From Department of Laboratory Medicine
Karolinska Institutet, Stockholm, Sweden

FROM BEDSIDE TO BENCH AND BACK: FUTURE OPTIONS FOR ANTIRETROVIRAL DRUGS IN NON-B HIV-1 SUBTYPES

Duncan Tazvinzwa Njenda

Karolinska Institutet

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From bedside to bench and back: Future options for antiretroviral drugs in non-B HIV-1 subtypes

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By

Duncan Tazvinzwa Njenda

Principal Supervisor:

Associate Professor Ujjwal Neogi
Karolinska Institutet
Department of Laboratory Medicine
Division of Clinical Microbiology

Co-supervisors:

Professor Susan Engelbrecht
Stellenbosch University, South Africa
Faculty of Medicine and
Health Sciences
Department of Pathology,
Division of Medical Virology

Professor Anders Sönnerborg
Karolinska Institutet, Sweden
Department of Laboratory Medicine
Division of Clinical Microbiology

Dr Graeme Brendon Jacobs
Stellenbosch University
Faculty of Medicine and
Health Sciences
Department of Pathology,
Division of Medical Virology

Professor Anna-Lena Spetz
Stockholm University
Department of Molecular Biosciences,
Wenner-Gren Institute

Opponent:

Associate Professor Zabrina Brumme
Simon Fraser University
Faculty of Health Sciences
British Columbia Centre for Excellence
in HIV/AIDS

Examination Board:

Associate Professor Maria (Mia) Brytting
The Public Health Agency of Sweden
Department of Microbiology
Division of Virology

Associate Professor Jonas Klingström
Karolinska Institutet
Department of Medicine
Center of infectious Medicine (CIM)

Dr Andre Gareth Loxton
Stellenbosch University, South Africa
Faculty of Medicine and Health Sciences
Division of Molecular Biology and
Human gentics

Professor Jan Stefan Gustav Schwartz
Lund University, Sweden
Department of Laboratory Medicine
Division of Medical Microbiology

Associate Professor Harold Marcotte
Karolinska Institutet
Department of Laboratory Medicine
Division of Clinical Immunology

I dedicate this to my family, friends, and most importantly,
to all the people that work for the betterment of people living with HIV.

"39:4.13 – Urantia Book There is no material reward for righteous living, but there is profound satisfaction – consciousness of achievement – and this transcends any conceivable material reward."

ABSTRACT

HIV-1 drug resistance remains a burden in low- and middle-income countries (LMIC). Regardless of the advances in antiretroviral (ARV) therapy, there is an increase in the trend of acquired and pre-treatment drug resistance mutations (DRM) in LMIC affected by diverse HIV-1 subtypes.

In the work presented in this thesis, I investigated the prevalence of HIV-1 acquired drug resistance (ADR) in South Africa from the period 2006-2014 (**Paper I**). Additionally, the *in vitro* protease- inhibitor (PI) drug sensitivity assay (DSA) response of HIV-1 subtype C (HIV-1C) derived recombinant viruses was compared to non-C viruses to understand their susceptibility profile to PI drugs. Results for the prevalence of ADR, specifically for patient sequences treated with a PI-based regimen (n=1043), was found to be <9%. No significant subtype-specific differences were seen for viruses tested in the DSA for Darunavir (DRV), Lopinavir (LPV) and Atazanavir (ATV) susceptibility. In **Paper II,** it was hypothesized that the *gag* gene could play a role in the response of PIs in the absence of known PI primary mutations. Hence to understand the role of Gag in impacting PI susceptibility and viral fitness, the *gag-p6* region was specifically investigated. The study showed an increase in viral fitness for HIV-1C viruses carrying the PYxE insertion in *gag-p6* when compared to the wild-type (WT) HIV-1C viruses. Furthermore, some PYxE-carrying viruses had low sensitivity to LPV and Tenofovir alafenamide (TAF) when tested in DSAs. Clinical data analysis, showed a higher pre-therapy viral load and a decrease in $CD4^+$ T-cell counts for patients harboring PYxE-carrying viruses when compared to WT. It was also essential to understand the inhibitory potential of most clinical (new and old) drugs used mainly to treat non-B HIV-1 subtypes. Hence, in **Paper III**, the newer antiretroviral drug 4'-Ethynyl-2'-Fluoro-2' deoxyadenosine (EFdA), was compared to TAF, first and second-generation non-nucleoside reverse transcriptase inhibitors (NNRTIs). It was demonstrated that EFdA has a high inhibitory potential independent of HIV-1 subtype and high antiviral activity against resistant viruses. However, HIV-1C viruses had a significantly reduced susceptibility to NNRTIs, specifically Rilpivirine and Etravirine. Finally in **Paper IV,** the drug susceptibility of Integrase strand transfer inhibitors (INSTIs) against diverse HIV-1 subtypes was investigated. Results indicated that INSTIs such as Dolutegravir (DRV), Bictegravir (BIC) and Cabotegravir (CAB) inhibited non-B subtypes significantly as compared to HIV-1B subtypes.

Finally, inferences suggest that subtype-specific differences play an essential role in influencing the ARV susceptibility which could further impact the treatment efficacy in sub-optimal adherence. To reduce the trend of increasing DRMs in non-B HIV-1 subtypes which are mainly dominating in LMICs, adherence support and viral load monitoring should be prioritized. A rapid adaptation of INSTIs and newer drugs that have long-acting potential is encouraged. However, pre-clinical studies and clinical trials that are mainly restricted to HIV-1B enrolled patients, should be inclusive of non-HIV-1B infected patients before the massive roll-out of INSTIs and newer drugs continues in non-HIV-1B dominated settings.

LIST OF SCIENTIFIC PAPERS

I. ***Njenda T. Duncan,*** Mikasi S. Given, Ambikan T. Anoop, Adetayo E. Obasa, Sarafianos G. Stefan, Sönnerborg Anders, Neogi Ujjwal, Engelbrecht Susan and Jacobs B. Graeme – *HIV-1 acquired drug resistance mutations in South Africa and phenotypic susceptibility in HIV-1 subtype C against protease inhibitors (Manuscript)*

II. van Domselaar Robert, ***Njenda T. Duncan***, Rao Rohit, Sönnerborg Ander, Singh Kamalendra, Neogi Ujjwal, (2019) *HIV-1 subtype 1 C with PYxE insertion has enhanced binding of Gag-p6 to host cell protein ALIX and increased replication fitness*

J. Virol. 93:e00077-19. DOI:10.1128/JVI.00077-19 [PMID: 3076057]

III. ***Njenda T. Duncan,*** Aralaggupe Shambhu, Singh, Kamalendra; Rao Rohit; Sönnerborg Anders, Sarafianos Stefan; Neogi Ujjwal, 2018. *Antiretroviral potency of 4'-Ethnyl-2'-Fluoro-2'-deoxyadenosine, Tenofovir alafenamide and second-generation non-nucleoside reverse-transcriptase inhibitors across diverse HIV-1 subtypes* J Antimicrob Chemother (2018); 73: 2721–2728 DOI: 10.1093/jac/dky256 [PMID: 30053052]

IV. Neogi Ujjwal, Singh Kamalendra, Aralaguppe G. Shambhu, Rogers C. Leonard, ***Njenda T. Duncan***, Sarafianos G. Stefan, Hejdeman Bo and Sönnerborg Anders, (2018). *Ex-vivo antiretroviral potency of newer integrase strand transfer inhibitors cabotegravir and bictegravir in HIV type 1 non-B subtypes.* AIDS. 2018 Feb 20;32(4).

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LIST OF RELATED SCIENTIFIC PAPERS NOT INCLUDED IN THESIS

I. Mikasi SG, Gichana JO, Van der Walt C, Brado D, Obasa AE, ***Njenda DT***, Messembe M, Lyonga E, Assoumou O, Cloete R, Ikomey GM, Jacobs GB. *HIV-1 Integrase Diversity and Resistance-Associated Mutations and Polymorphisms Among Integrase Strand Transfer Inhibitor-Naive HIV-1 Patients from Cameroon*. AIDS Res Hum Retroviruses. 2020 Jan 13.
doi: 10.1089/AID.2019.0264 PubMed [PMID: 31830799]

II. Hill KJ, Rogers LC, ***Njenda DT***, Burke DH, Sarafianos SG, Sönnerborg A, Neogi U,Singh K. *Strain-specific effect on biphasic DNA binding by HIV-1 integrase.*AIDS.2019 Mar 1;33(3):588-592.
doi: 10.1097/QAD.0000000000002078. PubMed [PMID:30475264];

III. Ikomey GM, Assoumou MCO, Gichana JO, ***Njenda DT***, Mikasi SG, Mesembe M, Lyonga E, Jacobs GB. *Observed HIV drug resistance associated mutations amongst naïve immunocompetent children in Yaoundé, Cameroon.* Germs. 2017 Dec 5;7(4):178-185. doi: 10.18683/germs.2017.1124. eCollection 2017 Dec.
PubMed [PMID: 29264355]

LIST OF ABBREVIATIONS

3TC	Lamivudine
ADR	Acquired drug resistance
AIDS	Acquired Immunodeficiency Syndrome
ARV	Antiretroviral
BIC	Bictegravir
CA	Capsid
CAB	Cabotegravir
cART	Combination antiretroviral therapy
DNA	Deoxyribonucleic Acid
DRM	Drug Resistance Mutation
DSA	Drug sensitivity assay
DTG	Dolutegravir
EfdA	4'-Ethynyl 2-fluro deoxyadenosine
ENV	Envelope
EVG	Elvitegravir
FDA	Food and Drug Administration
FTC	Emtricitabine
GRT	Genotypic resistance testing
HIC	High income countries
HIV	Human Immunodeficiency Virus
HIV-1B	Human Immunodeficiency virus type 1 subtype B
HIV-1C	Human Immunodeficiency virus type 1 subtype C
HTS	High throughput sequencing
IN	Integrase
INSTI	Integrase strand transfer inhibitor
LMIC	Low and middle-income countries
LTR	Long terminal repeats
MA	Matrix

NC	Nucleocapsid
NOP	Naturally occurring polymorphism
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside analogue Reverse Transcriptase Inhibitor
PDR	Pre-treatment drug resistance
PI	Protease inhibitors
RAL	Raltegravir
RNA	Ribose nucleic Acid
RT	Reverse transcriptase
SS	Sanger sequencing
TAF	Tenofovir alafenamide
TMC125/ETR	Etravirine
WHO	World Health Organization

CONTENTS

1 INTRODUCTION

Human immunodeficiency virus (HIV) drug resistance remains a challenge for the management of HIV-infected patients under combination antiretroviral therapy (cART) (1). Globally, Sub-Saharan Africa (SSA) is the region with the highest prevalence of HIV and with the most significant challenge in the management of HIV infections, as recently reported by the World Health Organization (WHO) (2). There has also been a substantial increase in the pre-treatment and acquired HIV-1 drug resistance rates across the 48 countries that comprise SSA. This chapter presents an overview of the replication cycle of HIV-1 and the different antiretroviral (ARV) drugs used to treat HIV-1. The end of the chapter, describes a conspectus of the epidemiology and drug resistance situation in South Africa and Sweden as the two main countries from which HIV-1 patient material was used for the different studies mentioned in this thesis. The samples obtained from both countries represent some of the HIV-1 non-B subtypes (HIV-1B) that are a burden to SSA and globally.

1.1 HIV-1 replication cycle

HIV-1 is an RNA retrovirus approximately 100-120nm in diameter and targets host immune cells for its replication (3). Genetically, HIV-1 consists of three structural genes (gag, *pol, and env*); two non-structural regulatory genes (*tat, rev*) and four non-structural accessory genes (*vif, vpr, vpu, and nef)* (4-6). The three structural genes – *gag, pol* and *env* – encode the Gag capsid proteins, viral enzymes (Protease, Reverse Transcriptase and Integrase) and Env proteins respectively, that constitute the infectious viral particle (Figure 1)(7).

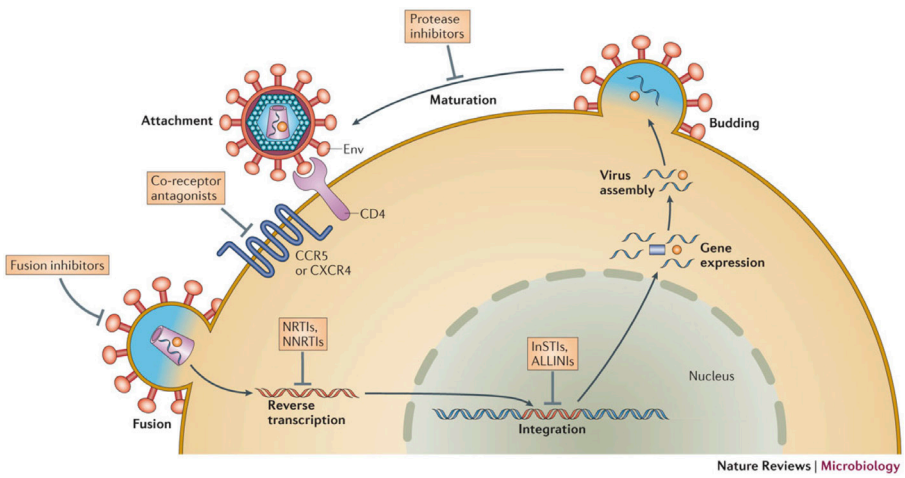

Figure 1. *HIV replication cycle and drug targets. [Image reproduced with permission from Springer Nature and adapted from Nature Reviews (7)]*

HIV transmission occurs via multiple routes that include heterosexual and homosexual intercourse, needle-stick injuries, transfusion with contaminated blood, and vertical transmission (Mother-to-child transmission- MTCT) (8-10).

Upon an individual's infection with HIV, the replication cycle of the virus initiates when its envelope glycoproteins (gp120 and 41) facilitate its attachment to the CD4 receptor (primarily present on T- lymphocytes) (11) and the co-receptor (CCR5/ CXCR-4) (12) on the host cell membrane. Attachment of the virus leads to a fusion-dependent event with the host cell membrane that allows docking of the viral RNA genome into the cytoplasm (13). The viral RNA is then reverse transcribed into double-stranded complementary DNA (cDNA), and the cDNA then associates with host factors as well as the viral Integrase enzyme to form the pre-integration complex (PIC) (14). The PIC is then transported into the cell nucleus where the viral integrase enzyme enacts a strand transfer reaction that results in the integration of the viral genome into the host cell genome (15). The presence of the viral protein Tat (Transactivator of transcription) and host cell transcription factors lead to the expression of viral mRNA. After post-transcriptional modification and translation of viral mRNA, viral proteins are produced (16).

Viral proteins are then transported to the inner membrane via interaction with the endosomal sorting complex required for transport (ESCRT) machinery (17). The assembly of immature virions begins near the host cell inner membrane before they bud off and through cleavage events by the viral protease, emerge as infectious particles to enter the plasma or can be transmitted from cell to cell (18) to start another round of infection. In an untreated HIV infected patient, the number of viral particles released in a day could be as high as 10000 particles per cell (19). Sometimes the virus may remain transcriptionally silent after integration in the infected cell and establishes a latent reservoir (primarily in CD4 central and transitional memory T-cells) (20). HIV-1 latency is the reason why HIV-1 patients receiving ARV treatment can not be completely cured (21).

1.2 HIV drug targets and ARV classes

The main steps of HIV viral replication include binding and entry, reverse transcription, integration, viral assembly, and budding. These steps form the basis for the targets of the 6 different ARV drug classes (Nucleos(t)ide reverse transcriptase inhibitors (NRTIs), Non- Nucleoside reverse transcriptase inhibitors (NNRTIs), Protease inhibitors (PIs), Integrase strand transfer inhibitors (INSTIs) and entry inhibitors (sub-divided as fusion inhibitors (FI) and inhibitors of co-receptor usage), Table 1. In total there are 31 United States of America (USA) Federal Drug Agency (FDA) approved HIV ARVs currently being marketed and the brand names of their co-formulated products are also given below in Figure 2 (22).

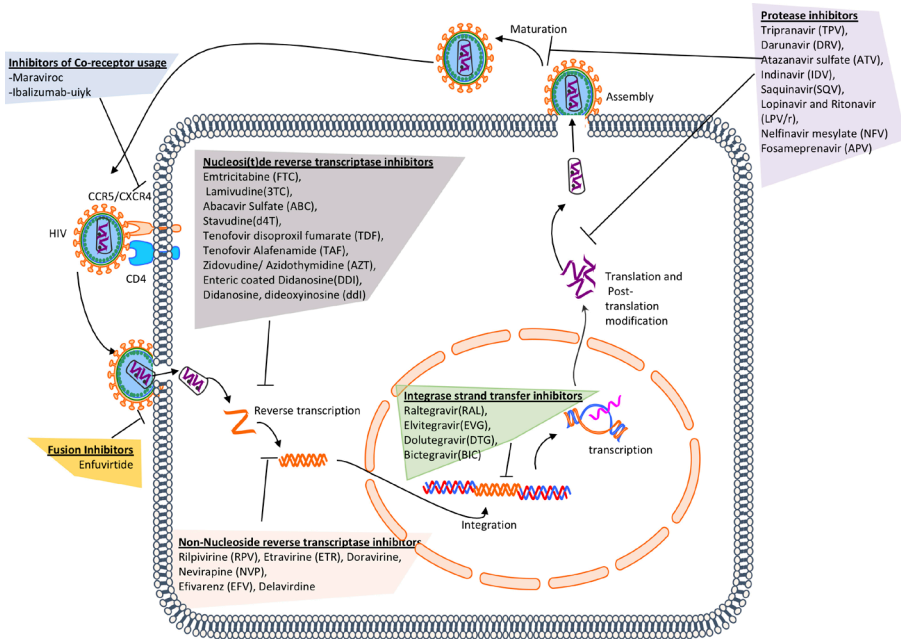

Figure 2. *FDA approved antiretroviral drugs for HIV treatment shown to act on different stages of the HIV-1 replication cycle*

Table 1. FDA approved Fixed-dose antiretroviral drugs for HIV currently marketed

Fixed-dose combinations	
Atripla (EFV+ FTC+TDF)	Prezcobix (DRV + Cobicistat)
Stribild (EVG + TDF + Cobicistat + FTC)	Trizivir (ABC +3TC +AZT)
Truvada (TDF + FTC)	Genvoya (EVG + cobicistat + FTC + TAF)
Triumeq (ABC + DTG + 3TC)	Biktarvy (BIC + FTC+TAF)
Complera (FTC + RPV + TDF)	Symtuza (DRV+ FTC +TAF + Cobicistat)
Combivir (3TC + AZT)	Dovato (DTG + 3TC)
Descovy (FTC + TAF)	Juluca (DTG + RPV)
Evotaz (ATV + Cobicistat)	Delstrigo (DOR + 3TC + TDF)
Epzicom (3TC + ABC)	Symfi (lo) (EFV +3TC +TDF)
Odefsey (FTC + TAF + RPV)	Cimduo (Temixys+ 3TC+TDF)

1.3 Mechanism of action of different ARVs

The classes of ARVs have different mechanisms of action that will be described below:

1.3.1 Nucleos(t)ide reverse transcriptase inhibitors (NRTIs)

NRTIs enter the cell by passive diffusion or are transported by a variety of carrier and ATP-dependent mediated membrane transporters. The most characterized membrane proteins for the transport of NRTIs are multidrug resistance proteins (MRPs) (23). Upon entering the cell cytoplasm, NRTIs are phosphorylated by the action of phosphotransferases and cellular kinases into triphosphate forms (24). NRTI triphosphate forms actively compete with the cell's natural dNTP substrates at HIV-1 RT's polymerase active site for incorporation into the growing cDNA strand synthesized by the HIV-1 RT enzyme. Once incorporated the triphosphate forms cause premature chain termination as they lack a 3'-OH group that would have facilitated the incorporation of the next dNTP substrate (25).

1.3.2 Non-Nucleoside reverse transcriptase inhibitors (NNRTIs)

NNRTIs do not have to be metabolized in the cell to gain activity against HIV-1. They gain cellular entry by passive diffusion as highly lipophilic molecules (26). They have been proposed to have three different mechanisms of action that express antiviral activity. The first classical mechanism is that of allosteric inhibition – NNRTIs bind in an HIV-RT pocket region that is 10Å away from the polymerase active site and cause a conformational change in HIV-RT affecting its ability to bind to the primer during reverse transcription (27).

The second mechanism (although controversial), involves inhibition effects on HIV-1 RT heterodimer formation. The inhibition restricts the enzyme's activity to simultaneous execute its polymerase and RNA degradation functions (26, 28). The third mechanism is believed to be an acceleration of cytoplasmic Gag-Pol processing by premature activation of the HIV-1 Protease enzyme. Premature activation of protease is hypothesized to occur when binding of NNRTIs to Gag-Pol proteins occurs, which then promotes oligomerization of the pol molecules by binding to the p66 subunit of HIV-RT. Premature cleavage of these Gag-Pol constructs restricts proteins required for viral particle assembly (26).

1.3.3 Protease Inhibitors (PIs)

PI cellular uptake can be via passive diffusion or mediated by Clathrin / Caveolae endocytic pathways (29). The tight binding interactions of PI on the dimer surface of the homodimer HIV-1 protease active site inhibits the enzyme's functioning to execute aspartic acid-mediated cleavage of its substrates (30). The result of

this inhibition affects the rate of viral assembly and can lead to the production of defective viral particles that are unable to establish another round of infection (31).

1.3.4 Integrase strand transfer inhibitors (INSTIs)

INSTI enter the cell by passive diffusion or endocytic adsorption. Once in the cytoplasm, they can bind to the integrase enzyme, but only access the enzyme's active site once there has been a conformational change that is only induced after the enzyme catalyzes the 3'-end processing reaction (32). The 3'-end processing reaction exposes a dinucleotide (CA) at the end of the viral cDNA that will eventually allow for integration into the host genome via the strand transfer reaction (32). To catalyze the strand transfer reaction in the nucleus, Integrase requires association with the host factor – lens epithelium-derived growth factor (LEDGF/p75) (33) and divalent magnesium ions to stabilize its active site. The magnesium ions are sequestrated by INSTIs that have a β-diketo acid group as part of their chemical structure. The β-diketo acid group in INSTIs has a high binding affinity for magnesium ions (34).

1.3.5 Fusion Inhibitors (FIs)

Fusion inhibitor (FI) drugs are delivered subcutaneously and have an extracellular mechanism of action upon entering the systemic system (35). FIs are in the form of short synthetic polypeptides (36 amino acids in length) with a short half-life (less than 4 hours), and specifically, act as structural analogs that bind to the heptad region (HR-2) of HIV-1 gp41. HR-2 associates with HR-1 during hairpin formation to form the 6-helix bundle that facilitates attachment of the host cell and viral membranes (36). Hence, FIs competitively interrupt the hairpin formation process that facilitates HIV-1 *env* gp41 glycoprotein folding, preventing entry of the virus (36).

1.3.6 Inhibitors of co-receptor usage (CCR5 antagonists)

CCR5 antagonists inhibit HIV-1 attachment by preventing the interaction of HIV-1 gp120 with CCR5 co-receptor used by CCR5 viral strains (R5-tropic) to enter the cell. The molecular antagonists bind in a side pocket region of the CCR5 molecule and cause a conformational change that disrupts the CCR5's molecular recognition of HIV-1 gp120 (37).

1.4 Biological basis and mechanism of drug resistance

HIV-1 has a high mutation rate determined to be $4.1 \pm 1.7 \times 10^{-3}$ per base per cell *in vivo* (38), and this translates to an arbitrary calculation of about 1-36 mutations occurring in each RNA genome target per round of replication. This is due to the

error-prone HIV-1 RT enzyme that lacks a 3'- 5' exonucleolytic proofreading (39). The error rate of the HIV-1 RT is one of the factors for the basis of HIV drug resistance – that is, the virus acquires mutations that enable it to grow in the presence of drugs. Besides, HIV-1 has a high recombination rate when the co-infection of a cell occurs with more than one variant (40). The high recombination rate facilitates an interchange of HIV genomic sequences one or all of which might harbor drug resistance mutations (41). HIV replicates as a pool of diverse viral strains termed as quasispecies that differ slightly in their genetic makeup in an individual (42). The sequence diversity of HIV has led to its systematical classification into two types (HIV-1 and HIV-2); groups (M, N, O, P), subtypes within groups and circulating recombinant forms that may all evolve different mechanisms of acquired resistance mutations and reduce the efficacy of ARV therapy (43).

1.5 HIV-1 resistance mechanisms against ARV drugs

HIV-1 drug resistance mutations can be described as primary (major) or secondary, which encompasses additional descriptive terms, such as minor and accessory or compensatory mutations (40). Most drug resistance mutations occur in the *pol* gene and those that are detected by standard genotypic tests confers resistance to 4 of the ARV drug classes (NRTIs, NNRTIs, PIs, and INSTIs). These 4 ARV drug classes are the main compounds in first and second-line therapy globally. The following section will only describe resistance mechanisms to these drug classes and exclude resistance mechanisms to other classes.

1.5.1 Resistance mechanisms to NRTIs

The major resistance mutations for NRTIs can be further classified into primer unblocking mutations or discriminatory mutations based on their occurrence near the HIV RT active site (40). Primer unblocking mutations, also referred to as thymidine analog mutations – TAMs, have an effect on ATP-mediated phosphorolytic excision of nucleosides, a process shown to reverse the incorporation of NRTIs (44). TAMs occur near the active site of HIV-1 RT enzyme and are selected by thymidine analogues AZT and D4T. TAMs occur in two pathways – type I or type II. The type I TAMs include M41L, L210W, T215Y show increased high-level of resistance to TDF, ABC and ddI, whereas the type II TAMs, which include D67N, K70R, T215F, K219Q/E are described for intermediate resistance to AZT and D4T(45).

Discriminatory mutations include clinically observed examples, such as K65R and M184V. K65R causes intermediate to high resistance to TDF, ddI, ABC, and d4T and low and intermediate resistance to 3TC and FTC. M184V causes high-level resistance to 3TC and FTC (46). Discriminatory mutations are so named as they enable a conformational change in the HIV-1 RT active site to 'discriminate' / or identify the NRTI from the cell's native dNTPs (40).

Minor and accessory or compensatory mutations for NRTIs depend on evolutionary selection forces that stem from the host immune system as well as drug pressures. The appearances of these mutations are usually assumed to restore viral fitness, which in turn, is usually affected when the virus acquires major mutations. One example is the A62V mutation that is a non-polymorphic mutation that occurs in HIV-1 A subtype and thought to compensate for viral fitness when it occurs with K65R (46). However, it was shown that A62V has no effect when it occurs with K65R and M184V in HIV-1C (Njenda DT, Master's Thesis, https://scholar.sun.ac.za/handle/10019.1/9866).

1.5.2 Resistance mechanisms to NNRTIs

The hydrophobic binding pocket for NNRTIs is formed by the amino acid residues L100, K101, K103, V106, T107, V108 V179, Y181, Y188, V189, G190, F227, W229, L234, and Y318 and mutations in the nucleotide positions of one of these sites can potentially cause resistance to NNRTIs (47).

K103N and Y181C are the most common mutations known to cause intermediate to high-level resistance against first (NVP, EFV) and second (ETR, RPV)-generation NNRTIs (46). K103N is an exception as it occurs outside the hydrophobic binding region. The K103N mechanism of action involves a hydrogen bond induced by its presence that prevents entry to the binding pocket region for NNRTIs (48). Minor mutations for NNRTIs usually occur from codon 225 to 318 of HIV-RT enzyme and usually occur with major mutations and have the impact of reducing the susceptibility of NNRTIs (47)

1.5.3 Resistance mechanisms to PIs

Resistance mutations to PIs occur less frequently in the protease gene in clinical practice (49). The development of key mutations to reduce the binding ability of PIs to the homodimer active site of the protease enzyme occurs near the substrate-binding cleft of the enzyme (50). These mutations have the effect of stretching the cavity of the active site and causing an unstable binding for the inhibitor (51). Common mutations that occur in the protease gene include M46L, I50V, D30N, G48V, V82A, I84V and L90M that have been shown to have clinically relevant HIV-1 drug resistance (52).

1.5.4 Resistance mechanisms to INSTIs

The catalytic core of the integrase enzyme is stabilized by the geometry of three residues termed the 'DDE' triad (amino acid residues D64, D116, and E152) (53). Resistance mechanisms to INSTIs, develop as a result of conformational changes induced by mutations that occur in the catalytic core, such as N155H and QI48H that increase the binding energy requirements for INSTIs. Other mutations (G118R,

Y143C/R) that have been described may impose steric hindrances to INSTIs such as EVG and RAL that have an oxadiazole group within their structure (54). Accessory mutations, such as R263K, may play a role in decreasing the viral DNA binding or may compensate for viral fitness, such as E138A and G140A (55). Other mechanisms of resistance that are currently being explored for INSTIs, such as DTG involve mutations in the LTR region (56). It is hypothesized that mutations in the LTR region could disrupt conventional integration, leading to linear forms of unintegrated viral DNA (possibly containing resistance mutations) that could still maintain a basal expression of the viral mRNA, that in turn will lead to viral production of INSTI resistant viruses (57). Overall, resistant mutations to INSTIs are not well characterized and require more research effort.

1.6 Drug resistance classification

Drug resistance mutations can be classified as pre-treatment drug resistance (also called pre-therapy or baseline drug resistance), and it refers to drug resistance mutations that are detected before a person starts therapy (2). Drug resistance mutations can also be classified as acquired/ therapy-induced resistance (resistance that develops when an individual is receiving ARV therapy over time (58). Pre-treatment drug resistance can be observed as possibility of two scenarios. The first being pre-treatment drug resistance as a result of a transmitted infectious resistant virus from one individual to another – termed as transmitted drug resistance (TDR) (58, 59). TDR can either be horizontal transmission via heterosexual/ homosexual intercourse or be vertical via mother to child transmission (MTCT) and may be hard to detect owing to the sensitive limit of standard genotypic assays and the possibility of reversion of drug resistance virus to wild- type forms (60). The second scenario involves pre-treatment drug resistance as a result of undocumented ARV therapy in individuals who present at the clinic as therapy-naïve HIV-1 infected patients. In most cases these individuals are initiated on treatment without genotypic testing, which is usually the standard of care in high-income countries (HIC), but not routine in LMIC (61).

1.7 Drug resistance in Sub Saharan Africa

HIV drug resistance remains a challenge for SSA. Figure 3 below presents a map of Sub-Saharan Africa as classified by the world bank with median estimates of pre-treatment and acquired drug resistance. Pre-treatment and therapy failure-associated/acquired drug resistance mutations reports published from 2010 to date are indicated in tables 3, 4 and 5 below for Northwest, west and Central Africa; Northeast and Eastern Africa and Southern Africa, respectively.

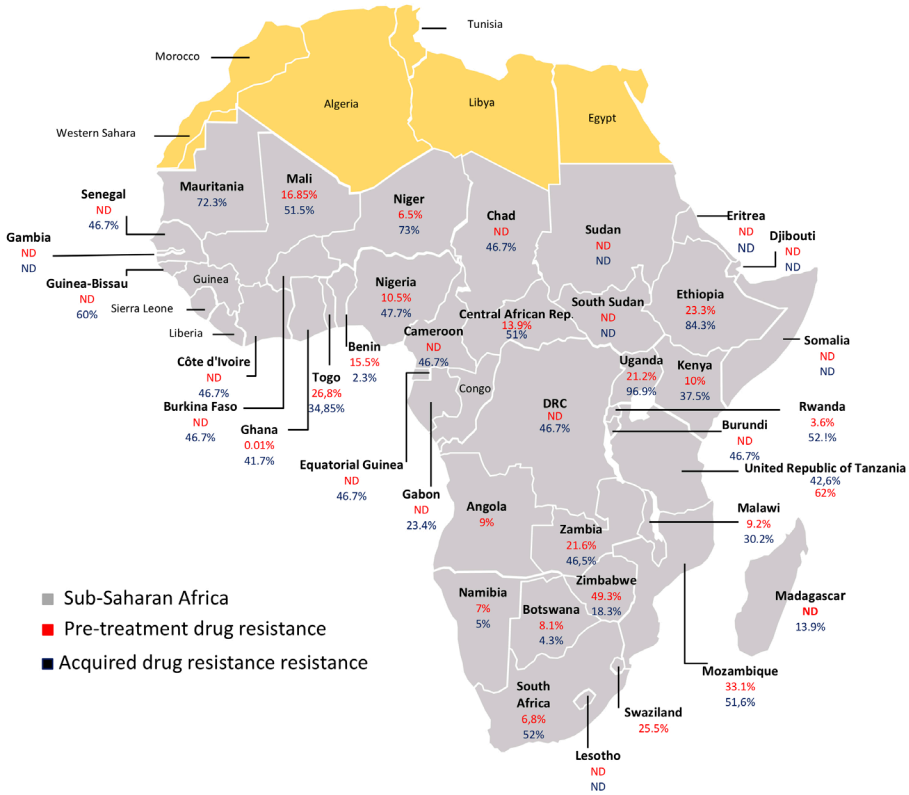

Figure 3. *Sub-Saharan Africa median estimates of pre-treatment drug resistance (in red) and therapy failure-associated (acquired drug resistance[ADR]) (in blue). Drug resistance range values are included in table 2-4 with references from the literature. ND = no data available*

1.7.1 Northwest, West and Central Africa

The expansion of antiretroviral programs has been a challenge for Sub-Saharan Africa countries in the northwest, west, and central region of Africa due to the high cost for acquisition of drugs, lack of virological monitoring and inadequate laboratory infrastructure (62). The presence of multiple HIV-1 subtypes adds to the problem of performing timeous screening of infected individuals as well as achieving sustained viral suppression due to the different observed efficacies that different ARVs have on non-B subtypes (63). A recent report that included 3736 sequences from central African countries (Burundi, Cameroon, Chad, Democratic Republic of Congo, Equatorial Guinea) and northwest and western African countries (Benin, Burkina Faso, Cote d'Ivoire, Senegal and Togo) had no indication of pre-treatment drug resistance, but indicated the frequency of acquired major resistance mutations

to NNRTIs and NRTIs to range from 5.5–88.9% (64). The previously mentioned report, had good concordance with data from the 2LADY- ANRS12169/EDCTP clinical trial. The trial had participant patients from Burkina Faso, Cameroon and Senegal and reported a 98.7% prevalence of at least one drug resistance mutation to first-line therapy (AZT/d4T+3TC + EFV/NVP) and a therapy failure rate of about 77%. Therapy failure due to drug resistance in the 2LADY- ANRS12169/EDCTP clinical trial was about 50 % of the therapy failure cases (65).

Table 2. PDR and ADR rates for northwest, west and central Africa

Country	Pre-treatment drug resistance	Acquired drug resistance
Benin	15.5% (66)	2.3% (67)
Cape Verde	3.4–12% (68)	10–47.8% (HIV-1); 17.6% (HIV-2)(68) (69)
Central African Republic	13.9%(70)	26–76% (71, 72)
Gabon	Data unavailable	Calculated to 23.4% (73)
Ghana	0.01% (74)	41.7% (75)
Equatorial Guinea	3.2–16.1% (76, 77)	12.7% (78)
Mali	7.9–25.8% (79, 80)	11–92% (81-83)
Mauritania	Data unavailable	72.3% (84)
Niger	6.5% (75)	73% (85)
Nigeria	<5–15.9% (86, 87)	1.6–93.8% (88-92)
Sierra Leone	Data unavailable	Data unavailable
Togo	26.8% (93)	18.1–51.6% (94)
Gambia	Data Unavailable	Data unavailable
Guinea-Bissau	Data Unavailable	60%(95)
Liberia	5.9% (96)	63–71% (97)

1.7.2 Northeast and Eastern Africa

Table 3. PDR and ADR rates for the northeast and eastern Africa

Country	Pre-treatment drug resistance	Acquired drug resistance
Kenya	1.1–10.93% (98–102)	7–68% (103–105)
Ethiopia	6.5–40% (106, 107)	81.8–86.7% (108, 109)
Eritrea	Data unavailable	Data unavailable
Somalia	Data unavailable	Data unavailable
Uganda	1.4–41 % (110-117)	93.8–100% (118–120)
Sudan	Data unavailable	Data unavailable
South Sudan	Data unavailable	Data unavailable
Rwanda	3.5–3.6 % (121)	9.1–95 % (122–124)

1.7.3 Southern Africa

Table 4. PDR and ADR rates for southern Africa

Country	Pre-treatment drug resistance	Therapy failure associated resistance
South Africa	4.6–9% (125-127)	52% (128)
Lesotho	Data unavailable	Data unavailable
Swaziland	9.1–41.9% (111)	Data unavailable
Mozambique	5.4–60.7% (111, 129)	10.1–93% (130–132)
Zimbabwe	23.9–74.7% (111)	6–30.6% (133, 134)
Botswana	3.5–5% (135, 136)	6.6–9.6% (136)
Namibia	7% (137)	5% (137)
Zambia	3-40.2% (138–140)	5–88% (141)
Angola	1.6–16.3% (142, 143)	Data unavailable
Malawi	<5–13.3% (144, 145)	20.3–40% (129, 146, 147)
Tanzania	2.2–82.9% (148–151)	34–90% (152–154)
Madagascar	Data unavailable	13.9% (155)
Mauritius	Data unavailable	Data unavailable
Comoros	Data unavailable	Data unavailable

1.7.4 Additional challenge for SSA to the success of ART therapy

The projected success of ARV therapy is envisioned under the UNAIDS 90-90-90 goals (156). However, there are a few additional problems that challenge the overall success of ART for Sub-Saharan Africa, and these include drug-drug interactions, adherence, limited development of research infrastructure and health care delivery systems, and increasing prevalence of diverse HIV-1 subtypes. These are briefly mentioned below:

1.7.6.1 Drug-Drug Interactions (DDI)

The treatment of opportunistic infections from bacteria and other viruses in HIV-1 infected patients compromises the effectiveness of ARV therapy via drug-drug interactions and this may lead to the emergence of drug resistance (157, 158).

This is particularly common in the Sub-Saharan region, where there are high prevalence rates of tuberculosis (TB), malaria, hepatitis and fungal infections (159). An example is the simultaneous administration of TB drugs (rifampicin – RMP, isoniazid – INH, pyrazinamide – PZA, ofloxacin, levofloxacin and moxifloxacin) with ARVs (160). Studies have shown that rifampicin is a potent inducer of CYP2B6 and CYP3A4 liver enzymes and its co-administration with ARVs, such as NVP and ATV, leads to a reduction in the plasma levels of the ARV drug (161, 162)

1.7.6.2 Adherence

Lack of drug stocks, ARV-related toxicities leading to adverse reactions and poverty characterize some of the problems that lead to non-adherence in HIV-1 infected patients in Sub-Saharan Africa (163). Access to ARVs from remote locations also plays a factor and studies have shown a relationship between the distances to nearby healthcare facilities from an HIV infected patient's residence relates to their probability to a loss- to- follow-up (LTFU) impacting their ability to access ARVs (164). None and sub-optimal adherence lead to the evolution of drug resistance and faster rates of progression towards virological failure (165). Other factors include influences of traditional medicine, faith-based healing, alcohol abuse and poverty (166) (which often leads to depression and food insecurity) are described in a meta-analysis study (167) as 'bottlenecks' for achieving high adherence in HIV-1 infected patients.

1.7.6.3 Limited infrastructure development

The development of viral load monitoring and genotypic resistance testing facilities is crucial for the success of early initiation of ARV therapy (168). However, most Sub-Saharan African countries are lagging in this endeavor. Given that there is an increasing level of HIV-1 diverse types that require efficient and timeous

detection of new HIV infections, the success and implementation of national ARV programs will largely rely on coordinated efforts of government support and expert contribution from healthcare providers (169).

1.8 HIV-1 prevalence and drug resistance in South Africa

South Africa has the largest antiretroviral program in the world. About 4.4 million individuals are receiving treatment and only 47% have achieved viral suppression below the detection limit as determined by clinical assays (170). HIV-1 subtype C is the dominating strain, but more and more circulating recombinant forms are emerging (171). HIV prevalence rate in South Africa remains one of the highest in the world and is approximately 19% for adults aged 15-49 nationally, with some remarkable differences between individual provinces in South Africa (Figure 4) (172).

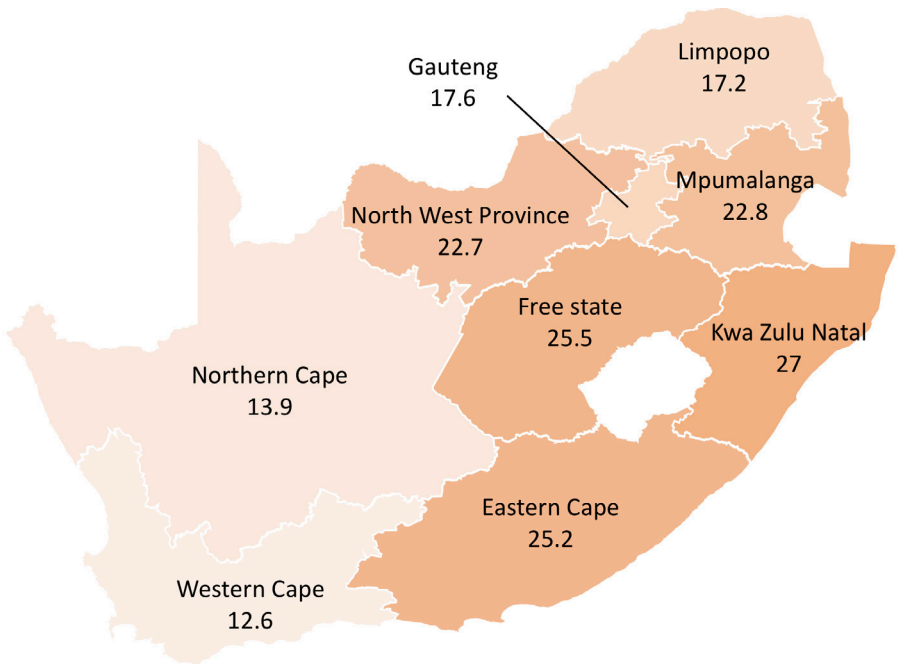

Figure 4. *HIV-1 prevalence by the province in South Africa (172)*

Pre-treatment drug resistance (PDR) has increased since the country's national ARV rollout program in 2004 and this increase, is specifically evident in HIV pregnant women receiving NNRTIs or a combination of NNRTIs and NRTIs (173).

The hardest-hit provinces in South Africa when it comes to PDR are Gauteng (162) and Eastern Cape (174), followed by Mpumalanga (175) and KwaZulu Natal (173) and lastly by Limpopo (176), Western Cape (127) and the Free State (177) (Figure 5).

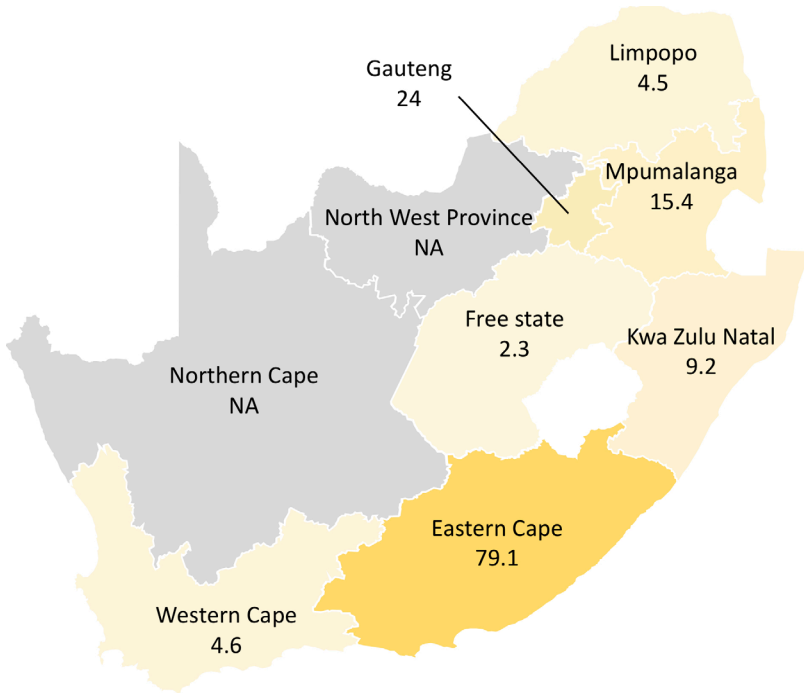

Figure 5. *HIV-1 PDR percentage rates by province in South Africa. NA = no data found/ available*

Projections for South Africa for HIV-1 infected patients requiring second-line protease inhibitor (PI)-based boosted treatment will increase to over 900000 by the year 2030 from the current estimate of about 128000 individuals that are now on PI-based therapy (178). The major challenge for managing HIV-1 patients on PI-based treatment is to make determinations for therapy failure, considering that major protease-resistant mutations are rarely present at treatment failure (179).

1.9 HIV-1 prevalence and drug resistance in Sweden

The HIV-1 burden in Sweden has reduced drastically since the introduction of combination therapy, and Sweden is the first country to reach the UNAIDS/WHO 90-90-90 goals (180). With a prevalence of less than 0.2%, Sweden is mainly dominated by HIV-1 Subtype B, though there is evidence that circulating recombinant forms are emerging and are being driven by specific transmission clusters (181). The central transmission cluster is with Men who have sex with other men (MSM) that are estimated to be around 100000 in Sweden (182).

The prevalence of HIV-1 drug resistance in Sweden remains low, but shows a significant increasing trend from less than 1.1% (183) to about 7.1 % with the majority of resistant carriers believed to be immigrants coming from SSA and MSM clusters (184). The majority of HIV-1 drug resistance is reported for patients who are using NRTI and NNRTI -based regimens and using ultra-sensitive genotypic resistance methods (107). This has led to recommendations for HIV-1 patients who have immigrated to Sweden, particularly from SSA to be initiated on PI-based regimen (184). However, the risk of virologic failure has been shown to be high in HIV non-B subtypes (185) and recommendations to initiate patients on PI regimens will still require close monitoring and frequent genotypic resistance testing in suspected cases of failure.

Overall, HIV-1 research in Sweden still has a significant role and is required to understand the dynamics of transmission of non-B HIV-1 subtypes as well as mechanisms of drug resistance that could ultimately change the HIV-1 Swedish epidemic in the foreseeable future.

The next chapter will focus on the specific role of gag and protease in affecting the development of HIV-1 resistance to ARV drugs.

2 ROLE OF GAG AND PROTEASE IN DRUG RESISTANCE

2.1 HIV-1 Group Antigen protein (Gag)

HIV-1 gag is a structural gene whose precursor (designated as gag $Pr55^{Gag}$) has a molecular weight of 55kDa and is cleaved into mature proteins – Matrix (MA), Capsid (CA), nucleocapsid proteins (NC), p6 and spacer proteins p1 and p7 by the viral protease encoded by HIV-1 pol gene (186). The Gag precursor is thought to have assembly and membrane targeting functions, whereas the mature proteins are involved in uncoating and disassembly (186). There are approximately 2000 copies of Gag in a single HIV-1 virus particle (187) that comprises about 50% of the viral mass (188).

HIV-1 resistance mechanisms to PIs have been proposed to include the development of mutations in the gag gene at protease cleavage sites (189). In total, there are 12 protease cleavage sites within the HIV genome, and 5 of them are in the *gag* gene (190, 191). Gag mutations at cleavage sites are hypothesized to cause an interaction with the active site of protease reducing the binding affinity of PIs and thereby allowing the enzyme to resume its proteolytic function (192-194). Gag cleavage site mutations reported include V128I (MA/CA); S373P,I376V (p2/NC); A431V and K436R (NC/p1) and P453A(p1/p6)(195) and are thought to increase PI resistance when occurring with primary drug resistance mutations in protease (189).

Another mechanism involves the development of *gag* mutations that act as secondary or compensatory mutations and restore viral fitness (189). One example of compensatory *gag* mutations, is a study involving the PI darunavir (DRV), the observation of gag mutations H219Q and I223V appeared to compensate for viral fitness in the presence of primary protease mutations (196). Other studies had shown that there is a minimal association of *gag* cleavage site mutations with primary resistance mutations and that *gag* non-cleavage mutations play a role (197). The most common *gag* non-cleavage site mutations characterized (mainly in the MA and CA regions) include T242N/S(198); T427D/N, E46V/K, Q369L(199); R76K, Y79F, and T81A(200). In a recent longitudinal study involving four patients from Spain who had been treated with PIs for nine years, the co-evolution of MA and CA non-cleavage site mutations was strongly associated with protease mutations (201) implying the role these mutations may have in impacting PI-based therapy.

In addition to the MA and CA regions, the C-terminal of *gag* involving the p6 has also been implicated in conferring resistance to ARVs in a similar mechanism that restores viral fitness. One example, is a study that examined the association of *gag* p6 mutations P5L/T and K27Q/N in enhancing p6 function in packaging pol molecules and reducing the potency of NRTI-based therapy against HIV CRF02_AG and subtype G (202). Further evidence of the role of *gag* p6 in resist-

ance and restoration of viral fitness is provided by studies that found amino acid motif duplications and insertions in the p6 gene.

The most common of these motifs in p6 that provide an escape mechanism for increased incorporation of RT molecules include PTAPP duplications (203, 204). More recent studies have described a PYxE insertion in *gag* p6 of HIV-1 subtype C that enhances viral replication and is associated with PI-based treatment failure (205, 206).

Overall, much remains unknown about cleavage, non-cleavage and c-terminal mutations in gag and the role they play. However, there is a strong link between *gag* and protease mutations, and this is particularly important as PIs are still used in the clinical setting and hence understanding patterns and mechanisms of resistance to PIs is crucial to ensure the management of HIV-1 patients.

2.2 HIV-1 protease

HIV-1 Protease is a homodimeric aspartic enzyme with a molecular weight of 21.6kDa that catalyzes the cleavage of Gag and Gag-Pol polyproteins (207). The catalytic site of the HIV-1 protease interacts with the protease inhibitor (PI). Mutations in the active site that lead to protease resistance against PIs are designated to be primary mutations, whereas those outside are termed to be secondary mutations (208). Primary mutations decrease the proteolytic function of protease reducing the replication capacity of the virus. Primary protease muations are affected by amino acid residues 25-32, 47-53 and 80-84 of Protease (209). Secondary or minor mutations can be pre-existing as naturally occurring polymorphisms (NOPs) or can develop after primary mutations have appeared in the presence of PI treatment (208). Most studies on the development of PI resistance have observed that secondary mutations have a compensatory effect on the fitness of protease instead of conferring resistance directly and occur with a frequency range of 40-45% for the entire Protease amino acid sequence (209). One example is a mutagenesis study the reported the impact of secondary mutations M36I and A71V when co-occurring with the primary mutation D30N to have more than 10-fold increase in resistance against the PI drugs nelfinavir and ritonavir (210).

Finally, it's unclear why specific mutations impact Protease extensively in some HIV-1 subtypes and not in others. For instance, the mutation L90M in combination with D30N and N88D had a more significant impact on increasing resistance to PI drugs in HIV-1C compared to HIV-1B (211). One explanation for this is that the plasticity of HIV-1 Protease allows for various permutations of mutations in both the primary and secondary sites to have an impact on the conformation of the active site and its hydrophobic interactions with the inhibitor (209). How this interaction differs amongst HIV -1 subtypes is a question yet to be fully answered.

The next chapter will look at some newer drugs that have the potential for use as long-acting drugs and alternatives to PIs for treatment in HIV-1 non-B subtypes.

3 NEWER ANTIRETROVIRAL DRUGS

3.1 EFdA

EFdA (4'-Ethynyl-2'-Fluoro-2' deoxyadenosine) also known as MK-8591/Islatravir is a result of a modification of one of 4'-ethynyl compounds described initially to act as NRTIs and shown to have potent activity against HIV-1, drug-resistant strains of HIV-1 (212) and more recently HIV-2 (213). Structurally, EFdA is distinct from other classical NRTIs in that it retains a 3-OH group in its ribose sugar and has an ethynyl and fluoro group (214). Chemical synthesis of EFdA has been improved over time from a yield of 2.5% to 37% (215). Currently, using less costly starting material and fewer purification steps, EFdA synthesis has an overall yield of 90% (216), allowing mass production of the drug form clinical applications.

Recent studies have confirmed that EFdA has multiple modes of HIV-1 inhibition and has been termed to be a translocation-defective reverse transcriptase inhibitor (TDRTI) based on one of its mode of inhibition. The first mode of inhibition involves the triphosphate form of EFdA (EFdA-TP) acting as a direct chain terminator, whereas the second mode involves EFdA-TP acting as a delayed chain terminator in a template sequence-dependent manner (217). The third mechanism of inhibition involves its monophosphate form (EFdA-MP) being incorporated in the primer used by HIV RT for the extension. The insertion of the monophosphate form in the primer leads to a mismatch that makes it hard for HIV RT to copy the template and to excise the monophosphate form of EFdA (217). As an attractive candidate ARV drug for clinical application, EFdA has been shown to have low toxicity and high resistance to deamination by adenosine deaminase (increasing its intracellular half-life(~17.5 to >72h)) – a characteristic that enhances its antiviral activity significantly more than other NRTIs (214). *In vitro* studies looking at the intestinal absorption and permeability of EFdA have shown that the drug mainly uses the paracellular route (characterized by compact spaces between cells and primarily used by small molecules for passive diffusion) (218). *In vivo*, EFdA has been shown to have efficacy in the gastrointestinal and female reproductive tracts of humanized bone marrow, liver and thymus mice (219). Additionally, it was also shown in rhesus macaques that EFdA had strong antiviral activity against simian immunodeficiency virus (SIV) for extended periods at sub-nanomolar concentrations (220).

EFdA has already entered human clinical trials. Phase I clinical trial evaluating the dosing and safety profiles of EFdA concluded that EFdA did not result in any adverse effects and could be tolerated even when administered up to 30mg per day (221). Phase II studies are being conducted as of the time of this writing. It is worth noting that the systematical evaluation of EFdA in comparison to NRTIs and NNRTIs has not been fully explored.

3.2 Bictegravir

WHO has recently advocated for the use of INSTIs as part of first-line therapy in low- and middle-income countries (2). It is worth noting that INSTI-based-regimens have been shown to have the same efficacy with PI-based regimens (222).

Bictegravir (BIC), a new INSTI drug was developed as a result of limitations observed with first-generation INSTI drugs Raltegravir (RAL) and Elvitegravir (EVG). These limitations include a low genetic barrier to resistance for all first-generation INSTIs, the requirement for twice-daily dosing specifically for RAL and the need for pharmacokinetic enhancement for EVG (223, 224). Problems with the second generation INSTI drug – Dolutegravir (DTG), that included drug-drug interactions, increase in patient serum creatine levels (223) and neural-tube defects in neonates (225) also encouraged the development of BIC. Structurally BIC has different substituted groups in its benzyl tail and a cross-linked bicyclic ring that reduces its chances to activate the pregnane X receptor (PXR) and lowers the risk of drug-drug interactions (224).

The mechanism of action of BIC involves blocking the active site of HIV-1 Integrase, consequently impairing its stand-transfer ability, but BIC acts as a weak inhibitor for the 3' processing activity (224). *In vitro* studies have reported BIC to have a longer half-life of dissociation from Integrase/DNA complexes compared to RAL, EVG and DTG (226). In clinical studies, the safety profile for BIC has been evaluated in a 10-day phase Ib study and it was shown that BIC was tolerated when doses of up to 100mg per day were administered and no INSTI associated mutations were observed (227). However, in a 24-week phase II study which compared the fixed-dose combination of BIC with FTC and TAF against DTG, FTC and TAF, reported an interesting observation on the long-term potential adverse effects of using BIC Though perhaps not statistically relevant one patient in the 24-week clinical trial taking BIC, FTC and TAF developed urticaria (228). However, HIV-1 prevalence and transmission resistance studies reported no clinical resistance development for BIC and have advocated for no baseline resistance testing for patients initiating BIC regimen as first-line therapy (229). Phase III clinical study of BIC containing regimen compared to DTG containing regimen indicated that BIC was not inferior to DTG and that it was equally tolerated with no adverse effects significantly related to its use (230). To date, HIV-1 subtype-specific differences for BIC have not been evaluated extensively.

3.3 Cabotegravir

Cabotegravir (CAB) (also known as GSK1265744) is an INSTI drug with the same mechanism of action as DTG and BIC. CAB is mainly degraded by uridine diphosphate glucuronosyltransferase (UGT) 1A1 and has an approximate half-life of 40 days (231).

The crystalline form of CAB has been used to develop the long-acting form that is administered subcutaneously as nanosuspension particles of the drug (231). An *in vitro* study assessed the potency of CAB against five different subtypes of HIV-1 derived from treatment-naive patients and reported a mean EC_{50} of 0.91nM (232). To add on, the previously mentioned study indicated that CAB had high EC_{50} and EC_{90} values to RAL-resistant viruses that contained G140S and Q148H integrase mutations. In contrast, it was shown in another study (233) that CAB had a lower EC_{50} compared RAL and EVG for G140S and Q148H-carrying viruses. On the other hand*, an in vivo* animal study showed that CAB long-acting administered prophylactically, protected macaques from SIV infection (234). Although a different study emphasized caution and pre-therapy testing before CAB is given as prophylaxis as in the study's SIV animal model, some macaques developed resistance to CAB and could not protect against viral infection (235).

Phase I clinical trials demonstrated that up to 30 mg of CAB could be tolerated with no adverse effects (236) and a supratherapeutic dose of up to 150mg every 12 hours given to healthy individuals, did not affect the cardiovascular system (237). Other phase 1 studies, have shown CAB had no adverse effect on the liver (238) and kidney function (239). A Phase II study reporting tolerability and pharmacokinetics of injectable CAB had a significant number of patients discontinuing treatment due to injection site reactions (ISR) (240). In the LATTE-2 phase II study, CAB was paired with RPV and proved to be as effective as EFV combined with two NRTIs (241, 242).

Regardless of support data, from clinical studies on the safety, tolerability, favorable pharmacokinetics, and antiviral efficacy of CAB, more research is required to understand the resistance mutations that can develop as a result of continued use of CAB. This will be crucial in situations where CAB long-acting-based treatment is given with other drugs treating co-infections as it is predicted that it might cause drug-drug interactions (243).

I will now present my findings for my thesis in the next chapter.

4 PRESENT FINDINGS

4.1 Thesis aims and objectives

ARV drug development and the majority of clinical trials has mainly been based on using HIV-1 subtype B (HIV-1B) infected patients. The majority of these patients are from high-income countries (HIC). The genetic and replicative capacity differences among subtypes can lead to the emergence of subtype-specific resistance mutations, which frequently also alter viral fitness (244-246). Substantial evidence supports the impact of genetic diversity on ART responses and drug-resistance pathways (247-249).

I hypothesized that HIV-1 subtype-specific differences can impact the susceptibility towards antiretroviral drugs that can affect the treatment efficacy and alter the drug resistant mutations pathways in non-B HIV-1 subtypes significantly.

The overall aim of my thesis is to investigate the potency and drug resistance susceptibility profile of clinically relevant old and new ARV drugs that can potentially be used as second-line therapy in non-B dominating countries. The specific aims include the following:

1. To understand the *ex vivo* potential of HIV-1C and non-C subtypes against PIs and type the prevalence of ADR in patients failing bPI-based therapy that is second-line treatment regimen in South Africa (**Paper I**).
2. To understand the role of PYxE-mutations that was identified by Neogi et al. (206) in viral-fitness and drug susceptibility. The PYxE (where x represents a lysine [K], a glutamine [Q] or an arginine [R]), occurs naturally in HIV-1C viruses from Eastern Africa (Ethiopia and Eretria) but occurs more in HIV-1C infections from India and South Africa (**Paper II**).
3. To investigate the virological and biochemical inhibitory potentials of a new reverse transcriptase inhibitor 4'-Ethnyl-2'-fluoro-2'-deoxyadenosine (EFdA) against broad-spectrum HIV-1 chimeric viruses of different subtypes (**Paper III**).
4. To understand the *ex vivo* antiretroviral potency of the newer INSTIs Cabotegravir and Bictegravir in non-B HIV-1 subtypes (**Paper IV**).

The studies aim to understand the potential subtype-specific differences in viral fitness and drug susceptibility against the drugs that target different stages of viral replication that includes, protease-mediated cleavage and maturation (**Paper I and II),** reverse transcription (**Paper III**) and integration (**Paper IV**) (Figure 6).

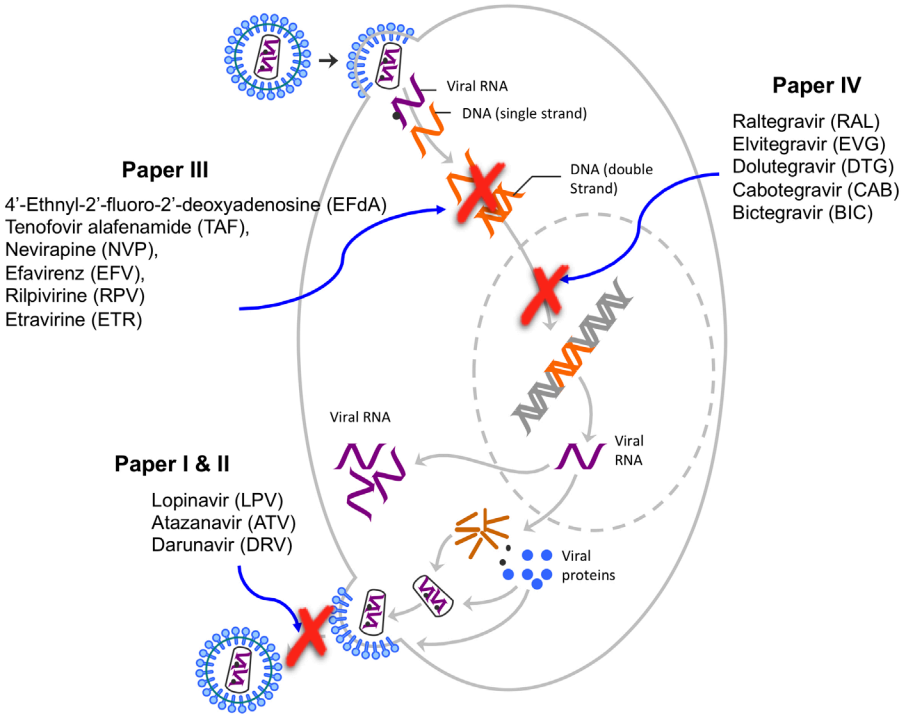

Figure 6. *Thesis overview*

4.2 Materials and methods (Papers I, II, III and IV)

4.2.1 Ethical considerations

Ethical clearance was granted by the Stellenbosch University Human research and ethics committee (HREC) under Ethics No. N15/08/71 for patient sequences used in **Paper I**. For **Paper II, III and IV** ethical clearance was granted by the Stockholm Regional Ethics Committee. The ethical permits were designated under the following numbers: 2005/1167-31/3 and 2014/928-31/2 for Paper-II; 2014/928-31/2 and 2013/1944-31/4 for Paper III; and 2006/1367-31/4 and 2014/928-31/2 for Paper IV.

4.2.2 Bioinformatics and statistical analysis

The goal of **Paper I** was to understand the acquired HIV-1 resistance patterns and prevalence in an HIV-1 pandemic dominated by HIV-1 subtype C. To this regard, data from the drug resistance Tygerberg (DRT) cohort containing HIV-1 *gag* and *protease* sequences of patients who had failed first-line and second-line treatment, were assessed for the prevalence of acquired drug resistance mutations (using the algorithms from version 8.8 of the HIV Stanford database(46)) and subtype (using

COMET HIV-1 subtype tool (250)). In **Paper II, III,** and **IV**, non- parametric statistical analysis was performed for drug sensitivity and viral growth kinetics assays using GraphPad Prism v6 and 8 (GraphPad Inc. La Jolla, USA).

4.2.3 RNA extraction and synthesis of recombinant viruses

Recombinant viruses (n=24) that were used in all papers**,** were derived from patient samples obtained from the Swedish InfCare cohort that includes patients who have migrated from other Sub-Saharan African countries (inclusive of South Africa) and Asia. Briefly, the recombinant viruses were derived by amplifying the *gag-pol* region using a previously described PCR protocol (251) and cloned into the pNL4.3 infectious vector backbone using BssHII and SalI restriction sites (Figure 7).

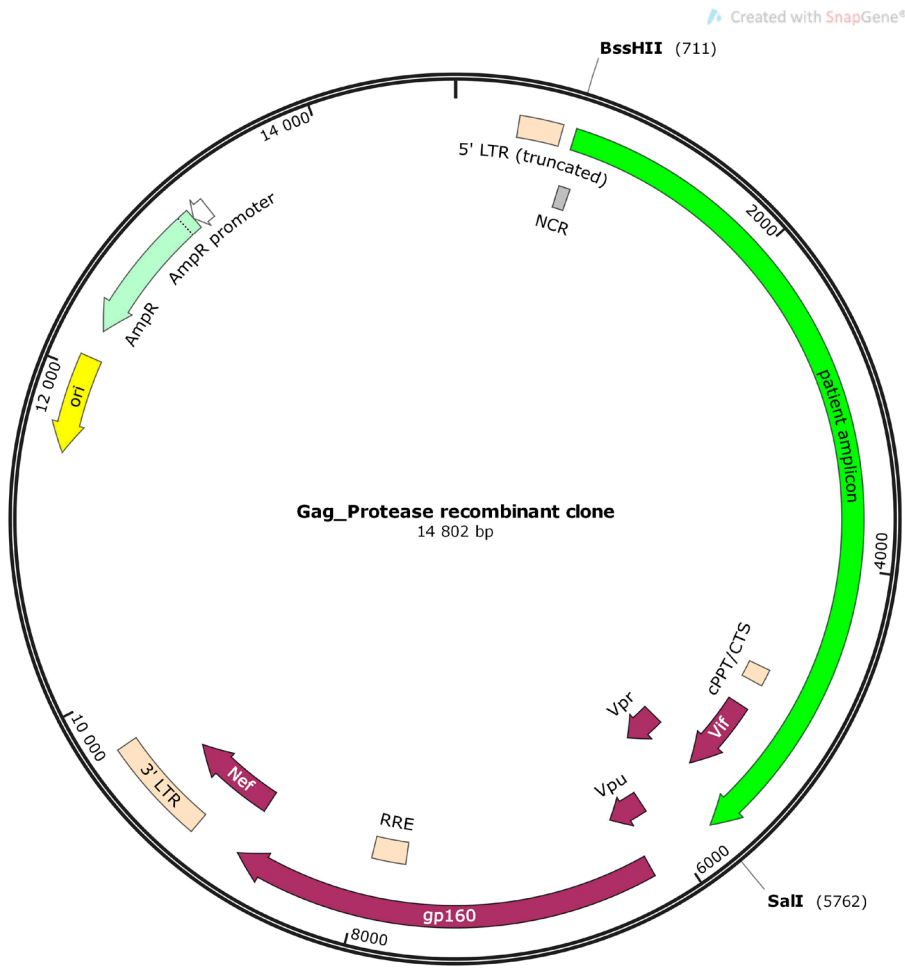

Figure 7. *Schematic of recombinant plasmids used in all papers presented in the thesis. Image generated using Snapgene software (SnapGene software from GSL Biotech; available at https://www.snapgene.com/)*

4.2.4 Sanger sequencing and analysis

Patient amplicons spanning the gag-pol of recombinant viruses from the Swedish cohort were sequenced using published primers as previously described (251). Sequence fragments were assembled using BioEdit sequence alignment editor (252), and phylogenetic analysis was done using Mega X (253).

4.2.5 Cell culture and reagents

For **Papers, I, II, III and IV,** recombinant plasmids were transfected in HEK293T cells to generate viral stocks. HEK293T cells were propagated in 10% fecal calf serum Dulbecco's modified Eagle medium (DMEM, Sigma, USA) supplemented media. TZM-bl reporter cells were also propagated and maintained with the cell culture media mentioned above. The TZM-bl reporter cell line is derived from HeLa cells and stably expresses high levels of CD4, CXCR4 and CCR5. In addition, the TZM-bl cell line has integrated copies of the luciferase gene under the control of the HIV-1 promoter gene that enables the indirect quantification of HIV-1 infection when the HIV-1 promoter is activated. To add on, a panel of NRTI and NNRTI-resistant viruses were obtained from the National Institutes of Health (AIDS reagent program, NIH, USA) and used in viral infectivity and drug sensitivity assays.

4.2.6 Viral Infectivity and drug sensitivity assay (DSA)

The following ARV drugs were purchased from Selleckchem, USA: for drugs used in the **Paper I** and **II:** ATV, DRV, and LPV; for **Paper III**: NVP, EFV, ETR, RPV and TAF. EFdA used in **Paper III** was provided by Professor Stefanos G. Sarafianos. For **Paper IV,** BIC and CAB were provided by ViiV Healthcare/GSK (Research) Triangle Park, North Carolina, USA) and Gilead Sciences (Foster City, California, USA), respectively. RAL, EVG, and DTG were purchased from Selleckchem, USA. The DSA was performed by determining the extent to which the antiretroviral drugs inhibited the replication of the reference virus (pNL4.3) and *gag-pol* derived recombinant viruses. The DSA was performed by having three technical replicates (triplicate) for each virus added on a 96-well cell culture plate. Each triplicate was added at a specified concentration within the dynamic range of the drug for the assay before the assay plate was incubated at 37˚C for 48 hours. End-point detection of the plate was done using Bright glo™ Luciferase assay kit (Promega, USA). The output at endpoint detection is relative light units (RLU) and is proportional to infectious virions produced in the DSA.The drug concentrations required for inhibiting virus replication by 50% (EC_{50}) is then calculated using the RLUs by a dose-response curve plotted using non-linear regression analysis in GraphPad Prism, version 6.07 (GraphPad Software, USA). The DSA described in all papers was performed at least three times and the output for the drug EC_{50}

results was used to compute the fold change value (FCV) for each virus relative to pNL4-3. PI DSA requires two rounds of infection in TZM-bl cells in contrast to RTI and INSTI drugs as shown in Figure 8**.**

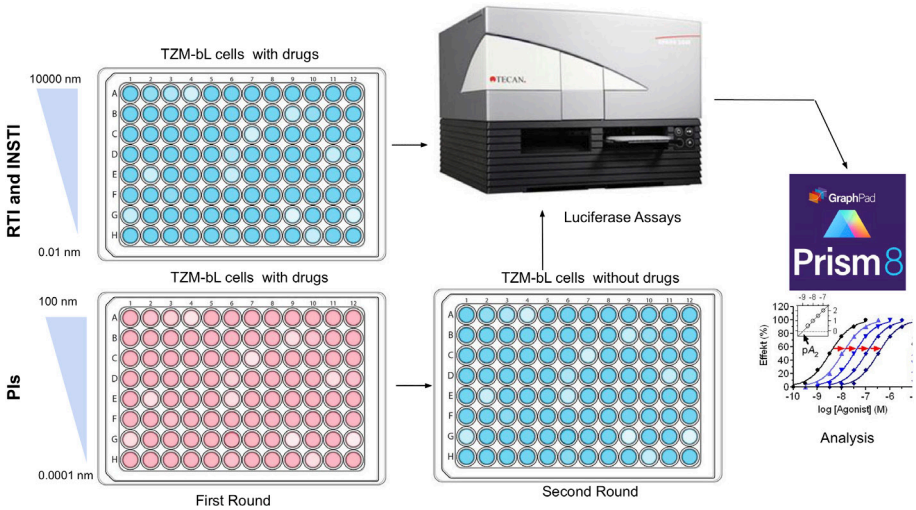

Figure 8. *Schematic representation of drug sensitivity assay (DSA).*

4.2.7 Viral growth kinetics assays (VGK)

Viral growth kinetic assay performed in **Paper II** was done using MT4-cells and the amount of p24 from supernatants collected on days 0, 3, 5- and 7-days post-infection was used as the end-point to calculate viral fitness relative to control reference virus, pNL4-3. Viral fitness was calculated using the formula described by Koval et al. (254) and graphical representation of the result was made using GraphPad Prism v6 (GraphPad Inc., La Jolla, CA, USA).

4.3 Results (Papers I, II, III and IV)

4.3.1 Paper I and II

In the **Paper I**, the prevalence of acquired drug resistance from 2006 to 2014 for South Africa for patients failing on protease inhibitor (PI) – based therapy and the phenotypic *in vitro* response to lopinavir (LPV), atazanavir (ATV) and darunavir (DRV) was evaluated. This was an additional analysis of an earlier study reported for South Africa (255). 1043 sequences from patients mainly failing on LPV/r-based therapy were analyzed for DRMs. The prevalence of PI mutations during the period was around 9%. While primary resistance mutations were detected for each class, the most frequent mutations were for PI – M46I, 154V, and V82A; for NRTI – M184V and for NNRTI – V106M, K103N, G190A and E138A (Figure 9).

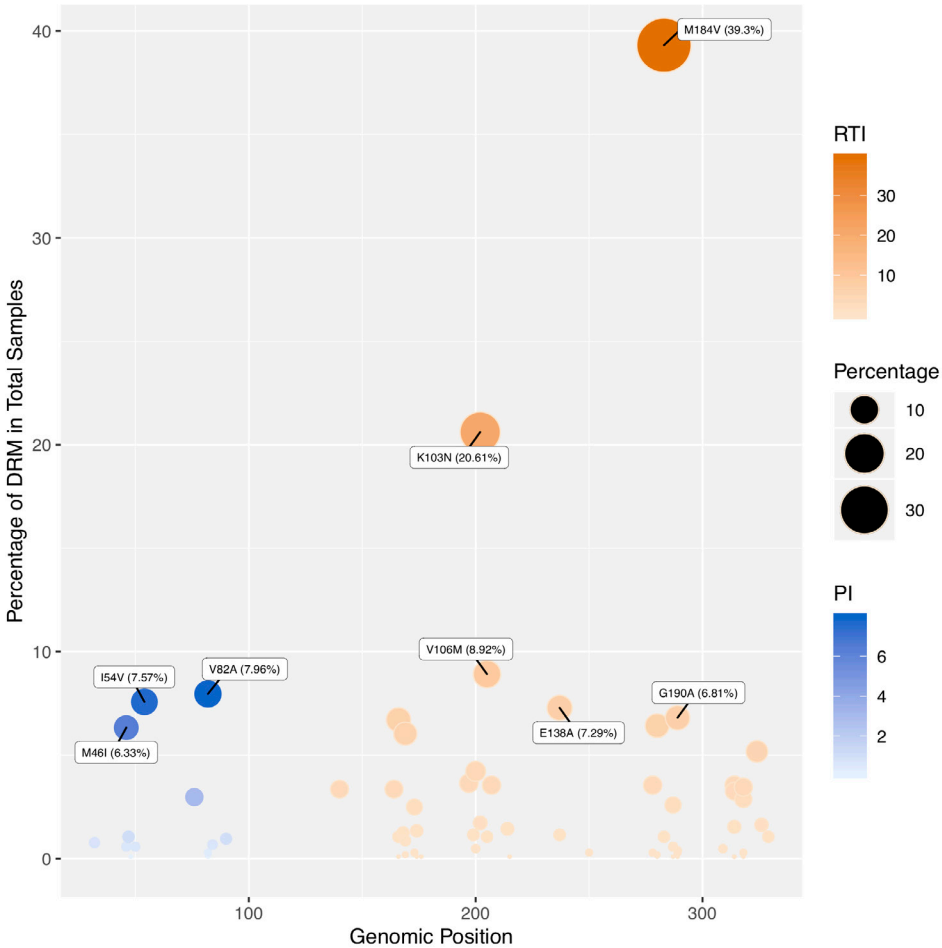

Figure 9. *Prevalence of ADR muations from PI-based therapy failure patients from South African*

The PI phenotypic DSA included HIV-1C derived chimeric viruses (n=13) compared to non-C (B =5 and A-like = 4 viruses; total n= 9) viruses representing patient samples from the Swedish InfCare database. Among the HIV-1C strains 62% (8/13) were from patients got infected in SSA including counties South Africa, Burundi, Ethiopia, Somalia and Kenya) The samples from the Swedish InfCare have diverse country origins and the phylogenetic relationship of the viruses is indicated in Figure 10.

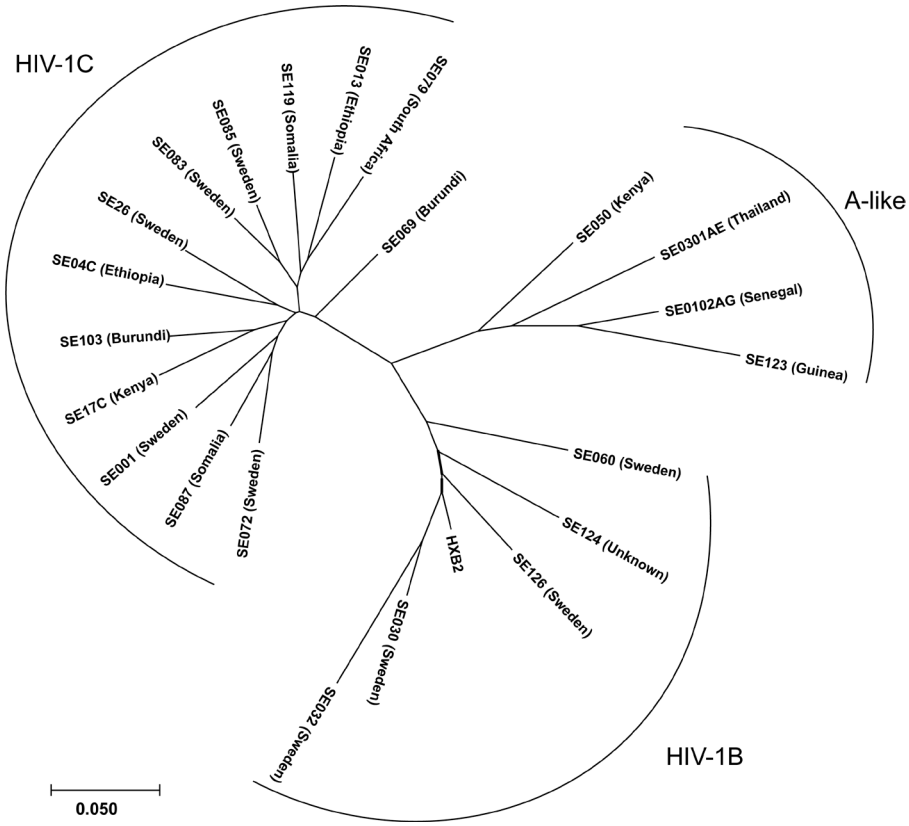

Figure 10. *Chimeric viruses used for PI DSA study. The prefix (SE =Sweden) indicates the origin of the recombinant virus and in bracket self-reported country of infection*

Phenotypic drug sensitivity results obtained in **Paper I** indicated that there were no subtype-specific differences found in HIV-1C and non-C viruses for LPV (p=0.948), ATV (p =0.067) and DRV (p=0.651). However, some HIV-1C viruses indicated a reduced response to all PI drugs (**Figure 11**). HIV drug resistance and sequence summary for chemeric viruses used in the PI DSA using HIV drug resistance algorithms from the Stanford HIV Database indicated no major and accessory mutations except for two HIV-1C viruses that have accessory PI mutations (K20T, G73C).

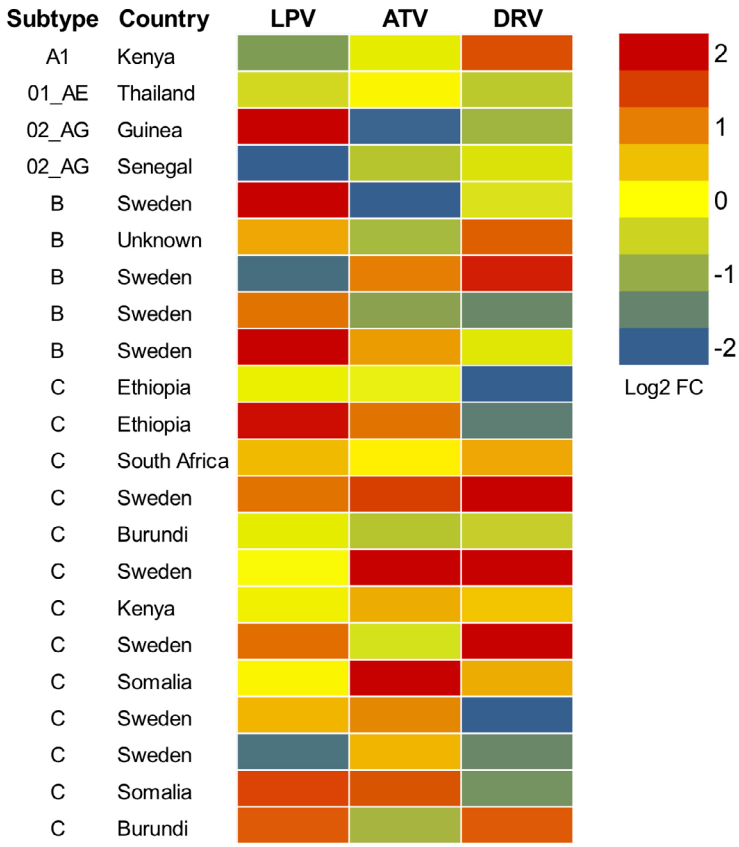

Figure 11. *Heat-map for chimeric viruses used in the Paper I showing DSA profile of LPV, ATV and DRV*

Given that chimeric viruses used in the **Paper I** was derived from treatment-naive patients whose viral protease gene did not contain major and accessory mutations it was reasonable to hypothesize that HIV-1 gag could influence the response to PI drugs. Hence, in **Paper II,** the gag gene was investigated,

In **Paper II**, the gag-p6 region in HIV-1C recombinant viruses was investigated to assess its impact on viral fitness. The gag-p6 late domain region of gag interacts with the host cell protein ALG-2-interacting protein X (ALIX) to facilitate active transport of gag to the internal surface of the cell membrane during HIV viral assembly and budding (17).

It was previously shown that HIV-1C has a unique PYxE (x being Arginine (R) or lysine (K) or glutamine (Q) amino acid residue) motif insertion in its gag-p6 gene where the YPx_nL motif has been naturally deleted in HIV-1C viruses and that the

PYxE motif is associated with a reduction in PI-based therapeutic response in some HIV-1C infected patients (206). In **Paper II,** the prevalence of PYKE in patient viral sequences from three HIV-1 cohorts (Swedish, German and Ethiopian) was higher than PYQE (~2 fold). In addition, clinical data from the Swedish cohort looking into patients specifically infected with HIV-1C revealed that the viral load and the $CD4^+$ T-cell count were higher and lower respectively, in patients carrying PYxE viruses compared to the wild-type (WT) HIV-1C. *In silico* simulation and co-localization experiments performed in **Paper II** aimed to previse and show the interaction of ALIX and gag (PYxE). The interaction with ALIX was especially enhanced with the presence of the PYxE motif compared to HIV-1C WT. Furthermore, in **Paper II,** it was shown that there is a high binding affinity between ALIX and gag (PYxE), and this affinity correlated well with the observed increased replication capacity of recombinant HIV-1C containing the PYxE motif when compared with non-C viruses. More specifically, viral growth differences were noted for HIV-1C viruses with PYKE, PYQE, no PYxE (i.e. WT) and when compared with the reference HIV-1B WT. Chimeric viruses with PTAPP duplication (PTAPPd) were also included in the assessment as this had been previously shown to impact fitness (256). The order of increased fitness was WT HIV-1B > PYKE > PYQE > PTAPPd> HIV-1C WT. In addition, the drug sensitivity profile of HIV-1C viruses containing PYKE and PYQE had reduced susceptibility to LPV. One of the PYKE viruses also displayed reduced sensitivity to TAF. All the viruses displayed high to medium susceptibility to NNRTIs and INSTIs tested (EC_{50} fold change <2).

4.3.2 Paper III

The results obtained in Paper I and Paper II indicated that HIV-1 subtype-specific differences could play a role in influencing ARV drug susceptibility. Hence the goal of **Paper III** was to understand and describe any HIV-1 subtype-specific differences to the response of clinically approved and newer ARV drugs. The specific aim investigated the *ex vivo* potency of the novel drug EFdA in comparison with first and second-generation NNRTIs – NVP, EFV and ETR, RPV respectively. In addition, TAF was also included as an approximate comparator of the same class to EFdA. The results obtained indicated that EFdA had a superior *ex vivo* potency compared to all other drugs used in the study in inhibiting all the recombinant viruses (n=24) tested independent of HIV-1 subtype. EFdA also inhibited RTI resistant viruses (n=9) better than TAF. Independent subtype inhibition of EFdA, was also confirmed by steady-state kinetic experiments that showed no difference in the incorporation efficiency of different phosphorylated forms of EFdA by different HIV-1 reverse transcriptase (RT) subtypes (01_AE, 02_AG, C and B). HIV-1 subtype representation for recombinant viruses used in DSA experiments included HIV-1B (n=6),) and HIV-1 A-like (02AG, 01_AE and A1 (n=4)) and HIV-1C (n=14). However, DSA data showed HIV-1C to have a significantly reduced susceptibility to RPV and ETR ($p = 0.017$ and $p= 0.004$ respectively).

The *in vitro* DSA data correlated well with the biochemical experiments performed in the study. More specifically, biochemical data was able to demonstrate molecular differences of the inhibitory potential of NNRTI drugs mentioned above, against different HIV-1 RT subtypes. HIV-1C RT was also shown to have reduced susceptibility to ETR and RPV compared to other HIV-1 non-C subtype RTs.

4.3.3 Paper IV

The observation of subtype-specific differences in Paper III with the use of drugs from the RTI class made it rational to continue the investigation of subtype-specific differences in the INSTI class. This was the primary goal of **Paper IV**. Recombinant viruses (n=24) described in Paper III were used to investigate the *in vitro* drug sensitivity of first (RAL, EVG) and second-generation (DTG, CAB, BIC) INSTI drugs. The DSA results obtained indicated that second-generation INSTIs have a subtype-independent *in vitro* potency except for DTG, which indicated to be more potent in non-B compared to HIV-1B subtypes. To add on, DTG, BIC, and CAB performed well in inhibiting A-like viruses compared to HIV-1C and B Subtypes. However, HIV-1C and HIV 02_AG had a significantly reduced susceptibility against first-generation drug EVG ($p<0.05$). RAL displayed the least potency against all subtypes tested. DSA data also compared very well with integrase strand-transfer activity biochemical experiments.

The secondary goal in **Paper IV** investigated the pattern of INSTI primary and acquired drug resistance mutations. To this regard, INSTI naïve (n=270) integrase sequences [representing HIV subtypes B (n=92), 01_AE (n=25), C (n=82), A1/A2 (n=22), CRFs (n=21), 02_AG (n=15) and other pure subtypes (n=13)] and INSTI experienced (n=96) individuals from a patient-cohort (collected from Swedish InfCare) were analyzed. Results in INSTI naïve patients indicated the frequency of INSTI primary mutations to be <1% (1/270) and INSTI accessory mutations to be 6.3% (17/270). In addition, the M50I polymorphic mutation was observed in 18% (49/270) of INSTI naïve patients. However, a prevalence of 24% (23/96) primary INSTI mutations was observed in INSTI-experienced patients. The most common mutations observed were N155H, Y143G/R, T66S, E92Q, Q148R, S147G, E138K, G140A/S. Lastly, there was no observable difference between non-B and HIV-1B subtypes in the occurrence of drug-resistant mutations.

5 DISCUSSION

HIV-1 continues to be a burden in low- and middle-income countries (LMIC) that are mainly infected with non-B HIV-1 subtypes. Regardless of the scaling-up of ARV rollout programs, efforts to contain the pandemic through early detection and virological monitoring of infected patients have not stopped the trend in increasing acquired drug resistance (ADR) (2, 257). The combined results of **Paper I, II, III, IV** make a persuasive argument to support the hypothesis proposed in this thesis that subtype-specific differences impact the outcomes of ARV therapy and that the impact is mainly detrimental to individuals infected with HIV-1 non-B subtypes.

In the **Paper I,** it was demonstrated that there is an increase in the prevalence of acquired drug resistance in South Africa from the period 2006 and 2014 and this is consistent with earlier reports for LMIC (257, 258). In addition, DSA results from the recombinant viruses (derived from the gag-pol gene of predominantly HIV-1C and non-C infected patients from the Swedish InfCare cohort)**,** indicate a wide range of PI susceptibility specifically for HIV-1C viruses and this agrees very well with what has been previously reported (259). The reduced sensitivity of some HIV-1C (though not significant) compared to non-C viruses for ATV and DRV in contrast to LPV could have multifactorial reasons. For Instance, it could be linked to the mechanism of binding. To explain further, even though most PIs function by binding in the active site of protease, DRV is an example of an exception. DRV has a unique mechanism of action of binding to protease that is not common for other PIs and this mechanism proposes that DRV can also bind on the surface of one of the enzymes' flexible flaps in the protease dimer (260). Notwithstanding the facts that DRV has a high binding affinity to HIV protease (approximately 100-fold than most PIs) (261), a high genetic barrier to resistance than most PIs (262) and that clinical studies have also shown the superiority of DRV against LPV (263) and ATV (264), there is evidence that DRV can be compromised by single mutations. For example, it has been shown that a single mutation in protease V32I can compromise the antiviral activity of DRV substantially (265). In addition, *in vitro* selection experiments have demonstrated the development of gag mutations that could affect DRV antiviral activity (196). Hence there might be mechanisms of drug resistance that could be specific to either DRV, ATV and LPV. Since viruses used in **Paper I,** lacked primary mutations in the protease gene and were derived from treatment-naïve patients, it may be rational to assume that naturally occurring polymorphisms (NOPs) or mutation(s) in HIV-1C gag could cause phenotypic resistance to PIs in a manner not yet investigated. Furthermore, it can be assumed that potential gag mutation(s) may not show cross-resistance to PI drugs and be specific in acting on the unique mechanisms each PI may have in binding to protease.

Paper II continued with the logic to speculate on the role that gag might play in influencing PI-based therapeutic response**.** The observed results for the replication fitness of HIV-1C viruses containing the PYxE motif in gag-p6 region compared to the wild- type (WT) HIV-1C reinforces the potential importance of gag. Other studies have shown similar results (205, 266)

In **Paper III**, EFdA was investigated with other NNRTIs and TAF for their *in vitro* potency and subtype-specific response. The broad and subtype-independent activity of EFdA was demonstrated compared to other drugs and EFdA can be recommended as a strong candidate for a long-acting drug that can be administered to HIV-1 patients. Proof of concept for the long-acting drug potential of EFdA has already been confirmed by a recent report (267) that demonstrated a reduction of viral load in animals injected with an implant of EFdA for more than six months. Results from **Paper III** give optimism to the future of ARV therapy for non-B subtypes. Considering the challenges HIV-1 infected patients face in adherence to existing clinical drugs (268), the potential of long-act formulations could revolutionize the progress of ARV therapy. However, RPV and ETR results were not encouraging for their use in an HIV-1C dominated setting. To add on, RPV already has a long-acting coformulation with INSTI drug – CAB, that has already entered clinical trials (269) and it is worth mentioning that the success of the long-acting formulation in a subtype C dominated setting will require close monitoring of HIV-1C patients. DRMs, even for those known to cause a reduction in fitness of HIV-1 viruses when treated with RPV, can be compensated by single mutations that evolve rapidly, leading to the emergence of fit RPV-resistant viruses. For instance, it is already known, that the single mutation E138A/K (which reduces the fitness of RPV-resistant HIV-1C viruses) can be compensated by the development of N348I mutation in the connection domain of RT leading to a E138A/K fit resistant virus (270). Hence regimens containing RPV may not be suitable in an HIV-1C dominated setting.

To conclude, the World Health Organisation (WHO) has recommended the use of INSTIs as part of the regimen for first-line therapy in LMIC (2). Results from **Paper IV**, suggest that second-generation INSTIs are a better option for non-B subtypes. However, the occurrence of naturally occurring polymorphisms (NOPs), such as M50I, could compromise the efficacy of second-generation INSTIs when they occur with known mutations, such as R263K, as recently shown (271). The observation of NOPs in HIV-1C integrase that have the potential to impact the efficacy of INSTIs was also reported recently for integrase sequences analyzed from South Africa (272). However, more mechanistic studies are needed to demonstrate the association of NOPs with major INSTI mutations and evaluate the potency of INSTIs in treating HIV-1 non-B subtypes.

6 CONCLUSION AND RECOMMENDATIONS

The following conclusions and recommendations can be drawn from findings in this thesis:

- The prevalence of acquired drug resistance in South Africa is increasing, and more specifically, the prevalence is increasing for NNRTI and NRTI mutations. However, the majority of patients who failed on PI-based second-line treatment did not have any PI DRMs. Therefore it is recommended that patients receiving PI-based regimens should be monitored closely for adherence.
- Mutations in the gene or changes in the gag-p6 region could significantly limit the options of second-line treatment involving boosted PI in the presence or absence of known protease mutations. More research, involving mechanistic studies is recommended to identify the role of gag mutations or naturally occurring polymorphisms (NOPs) that affect PI susceptibility. Once the mutations are established, genotypic testing assays can be expanded to include the gag gene.
- EFdA is a strong candidate to inhibit non-B subtypes and a good candidate as a long-acting drug to overcome problems associated with adherence that can hamper the success of ARV therapy and lead to the emergence of drug resistance. Furthermore, it is recommended that the use of RPV-based regimens in an HIV-1C dominated setting should be closely monitored and possibly avoided in patients who are in high-risk groups, such people who inject drugs (PWID), men who have sex with other men (MSM) and sex workers, where there is increased risk of transmitting resistant viruses.
- Finally, it can be concluded that second-generation INSTIs have a high inhibitory potential than first-generation INSTI drugs and, more specifically, in non-B subtypes (such as A-like viruses). However, it is recommended that routine monitoring and adherence support be given to INSTI HIV-1 patients for the surveillance of NOPs that could synergize with know INSTI mutations and lead to the emergence of resistance.

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8 REFERENCES

1. Cortez KJ, Maldarelli F. Clinical management of HIV drug resistance. Viruses. 2011;3(4):347-78.
2. Organization WH. WHO | HIV drug resistance report 2019. WHO. 2019.
3. Lederman MM, Rodriguez B, S. S. Immunopathogenesis of HIV infection. University of Califonia, San Francisco2004.
4. Haseltine WA. Molecular biology of the human immunodeficiency virus type 1. Faseb j. 1991;5(10):2349-60.
5. Joshi S, Joshi RL. Molecular biology of human immunodeficiency virus type-1. Transfus Sci. 1996;17(3):351-78.
6. Zeichner SL. The molecular biology of HIV. Insights into pathogenesis and targets for therapy. Clin Perinatol. 1994;21(1):39-73.
7. Laskey SB, Siliciano RF. A mechanistic theory to explain the efficacy of antiretroviral therapy. Nature Reviews Microbiology. 2014;12:772.
8. Dhurat R, Manglani M, Sharma R, Shah NK. Clinical spectrum of HIV infection. Indian Pediatr. 2000;37(8):831-6.
9. Mayer KH, Anderson DJ. Heterosexual HIV transmission. Infect Agents Dis. 1995;4(4):273-84.
10. Programs NRCUaIoMUPoNEaBD, Normand J, Vlahov D, Moses LE. The Epidemiology of HIV and AIDS. 1995.
11. Bour S, Geleziunas R, Wainberg MA. The human immunodeficiency virus type 1 (HIV-1) CD4 receptor and its central role in promotion of HIV-1 infection. Microbiol Rev. 1995;59(1):63-93.
12. Bleul CC, Wu L, Hoxie JA, Springer TA, Mackay CR. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. Proc Natl Acad Sci U S A. 1997;94(5):1925-30.
13. Wilen CB, Tilton JC, Doms RW. HIV: cell binding and entry. Cold Spring Harb Perspect Med. 2012;2(8).
14. Kotova S, Li M, Dimitriadis EK, Craigie R. Nucleoprotein intermediates in HIV-1 DNA integration visualized by atomic force microscopy. J Mol Biol. 2010;399(3):491-500.
15. Craigie R. The molecular biology of HIV integrase. Future virology. 2012;7(7):679-86.

16. Karn J, Stoltzfus CM. Transcriptional and posttranscriptional regulation of HIV-1 gene expression. Cold Spring Harb Perspect Med. 2012;2(2):a006916.

17. Meng B, Ip NC, Prestwood LJ, Abbink TE, Lever AM. Evidence that the endosomal sorting complex required for transport-II (ESCRT-II) is required for efficient human immunodeficiency virus-1 (HIV-1) production. Retrovirology. 2015;12:72.

18. Mothes W, Sherer NM, Jin J, Zhong P. Virus Cell-to-Cell Transmission. Journal of Virology. 2010;84(17):8360.

19. De Boer RJ, Ribeiro RM, Perelson AS. Current estimates for HIV-1 production imply rapid viral clearance in lymphoid tissues. PLoS Comput Biol. 2010;6(9):e1000906.

20. Lee GQ, Lichterfeld M. Diversity of HIV-1 reservoirs in CD4+ T-cell subpopulations. Curr Opin HIV AIDS. 2016;11(4):383-7.

21. Dahabieh MS, Battivelli E, Verdin E. Understanding HIV latency: the road to an HIV cure. Annual review of medicine. 2015;66:407-21.

22. U.S Federal drug Agency (FDA). FDA-Approved HIV Medicines Understanding HIV/AIDS. @AIDSinfo; 2019 [cited 2019 2 August]. Available from: https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/21/58/fda-approved-hiv-medicines.

23. Russel FG, Koenderink JB, Masereeuw R. Multidrug resistance protein 4 (MRP4/ABCC4): a versatile efflux transporter for drugs and signalling molecules. Trends Pharmacol Sci. 2008;29(4):200-7.

24. Hurwitz SJ, Schinazi RF. Practical Considerations For Developing Nucleoside Reverse Transcriptase Inhibitors. Drug discovery today Technologies. 2012;9(3):e183-e93.

25. De Clercq E. Antiviral therapy for human immunodeficiency virus infections. Clinical Microbiology Reviews. 1995;8(2):200.

26. Sluis-Cremer N, Tachedjian G. Mechanisms of inhibition of HIV replication by non-nucleoside reverse transcriptase inhibitors. Virus Res. 2008;134(1-2):147-56.

27. Usach I, Melis V, Peris JE. Non-nucleoside reverse transcriptase inhibitors: a review on pharmacokinetics, pharmacodynamics, safety and tolerability. J Int AIDS Soc. 2013;16:1-14.

28. Figueiredo A, Moore KL, Mak J, Sluis-Cremer N, de Bethune MP, Tachedjian G. Potent nonnucleoside reverse transcriptase inhibitors target HIV-1 Gag-Pol. PLoS Pathog. 2006;2(11):e119.

29. He Y, Xia DN, Li QX, Tao JS, Gan Y, Wang C. Enhancement of cellular uptake, transport and oral absorption of protease inhibitor saquinavir by nanocrystal formulation. Acta Pharmacol Sin. 2015;36(9):1151-60.

30. Lefebvre E, Schiffer CA. Resilience to resistance of HIV-1 protease inhibitors: profile of darunavir. AIDS reviews. 2008;10(3):131-42.

31. Adamson CS. Protease-Mediated Maturation of HIV: Inhibitors of Protease and the Maturation Process. Mol Biol Int. 2012;2012:604261.

32. Mouscadet J-F, Tchertanov L. Raltegravir: molecular basis of its mechanism of action. European journal of medical research. 2009;14 Suppl 3(Suppl 3):5-16.

33. Anstett K, Brenner B, Mesplede T, Wainberg MA. HIV drug resistance against strand transfer integrase inhibitors. Retrovirology. 2017;14:16.

34. Marchand C, Zhang X, Pais GC, Cowansage K, Neamati N, Burke TR, Jr., et al. Structural determinants for HIV-1 integrase inhibition by beta-diketo acids. J Biol Chem. 2002;277(15):12596-603.

35. Hardy H, Skolnik PR. Enfuvirtide, a new fusion inhibitor for therapy of human immunodeficiency virus infection. Pharmacotherapy. 2004;24(2):198-211.

36. Manfredi R, Sabbatani S. A novel antiretroviral class (fusion inhibitors) in the management of HIV infection. Present features and future perspectives of enfuvirtide (T-20). Curr Med Chem. 2006;13(20):2369-84.

37. Briz V, Poveda E, Soriano V. HIV entry inhibitors: mechanisms of action and resistance pathways. J Antimicrob Chemother. 2006;57(4):619-27.

38. Cuevas JM, Geller R, Garijo R, Lopez-Aldeguer J, Sanjuan R. Extremely High Mutation Rate of HIV-1 In Vivo. PLoS Biol. 2015;13(9):e1002251.

39. Hu WS, Hughes SH. HIV-1 reverse transcription. Cold Spring Harb Perspect Med. 2012;2(10).

40. Tang MW, Shafer RW. HIV-1 antiretroviral resistance: scientific principles and clinical applications. Drugs. 2012;72(9):e1-25.

41. Kouyos RD, Fouchet D, Bonhoeffer S. Recombination and drug resistance in HIV: population dynamics and stochasticity. Epidemics. 2009;1(1):58-69.

42. Domingo E, Sheldon J, Perales C. Viral quasispecies evolution. Microbiol Mol Biol Rev. 2012;76(2):159-216.

43. Hemelaar J, Gouws E, Ghys PD, Osmanov S. Global trends in molecular epidemiology of HIV-1 during 2000-2007. Aids. 2011;25(5):679-89.

44. Mayers DL SJ, Ouellette M, Kaye KS, Marchaim D. Antimicrobial Drug Resistance – Clinical and Epidemiological Aspects, Volume 2 | Douglas L. Mayers | Springer2019.

45. Sarafianos SG, Marchand B, Das K, Himmel DM, Parniak MA, Hughes SH, et al. Structure and function of HIV-1 reverse transcriptase: molecular mechanisms of polymerization and inhibition. J Mol Biol. 2009;385(3):693-713.

46. Rhee SY, Gonzales MJ, Kantor R, Betts BJ, Ravela J, Shafer RW. Human immunodeficiency virus reverse transcriptase and protease sequence database. Nucleic Acids Res. 2003;31(1):298-303.

47. Mackie N. Resistance to non-nucleoside reverse transcriptase inhibitors. In: Geretti AM, editor. Antiretroviral Resistance in Clinical Practice. London: Mediscript.

48. Lai M-T, Munshi V, Lu M, Feng M, Hrin-Solt R, McKenna PM, et al. Mechanistic Study of Common Non-Nucleoside Reverse Transcriptase Inhibitor-Resistant Mutations with K103N and Y181C Substitutions. Viruses. 2016;8(10):263.

49. Barber TJ, Harrison L, Asboe D, Williams I, Kirk S, Gilson R, et al. Frequency and patterns of protease gene resistance mutations in HIV-infected patients treated with lopinavir/ritonavir as their first protease inhibitor. J Antimicrob Chemother. 2012;67(4):995-1000.

50. Maarseveen Nv, Boucher C. Resistance to protease inhibitors [Text]: Mediscript; 2006. Available from: https://www.ncbi.nlm.nih.gov/pubmed/.

51. Ghosh AK, Osswald HL, Prato G. Recent Progress in the Development of HIV-1 Protease Inhibitors for the Treatment of HIV/AIDS. J Med Chem. 2016;59(11):5172-208.

52. Patick AK, Potts KE. Protease inhibitors as antiviral agents. Clin Microbiol Rev. 1998;11(4):614-27.

53. Pommier Y, Johnson AA, Marchand C. Integrase inhibitors to treat HIV/AIDS. Nat Rev Drug Discov. 2005;4(3):236-48.

54. Mesplede T, Quashie PK, Wainberg MA. Resistance to HIV integrase inhibitors. Curr Opin HIV AIDS. 2012;7(5):401-8.

55. Dow DE, Bartlett JA. Dolutegravir, the Second-Generation of Integrase Strand Transfer Inhibitors (INSTIs) for the Treatment of HIV. Infect Dis Ther. 2014;3(2):83-102.

56. Thierry E, Deprez E, Delelis O. Different Pathways Leading to Integrase Inhibitors Resistance. Front Microbiol. 2016;7:2165.

57. Malet I, Subra F, Charpentier C, Collin G, Descamps D, Calvez V, et al. Mutations Located outside the Integrase Gene Can Confer Resistance to HIV-1 Integrase Strand Transfer Inhibitors. MBio. 2017;8(5).

58. Kuritzkes DR. Drug resistance in HIV-1. Current opinion in virology. 2011;1(6):582-9.

59. Pennings PS. HIV Drug Resistance: Problems and Perspectives. Infectious disease reports. 2013;5(Suppl 1):e5-e.

60. Steegen K, Bronze M, Papathanasopoulos MA, van Zyl G, Goedhals D, Van Vuuren C, et al. Prevalence of Antiretroviral Drug Resistance in Patients Who Are Not Responding to Protease Inhibitor-Based Treatment: Results From the First National Survey in South Africa. J Infect Dis. 2016;214(12):1826-30.

61. Prosperi MCF, Rosen-Zvi M, Altmann A, Zazzi M, Di Giambenedetto S, Kaiser R, et al. Antiretroviral therapy optimisation without genotype resistance testing: a perspective on treatment history based models. PloS one. 2010;5(10):e13753-e.

62. Jespersen S, Hønge BL, Oliveira I, Medina C, da Silva Té D, Correia FG, et al. Challenges facing HIV treatment in Guinea-Bissau: the benefits of international research collaborations. Bulletin of the World Health Organization. 2014;92(12):909-14.

63. Wainberg MA, Brenner BG. The Impact of HIV Genetic Polymorphisms and Subtype Differences on the Occurrence of Resistance to Antiretroviral Drugs. Mol Biol Int. 2012;2012:256982.

64. Villabona-Arenas CJ, Vidal N, Guichet E, Serrano L, Delaporte E, Gascuel O, et al. In-depth analysis of HIV-1 drug resistance mutations in HIV-infected individuals failing first-line regimens in West and Central Africa. Aids. 2016;30(17):2577-89.

65. Guichet E, Aghokeng A, Serrano L, Bado G, Toure-Kane C, Eymard-Duvernay S, et al. Short Communication: High Viral Load and Multidrug Resistance Due to Late Switch to Second-Line Regimens Could Be a Major Obstacle to Reach the 90-90-90 UNAIDS Objectives in Sub-Saharan Africa. AIDS Res Hum Retroviruses. 2016;32(12):1159-62.

66. Chamberland A, Montreal Uo, Diabaté S, Montreal Uo, Sylla M, Montreal Uo, et al. Transmission of HIV-1 drug resistance in Benin could jeopardise future treatment options. Sexually Transmitted Infections. 2019;88(3):179-83.

67. Jerome CS, Agonnoude M, Sopoh GE, Bah-Chabi AI, de Souza A, Bachabi M, et al. Sociodemographic, lifestyle and therapeutic predictors of 2- year survival in HIV-infected persons receiving antiretroviral therapy in Benin. J Public Health Afr. 2017;8(1):651.

68. Oliveira V, Bartolo I, Borrego P, Rocha C, Valadas E, Barreto J, et al. Genetic diversity and drug resistance profiles in HIV type 1- and HIV type 2-infected patients from Cape Verde Islands. AIDS Res Hum Retroviruses. 2012;28(5):510-22.

69. de P-A, II, Guimaraes ML, Bello G, Vicente AC, Morgado MG. Profile of the HIV epidemic in Cape Verde: molecular epidemiology and drug resistance mutations among HIV-1 and HIV-2 infected patients from distinct islands of the archipelago. PLoS One. 2014;9(4):e96201.

70. Charpentier C, Gody JC, Tisserand P, Matta M, Pere H, Fournier J, et al. Surveillance of antiretroviral drug resistance mutations in untreated young children living in the Central African Republic. Antivir Ther. 2011;16(8):1347-50.

71. Charpentier C, Gody JC, Mbitikon O, Moussa S, Matta M, Pere H, et al. Virological response and resistance profiles after 18 to 30 months of first- or second-/third-line antiretroviral treatment: a cross-sectional evaluation in HIV type 1-infected children living in the Central African Republic. AIDS Res Hum Retroviruses. 2012;28(1):87-94.

72. Pere H, Charpentier C, Mbelesso P, Dandy M, Matta M, Moussa S, et al. Virological response and resistance profiles after 24 months of first-line antiretroviral treatment in adults living in Bangui, Central African Republic. AIDS Res Hum Retroviruses. 2012;28(4):315-23.

73. Liégeois F, Vella C, Eymard-Duvernay S, Sica J, Makosso L, Mouinga-Ondémé A, et al. Virological failure rates and HIV-1 drug resistance patterns in patients on first-line antiretroviral treatment in semirural and rural Gabon. Journal of the International AIDS Society. 2012;15(2):17985-.

74. Nii-Trebi NI, Ibe S, Barnor JS, Ishikawa K, Brandful JAM, Ofori SB, et al. HIV-1 Drug-Resistance Surveillance among Treatment-Experienced and -Naïve Patients after the Implementation of Antiretroviral Therapy in Ghana. PLOS ONE. 2013;8(8):e71972.

75. Charpentier C, Bellecave P, Cisse M, Mamadou S, Diakite M, Peytavin G, et al. High prevalence of antiretroviral drug resistance among HIV-1-untreated patients in Guinea-Conakry and in Niger. Antivir Ther. 2011;16(3):429-33.

76. Lavu E, Kave E, Mosoro E, Markby J, Aleksic E, Gare J, et al. High Levels of Transmitted HIV Drug Resistance in a Study in Papua New Guinea. PLoS One. 2017;12(2):e0170265.

77. Alvarez P, Fernández McPhee C, Prieto L, Martín L, Obiang J, Avedillo P, et al. HIV-1 Variants and Drug Resistance in Pregnant Women from Bata (Equatorial Guinea): 2012-2013. PloS one. 2016;11(10):e0165333-e.

78. Gare J, Ryan CE, David M, Timbi D, Kaima P, Kombati Z, et al. Presence of HIV drug resistance in antiretroviral therapy-naive and -experienced patients from Papua New Guinea. J Antimicrob Chemother. 2014;69(8):2183-6.

79. Crowell CS, Maiga AI, Sylla M, Taiwo B, Kone N, Oron AP, et al. High Rates of Baseline Drug Resistance and Virologic Failure Among ART-naive HIV-infected Children in Mali. The Pediatric infectious disease journal. 2017;36(11):e258-e63.

80. Maiga AI, Fofana DB, Maiga AC, Diallo F, Ait-Arkoub Z, Daou F, et al. Transmitted antiretroviral drug resistance in newly HIV-infected and untreated patients in Ségou and Bamako, Mali. AIDS research and human retroviruses. 2013;29(1):182-6.

81. Diouara AA, Ndiaye HD, Guindo I, Bangoura N, Cisse M, Edmond T, et al. Antiretroviral treatment outcome in HIV-1-infected patients routinely followed up in capital cities and remote areas of Senegal, Mali and Guinea-Conakry. J Int AIDS Soc. 2014;17:19315.

82. Germanaud D, Derache A, Traore M, Madec Y, Toure S, Dicko F, et al. Level of viral load and antiretroviral resistance after 6 months of non-nucleoside reverse transcriptase inhibitor first-line treatment in HIV-1-infected children in Mali. J Antimicrob Chemother. 2010;65(1):118-24.

83. Fofana DB, Soulie C, Balde A, Lambert-Niclot S, Sylla M, Ait-Arkoub Z, et al. High level of HIV-1 resistance in patients failing long-term first-line antiretroviral therapy in Mali. J Antimicrob Chemother. 2014;69(9):2531-5.

84. Fall-Malick FZ, Tchiakpe E, Ould Soufiane S, Diop-Ndiaye H, Mouhamedoune Baye A, Ould Horma Babana A, et al. Drug resistance mutations and genetic diversity in adults treated for HIV type 1 infection in Mauritania. J Med Virol. 2014;86(3):404-10.

85. de Truchis P, Le MP, Daou M, Madougou B, Nouhou Y, Moussa Saley S, et al. High efficacy of first-line ART in a West African cohort, assessed by dried blood spot virological and pharmacological measurements. J Antimicrob Chemother. 2016;71(11):3222-7.

86. Boerma RS, Boender TS, Sigaloff KC, Rinke de Wit TF, van Hensbroek MB, Ndembi N, et al. High levels of pre-treatment HIV drug resistance and treatment failure in Nigerian children. J Int AIDS Soc. 2016;19(1):21140.

87. Imade GE, Sagay AS, Chaplin B, Chebu P, Musa J, Okpokwu J, et al. Short communication: Transmitted HIV drug resistance in antiretroviral-naive pregnant women in north central Nigeria. AIDS Res Hum Retroviruses. 2014;30(2):127-33.

88. Rottinghaus EK, Ugbena R, Diallo K, Bassey O, Azeez A, Devos J, et al. Dried blood spot specimens are a suitable alternative sample type for HIV-1 viral load measurement and drug resistance genotyping in patients receiving first-line antiretroviral therapy. Clin Infect Dis. 2012;54(8):1187-95.

89. Hamers RL, Wallis CL, Kityo C, Siwale M, Mandaliya K, Conradie F, et al. HIV-1 drug resistance in antiretroviral-naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study. Lancet Infect Dis. 2011;11(10):750-9.

90. Crawford KW, Wakabi S, Kibuuka H, Magala F, Keshinro B, Okoye I, et al. Short communication: east meets west: a description of HIV-1 drug resistance mutation patterns of patients failing first line therapy in PEPFAR clinics from Uganda and Nigeria. AIDS Res Hum Retroviruses. 2014;30(8):796-9.

91. Babajide K, Ayemoba O, Terfa K, Ake J, Crowell TA, Adamu Y, et al. Virological Suppression and Patterns of Resistance Amongst Patients on Antiretroviral Therapy at 4 Nigerian Military Hospitals. Curr HIV Res. 2017;15(2):146-51.

92. Chaplin B, Imade G, Onwuamah C, Odaibo G, Audu R, Okpokwu J, et al. Distinct Pattern of Thymidine Analogue Mutations with K65R in Patients Failing Tenofovir-Based Antiretroviral Therapy. AIDS Res Hum Retroviruses. 2018;34(2):228-33.

93. Salou M, Butel C, Konou AA, Ekouevi DK, Vidal N, Dossim S, et al. High Rates of Drug Resistance Among Newly Diagnosed HIV-infected Children in the National Prevention of Mother-to-child Transmission Program in Togo. Pediatr Infect Dis J. 2016;35(8):879-85.

94. Konou AA, Salou M, Vidal N, Kodah P, Kombate D, Kpanla P, et al. Virological outcome among HIV-1 infected patients on first-line antiretroviral treatment in semi-rural HIV clinics in Togo. AIDS Res Ther. 2015;12:38.

95. Jespersen S, Tolstrup M, Honge BL, Medina C, Te Dda S, Ellermann-Eriksen S, et al. High level of HIV-1 drug resistance among patients with HIV-1 and HIV-1/2 dual infections in Guinea-Bissau. Virol J. 2015;12:41.

96. Loubet P, Charpentier C, Visseaux B, Nuta C, Adu E, Chapplain JM, et al. Short communication: Prevalence of HIV-1 transmitted drug resistance in Liberia. AIDS Res Hum Retroviruses. 2014;30(9):863-6.

97. Loubet P, Charpentier C, Visseaux B, Borbor A, Nuta C, Adu E, et al. Prevalence of HIV-1 drug resistance among patients failing first-line ART in Monrovia, Liberia: a cross-sectional study. J Antimicrob Chemother. 2015;70(6):1881-4.

98. Silverman RA, Beck IA, Kiptinness C, Levine M, Milne R, McGrath CJ, et al. Prevalence of Pre-antiretroviral-Treatment Drug Resistance by Gender, Age, and Other Factors in HIV-Infected Individuals Initiating Therapy in Kenya, 2013-2014. J Infect Dis. 2017;216(12):1569-78.

99. Chung MH, Silverman R, Beck IA, Yatich N, Dross S, McKernan-Mullin J, et al. Increasing HIV-1 pretreatment drug resistance among antiretroviral-naive adults initiating treatment between 2006 and 2014 in Nairobi, Kenya. Aids. 2016;30(10):1680-2.

100. Onywera H, Maman D, Inzaule S, Auma E, Were K, Fredrick H, et al. Surveillance of HIV-1 pol transmitted drug resistance in acutely and recently infected antiretroviral drug-naive persons in rural western Kenya. PLoS One. 2017;12(2):e0171124.

101. Hassan AS, Mwaringa SM, Obonyo CA, Nabwera HM, Sanders EJ, Rinke de Wit TF, et al. Low prevalence of transmitted HIV type 1 drug resistance among antiretroviral-naive adults in a rural HIV clinic in Kenya. AIDS research and human retroviruses. 2013;29(1):129-35.

102. Sigaloff KC, Mandaliya K, Hamers RL, Otieno F, Jao IM, Lyagoba F, et al. Short communication: High prevalence of transmitted antiretroviral drug resistance among newly HIV type 1 diagnosed adults in Mombasa, Kenya. AIDS Res Hum Retroviruses. 2012;28(9):1033-7.

103. Kiptoo M, Brooks J, Lihana RW, Sandstrom P, Ng'ang'a Z, Kinyua J, et al. HIV-1 drug resistance-associated mutations among HIV-1 infected drug-naïve antenatal clinic attendees in rural Kenya. BMC infectious diseases. 2013;13:517-.

104. Hassan AS, Nabwera HM, Mwaringa SM, Obonyo CA, Sanders EJ, Rinke de Wit TF, et al. HIV-1 virologic failure and acquired drug resistance among first-line antiretroviral experienced adults at a rural HIV clinic in coastal Kenya: a cross-sectional study. AIDS Res Ther. 2014;11(1):9.

105. Kantor R, DeLong A, Balamane M, Schreier L, Lloyd RM, Jr., Injera W, et al. HIV diversity and drug resistance from plasma and non-plasma analytes in a large treatment programme in western Kenya. Journal of the International AIDS Society. 2014;17(1):19262-.

106. Huruy K, Maier M, Mulu A, Liebert UG. Limited increase in primary HIV-1C drug resistance mutations in treatment naive individuals in Ethiopia. J Med Virol. 2015;87(6):978-84.

107. Ekici H, Amogne W, Aderaye G, Lindquist L, Sonnerborg A, Abdurahman S. Minority drug-resistant HIV-1 variants in treatment naive East-African and Caucasian patients detected by allele-specific real-time PCR. PLoS One. 2014;9(10):e111042.

108. Mulu A, Maier M, Liebert UG. Low Incidence of HIV-1C Acquired Drug Resistance 10 Years after Roll-Out of Antiretroviral Therapy in Ethiopia: A Prospective Cohort Study. PLoS One. 2015;10(10):e0141318.

109. Abdissa A, Yilma D, Fonager J, Audelin AM, Christensen LH, Olsen MF, et al. Drug resistance in HIV patients with virological failure or slow virological response to antiretroviral therapy in Ethiopia. BMC infectious diseases. 2014;14:181-.

110. Kityo C, Boerma RS, Sigaloff KCE, Kaudha E, Calis JCJ, Musiime V, et al. Pretreatment HIV drug resistance results in virological failure and accumulation of additional resistance mutations in Ugandan children. J Antimicrob Chemother. 2017;72(9):2587-95.

111. Jordan MR, Penazzato M, Cournil A, Vubil A, Jani I, Hunt G, et al. Human Immunodeficiency Virus (HIV) Drug Resistance in African Infants and Young Children Newly Diagnosed With HIV: A Multicountry Analysis. Clin Infect Dis. 2017;65(12):2018-25.

112. Reynolds SJ, Ssempijja V, Galiwango R, Ndyanabo A, Nakigozi G, Lyagoba F, et al. Low Rates of Transmitted Drug Resistance Among Newly Identified HIV-1 Seroconverters in Rural Rakai, Uganda. AIDS Res Hum Retroviruses. 2017;33(5):448-51.

113. Lee GQ, Bangsberg DR, Muzoora C, Boum Y, Oyugi JH, Emenyonu N, et al. Prevalence and virologic consequences of transmitted HIV-1 drug resistance in Uganda. AIDS Res Hum Retroviruses. 2014;30(9):896-906.

114. Nazziwa J, Njai HF, Ndembi N, Birungi J, Lyagoba F, Gershim A, et al. Short communication: HIV type 1 transmitted drug resistance and evidence of transmission clusters among recently infected antiretroviral-naive individuals from Ugandan fishing communities of Lake Victoria. AIDS Res Hum Retroviruses. 2013;29(5):788-95.

115. Ssemwanga D, Kapaata A, Lyagoba F, Magambo B, Nanyonjo M, Mayanja BN, et al. Low drug resistance levels among drug-naive individuals with recent HIV type 1 infection in a rural clinical cohort in southwestern Uganda. AIDS Res Hum Retroviruses. 2012;28(12):1784-7.

116. Ssemwanga D, Ndembi N, Lyagoba F, Magambo B, Kapaata A, Bukenya J, et al. Transmitted antiretroviral drug resistance among drug-naive female sex workers with recent infection in Kampala, Uganda. Clin Infect Dis. 2012;54 Suppl 4:S339-42.

117. Ndembi N, Hamers RL, Sigaloff KC, Lyagoba F, Magambo B, Nanteza B, et al. Transmitted antiretroviral drug resistance among newly HIV-1 diagnosed young individuals in Kampala. Aids. 2011;25(7):905-10.

118. Towler WI, Barlow-Mosha L, Church JD, Bagenda D, Ajuna P, Mubiru M, et al. Analysis of drug resistance in children receiving antiretroviral therapy for treatment of HIV-1 infection in Uganda. AIDS research and human retroviruses. 2010;26(5):563-8.

119. von Braun A, Sekaggya-Wiltshire C, Scherrer AU, Magambo B, Kambugu A, Fehr J, et al. Early virological failure and HIV drug resistance in Ugandan adults co-infected with tuberculosis. AIDS Res Ther. 2017;14(1):1.

120. Sigaloff KC, Kayiwa J, Musiime V, Calis JC, Kaudha E, Mukuye A, et al. Short communication: high rates of thymidine analogue mutations and dual-class resistance among HIV-infected Ugandan children failing first-line antiretroviral therapy. AIDS Res Hum Retroviruses. 2013;29(6):925-30.

121. Ndahimana J, Riedel DJ, Mwumvaneza M, Sebuhoro D, Uwimbabazi JC, Kubwimana M, et al. Drug resistance mutations after the first 12 months on antiretroviral therapy and determinants of virological failure in Rwanda. Trop Med Int Health. 2016;21(7):928-35.

122. Rusine J, Asiimwe-Kateera B, van de Wijgert J, Boer KR, Mukantwali E, Karita E, et al. Low primary and secondary HIV drug-resistance after 12 months of antiretroviral therapy in human immune-deficiency virus type 1 (HIV-1)-infected individuals from Kigali, Rwanda. PLoS One. 2013;8(8):e64345.

123. Ndahimana J, Riedel DJ, Muhayimpundu R, Nsanzimana S, Niyibizi G, Mutaganzwa E, et al. HIV drug resistance mutations among patients failing second-line antiretroviral therapy in Rwanda. Antivir Ther. 2016;21(3):253-9.

124. Mutwa PR, Boer KR, Rusine J, Muganga N, Tuyishimire D, Schuurman R, et al. Long-term effectiveness of combination antiretroviral therapy and prevalence of HIV drug resistance in HIV-1-infected children and adolescents in Rwanda. Pediatr Infect Dis J. 2014;33(1):63-9.

125. Steegen K, Carmona S, Bronze M, Papathanasopoulos MA, van Zyl G, Goedhals D, et al. Moderate Levels of Pre-Treatment HIV-1 Antiretroviral Drug Resistance Detected in the First South African National Survey. PLoS One. 2016;11(12):e0166305.

126. Manasa J, Danaviah S, Lessells R, Elshareef M, Tanser F, Wilkinson E, et al. Increasing HIV-1 Drug Resistance Between 2010 and 2012 in Adults Participating in Population-Based HIV Surveillance in Rural KwaZulu-Natal, South Africa. AIDS Res Hum Retroviruses. 2016;32(8):763-9.

127. Jacobs GB, Wilkinson E, Isaacs S, Spies G, de Oliveira T, Seedat S, et al. HIV-1 subtypes B and C unique recombinant forms (URFs) and transmitted drug resistance identified in the Western Cape Province, South Africa. PLoS One. 2014;9(6):e90845.

128. Kanthula R, Rossouw TM, Feucht UD, van Dyk G, Beck IA, Silverman R, et al. Persistence of HIV drug resistance among South African children given nevirapine to prevent mother-to-child-transmission. Aids. 2017;31(8):1143-8.

129. Mancinelli S, Galluzzo CM, Andreotti M, Liotta G, Jere H, Sagno JB, et al. Virological Response and Drug Resistance 1 and 2 Years Post-Partum in HIV-Infected Women Initiated on Life-Long Antiretroviral Therapy in Malawi. AIDS Res Hum Retroviruses. 2016;32(8):737-42.

130. Vaz P, Augusto O, Bila D, Macassa E, Vubil A, Jani IV, et al. Surveillance of HIV drug resistance in children receiving antiretroviral therapy: a pilot study of the World Health Organization's generic protocol in Maputo, Mozambique. Clin Infect Dis. 2012;54 Suppl 4:S369-74.

131. De Luca A, Sidumo ZJ, Zanelli G, Magid NA, Luhanga R, Brambilla D, et al. Accumulation of HIV-1 drug resistance in patients on a standard thymidine analogue-based first line antiretroviral therapy after virological failure: implications for the activity of next-line regimens from a longitudinal study in Mozambique. BMC Infect Dis. 2017;17(1):605.

132. Tilghman M, Tsai D, Buene TP, Tomas M, Amade S, Gehlbach D, et al. Pooled Nucleic Acid Testing to Detect Antiretroviral Treatment Failure in HIV-Infected Patients in Mozambique. J Acquir Immune Defic Syndr. 2015;70(3):256-61.

133. Chimukangara B, Manasa J, Mitchell R, Nyabadza G, Katzenstein D, Masimirembwa C. Community Based Antiretroviral Treatment in Rural Zimbabwe. AIDS Res Hum Retroviruses. 2017;33(12):1185-91.

134. Makadzange AT, Higgins-Biddle M, Chimukangara B, Birri R, Gordon M, Mahlanza T, et al. Clinical, Virologic, Immunologic Outcomes and Emerging HIV Drug Resistance Patterns in Children and Adolescents in Public ART Care in Zimbabwe. PLoS One. 2015;10(12):e0144057.

135. Rowley CF, MacLeod IJ, Maruapula D, Lekoko B, Gaseitsiwe S, Mine M, et al. Sharp increase in rates of HIV transmitted drug resistance at antenatal clinics in Botswana demonstrates the need for routine surveillance. J Antimicrob Chemother. 2016;71(5):1361-6.

136. Bussmann H, de la Hoz Gomez F, Roels TH, Wester CW, Bodika SM, Moyo S, et al. Prevalence of transmitted HIV drug resistance in Botswana: lessons learned from the HIVDR-Threshold Survey conducted among women presenting for routine antenatal care as part of the 2007 national sentinel survey. AIDS Res Hum Retroviruses. 2011;27(4):365-72.

137. Hong SY, Jonas A, DeKlerk M, Shiningavamwe A, Desta T, Badi A, et al. Population-based surveillance of HIV drug resistance emerging on treatment and associated factors at sentinel antiretroviral therapy sites in Namibia. J Acquir Immune Defic Syndr. 2015;68(4):463-71.

138. Poppe LK, Chunda-Liyoka C, Kwon EH, Gondwe C, West JT, Kankasa C, et al. HIV drug resistance in infants increases with changing prevention of mother-to-child transmission regimens. Aids. 2017;31(13):1885-9.

139. Price MA, Wallis CL, Lakhi S, Karita E, Kamali A, Anzala O, et al. Transmitted HIV type 1 drug resistance among individuals with recent HIV infection in East and Southern Africa. AIDS Res Hum Retroviruses. 2011;27(1):5-12.

140. Hamers RL, Siwale M, Wallis CL, Labib M, van Hasselt R, Stevens WS, et al. HIV-1 drug resistance mutations are present in six percent of persons initiating antiretroviral therapy in Lusaka, Zambia. J Acquir Immune Defic Syndr. 2010;55(1):95-101.

141. Seu L, Mulenga LB, Siwingwa M, Sikazwe I, Lambwe N, Guffey MB, et al. Characterization of HIV drug resistance mutations among patients failing first-line antiretroviral therapy from a tertiary referral center in Lusaka, Zambia. J Med Virol. 2015;87(7):1149-57.

142. Bartolo I, Zakovic S, Martin F, Palladino C, Carvalho P, Camacho R, et al. HIV-1 diversity, transmission dynamics and primary drug resistance in Angola. PLoS One. 2014;9(12):e113626.

143. Afonso JM, Bello G, Guimaraes ML, Sojka M, Morgado MG. HIV-1 genetic diversity and transmitted drug resistance mutations among patients from the North, Central and South regions of Angola. PLoS One. 2012;7(8):e42996.

144. Wadonda-Kabondo N, Banda R, Moyo K, M'Bang'ombe M, Chiwaula M, Porter C, et al. Prevalence of transmitted HIV drug resistance among newly diagnosed antiretroviral therapy-naive pregnant women in Lilongwe and Blantyre, Malawi. Clin Infect Dis. 2012;54 Suppl 4:S324-7.

145. Bansode VB, Travers SA, Crampin AC, Ngwira B, French N, Glynn JR, et al. Reverse transcriptase drug resistance mutations in HIV-1 subtype C infected patients on ART in Karonga District, Malawi. AIDS Res Ther. 2011;8(1):38.

146. Hosseinipour M, Nelson JAE, Trapence C, Rutstein SE, Kasende F, Kayoyo V, et al. Viral Suppression and HIV Drug Resistance at 6 Months Among Women in Malawi's Option B+ Program: Results From the PURE Malawi Study. J Acquir Immune Defic Syndr. 2017;75 Suppl 2:S149-s55.

147. Luebbert J, Tweya H, Phiri S, Chaweza T, Mwafilaso J, Hosseinipour MC, et al. Virological failure and drug resistance in patients on antiretroviral therapy after treatment interruption in Lilongwe, Malawi. Clin Infect Dis. 2012;55(3):441-8.

148. Vairo F, Nicastri E, Liuzzi G, Chaula Z, Nguhuni B, Bevilacqua N, et al. HIV-1 drug resistance in recently HIV-infected pregnant mother's naive to anti-retroviral therapy in Dodoma urban, Tanzania. BMC Infect Dis. 2013;13:439.

149. Hawkins C, Ulenga N, Liu E, Aboud S, Mugusi F, Chalamilla G, et al. HIV virological failure and drug resistance in a cohort of Tanzanian HIV-infected adults. J Antimicrob Chemother. 2016;71(7):1966-74.

150. Meini G, Balestrieri M, Cianchino S, Tacconi D, Rossi de Gasperis M, Concato C, et al. Frequent detection of antiretroviral drug resistance in HIV-1-infected orphaned children followed at a donor-funded rural pediatric clinic in Dodoma, Tanzania. AIDS Res Hum Retroviruses. 2015;31(4):448-51.

151. Fogel J, Li Q, Taha TE, Hoover DR, Kumwenda NI, Mofenson LM, et al. Initiation of antiretroviral treatment in women after delivery can induce multiclass drug resistance in breastfeeding HIV-infected infants. Clin Infect Dis. 2011;52(8):1069-76.

152. Muri L, Gamell A, Ntamatungiro AJ, Glass TR, Luwanda LB, Battegay M, et al. Development of HIV drug resistance and therapeutic failure in children and adolescents in rural Tanzania: an emerging public health concern. Aids. 2017;31(1):61-70.

153. Ngarina M, Kilewo C, Karlsson K, Aboud S, Karlsson A, Marrone G, et al. Virologic and immunologic failure, drug resistance and mortality during the first 24 months postpartum among HIV-infected women initiated on antiretroviral therapy for life in the Mitra plus Study, Dar es Salaam, Tanzania. BMC Infect Dis. 2015;15:175.

154. Shao ER, Kifaro EG, Chilumba IB, Nyombi BM, Moyo S, Gaseitsiwe S, et al. HIV-1 drug mutations in children from northern Tanzania. J Antimicrob Chemother. 2014;69(7):1928-32.

155. Andriantsimietry SH, Razanakolona RL. Importance of Madagascar's HIV/AIDS reference laboratory. Med Mal Infect. 2016;46(3):146-9.

156. UNAIDS. 90–90–90 – An ambitious treatment target to help end the AIDS epidemic. 2017 [cited 2019 2 August]. Available from: https://www.unaids.org/en/resources/documents/2017/90-90-90.

157. Kumar S, Rao PS, Earla R, Kumar A. Drug-drug interactions between antiretroviral therapies and drugs of abuse in HIV systems. Expert Opin Drug Metab Toxicol. 2015;11(3):343-55.

158. Foy M, Sperati CJ, Lucas GM, Estrella MM. Drug interactions and antiretroviral drug monitoring. Curr HIV/AIDS Rep. 2014;11(3):212-22.

159. Low A, Gavriilidis G, Larke N, MR BL, Drouin O, Stover J, et al. Incidence of Opportunistic Infections and the Impact of Antiretroviral Therapy Among HIV-Infected Adults in Low- and Middle-Income Countries: A Systematic Review and Meta-analysis. Clin Infect Dis. 2016;62(12):1595-603.

160. Rabie H, Frigati L, Hesseling AC, Garcia-Prats AJ. Tuberculosis: opportunities and challenges for the 90-90-90 targets in HIV-infected children. J Int AIDS Soc. 2015;18(Suppl 6):20236.

161. Ribera E, Pou L, Lopez RM, Crespo M, Falco V, Ocana I, et al. Pharmacokinetic interaction between nevirapine and rifampicin in HIV-infected patients with tuberculosis. J Acquir Immune Defic Syndr. 2001;28(5):450-3.

162. Mallolas J, Sarasa M, Nomdedeu M, Soriano A, Lopez-Pua Y, Blanco JL, et al. Pharmacokinetic interaction between rifampicin and ritonavir-boosted atazanavir in HIV-infected patients. HIV Med. 2007;8(2):131-4.

163. Shubber Z, Mills EJ, Nachega JB, Vreeman R, Freitas M, Bock P, et al. Patient-Reported Barriers to Adherence to Antiretroviral Therapy: A Systematic Review and Meta-Analysis. PLoS Med. 2016;13(11):e1002183.

164. Lankowski AJ, Siedner MJ, Bangsberg DR, Tsai AC. Impact of geographic and transportation-related barriers on HIV outcomes in sub-Saharan Africa: a systematic review. AIDS Behav. 2014;18(7):1199-223.

165. Nachega JB, Marconi VC, van Zyl GU, Gardner EM, Preiser W, Hong SY, et al. HIV treatment adherence, drug resistance, virologic failure: evolving concepts. Infect Disord Drug Targets. 2011;11(2):167-74.

166. Chop E, Duggaraju A, Malley A, Burke V, Caldas S, Yeh PT, et al. Food insecurity, sexual risk behavior, and adherence to antiretroviral therapy among women living with HIV: A systematic review. Health Care Women Int. 2017;38(9):927-44.

167. Moshabela M, Bukenya D, Darong G, Wamoyi J, McLean E, Skovdal M, et al. Traditional healers, faith healers and medical practitioners: the contribution of medical pluralism to bottlenecks along the cascade of care for HIV/AIDS in Eastern and Southern Africa. Sex Transm Infect. 2017;93(Suppl 3).

168. Lahuerta M, Ue F, Hoffman S, Elul B, Kulkarni SG, Wu Y, et al. The problem of late ART initiation in Sub-Saharan Africa: a transient aspect of scale-up or a long-term phenomenon? J Health Care Poor Underserved. 2013;24(1):359-83.

169. Nansseu JR, Bigna JJ. Antiretroviral therapy related adverse effects: Can sub-Saharan Africa cope with the new "test and treat" policy of the World Health Organization? Infect Dis Poverty. 2017;6(1):24.

170. UNAIDS. Country Factsheets, South Africa. 2017 [cited 2019 7 May]. Available from: http://www.unaids.org/en/regionscountries/countries/southafrica.

171. Jacobs GB, Wilkinson E, Isaacs S, Spies G, de Oliveira T, Seedat S, et al. HIV-1 Subtypes B and C Unique Recombinant Forms (URFs) and Transmitted Drug Resistance Identified in the Western Cape Province, South Africa. Plos One. 2014;9(3):14.

172. Kanabus A. HIV statistics for South Africa | Prevalence, incidence, ARVs. 2019 [cited 2019 2 August]. Available from: https://www.tbfacts.org/hiv-statistics-south-africa/.

173. Chimukangara B, benjiechim@yahoo.com, KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP) DoLMMS, University of KwaZulu-Natal, Durban, South Africa, Centre for the AIDS Programme of Research in South Africa (CAPRISA) DDMRI, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa, Department of Virology NHLS, University of KwaZulu-Natal, Durban, South Africa, Lessells RJ, et al. Trends in Pretreatment HIV-1 Drug Resistance in Antiretroviral Therapy-naive Adults in South Africa, 2000–2016: A Pooled Sequence Analysis. EClinicalMedicine. 2019;9:26-34.

174. Adeniyi OV, Obi CL, Ter Goon D, Iweriebor B, Ajayi AI, Lambert J, et al. HIV-1 drug resistance surveillance among parturient women on anti-retroviral therapy in the Eastern Cape, South Africa: Implications for elimination of mother-to-child transmission. Journal of Clinical Virology. 2019;117:89-95.

175. Msimanga PW, Vardas E, Engelbrecht S. HIV-1 diversity in an antiretroviral treatment naive cohort from Bushbuckridge, Mpumalanga Province, South Africa. Virology Journal. 2015;12:13.

176. Iweriebor BC, Mavhandu LG, Masebe T, Rekosh D, Hammarskjold ML, Mphahlele JM, et al. Molecular epidemiology of HIV in two highly endemic areas of northeastern South Africa. Archives of Virology. 2012;157(3):455-65.

177. Huang KHG, Goedhals D, Fryer H, van Vuuren C, Katzourakis A, De Oliveira T, et al. Prevalence of HIV type-1 drug-associated mutations in pre-therapy patients in the Free State, South Africa. Antiviral Therapy. 2009;14(7):975-84.

178. Estill J, Ford N, Salazar-Vizcaya L, Haas AD, Blaser N, Habiyambere V, et al. The need for second-line antiretroviral therapy in adults in sub-Saharan Africa up to 2030: a mathematical modelling study. Lancet HIV. 2016;3(3):e132-9.

179. Sutherland KA, Goodall RL, McCormick A, Kapaata A, Lyagoba F, Kaleebu P, et al. Gag-Protease Sequence Evolution Following Protease Inhibitor Monotherapy Treatment Failure in HIV-1 Viruses Circulating in East Africa. Aids Research and Human Retroviruses. 2015;31(10):1032-7.

180. Gisslén M, Svedhem V, Lindborg L, Flamholc L, Norrgren H, Wendahl S, et al. Sweden, the first country to achieve the Joint United Nations Programme on HIV/AIDS (UNAIDS)/World Health Organization (WHO) 90-90-90 continuum of HIV care targets. HIV Medicine. 2017;18(4):305-7.

181. Esbjornsson J, Mild M, Audelin A, Fonager J, Skar H, Jorgensen LB, et al. HIV-1 transmission between MSM and heterosexuals, and increasing proportions of circulating recombinant forms in the Nordic Countries. Virus Evolution. 2016;2(1):10.

182. UNAIDS. Country factsheets: Sweden|2018. 2019 [cited 2019 6 August]. Available from: https://www.unaids.org/en/regionscountries/countries/sweden.

183. Bontell I, Haggblom A, Bratt G, Albert J, Sonnerborg A. Trends in antiretroviral therapy and prevalence of HIV drug resistance mutations in Sweden 1997-2011. PLoS One. 2013;8(3):e59337.

184. Andersson EN, Flamholc A, Gisslén L, Norrgren MHB, Svedhem H, Wendahl V, et al. A. Increasing prevalence of transmitted drug resistance mutations in patients diagnosed with HIV in Sweden 2010–2014. In. 2015.

185. Häggblom A, Svedhem V, Singh K, Sönnerborg A, Neogi U. Virological failure in patients with HIV-1 subtype C receiving antiretroviral therapy: an analysis of a prospective national cohort in Sweden. The Lancet HIV. 2016;3(4):e166-e74.

186. Freed EO. HIV-1 Gag Proteins: Diverse Functions in the Virus Life Cycle. Virology. 1998;251(1):1-15.

187. Scarlata S, Carter C. Role of HIV-1 Gag domains in viral assembly. Biochimica et Biophysica Acta (BBA) – Biomembranes. 2003;1614(1):62-72.

188. Bell NM, Lever AM. HIV Gag polyprotein: processing and early viral particle assembly. Trends Microbiol. 2013;21(3):136-44.

189. Clavel F, Mammano F. Role of Gag in HIV Resistance to Protease Inhibitors. Viruses. 2010;2(7):1411-26.

190. Müller B, Anders M, Akiyama H, Welsch S, Glass B, Nikovics K, et al. HIV-1 Gag Processing Intermediates Trans-dominantly Interfere with HIV-1 Infectivity. The Journal of biological chemistry. 2009;284:29692-703.

191. de Oliveira T, Engelbrecht S, Janse van Rensburg E, Gordon M, Bishop K, zur Megede J, et al. Variability at human immunodeficiency virus type 1 subtype C protease cleavage sites: an indication of viral fitness? J Virol. 2003;77(17):9422-30.

192. Dam E, Quercia R, Glass B, Descamps D, Launay O, Duval X, et al. Gag mutations strongly contribute to HIV-1 resistance to protease inhibitors in highly drug-experienced patients besides compensating for fitness loss. PLoS pathogens. 2009;5(3):e1000345-e.

193. Weber IT, Agniswamy J. HIV-1 Protease: Structural Perspectives on Drug Resistance. Viruses. 2009;1(3):1110-36.

194. van Maarseveen NM, Andersson D, Lepšík M, Fun A, Schipper PJ, de Jong D, et al. Modulation of HIV-1 Gag NC/p1 cleavage efficiency affects protease inhibitor resistance and viral replicative capacity. Retrovirology. 2012;9:29-.

195. Fun A, Wensing AM, Verheyen J, Nijhuis M. Human Immunodeficiency Virus Gag and protease: partners in resistance. Retrovirology. 2012;9:63.

196. Koh Y, Amano M, Towata T, Danish M, Leshchenko-Yashchuk S, Das D, et al. Selection of Highly Darunavir-Resistant and Replication-Competent HIV-1 Variants by Using a Mixture of Clinical HIV-1 Isolates Resistant to Multiple Conventional Protease Inhibitors. Journal of Virology. 2010;84(22):11961.

197. Coetzer M, Ledingham L, Diero L, Kemboi E, Orido M, Kantor R. Gp41 and Gag amino acids linked to HIV-1 protease inhibitor-based second-line failure in HIV-1 subtype A from Western Kenya. 2017.

198. Gijsbers EF, van Nuenen AC, Schuitemaker H, Kootstra NA. Gag sequence variation in a human immunodeficiency virus type 1 transmission cluster influences viral replication fitness. J Gen Virol. 2013;94(Pt 2):354-9.

199. Kletenkov K, Hoffmann D, Boni J, Yerly S, Aubert V, Schoni-Affolter F, et al. Role of Gag mutations in PI resistance in the Swiss HIV cohort study: bystanders or contributors? J Antimicrob Chemother. 2017;72(3):866-75.

200. Parry CM, Kolli M, Myers RE, Cane PA, Schiffer C, Pillay D. Three residues in HIV-1 matrix contribute to protease inhibitor susceptibility and replication capacity. Antimicrob Agents Chemother. 2011;55(3):1106-13.

201. Codoner FM, Pena R, Blanch-Lombarte O, Jimenez-Moyano E, Pino M, Vollbrecht T, et al. Gag-protease coevolution analyses define novel structural surfaces in the HIV-1 matrix and capsid involved in resistance to Protease Inhibitors. Sci Rep. 2017;7(1):3717.

202. Ojesina AI, Chaplin B, Sankalé J-L, Murphy R, Idigbe E, Adewole I, et al. Interplay of Reverse Transcriptase Inhibitor Therapy and Gag p6 Diversity in HIV Type 1 Subtype G and CRF02_AG. AIDS Research and Human Retroviruses. 2008;24(9):1167-74.

203. Soriano V, de Mendoza C. Genetic mechanisms of resistance to NRTI and NNRTI. HIV Clin Trials. 2002;3(3):237-48.

204. Sharma S, Aralaguppe SG, Abrahams M-R, Williamson C, Gray C, Balakrishnan P, et al. The PTAP sequence duplication in HIV-1 subtype C Gag p6 in drug-naive subjects of India and South Africa. BMC infectious diseases. 2017;17(1):95-.

205. Aralaguppe SG, Winner D, Singh K, Sarafianos SG, Quiñones-Mateu ME, Sönnerborg A, et al. Increased replication capacity following evolution of PYxE insertion in Gag-p6 is associated with enhanced virulence in HIV-1 subtype C from East Africa. Journal of Medical Virology. 2017;89(1):106-11.

206. Neogi U, Rao SD, Bontell I, Verheyen J, Rao VR, Gore SC, et al. Novel tetra-peptide insertion in Gag-p6 ALIX-binding motif in HIV-1 subtype C associated with protease inhibitor failure in Indian patients. AIDS (London, England). 2014;28(15):2319-22.

207. Kohl NE, Emini EA, Schleif WA, Davis LJ, Heimbach JC, Dixon RA, et al. Active human immunodeficiency virus protease is required for viral infectivity. Proceedings of the National Academy of Sciences of the United States of America. 1988;85(13):4686-90.

208. Adamson CS. Protease-Mediated Maturation of HIV: Inhibitors of Protease and the Maturation Process. Molecular biology international. 2012;2012:604261-.

209. Ali A, Bandaranayake RM, Cai Y, King NM, Kolli M, Mittal S, et al. Molecular Basis for Drug Resistance in HIV-1 Protease. Viruses. 2010;2(11):2509-35.

210. Clemente JC, Hemrajani R, Blum LE, Goodenow MM, Dunn BM. Secondary Mutations M36I and A71V in the Human Immunodeficiency Virus Type 1 Protease Can Provide an Advantage for the Emergence of the Primary Mutation D30N. Biochemistry. 2003;42(51):15029-35.

211. Coman RM, Robbins AH, Fernandez MA, Gilliland CT, Sochet AA, Goodenow MM, et al. The contribution of naturally occurring polymorphisms in altering the biochemical and structural characteristics of HIV-1 subtype C protease. Biochemistry. 2008;47(2):731-43.

212. Kodama EI, Kohgo S, Kitano K, Machida H, Gatanaga H, Shigeta S, et al. 4'-Ethynyl nucleoside analogs: potent inhibitors of multidrug-resistant human immunodeficiency virus variants in vitro. Antimicrob Agents Chemother. 2001;45(5):1539-46.

213. Wu VH, Smith RA, Masoum S, Raugi DN, Ba S, Seydi M, et al. MK-8591 (4'-Ethynyl-2-Fluoro-2'-Deoxyadenosine) Exhibits Potent Activity against HIV-2 Isolates and Drug-Resistant HIV-2 Mutants in Culture. Antimicrob Agents Chemother. 2017;61(8).

214. Nakata H, Amano M, Koh Y, Kodama E, Yang G, Bailey CM, et al. Activity against human immunodeficiency virus type 1, intracellular metabolism, and effects on human DNA polymerases of 4'-ethynyl-2-fluoro-2'-deoxyadenosine. Antimicrob Agents Chemother. 2007;51(8):2701-8.

215. Fukuyama K, Ohrui H, Kuwahara S. Synthesis of EFdA via a diastereoselective aldol reaction of a protected 3-keto furanose. Org Lett. 2015;17(4):828-31.

216. McLaughlin M, Kong J, Belyk KM, Chen B, Gibson AW, Keen SP, et al. Enantioselective Synthesis of 4'-Ethynyl-2-fluoro-2'-deoxyadenosine (EFdA) via Enzymatic Desymmetrization. Org Lett. 2017;19(4):926-9.

217. Michailidis E, Huber AD, Ryan EM, Ong YT, Leslie MD, Matzek KB, et al. 4'-Ethynyl-2-fluoro-2'-deoxyadenosine (EFdA) inhibits HIV-1 reverse transcriptase with multiple mechanisms. J Biol Chem. 2014;289(35):24533-48.

218. Zhang W, Parniak MA, Sarafianos SG, Empey PE, Rohan LC. In vitro transport characteristics of EFdA, a novel nucleoside reverse transcriptase inhibitor using Caco-2 and MDCKII cell monolayers. Eur J Pharmacol. 2014;732:86-95.

219. Shanmugasundaram U, Kovarova M, Ho PT, Schramm N, Wahl A, Parniak MA, et al. Efficient Inhibition of HIV Replication in the Gastrointestinal and Female Reproductive Tracts of Humanized BLT Mice by EFdA. PLOS ONE. 2016;11(7):e0159517.

220. Stoddart CA, Galkina SA, Joshi P, Kosikova G, Moreno ME, Rivera JM, et al. Oral administration of the nucleoside EFdA (4'-ethynyl-2-fluoro-2'-deoxyadenosine) provides rapid suppression of HIV viremia in humanized mice and favorable pharmacokinetic properties in mice and the rhesus macaque. Antimicrob Agents Chemother. 2015;59(7):4190-8.

221. National Institutes of Health U. A Study of Islatravir (MK-8591) in Anti-Retroviral Therapy-Naive, Human Immunodeficiency Virus-1 Infected Participants (MK-8591-003) – Study Results – ClinicalTrials.gov. 2019 [cited 2019 28 August]. Available from: https://clinicaltrials.gov/ct2/show/results/NCT02217904.

222. Sörstedt E, Carlander C, Flamholc L, Hejdeman B, Svedhem V, Sönnerborg A, et al. Effect of dolutegravir in combination with Nucleoside Reverse Transcriptase Inhibitors (NRTIs) on people living with HIV who have pre-existing NRTI mutations. International Journal of Antimicrobial Agents. 2018;51(5):733-8.

223. SE Lazerwith RC, X Chen, G Chin, MC Desai, S Eng, R Jacques, M Ji, G Jones, H Martin, C McMahon, M Mish, P Morganelli, J Mwangi, H-J Pyun, U Schmitz, G Stepan, J Szwarcberg, J Tang, M Tsiang, J Wang, K Wang, K White, L Wiser, J Zack, H Jin, Gilead Sciences FC, CA. Discovery of Bictegravir (GS-9883), a Novel, Unboosted, Once-Daily HIV-1 Integrase Strand Transfer Inhibitor (INSTI) with Improved Pharmacokinetics and In Vitro Resistance Profile. 2019 [cited 2019 28 August]. Available from: http://www.natap.org/2016/HIV/062016_05.htm.

224. Tsiang M, Jones GS, Goldsmith J, Mulato A, Hansen D, Kan E, et al. Antiviral Activity of Bictegravir (GS-9883), a Novel Potent HIV-1 Integrase Strand Transfer Inhibitor with an Improved Resistance Profile. Antimicrobial Agents and Chemotherapy. 2016;60(12):7086-97.

225. Zash R, Makhema J, Shapiro RL. Neural-Tube Defects with Dolutegravir Treatment from the Time of Conception. The New England journal of medicine. 2018;379(10):979-81.

226. Poster Abstracts. HIV Medicine. 2017;18(S1):14-70.

227. Gallant JE, Thompson M, DeJesus E, Voskuhl GW, Wei X, Zhang H, et al. Antiviral Activity, Safety, and Pharmacokinetics of Bictegravir as 10-Day Monotherapy in HIV-1-Infected Adults. J Acquir Immune Defic Syndr. 2017;75(1):61-6.

228. Sax PE, DeJesus E, Crofoot G, Ward D, Benson P, Dretler R, et al. Bictegravir versus dolutegravir, each with emtricitabine and tenofovir alafenamide, for initial treatment of HIV-1 infection: a randomised, double-blind, phase 2 trial. The Lancet HIV. 2017;4(4):e154-e60.

229. Alvarez M, Casas P, de Salazar A, Chueca N, Guerrero-Beltran C, Rodriguez C, et al. Surveillance of transmitted drug resistance to integrase inhibitors in Spain: implications for clinical practice. Journal of Antimicrobial Chemotherapy. 2019;74(6):1693-700.

230. Stellbrink HJ, Arribas JR, Stephens JL, Albrecht H, Sax PE, Maggiolo F, et al. Co-formulated bictegravir, emtricitabine, and tenofovir alafenamide versus dolutegravir with emtricitabine and tenofovir alafenamide for initial treatment of HIV-1 infection: week 96 results from a randomised, double-blind, multicentre, phase 3, non-inferiority trial. Lancet HIV. 2019;6(6):e364-e72.

231. Trezza C, Ford SL, Spreen W, Pan R, Piscitelli S. Formulation and pharmacology of long-acting cabotegravir. Curr Opin HIV AIDS. 2015;10(4):239-45.

232. Karmon SL, Mohri H, Spreen W, Markowitz M. GSK1265744 demonstrates robust in vitro activity against various clades of HIV-1. J Acquir Immune Defic Syndr. 2015;68(3):e39-41.

233. Yoshinaga T, Kobayashi M, Seki T, Miki S, Wakasa-Morimoto C, Suyama-Kagitani A, et al. Antiviral characteristics of GSK1265744, an HIV integrase inhibitor dosed orally or by long-acting injection. Antimicrob Agents Chemother. 2015;59(1):397-406.

234. Andrews CD, Bernard LS, Poon AY, Mohri H, Gettie N, Spreen WR, et al. Cabotegravir long acting injection protects macaques against intravenous challenge with SIVmac251. Aids. 2017;31(4):461-7.

235. Radzio-Basu J, Council O, Cong ME, Ruone S, Newton A, Wei X, et al. Drug resistance emergence in macaques administered cabotegravir long-acting for pre-exposure prophylaxis during acute SHIV infection. Nat Commun. 2019;10(1):2005.

236. Patel P, Ford SL, Lou Y, Bakshi K, Tenorio AR, Zhang Z, et al. Effect of a High-Fat Meal on the Pharmacokinetics of the HIV Integrase Inhibitor Cabotegravir. Clin Pharmacol Drug Dev. 2019;8(4):443-8.

237. Lou Y, Buchanan AM, Chen S, Ford SL, Gould E, Margolis D, et al. Effect of Cabotegravir on Cardiac Repolarization in Healthy Subjects. Clin Pharmacol Drug Dev. 2016;5(6):509-16.

238. Shaik JSB, Ford SL, Lou Y, Zhang Z, Bakshi KK, Tenorio AR, et al. A Phase 1 Study to Evaluate the Pharmacokinetics and Safety of Cabotegravir in Patients With Hepatic Impairment and Healthy Matched Controls. Clin Pharmacol Drug Dev. 2019;8(5):664-73.

239. Parasrampuria R, Ford SL, Lou Y, Fu C, Bakshi KK, Tenorio AR, et al. A Phase I Study to Evaluate the Pharmacokinetics and Safety of Cabotegravir in Adults With Severe Renal Impairment and Healthy Matched Control Participants. Clin Pharmacol Drug Dev. 2019;8(5):674-81.

240. Landovitz RJ, Li S, Grinsztejn B, Dawood H, Liu AY, Magnus M, et al. Safety, tolerability, and pharmacokinetics of long-acting injectable cabotegravir in low-risk HIV-uninfected individuals: HPTN 077, a phase 2a randomized controlled trial. PLoS Med. 2018;15(11):e1002690.

241. Margolis DA, Brinson CC, Smith GHR, de Vente J, Hagins DP, Eron JJ, et al. Cabotegravir plus rilpivirine, once a day, after induction with cabotegravir plus nucleoside reverse transcriptase inhibitors in antiretroviral-naive adults with HIV-1 infection (LATTE): a randomised, phase 2b, dose-ranging trial. Lancet Infect Dis. 2015;15(10):1145-55.

242. Margolis DA, Gonzalez-Garcia J, Stellbrink HJ, Eron JJ, Yazdanpanah Y, Podzamczer D, et al. Long-acting intramuscular cabotegravir and rilpivirine in adults with HIV-1 infection (LATTE-2): 96-week results of a randomised, open-label, phase 2b, non-inferiority trial. Lancet. 2017;390(10101):1499-510.

243. Rajoli RKR, Curley P, Chiong J, Back D, Flexner C, Owen A, et al. Predicting Drug-Drug Interactions Between Rifampicin and Long-Acting Cabotegravir and Rilpivirine Using Physiologically Based Pharmacokinetic Modeling. J Infect Dis. 2019;219(11):1735-42.

244. Singh K, Flores JA, Kirby KA, Neogi U, Sonnerborg A, Hachiya A, et al. Drug resistance in non-B subtype HIV-1: impact of HIV-1 reverse transcriptase inhibitors. Viruses. 2014;6(9):3535-62.

245. Sunpath H, Wu B, Gordon M, Hampton J, Johnson B, Moosa MY, et al. High rate of K65R for antiretroviral therapy-naive patients with subtype C HIV infection failing a tenofovir-containing first-line regimen. Aids. 2012;26(13):1679-84.

246. Kantor R, Smeaton L, Vardhanabhuti S, Hudelson SE, Wallis CL, Tripathy S, et al. Pretreatment HIV Drug Resistance and HIV-1 Subtype C Are Independently Associated With Virologic Failure: Results From the Multinational PEARLS (ACTG A5175) Clinical Trial. Clin Infect Dis. 2015;60(10):1541-9.

247. Brenner B, Turner D, Oliveira M, Moisi D, Detorio M, Carobene M, et al. A V106M mutation in HIV-1 clade C viruses exposed to efavirenz confers cross-resistance to non-nucleoside reverse transcriptase inhibitors. Aids. 2003;17(1):F1-5.

248. Theys K, Vercauteren J, Snoeck J, Zazzi M, Camacho RJ, Torti C, et al. HIV-1 subtype is an independent predictor of reverse transcriptase mutation K65R in HIV-1 patients treated with combination antiretroviral therapy including tenofovir. Antimicrob Agents Chemother. 2013;57(2):1053-6.

249. Brenner BG, Coutsinos D. The K65R mutation in HIV-1 reverse transcriptase: genetic barriers, resistance profile and clinical implications. HIV Ther. 2009;3(6):583-94.

250. Struck D, Lawyer G, Ternes A-M, Schmit J-C, Bercoff DP. COMET: adaptive context-based modeling for ultrafast HIV-1 subtype identification. Nucleic acids research. 2014;42(18):e144-e.

251. Grossmann S, Nowak P, Neogi U. Subtype-independent near full-length HIV-1 genome sequencing and assembly to be used in large molecular epidemiological studies and clinical management. Journal of the International AIDS Society. 2015;18(1):20035-.

252. Hall TA. BioEdit A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41, 95-98. - References - Scientific Research Publishing. Nucl Acids Symp Ser. 1999;41:95-8.

253. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol. 2018;35(6):1547-9.

254. Koval CE, Dykes C, Wang J, Demeter LM. Relative replication fitness of efavirenz-resistant mutants of HIV-1: Correlation with frequency during clinical therapy and evidence of compensation for the reduced fitness of K103N + L100I by the nucleoside resistance mutation L74V. Virology. 2006;353(1):184-92.

255. Van Zyl GU, Liu TF, Claassen M, Engelbrecht S, de Oliveira T, Preiser W, et al. Trends in Genotypic HIV-1 Antiretroviral Resistance between 2006 and 2012 in South African Patients Receiving First- and Second-Line Antiretroviral Treatment Regimens. PLoS One. 2013;8(6):e67188.

256. Sharma S, Arunachalam PS, Menon M, Ragupathy V, Satya RV, Jebaraj J, et al. PTAP motif duplication in the p6 Gag protein confers a replication advantage on HIV-1 subtype C. The Journal of biological chemistry. 2018;293(30):11687-708.

257. Gupta RK, Jordan MR, Sultan BJ, Hill A, Davis DHJ, Gregson J, et al. Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. Lancet (London, England). 2012;380(9849):1250-8.

258. Hamers RL, Rinke de Wit TF, Holmes CB. HIV drug resistance in low-income and middle-income countries. Lancet HIV. 2018;5(10):e588-e96.

259. Sutherland KA, Collier DA, Claiborne DT, Prince JL, Deymier MJ, Goldstein RA, et al. Wide variation in susceptibility of transmitted/founder HIV-1 subtype C Isolates to protease inhibitors and association with in vitro replication efficiency. Sci Rep. 2016;6:38153.

260. Kovalevsky AY, Liu F, Leshchenko S, Ghosh AK, Louis JM, Harrison RW, et al. Ultra-high resolution crystal structure of HIV-1 protease mutant reveals two binding sites for clinical inhibitor TMC114. J Mol Biol. 2006;363(1):161-73.

261. Dierynck I, De Wit M, Gustin E, Keuleers I, Vandersmissen J, Hallenberger S, et al. Binding kinetics of darunavir to human immunodeficiency virus type 1 protease explain the potent antiviral activity and high genetic barrier. Journal of virology. 2007;81(24):13845-51.

262. Aoki M, Das D, Hayashi H, Aoki-Ogata H, Takamatsu Y, Ghosh AK, et al. Mechanism of Darunavir (DRV)'s High Genetic Barrier to HIV-1 Resistance: A Key V32I Substitution in Protease Rarely Occurs, but Once It Occurs, It Predisposes HIV-1 To Develop DRV Resistance. mBio. 2018;9(2).

263. Mills AM, Nelson M, Jayaweera D, Ruxrungtham K, Cassetti I, Girard PM, et al. Once-daily darunavir/ritonavir vs. lopinavir/ritonavir in treatment-naive, HIV-1-infected patients: 96-week analysis. Aids. 2009;23(13):1679-88.

264. Antoniou T, Szadkowski L, Walmsley S, Cooper C, Burchell AN, Bayoumi AM, et al. Comparison of atazanavir/ritonavir and darunavir/ritonavir based antiretroviral therapy for antiretroviral naive patients. BMC Infect Dis. 2017;17(1):266.

265. Aoki M, Das D, Hayashi H, Aoki-Ogata H, Takamatsu Y, Ghosh AK, et al. Mechanism of Darunavir (DRV)'s High Genetic Barrier to HIV-1 Resistance: A Key V32I Substitution in Protease Rarely Occurs, but Once It Occurs, It Predisposes HIV-1 To Develop DRV Resistance. mBio. 2018;9(2):e02425-17.

266. Chaturbhuj D, Patil A, Gangakhedkar R. PYRE insertion within HIV-1 subtype C p6-Gag functions as an ALIX-dependent late domain. Scientific Reports. 2018;8(1):8917.

267. Barrett SE, Teller RS, Forster SP, Li L, Mackey MA, Skomski D, et al. Extended-Duration MK-8591-Eluting Implant as a Candidate for HIV Treatment and Prevention. Antimicrobial Agents and Chemotherapy. 2018;62(10):e01058-18.

268. Genberg BL, Lee Y, Rogers WH, Wilson IB. Four types of barriers to adherence of antiretroviral therapy are associated with decreased adherence over time. AIDS and behavior. 2015;19(1):85-92.

269. Fernandez C, van Halsema CL. Evaluating cabotegravir/rilpivirine long-acting, injectable in the treatment of HIV infection: emerging data and therapeutic potential. HIV/AIDS (Auckland, NZ). 2019;11:179-92.

270. Xu HT, Colby-Germinario SP, Asahchop EL, Oliveira M, McCallum M, Schader SM, et al. Effect of mutations at position E138 in HIV-1 reverse transcriptase and their interactions with the M184I mutation on defining patterns of resistance to nonnucleoside reverse transcriptase inhibitors rilpivirine and etravirine. Antimicrob Agents Chemother. 2013;57(7):3100-9.

271. Wares M, Mesplède T, Quashie PK, Osman N, Han Y, Wainberg MA. The M50I polymorphic substitution in association with the R263K mutation in HIV-1 subtype B integrase increases drug resistance but does not restore viral replicative fitness. Retrovirology. 2014;11:7-.

272. Brado D, Obasa AE, Ikomey GM, Cloete R, Singh K, Engelbrecht S, et al. Analyses of HIV-1 integrase sequences prior to South African national HIV-treatment program and availability of integrase inhibitors in Cape Town, South Africa. Scientific Reports. 2018;8(1):4709.