarification or more information about specific mutations and their clinical impact. Polymorphisms associated with impaired treatment responses that occur in otherwise wild-type viruses should not be used in epidemiologic analyses to identify transmitted HIV-1 drug resistance.

## Clinical Context

The figures are designed for practitioners to use in identifying key mutations associated with antiretroviral drug resistance and in making therapeutic decisions. In the context of making clinical decisions regarding antiretroviral therapy, evaluating the results of HIV-1 genotypic testing includes: (1) assessing whether the pattern or absence of a pattern in the mutations is consistent with the patient's antiretroviral therapy history; (2) recognizing that in the absence of drug (selection pressure), resistant strains may be present at levels below the limit of detection of the test (analyzing stored samples, collected under selection pressure, could be useful in this setting); and (3) recognizing that virologic failure of the first regimen typically involves HIV-1 isolates with resistance to only 1 or 2 of the drugs in the regimen (in this setting, resistance develops most commonly to lamivu-

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Appendix A

dine or emtricitabine or the nonnucleoside analogue reverse transcriptase inhibitors [NNRTIs]).

The absence of detectable viral resistance after treatment failure may result from any combination of the following factors: the presence of drug-resistant minority viral populations, a prolonged interval between the time of antiretroviral drug discontinuation and genotypic testing, nonadherence to medications, laboratory error, lack of current knowledge of the association of certain mutations with drug resistance, the occurrence of relevant mutations outside the regions targeted by routine resistance assays, drug-drug interactions leading to subtherapeutic drug levels, and possibly compartmental issues, indicating that drugs may not reach optimal levels in specific cellular or tissue reservoirs.

For more in-depth reading and an extensive reference list, see the 2008 IAS–USA panel recommendations for resistance testing[8] and 2012 IAS–USA panel recommendations for antiretroviral therapy.[9] Updates are posted periodically at www.iasusa.org.

## Comments

Please send your evidence-based comments, including relevant reference citations, to the **journal"at"iasusa.org** or by fax at 415-544-9401.

## Reprint Requests

The Drug Resistance Mutations Group welcomes interest in the mutations figures as an educational resource for practitioners and encourages dissemination of the material to as broad an audience as possible. However, permission is required to reprint the figures and **no alterations in format or the content can be made**.

Requests to reprint the material should include the name of the publisher or sponsor, the name or a description of the publication in which you wish to reprint the material, the funding organization(s), if applicable, and the intended audience. Requests to make any minimal adaptations of the material should include the former, plus a detailed explanation of the adaptation(s) and, if possible, a copy of the proposed adaptation. To ensure the integrity of the mutations figures, IAS–USA policy is to grant permission for only minor, preapproved adaptations of the figures (eg, an adjustment in size). Minimal adaptations only will be considered; no alterations of the content of the figures or user notes will be permitted.

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

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

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

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

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

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

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

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

| | K | L | Y | M |
|---|---|---|---|---|
| Abacavir[f,g] | 65 | 74 | 115 | 184 |
| | R | V | F | V |
| Didanosine[g,h] | 65 | 74 | | |
| | R | V | | |
| Emtricitabine | 65 | | | 184 |
| | R | | | V |
| | | | | I |
| Lamivudine | 65 | | | 184 |
| | R | | | V |
| | | | | I |

| | M | K | D | K | L | T | K |
|---|---|---|---|---|---|---|---|
| Stavudine[d,e,g,i,j,k] | 41 | 65 | 67 | 70 | 210 | 215 | 219 |
| | L | R | N | R | W | Y | Q |
| | | | | | | F | E |

| | K | K |
|---|---|---|
| Tenofovir[l] | 65 | 70 |
| | R | E |

| | M | D | K | L | T | K |
|---|---|---|---|---|---|---|
| Zidovudine[d,e,j,k] | 41 | 67 | 70 | 210 | 215 | 219 |
| | L | N | R | W | Y | Q |
| | | | | | F | E |

### Nonnucleoside Analogue Reverse Transcriptase Inhibitors (NNRTIs)[a,m]

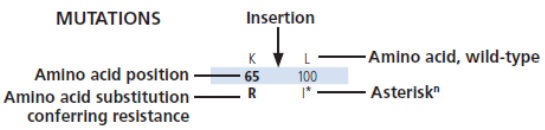
| | L | K | K | V | V | Y | Y | G | P | M |
|---|---|---|---|---|---|---|---|---|---|---|
| Efavirenz | 100 | 101 | 103 | 106 | 108 | 181 | 188 | 190 | 225 | 230 |
| | I | P | N | M | I | C | L | S | H | L |
| | | | S | | | I | | A | | |

| | V | A | L | K | V | E | V | Y | G | M |
|---|---|---|---|---|---|---|---|---|---|---|
| Etravirine[n] | 90 | 98 | 100 | 101 | 106 | 138 | 179 | 181 | 190 | 230 |
| | I | G | I* | E | I | A | D | C* | S | L |
| | | | | H | | G | F | I* | A | |
| | | | | P* | | K | T | V* | | |
| | | | | | | Q | | | | |

| | L | K | K | V | V | Y | Y | G | M |
|---|---|---|---|---|---|---|---|---|---|
| Nevirapine | 100 | 101 | 103 | 106 | 108 | 181 | 188 | 190 | 230 |
| | I | P | N | A | I | C | C | A | L |
| | | | S | M | | I | L | | |
| | | | | | | | H | | |

| | K | E | V | Y | Y | H | F | M |
|---|---|---|---|---|---|---|---|---|
| Rilpivirine[o] | 101 | 138 | 179 | 181 | 188 | 221 | 227 | 230 |
| | E | A | L | C | L | Y | C | I |
| | P | G | | I | | | | L |
| | | K | | V | | | | |
| | | Q | | | | | | |
| | | R | | | | | | |

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



Appendix A

## MUTATIONS IN THE PROTEASE GENE ASSOCIATED WITH RESISTANCE TO PROTEASE INHIBITORS[p,q,r]

| | L | G | K | L | V | L | E | M | M | G | I | F | I | D | I | I | A | G | V | I | I | N | L | I |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Atazanavir +/- ritonavir[s] | 10 | 16 | 20 | 24 | 32 | 33 | 34 | 36 | 46 | 48 | **50** | 53 | 54 | 60 | 62 | 64 | 71 | 73 | 82 | **84** | 85 | **88** | 90 | 93 |
| | I | E | R | I | I | I | Q | I | I | V | L | L | L | E | V | L | V | C | A | V | V | V | S | M | L |
| | F | | M | | | F | | L | L | | | Y | V | | | M | I | S | T | | | | | | M |
| | V | | I | | | V | | V | | | | | M | | | V | T | T | F | | | | | | |
| | C | | T | | | | | | | | | | T | | | | L | A | I | | | | | | |
| | | | V | | | | | | | | | | A | | | | | | | | | | | | |

| | V | | | | V | L | | | I | | I | | I | | | | T | L | | I | | L | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Darunavir/ ritonavir[t] | 11 | | | | 32 | 33 | | | **47** | | **50** | | **54** | | | | 74 | **76** | | **84** | | 89 | | |
| | I | | | | I | F | | | V | | V | | M | | | | P | V | | V | | V | | |
| | | | | | | | | | | | | | L | | | | | | | | | | | |

| | L | | | | V | | | | M | I | I | | I | | | | G | L | V | I | | L | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fosamprenavir/ ritonavir | 10 | | | | 32 | | | | 46 | 47 | **50** | | 54 | | | | 73 | 76 | 82 | **84** | | 90 | | |
| | F | | | | I | | | | I | V | V | | L | | | | S | V | A | V | | M | | |
| | I | | | | | | | | L | | | | V | | | | | | F | | | | | |
| | R | | | | | | | | | | | | M | | | | | | S | | | | | |
| | V | | | | | | | | | | | | | | | | | | T | | | | | |

| | L | | K | L | V | | M | M | | | I | | | | | A | G | L | V | V | I | | L | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Indinavir/ ritonavir[u] | 10 | | 20 | 24 | 32 | | 36 | **46** | | | 54 | | | | | 71 | 73 | 76 | 77 | **82** | **84** | | 90 | |
| | I | | M | I | I | | I | I | | | V | | | | | V | S | V | I | A | V | | M | |
| | R | | R | | | | | L | | | | | | | | T | A | | | F | | | | |
| | V | | | | | | | | | | | | | | | | | | | T | | | | |

| | L | | K | L | V | L | | | M | I | | I | F | I | | | A | G | | V | I | | L | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lopinavir/ ritonavir[v] | 10 | | 20 | 24 | **32** | 33 | | | 46 | **47** | 50 | 53 | 54 | | 63 | | 71 | 73 | | **76** | 82 | 84 | | 90 | |
| | F | | M | I | I | F | | | I | V | V | L | V | | P | | V | S | | V | A | V | | M | |
| | I | | R | | | | | | L | A | | | L | | | | T | | | | F | | | | |
| | R | | | | | | | | | | | | A | | | | | | | | T | | | | |
| | V | | | | | | | | | | | | M | | | | | | | | S | | | | |
| | | | | | | | | | | | | | T | | | | | | | | | | | | |
| | | | | | | | | | | | | | S | | | | | | | | | | | | |

| | L | | | D | | | M | M | | | | | | | | A | | | V | V | I | N | L | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nelfinavir[u,w] | 10 | | | **30** | | | 36 | 46 | | | | | | | | 71 | | | 77 | 82 | 84 | 88 | **90** | |
| | F | | | N | | | I | I | | | | | | | | V | | | I | A | V | D | M | |
| | I | | | | | | | L | | | | | | | | T | | | | F | | S | | |
| | | | | | | | | | | | | | | | | | | | | T | | | | |
| | | | | | | | | | | | | | | | | | | | | S | | | | |

| | L | | L | | | | | | | G | | I | | | I | | A | G | | V | V | I | | L | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Saquinavir/ ritonavir[u] | 10 | | 24 | | | | | | | **48** | | 54 | | | 62 | | 71 | 73 | | 77 | 82 | 84 | | **90** | |
| | I | | I | | | | | | | V | | V | | | V | | V | S | | I | A | V | | M | |
| | R | | | | | | | | | | | L | | | | | T | | | | F | | | | |
| | V | | | | | | | | | | | | | | | | | | | | T | | | | |
| | | | | | | | | | | | | | | | | | | | | | S | | | | |

| | L | | | | L | | M | | K | M | I | | | I | Q | | H | | T | | V | N | I | | L | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tipranavir/ ritonavir[x] | 10 | | | | 33 | | 36 | | 43 | 46 | **47** | | | 54 | **58** | | 69 | | **74** | | **82** | 83 | 84 | | 89 | |
| | V | | | | F | | I | | T | L | V | | | A | E | | K | | P | | L | D | V | | I | |
| | | | | | | | L | | | | | | | M | | | R | | | | T | | | | M | |
| | | | | | | | V | | | | | | | V | | | | | | | | | | | V | |

## MUTATIONS IN THE ENVELOPE GENE ASSOCIATED WITH RESISTANCE TO ENTRY INHIBITORS

| | G | I | V | Q | Q | N | N |
|---|---|---|---|---|---|---|---|
| Enfuvirtide[y] | **36** | **37** | **38** | **39** | **40** | **42** | **43** |
| | **D** | **V** | **A** | **R** | **H** | **T** | **D** |
| | **S** | | **M** | | | | |
| | | | **E** | | | | |
| Maraviroc[z] | See User Note | | | | | | |

## MUTATIONS IN THE INTEGRASE GENE ASSOCIATED WITH RESISTANCE TO INTEGRASE STRAND TRANSFER INHIBITORS

| | E | G | Q |
|---|---|---|---|
| Dolutegravir[aa] | **138** | **140** | **148** |
| | A | **S** | **H** |
| | K | A | |

| | T | E | T | S | Q | N |
|---|---|---|---|---|---|---|
| Elvitegravir[bb] | **66** | **92** | **97** | **147** | **148** | **155** |
| | **I** | **Q** | A | **G** | **R** | **H** |
| | A | G | | | H | |
| | K | | | | K | |

| | L | E | T | E | G | Y | Q | N |
|---|---|---|---|---|---|---|---|---|
| Raltegravir[cc] | **74** | **92** | **97** | **138** | **140** | **143** | **148** | **155** |
| | M | Q | A | A | A | **R** | **H** | **H** |
| | | | | K | S | **H** | **K** | |
| | | | | | | **C** | **R** | |

Appendix A

## User Notes

**a.** Some nucleoside (or nucleotide) analogue reverse transcriptase inhibitor (nRTI) mutations, like T215Y and H208Y,[1] may lead to viral hypersusceptibility to the non-nucleoside analogue reverse transcriptase inhibitors (NNRTIs), including etravirine,[2] in nRTI-treated individuals. The presence of these mutations may improve subsequent virologic response to NNRTI-containing regimens (nevirapine or efavirenz) in NNRTI-naive individuals,[3-7] although no clinical data exist for improved response to etravirine in NNRTI-experienced individuals. Mutations at the C-terminal reverse transcriptase domains (amino acids 293-560) outside of regions depicted on the figure bars may prove to be important for nRTI and NNRTI HIV-1 drug resistance. The clinical relevance of these connection domain mutations arises mostly in conjunction with thymidine analogue-associated mutations (TAMs) and M184V and have not been associated with increased rates of virologic failure of etravirine or rilpivirine in clinical trials.[8-10]

**b.** The 69 insertion complex consists of a substitution at codon 69 (typically T69S) and an insertion of 2 or more amino acids (S-S, S-A, S-G, or others). The 69 insertion complex is associated with resistance to all nRTIs currently approved by the US FDA when present with 1 or more TAMs at codons 41, 210, or 215.[11] Some other amino acid changes from the wild-type T at codon 69 without the insertion may be associated with broad nRTI resistance.

**c.** Tenofovir retains activity against the Q151M complex of mutations.[11] Q151M is the most important mutation in the complex (ie, the other mutations in the complex [A62V, V75I, F77L, and F116Y] in isolation may not reflect multidrug resistance).

**d**. Mutations known to be selected by TAMs (ie, M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E) also confer reduced susceptibility to all currently approved nRTIs.[12] The degree to which cross-resistance is observed depends on the specific mutations and number of mutations involved.[13-16]

**e.** Although reverse transcriptase changes associated with the E44D and V118I mutations may have an accessory role in increased resistance to nRTIs in the presence of TAMs, their clinical relevance is very limited.[17-19]

**f.** The M184V mutation alone does not appear to be associated with a reduced virologic response to abacavir in vivo. When associated with TAMs, M184V increases abacavir resistance.[20,21]

**g.** As with tenofovir, the K65R mutation may be selected by didanosine, abacavir, or stavudine (particularly in patients with nonsubtype-B clades) and is associated with decreased viral susceptibility to these drugs.[20,22,23] Data are lacking on the potential negative impact of K65R on clinical response to didanosine.

**h.** The presence of 3 of the following mutations—M41L, D67N, L210W, T215Y/F, K219Q/E—is associated with resistance to didanosine.[24] The presence of K70R or M184V alone does not decrease virologic response to didanosine.[25]

**i.** K65R is selected frequently (4% – 11%) in patients with nonsubtype-B clades for whom stavudine-containing regimens are failing in the absence of tenofovir.[26,27]

**j.** The presence of M184V appears to delay or prevent emergence of TAMs.[28] This effect may be overcome by an accumulation of TAMs or other mutations.

**k.** The T215A/C/D/E/G/H/I/L/N/S/V substitutions are revertant mutations at codon 215 that confer increased risk of virologic failure of zidovudine or stavudine in antiretroviral-naive patients.[29,30] The T215Y mutant may emerge quickly from one of these mutations in the presence of zidovudine or stavudine.[31]

**l.** The presence of K65R is associated with a reduced virologic response to tenofovir.[11] A reduced response also occurs in the presence of 3 or more TAMs inclusive of either M41L or L210W.[11] The presence of TAMs or combined treatment with zidovudine prevents the emergence of K65R in the presence of tenofovir.[32-34]

**m.** The sequential use of nevirapine and efavirenz (in either order) is not recommended because of cross-resistance between these drugs.[35]

**n**. Resistance to etravirine has been extensively studied only in the context of coadministration with darunavir/ritonavir. In this context, mutations associated with virologic outcome have been assessed and their relative weights (or magnitudes of impact) assigned. In addition, phenotypic cutoff values have been calculated, and assessment of genotype-phenotype correlations from a large clinical database have determined relative importance of the various mutations. These 2 approaches are in agreement for many, but not all, mutations and weights.[36-38] Asterisks (*) are used to emphasize higher relative weights with regard to reduced susceptibility and reduced clinical response compared with other etravirine mutations.[39] The single mutations L100I*, K101P*, and Y181C*/I*/V* reduce clinical utility. The presence of K103N alone does not affect etravirine response.[40] Accumulation of several mutations results in greater reductions in susceptibility and virologic response than do single mutations.[41-43]

**o.** Fifteen mutations have been associated with decreased rilpivirine susceptibility (K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, H221Y, F227C, and M230I/L).[44-46] A 16th mutation, Y188L, reduces rilpivirine susceptibility 6 fold.[47] K101P and Y181I/V reduce rilpivirine susceptibility approximately 50 fold and 15 fold, respectively, but are uncommonly observed in patients receiving rilpivirine.[48-50] K101E, E138K, and Y181C, each of which reduces rilpivirine susceptibility 2.5 fold to 3 fold, occur commonly in patients receiving rilpivirine. E138K and to a lesser extent K101E usually occur in combination with the nRTI resistance mutation M184I, which alone does not reduce rilpivirine susceptibility. When M184I is combined with E138K or K101E, rilpivirine susceptibility is reduced approximately 7 fold and 4.5 fold, respectively.[50-53]

**p.** Often, numerous mutations are necessary to substantially impact virologic response to a ritonavir-boosted protease inhibitor (PI).[54] In some specific circumstances, atazanavir might be used unboosted. In such cases, the mutations that are selected are the same as with ritonavir-boosted atazanavir, but the relative frequency of mutations may differ.

**q**. Resistance mutations in the protease gene are classified as "major" or "minor."

Major mutations in the protease gene (positions in **bold** type) are defined as those selected first in the presence of the drug or those substantially reducing drug susceptibility. These mutations tend to be the primary contact residues for drug binding.

Minor mutations generally emerge later than major mutations and by themselves do not have a substantial effect on phenotype. They may improve replication of viruses containing major mutations. Some minor mutations are present as common polymorphic changes in HIV-1 nonsubtype-B clades.

**r.** Ritonavir is not listed separately, as it is currently used only at low dose as a pharmacologic booster of other PIs.

**s.** Many mutations are associated with atazanavir resistance. Their impacts differ, with I50L, I84V, and N88S having the greatest effect. Higher atazanavir levels obtained with ritonavir boosting increase the number of mutations required for loss of activity. The presence of M46I plus L76V might increase susceptibility to atazanavir when no other related mutations are present.[55]

**t.** HIV-1 RNA response to ritonavir-boosted darunavir correlates with baseline susceptibility and the presence of several specific PI mutations. Reductions in response are associated with increasing numbers of the mutations indicated in the figure bar. The negative impact of the protease mutations I47V, I54M, T74P, and I84V and the positive impact of

Appendix A

the protease mutation V82A on virologic response to darunavir/ritonavir were shown in 2 data sets independently.[56,57] Some of these mutations appear to have a greater effect on susceptibility than others (eg, I50V vs V11I). A median darunavir phenotypic fold-change greater than 10 (low clinical cutoff) occurs with 3 or more of the 2007 IAS–USA mutations listed for darunavir[58] and is associated with a diminished virologic response.[59]

**u.** The mutations depicted on the figure bar cannot be considered comprehensive because little relevant research has been reported in recent years to update the resistance and cross-resistance patterns for this drug.

**v.** In PI-experienced patients, the accumulation of 6 or more of the mutations indicated on the figure bar is associated with a reduced virologic response to lopinavir/ritonavir.[60,61] The product information states that accumulation of 7 or 8 mutations confers resistance to the drug.[62] However, there is emerging evidence that specific mutations, most notably I47A (and possibly I47V) and V32I, are associated with high-level resistance.[63-65] The addition of L76V to 3 PI resistance-associated mutations substantially increases resistance to lopinavir/ritonavir.[55]

**w.** In some nonsubtype-B HIV-1, D30N is selected less frequently than are other PI mutations.[66]

**x.** Clinical correlates of resistance to tipranavir are limited by the paucity of clinical trials and observational studies of the drug. The available genotypic scores have not been validated on large, diverse patient populations. The presence of mutations L24I, I50L/V, F53Y/L/W, I54L, and L76V have been associated with improved virologic response to tipranavir in some studies.[67-69]

**y.** Resistance to enfuvirtide is associated primarily with mutations in the first heptad repeat (HR1) region of the gp41 envelope gene. However, mutations or polymorphisms in other regions of the envelope (eg, the HR2 region or those yet to be identified) as well as coreceptor usage and density may affect susceptibility to enfuvirtide.[70-72]

**z.** The activity of CC chemokine receptor 5 (CCR5) antagonists is limited to patients with virus that uses only CCR5 for entry (R5 virus). Viruses that use both CCR5 and CXC chemokine receptor 4 (CXCR4; termed dual/mixed [D/M] virus) or only CXCR4 (X4 virus) do not respond to treatment with CCR5 antagonists. Virologic failure of these drugs frequently is associated with outgrowth of D/M or X4 virus from a preexisting minority population present at levels below the limit of assay detection. Mutations in HIV-1 gp120 that allow the virus to bind to the drug-bound form of CCR5 have been described in viruses from some patients whose virus remained R5 after virologic failure of a CCR5 antagonist. Most of these mutations are found in the V3 loop, the major determinant of viral tropism. There is as yet no consensus on specific signature mutations for CCR5 antagonist resistance, so they are not depicted in the figure. Some CCR5 antagonist-resistant viruses selected in vitro have shown mutations in gp41 without mutations in V3;[73] the clinical significance of such mutations is not yet known.

**aa.** Cross-resistance studies with raltegravir- and elvitegravir-resistant viruses in vitro indicate that Q148H and G140S in combination with mutations L74I/M, E92Q, T97A, E138A/K, G140A, or N155H are associated with 5-fold to 20-fold reduced dolutegravir susceptibility[74] and reduced virologic suppression in patients.[75-81] Results of the phase III dolutegravir study in antiretroviral treatment-naive patients are expected to provide additional resistance information.

**bb.** Six elvitegravir codon mutations have been observed in integrase strand transfer inhibitor treatment-naive and -experienced patients in whom therapy is failing.[82-88] T97A results in only a 2-fold change in elvitegravir susceptibility and may require additional mutations for resistance.[85,86] The sequential use of elvitegravir and raltegravir (in either order) is not recommended because of cross-resistance between these drugs.[85]

**cc.** Raltegravir failure is associated with integrase mutations in at least 3 distinct, but not exclusive, genetic pathways defined by 2 or more mutations including (1) a signature (major) mutation at Q148H/K/R, N155H, or Y143R/H/C; and (2) 1 or more additional minor mutations. Minor mutations described in the Q148H/K/R pathway include L74M plus E138A, E138K, or G140S. The most common mutational pattern in this pathway is Q148H plus G140S, which also confers the greatest loss of drug susceptibility. Mutations described in the N155H pathway include this major mutation plus either L74M, E92Q, T97A, E92Q plus T97A, Y143H, G163K/R, V151I, or D232N.[89] The Y143R/H/C mutation is uncommon.[90-94] E92Q alone reduces susceptibility to elvitegravir more than 20 fold and causes limited (<5 fold) cross resistance to raltegravir.[84,95-97] N155H mutants tend to predominate early in the course of raltegravir failure but are gradually replaced by viruses with higher resistance, often bearing mutations G140S plus Q148H/R/K, with continuing raltegravir treatment.[90]

## References to the User Notes


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# Appendix B: Approval from the Witwatersrand scientific review committee

Appendix B

Postgraduate Office, Faculty of Health Sciences
Wits Medical School, 7 York Road, PARKTOWN, 2193, Johannesburg • Tel: (011) 717 2000 • Fax: (011) 717 2119

**APPOINTMENT OF SUPERVISOR/S** OF RESEARCH REPORT, DISSERTATION OR THESIS

**Motivation / Reason for Appointment:**
Protocol review

**Recommendation of Division / Department / School:**

**Student** Surname and Initials: NGABIRE P. Student Number: 397339
Degree: MB ChB Div / Dept / School: PEDIATRICS
Title: "Descriptive study of HIV drug resistance genotyping test in a pediatric public sector in Johannesburg"

**Supervisor 1**: Karl-Günter TECHNAU
Supervisor Qualifications: MBBCH MSc (Med) Dip HIV Man DCH
Supervisor Department: Paediatrics - Rahima Moosa Hospital
Supervisor Telephone: 082687 3633 E-mail: karltechnau@gmail.com

**Supervisor 2**: Carole Wallis
Supervisor Qualifications: MScMed PhD
Supervisor Department: External
Supervisor Telephone: 011 358 0816 E-mail: Carole.Wallis@lancet.co.za

**Student** Signature: Supervisor 1 Signature: Ueckman
Supervisor 2 Signature: Swally

**RECOMMENDATION BY HEAD OF DIVISION / DEPARTMENT / SCHOOL:**

Ueckman KG (Surname and Initials) (Signature) 26. 2. 2013 (Date)

**APPROVAL BY ASSESSOR GROUP:**
(On behalf of the FGSC) (Signature) 26/2/'13 (Date)

*FOR CIRCULATION TO THE FGSC*

# Appendix C: Human research ethics committee clearance certificate

Appendix C

R14/49 Dr Phocas Ngabire

## HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

## CLEARANCE CERTIFICATE NO. M130222

| | |
|---|---|
| **NAME: (Principal Investigator)** | Dr Phocas Ngabire |
| **DEPARTMENT:** | Department of Paediatrics & Child Health<br>Rahima Moosa Mother & Child Hospital |
| **PROJECT TITLE:** | Descriptive Study of HIV Drug Resistance Genotyping Test in Test in the Paediatric Public Sector in Johannesburg |
| **DATE CONSIDERED:** | 22/02/2013 |
| **DECISION:** | Approved unconditionally |
| **CONDITIONS:** | |
| **SUPERVISOR:** | Dr Karl Technau |
| **APPROVED BY:** | Professor PE Cleaton-Jones, Chairperson, HREC (Medical) |

**DATE OF APPROVAL:** 22/02/2013

**This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.**

**DECLARATION OF INVESTIGATORS**

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

**PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES**

## Appendix D: Approval from the CEO, RMMCH

Appendix D

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

RAHIMA MOOSA MOTHER AND CHILD HOSPITAL

RAHIMA MOOSA MOTHER AND CHILD HOSPITAL
Enquiries: Mrs. S. Jordaan
Tel: (011) 470 – 9030/4
Fax: (011) 477 4117

716 Magaliesberg Street
PARKTOWN
2193

Re: "Experience of HIV drug resistance genotype testing in a Public Sector paediatric population in Johannesburg"

Dear Dr. Phocas Ngabire

Permission is granted for you to conduct the above survey as indicated in your request:

1. The Rahima Moosa hospital will not in anyway incur or inherit costs as a result of the said study.
2. Your study shall not disrupt services at the study site.
3. Strict confidentiality shall be observed at all times.
4. Informed consent shall be solicited from patients participating in your study.
5. NO file should leave the records department and/or the hospital premises.

Arrangement will be made with recordkeeping clerks so that you could occupy space in their department.

Kindly forward this office with the results of your research on completion of it.

I, Dr P. NGABIRE accept the terms and conditions set-in this document

sign _____ date 01 Feb 2013

Yours sincerely,

CHIEF EXECUTIVE OFFICER
SJ/cj. 2013-01-31