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Explore the Patterns of Resistance Mutations to Antiretroviral Drugs in Treated Patients Infected with HIV in Northern Oman

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Abstract:

Background: The effectiveness of antiretroviral therapies (ART) made it possible for people with HIV to live long and healthy lives. However, a significant threat to the progress obtained thus far in ART is posed by rapid mutation patterns of HIV, which confer its resistance to ART.

Aim: This study aims to explore the patterns of resistance mutations to antiretroviral drugs, in Northern Oman, in different population groups including wife and husband (couples), singles and homosexual and to

Method: A retrospective cross-sectional design was selected to carry out this investigation. Data from 193 HIV positive patientsfrom Northern Oman health institutions, Suhar hospital (tertiary hospital) and Suhar polyclinic (Secondary care) were used in this study. A total of 82 participants had a genomic assay (genotype) analysis done and were included for mutation and resistance assessment, which included the number of ART had resistance or susceptible, kind of mutations and its frequencies. Ethical approval had been obtained from Robert Gordon University committee and the Central Ethical approval committee in Oman. Data of CD_4 and VL was analysed via descriptive statistics and Wilcoxon matched-pairs test.

Results: The majority of the HIV positive patients were married (n=144 out of 193, approx. 74.6%), in the age group of 31-45. The highest rates of transmitted HIV infection were found to be associated with sexual behaviour (n= 106 out of 193, approx. 54.9%), this figure included different population groups (single and married, males and females, in different ages). Mutation and resistance patterns were found to be increased in patients undergoing NRTI and NNRTI ARTs. Population descriptive and genomic assays mimic those seen in other HIV positive populations. Different strategies had been recommended by the WHO such as combination therapies of new ART and patient's adherence follow up.

Conclusion: Resistance mutations to ART developed fast post 6 months of initial treatments in the study population. This concluded, there was a huge gap between the initial treatment and the most recent progression results and this gap should be indicated.

Keywords: AIDs, HIV Resistance, Genotype, ARTs

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Abbreviations	Descriptions
AIDS	Acquired Immune Deficiency Syndrome
HIV	Human Immunodeficiency Virus
ART	Antiretroviral Therapy
NRTIs	Nucleotide Reverse Transcriptase Inhibitors
NNRTIs	Non-Nucleotide Reverse Transcriptase Inhibitors
PIs	Protease Inhibitors
INIs	Integrase Inhibitors
CCR5	C-C motif chemokine receptor
PVL	Plasma Viral Load
CD_4	Glycoprotein found in the surface of immune cells such as T helper cells.
PDR	Pre-treatment Drug Resistance testing

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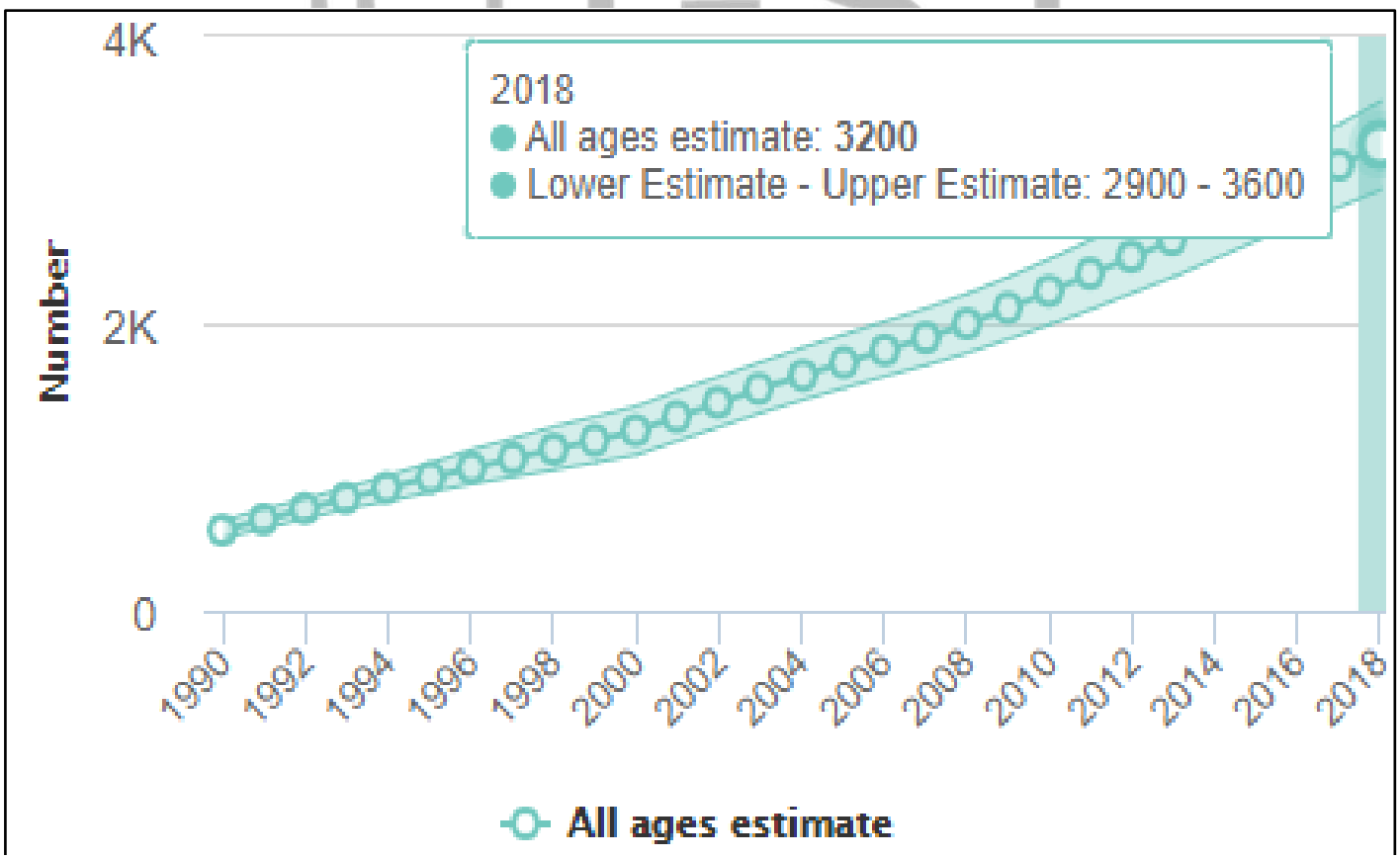
1. Introduction

Currently, an estimated number of 36.9 million people across the globe live with HIV (human immunodeficiency virus) or AIDS (acquired immunodeficiency syndrome). Out of this number, a total of 1.8 million are children.[1, 2]With advances in treatment for these conditions, death rates caused by HIV have considerably dropped from 1.9 million in 2004 to 940.000 in 2017. These effects have been attributed to increased access to ART (antiretroviral therapy).[2] However, drug-resistance poses a significant threat to the reduction of mortality associated with HIV resistance.[3, 4]

In Oman, the first case of HIV was reported in 1984. Since then, to 2015, recent estimates [5]note that the number of people living with HIV in Oman is 2879. These people are predominantly male, and the majority of both genders are young people aged 20-49 years old. The highest rates of infection have been reported in the Musandam governorate. From the total number of infections in Oman, 66.8% are attributed to unprotected sex, 8.3% to mother-to-child transmissions, 4.3% of cases of infection have been associated with intravenous drug abuse and 3.2% to blood transfusions. A significant rate of 17.4% of infections has no known cause of infection.[5] By 2018, statistics provided by UNAIDS [6]for Oman indicate an increase in the number of HIV cases, with estimates set at 2900-3600 people, with an average reported case of 3200. Infected women are reported to be less than 1000. There is no reported data for children aged 0 to 14 years. These data are illustrated in Figure 1 below as provided by UNAIDS. [6]

Figure 1 HIV Estimates in Oman:



UNAIDS (2018). HIV Prevalence in Oman [online]

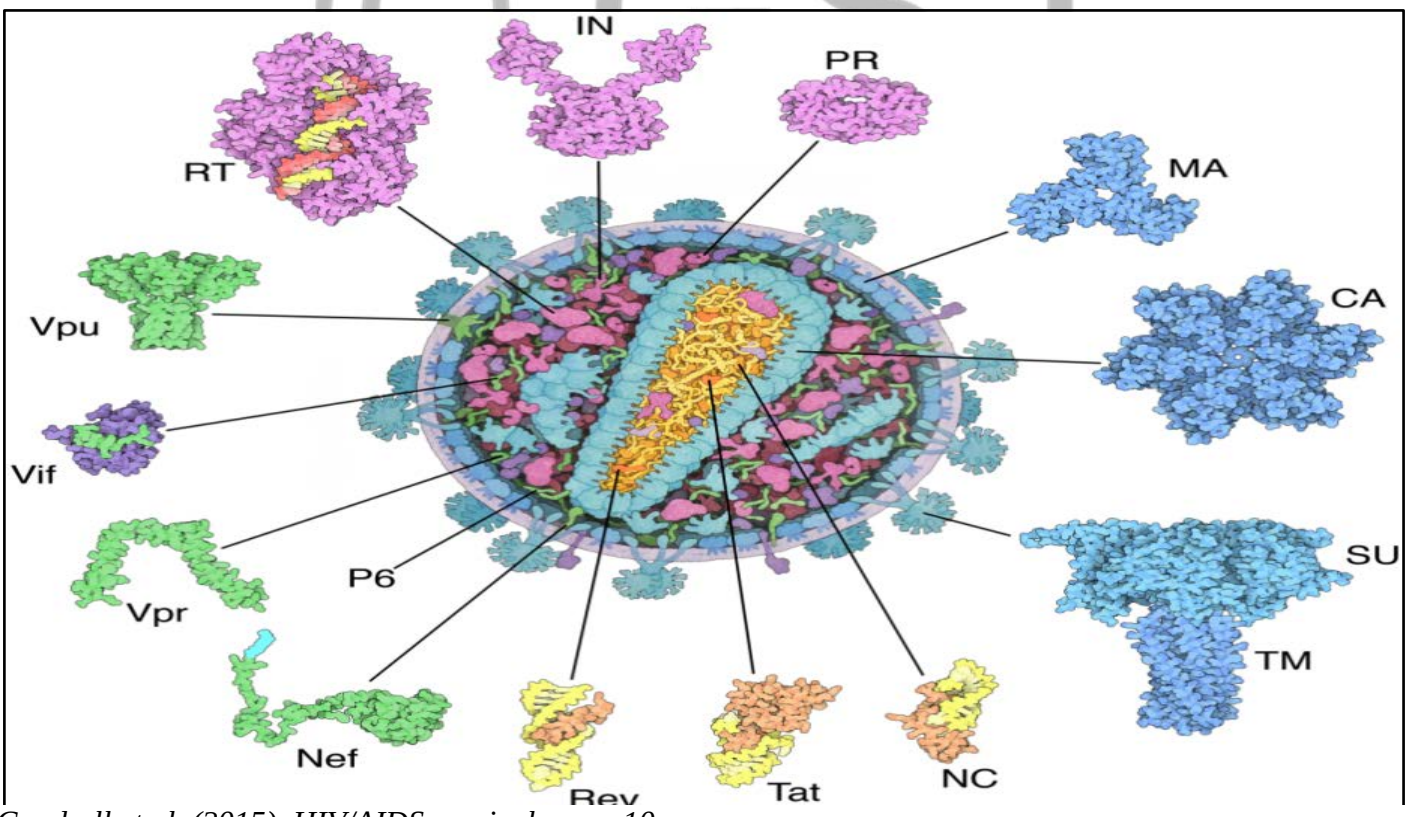
1.1. HIV Structure and Detection of Mutation.

HIV belongs to the Lentivirus species, which is a subgroup of the Retrovirus family. Two species are distinguished, HIV-1 and HIV-2. HIV-2 is a less virulent strand, mostly confined in West Africa due to its reduced potential for infection. On a global scale, major concerns with infection and AIDS are connected with HIV-1, a highly virulent virus.[7]

The HIV-1 structure is spherical, with an approximated diameter of 120nm, consisting of two copies of +RNA, which contain the nine genes of the virus (gag, pol, env, tat, rev, nef, vif, vpr, vpu) encoding a total of 19 proteins.[8, 9] These genes are encapsulated by a conical capsid constructed from 2000 copies of the p24 viral protein. Additional nucleocapsid proteins and enzymes that aid in replication and transcription are present. These include reverse transcriptase (RT), protease (Pro), as well as ribonuclease (RNase H) and integrase (IN). A matrix of viral protein p17 and the viral envelope ensure the integral structure of the virus.[10] An image of the virus as presented along with relevant proteins is presented in Figure 2 below.[11]

The most common way of resistance mutation in HIV-1 patients is a genotype test, which is one of the earliest gene sequencing tests. Its more complex test comparing to other typical antimicrobial susceptibility tests, but it can detect mutations at any level [Low, intermediate, high] [38]. *Appendix 1* shows, an example of genotype test results.

Figure 2 HIV-1 Structure



Goodsell et al. (2015), HIV/AIDS curriculum, p. 10.

1.2. HIV Treatment Protocols

As previously described, several lines of treatment are available for HIV. These therapies focus on disrupting the replication process of the virus in various stages. International guidelines [17, 18] recommend combinations of NRTIs, NNRTIs, PIs, fusion inhibitors, CCR5 antagonists, IN inhibitors and pharmacokinetic enhancers. Recently, the FDA [18] approved the use of a post-attachment inhibitor. Treatment regimens are recommended based on age and health status. These recommendations are listed in **Table 1 below**.

Table 1 International Recommendations for First-Line ART and Alternative First-Line ART

Groups	**First-line ART**	**Alternative First-Line ART**
Adults	• TDF+3TC (or FTC) +EFV	• AZT+3TC+EFV (or NVP) • TDF+3TC (or FTC) +DTG[c] • TDF+3TC (or FTC) +EFV_{400} [c, e] • TDF+3TC (or FTC) +NVP
Adolescents	• TDF+3TC (orFTC)+EFV	• AZT+3TC+EFV (or NVP) • TDF (or ABC) +3TC (orFTC)+DTG[c,d] • TDF (or ABC) + 3TC (orFTC)+EFV_{400} [c, d,e] • TDF (or ABC) + 3TC (orFTC)+NVP
Pregnant and Breastfeeding Women	• TDF+3TC (or FTC) +EFV	• AZT+3TC+EFV (or NVP) • TDF+3TC (or FTC) +NVP
Children from 3 to Less Than 10 Years	• ABC+3TC+EFV	• ABC+3TC+NVP • AZT+3TC+EFV (or NVP) • TDF+3TC (or FTC) +EFV (or NVP)
Children Less Than 3 Years	• ABC (or AZT) +3TC+LPV/r	• ABC (or AZT) +3TC+NVP
For adults and adolescents, d4T should be discontinued as an option in first-line treatment. [b] ABC or boosted protease inhibitors (ATV/r, DRV/r, LPV/r) can be used in special circumstances. [c]Safety and efficacy data on the use of DTG and EFV_{400} in pregnant women, people with HIV/TB co-infection and adolescents younger than 12 years of age are not yet established. [d] Conditional recommendation, moderate-quality evidence. [e]EFV at a lower dose (400 mg/day).		
3TC (Lamivudine), ABC (Abacavir), AZT (Zidovudine), DRV (Darunavir), DTG (Dolutegravir), EFV (Efavirenz), FTC (Emtricitabine), LPV (Lopinavir), NVP (Nevirapine), r (Ritonavir), TDF (Tenofovir).		

National recommendations in Oman also provide ART according to age and health status albeit fewer combinations are available by contrast with international guidelines. These recommendations are listed in **Table 2.**[19]

Table 2 National Recommendations for First-Line ART

Groups	First-Line	Alternative
Adults and Adolescents	• TDF+FTC+EFV	• AZT+3TC+EFV • TDF+FTC+ATV/r • AZT+3TC+ATV/r
Pregnant Women	• TDF+FTC+EFV	• TDF+FTC+ATV/r • TDF+FTC+LPV/r • AZT+3TC+EFV • AZT+3TC+ATV/r • AZT+3TC+LPV/r

Nothing that resistance is likely to occur in patients with first-line ART or alternatives national guidelines. National guidelines [(19)] also recommend several combinations of antiviral medication as the second and third line of treatment. These recommendations are listed in **Table 3.**

Table 3 National Recommendations for Second-Line and Third-Line ART

Groups / Regimens	Second -Line Regimens
Adults& Adolescents (≥ 13 years) And Pregnant Women.	• AZT+ 3TC+ LPV/r • AZT+3TC+DRV/r
Children less than 13 years	• The decision should be made by Paediatrician (Infectious Disease Department, Royal Hospital).
Third-Line Regimens	
• Integrase inhibitors (e.g. DTG, RGV). • Second generation NNRTIs (e.g. ETR, RPV). • Second generation PIs (e.g.Atazanavir (ATV). • Entry Inhibitors are known as Fusion inhibitors FIs (e.g. Maraviroc (MRC), Enfuvirtide (ENF). ❖ It should be started by an HIV specialist in AL-Nahdha, Royal, and Sultan Qaboos University Hospitals.	

As it can be observed from data presented in **Table 1** and respectively **Table 2** and **Table 3**, available regimes are limited in Oman by contrast with the international recommendation. However, considering population polymorphism and rates of mutation resistance to current regimens is highly likely to occur.**(12)** This may imply that Oman would require an update of current regimens in line with international standards.

1.3. Other studies

[3]Abbas. U.L, et al. Drug resistance from preferred antiretroviral regimens for HIV infection in South Africa: A modelling study. *PloS one*, 2019; 1-17.

This study has been done in KwaZulu Natal, South Africa. In this paper, the Author found and highlighted such important points. The major resistance mutations in NRTIs were M184V and K65R. The numbers stand for the position of the codon and the letters for the amino acid substrates. The author said, WHO first-line recommendations preferred regimens could lead to substantial drug resistance and up to date, these regimens are still used in practice. Moreover, this paper highlighted the impact of the patient'sadherence and acquired resistance on virological failure. Also, M184V was selected by Zidovudine (AZT). This paper mainly concerned about the first line WHO-recommended regimens only did not include other second and alternative regimens**.** In addition, the correlation of CD4 and VL [Important parameters in HIV-1 testing] with the resistance mutations.

In this paper, the aims were not clear enough to answer the research question. In addition, the author did not mention the study population sample size, exclusion and inclusion, and the ethical consideration in this study. Justification for the selected methods was stated clearly. Furthermore, data collection was appropriate. The data were analysis sufficiently, a clear statement of findings, and robust qualitative insights. Limitations of this study were included.

[4] Hosseinipour.M.C, et al. Emergence of HIV drug resistance during the first and second line of antiretroviral therapy in resource-limited settings. *The journal of infectious disease*, 2013; 207(S2), S49-56.

In this paper, HI drug resistance primarily due to M184V and NNRTIs mutations which had been identified in 60%-70% of the study population. Moreover, adherence, as stated in the previous article, found to be the major factor behind drug resistance. Another common point between this article and the previous one, the WHO first-line recommended regimens were associated with a high risk of failure. Finally, this paper concluded, optimizing patient's adherence, and good treatment monitoring is crucial to prevent drug resistance**.**

In this study, the aim was clear and the time frame too. In addition, research design and recruitment strategies were appropriate. Furthermore, the aim was addressed through robust data collection procedures. The author did not include any ethical consideration and exclusion and inclusion criteria.

[25] Hamers .R.L, et al. Patterns of HIV-1drug resistance after first-line antiretroviral therapy (ART) failure in Sub-Saharan African countries: Implication for second ART strategies. *HIV/AIDs*, 2012; 54(11), 1660-9.

This study was conducted in 13 clinical sites in 6 African countries [Kenya, Nigeria, South Africa, Uganda, Zambia, and Zimbabwe], which included a total of 2755 participants. Out of the total number, only 142

genotype assays had been done. HIV-1 sequences were established for those 142 virologically failed ART patients. The most common drug resistance mutations were M184V (53.5%), K103N (28.9%), and Y181C (15.5%). Zidovudine (AZT) and Nevirapine were the most frequent resistance mutations. Furthermore, M184V was selected by Zidovudine and K103N selected by Efavirenz and Nevirapine. In this paper, it was mentioned that the use of Zidovudine rather than tenofovir may be preferred in the second line to limit the resistance incidences and second-generation NNRTIs (Rilpivirine or Etravirine) as a second line in a deprived area. This study had limitations such as the number of genotype assays,it's difficult to track resistance mutation with a small number of tests. Moreover, there were challenges in blood sampling post ART initiation which may limit the accuracy of this study.

In this paper, the author gave a clear description of the study's aims, population number, sites of this study, and methods.Exclusion, inclusion criteria, time frame, and ethical considerations were included. This study was robust and ideal for any HIV-1 ART resistance mutations in future researches.

1.4. Rationale and Aim of the Study

Considering the potential for mutation of HIV, it is likely that patterns of resistance may develop in the HIV infected population in Oman. Recognising this as an issue is important because ART resistance sets at peril the lives of people infected with HIV and can also contribute to the spread of infection by allowing significant viral replication and enhancing the odds of transmission.

1.5. Research aim

Explore the patterns of resistance mutations to antiretroviral drugs, in Northern Oman, in different population groups including wife and husband (couples), singles and homosexuals and to characterise the specific strategies used to manage such cases.

1.6. Objectives

In different population groups of HIV-1-infected patients:

1. Determine the incidence, prevalence, and types of resistant mutation to Antiretroviral Therapy (ART).
2. Characterise the strategies used for the management of the resistant mutation to ART including the impact on medication selection.
3. Make recommendations for the identification and management of such patients.

2. Methodology

This study will follow a quantitative methodology, relying on the positivist research paradigm. As a research philosophy, positivism argues that a phenomenon investigated can be quantified to be understood. Additionally, positivism postulates that there is only one reality, and this can be understood through an objective mathematical approach.[20] Quantitative research derives from the positivist research philosophy

and employs quantitative methods of data collection and analysis to reach the established aim of the research.[21]

2.1. Research Design

A retrospective study design has been selected to reach the aim of this study. A retrospective study is carried out when the outcome of interest has already occurred at the time in which the study is initiated.[22] This type of design can enable the investigator to formulate a hypothesis about the outcome and the exposure and to further investigate emerging relations between these elements. However, causality should not be implied from a retrospective design. Differently stated, although associations between the outcome and exposure elements are found, these do not imply that the outcome has been caused by these elements.[23] Hence, an association does not imply causation.

2.2. Setting

Secondary data was collected from two governmental health institutions in Oman. The primary site was a tertiary hospital (Suhar Hospital) and the secondary site was the Suhar Polyclinic. Both healthcare institutions are located in the North part of Oman. The time frame for the collected data varied from the oldest (2005) to the most recent (2019).

2.3. Sampling Procedures

The available databases from the selected hospital sites were searched for data related to HIV patients. Participant selection was done via purposive sampling (all HIV positive participants had been selected, then I excluded some participants based on the exclusion criteria). This type of procedure involves selecting participants based on a series of pre-defined inclusion criteria.[24] These criteria have been established in line with the research questions and specific HIV resistance patterns to ART. The inclusion and exclusion criteria for participants are listed in **Table 4** below.

Table 4 Inclusion and Exclusion Criteria for Participants

Inclusion Criteria	**Exclusion Criteria**
Patients aged ≥15 years.	Patients who passed away (access to their medical records is denied by the system).
Patients on first, second, or other alternative ART regimens, mono or combination therapies.	Patients who have no treatment records and laboratory investigations e.g. CD4, PVL or genotype assay results in both Suhar hospital and Suhar polyclinic
Patients that were on ART for at least six months.	
Patients that have records for the biochemical and genetic analysis of interest.	

The application of these criteria resulted in a total number of 193 participants (*n=193*) have been included, and 57 have been excluded due to a lack of information, referral to Royal hospital (Muscat), and passed away patients.

2.4. Variables

The study collected socio-demographic data, symptomatology data, mode of transmission, treatment line, biochemical data, and genetic assay data. Socio-demographic variables included: gender, marital status, and age. Mode of transmission data included: sexual transmission (bisexual, heterosexual, homosexual), IV drug abuse, transfusion, vertical and unknown cause. Symptom data will be recorded as a binary variable of symptomatic or asymptomatic. Treatment line variables will include first-line, second-line, and third-line treatments. Biochemical variables included CD4+ counts and PLV (viral load). Genotype assay variables focused on identifying *gag-pol* mutations and determining virus susceptibility of resistance to PIs, NRTIs, and NRRTIs. Genotypic drug resistance was defined as the presence of ≥1 major amino acid substitution.**(25)** Sample stratification will be carried out based on all variables tested.

2.5. Data collection

Different kinds of literature had been reviewed to gather the most important data (variables) as stated above that can help to answer thesis questions, aims, and objectives. Later on, Microsoft excel sheet had been created to arrange all these variables. Following this step, authorisation had been obtained from the Suhar Hospital HOD to carry out the data collection from the hospital system (ALSHEFFA). I logged into the system using my account as I am a registered pharmacist at that hospital. This system allowed me to get access to patient records, referral letters, past medical history, and Lab investigations. At Suhar polyclinic, my colleague helped me to collect data using the same datasheet. After that, the collected data had been checked against accuracy. All uncompleted and wired entries had been removed. Then my colleagues did a double-check for all entries. Finally, the two datasheets for both health institutions had been combined into one datasheet preparing it for the analysis.

2.6. Data Analysis

Data were analysed using IBM Statistical Package for Social Sciences (SPSS) v. 25. Descriptive statistics will be used to present data related to socio-demographic variables and biochemical variables. Wilcoxon matched-pairs test, which is a non-parametric statistical hypothesis test, used to compare two related samples, match sample, and repeated measurements for a single sample to elicit whether the mean ranks differ[(34)]. It wascarried out between pre and post the first attempt of ART for CD4+ and PLV results. This conducted to determine if there is any statistically significant difference between pre and post the first attempt after 6 months of treatment. In this case, the test statistic Z-value [Numerical measurement which determines the relationship between means of a group of values. If Z-score is (0), it indicates that the data

point's score similar to the mean score, and if it equal (1) indicates a value that is one SD from the mean. Z-Value positive (score above the mean), negative (score below the mean)] [(35)]had considered with a level of significance set at $p<0.05$. The null hypothesis and alternative hypothesis in this case are:

H0. There is no statistically significant difference between CD4+, PVL pre and post the first attempt of ART.

H1: There is a statistically significant difference between CD4+, PVL pre and post the first attempt of ART.

Moreover, genotype assay results will be presented in two graphs one graph will present the latest genotype assay results (Resistance, susceptible or non-applicable) and the second one will present the major ART resistance mutations for the latest genotype assay.

2.7. Ethics

Ethical approval for this study was secured from the PALS Ethical Panel, Robert Gordon University and from the appropriate local ethical committee in Oman. All patient data will be kept confidential under password protection. Data security issues will be reported to the local supervisor for proper action.

3. Results

3.1. Baseline characteristics of the study population (n=193).

A total number of 193 participants undergoing ART treatments were included in the study, from which 113 underwent treatment with first-line ART (*n=113*), 65 underwent treatment with second-line ART (*n-65*) and 15 underwent treatment with third-line ART (*n=15*). All results are listed in **Table 5** below. The majority of participants in all lines of treatment were male (59.29%, 61.53%, and 73.33%) and married (69.02%, 81, 53% and 86.66%). The most predominant age group in all treatment lines was 31-45 years old, with individuals over 60 years old constituting the lowest group. These results are similar to previously reported data on socio-demographic characteristics of the HIV population in Oman.**(6, 5)**

The majority of participants in all groups had the CD_4 count ≥500/µl. In the second line of treatment, 26.15% of participants had a CD_4 count of 499-200/µl and <200/µl respectively. In the third line of treatment, 26.66% of participants had a CD_4 count of 499-200/µl and <200/µl respectively. Overall, the total number of 34 participants in all treatment groups had a CD_4 count of <200/µl. This indicates that 18.13% of patients in the whole sample had progressed to stage 3 HIV. The same percentage of patients presented a CD_4 count of 499-200/µl, indicating that these patients are at risk of developing AIDS. Hence, a total of 36.26% of participants were either at high risk of developing AIDS or had already developed the condition.

A significant number of patients had a high viral load, with 15.92%, 40% and 46.66% of patients across treatment groups showing a VL of ≥10000/ml. Only 83 patients were undetectable in the first line of

treatment, 22 in the second line of treatment and 4 in the third line of treatment. Corroborated by the results obtained for CD_4 counts, these data indicate an increasing resistance development towards all current lines of treatment.

Table 5: Baseline characteristics of the study population (*n*=193)

Treatment stages / Features	Patients on Fist Line ART (**113**)	Patients on Second Line ART (**65**)	Patients on Third Line ART (**15**)
Gender:			
Male	**67** (59.29 %)	**40** (61.53 %)	**11** (73.33 %)
Female	**46** (40.70 %)	**25** (38.46 %)	**4** (26.66 %)
Marital status:			
Married	**78** (69.02 %)	**53** (81.53 %)	**13** (86.66 %)
Single	**35** (30.97 %)	**12** (18.46 %)	**2** (13.33 %)
Divorced	**0** (0 %)	**0** (0 %)	**0** (0 %)
Age:			
15-30 years	**32** (28.31 %)	**16** (24.61 %)	**3** (20 %)
31-45 years	**57** (50.44 %)	**32** (49.23 %)	**6** (40 %)
46-60 years	**20** (17.69 %)	**16** (24.61 %)	**6** (40 %)
>60 years	**4** (3.53 %)	**1** (1.53 %)	**0** (0 %)
Mode of Transmission:			
Bisexual	**49** (43.36 %)	**31** (47.69 %)	**8** (53.33 %)
Heterosexual	**12** (10.61 %)	**5** (7.69 %)	**1** (6.66 %)
Homosexual	**7** (6.19 %)	**4** (6.15 %)	**2** (13.33 %)
IV drug abuse	**17** (15.04 %)	**7** (10.76 %)	**3** (20 %)
Transfusion	**7** (6.19 %)	**3** (4.61 %)	**1** (6.66 %)
Vertical	**0** (0 %)	**0** (0 %)	**0** (0 %)
Unknown	**21** (18.58 %)	**15** (23.07 %)	**0** (0 %)
Symptoms:			
Asymptomatic	**96** (84.95 %)	**47** (72.30 %)	**8** (53.33 %)
Symptomatic	**17** (15.04 %)	**18** (27.69 %)	**7** (46.66 %)
CD_4 (cell/µl) Counts:			
≥ 500	**87** (76.99 %)	**31** (47.69 %)	**7** (46.66 %)
499-200	**13** (11.50 %)	**17** (26.15 %)	**4** (26.66 %)
< 200	**13** (11.50 %)	**17** (26.15 %)	**4** (26.66 %)
Viral load (copies/ ml)			
≥10000 (High viral load)	**18** (15.92 %)	**26** (40 %)	**7** (46.66 %)
≤ 500 (Low viral load)	**12** (10.61 %)	**17** (26.15 %)	**4** (26.66 %)
<50 (Undetectable)	**83** (73.45 %)	**22** (33.84 %)	**4** (26.66 %)
Genotype assay			
Done	**23** (20.35 %)	**44** (67.69 %)	**15** (100 %)
Not Done	**90** (79.64 %)	**21** (32.30 %)	**0** (0 %)
ART resistance			

results			
***NRTIs (*n*=492)	**22** (4.47 %)	**125** (25.40 %)	**58** (11.78 %)
*NNRTIs (*n*= 246)	**21** (8.53 %)	**82** (33.33 %)	**24** (9.75 %)
*PIs (*n*= 492)	**17** (3.45 %)	**31** (6.30 %)	**9** (1.82 %)
*INSTIs (*n*= 164)	**0** (0 %)	**0** (0 %)	**0** (0%)

*Test results for each single medicine in this class in each single genotype assay attempt

3.2. Wilcoxon Signed Ranks Test [Wilcoxon matched-pairs test].

To determine if the ART was effective in the initial line of treatment, a Wilcoxon matched-pairs testanalysis was conducted using CD_4 and VL data from the whole sample (*n=193*) at baseline (no ART) and 6 months post ART.There are two major Laboratory parameters, CD_4 and VL. The effect of ART can be determined through these parameters i.e. increase the level of CD_4 and reduce the level of VL. [(36)]

Table 6: Wilcoxon Signed Ranks Test output from SPSS (*n*=193).

Ranks

		N	Mean Rank	Sum of Ranks
VL pre-treatment (copies/ml) - VL after first attempt (copies/ml)	Negative Ranks	5[a]	56.70	283.50
	Positive Ranks	188[b]	98.07	18437.50
	Ties	0[c]		
	Total	193		
CD4 Pre-treatment (µl) - CD4 after first attempt (µl)	Negative Ranks	174[d]	103.96	18089.50
	Positive Ranks	19[e]	33.24	631.50
	Ties	0[f]		
	Total	193		

a. VL pre-treatment (copies/ml) < VL after first attempt (copies/ml)

b. VL pre-treatment (copies/ml) > VL after first attempt (copies/ml)

c. VL pre-treatment (copies/ml) = VL after first attempt (copies/ml)

d. CD4 Pre-treatment (µl) < CD4 after first attempt (µl)

e. CD4 Pre-treatment (µl) > CD4 after first attempt (µl)

f. CD4 Pre-treatment (µl) = CD4 after first attempt (µl)

Test Statistics[a]

	VL pre-treatment (copies/ml) VL after first attempt (copies/ml)	CD4 Pre-treatment (µl) - CD4 after first attempt (µl)
Z	-11.682[b]	-11.234[c]
Asymp. Sig. (2-tailed)	.000	.000

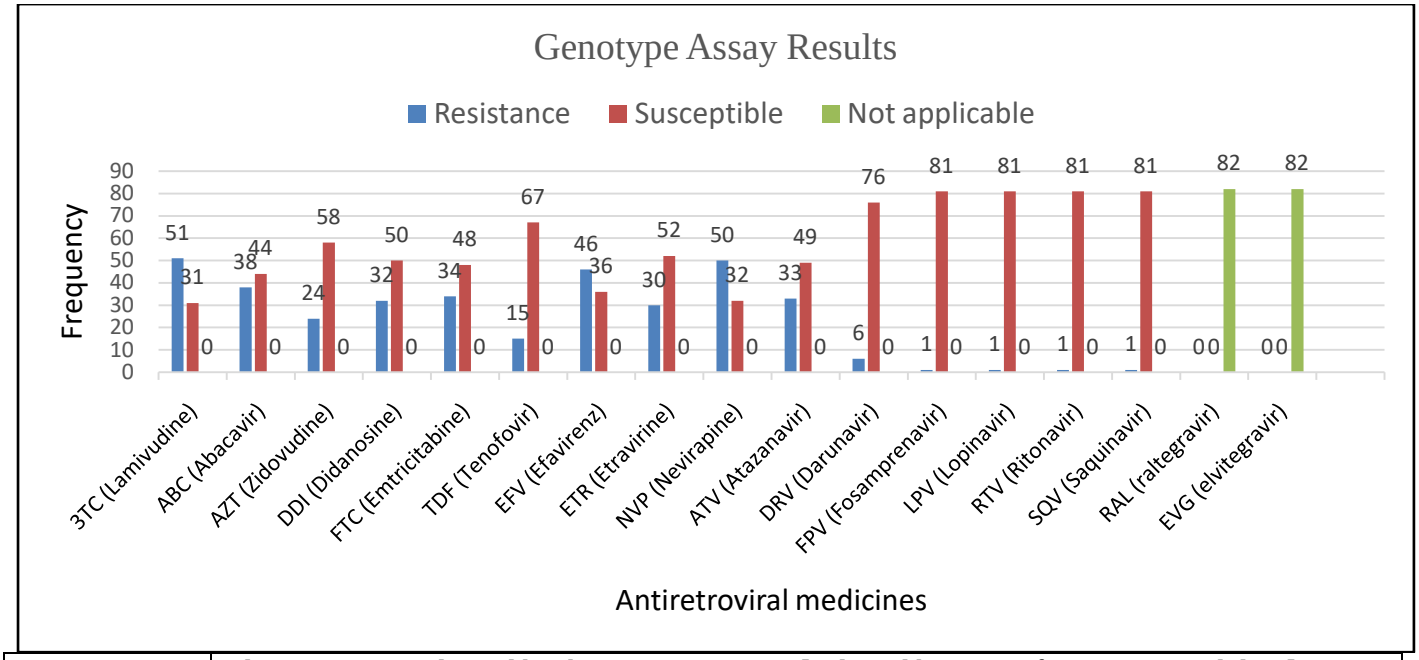
a. Wilcoxon Signed Ranks Test
b. Based on negative ranks.
c. Based on positive ranks.

As can be seen in **Table 6**, 5 participants out of 193 had a negative rank when theVL pre-treatment < VL after the first attempt and 188 out of 193 had a positive rank when the VL pre-treatment > VL after the first attempt. On the other hand, there were 174 out of 193 participantshad negative ranks when the CD4 Pre-treatment < CD4 after the first attempt and 19had positive ranks whenCD4 Pre-treatment > CD4 after the first attempt. In the lower part of table 6 illustrates, the test statistic Z= -11.682 forVL pre-treatment VL after first attempt with p-value <0.05 (0.000), and Z = - 11.234 for CD4 Pre-treatment -CD4 after first attempt with p-value < 0.05 (0.000). A Wilcoxon signed-rank test showed that a 6 months ART had elicited a statistically significant change in the VL and CD_4 values. Therefore, a null hypothesis was rejected and the alternative hypothesis accepted.

3.3. Genotype assay

To indicate the patterns and type of resistance mutations genotype tests had been done. From the total number of participants 193, only 82 had genotyping assays done. Data from these participants were further used to extract ART resistance results. NRTIs resistance was present in 4.47% of the participants on the first line of treatment, 25.40% of people in the second line of treatment and 11.78% of people in the third line of treatment. Overall, people in the first line of treatment exhibited lower rates of resistance for NRTI, NNRTI, PI, and INSTI. Most predominant mutations were seen in the second line of treatment where 25.40% of people had an NRTI resistance, and 33.33% had an NNRTI resistance. In the third line of treatment, NRTI resistance was predominant as can be seen in **Table 5** above. Genotype assay results are displayed in **Figures 3 and 4**.

Figure 3:Resistance and Susceptibility Patterns (*N*=82)(2018-2019).



Resistance	Clear mutation indicated by the genotype assays [Selected by a specific antiretroviral drug]
Susceptible	The HIV virus is liable to be influenced by a particular drug
Not applicable	Not done

The initial analysis for the genotype assay results showed that 3TC had the most frequent resistance rate 51 and 31 susceptible out of 82 equivalents to 62.19 % resistance and 37.80 % susceptible, followed by NVP and EFV. FPV, LPV, RTV, and SQV had the least frequent resistance rate**Figure 3**.

As patterns of resistance increase, the CD_4 count decrease and VL increase as a signal of ineffective therapy**.** [13, 14] As determined by descriptive analysis, people in the second line of treatment exhibited poorer CD_4 counts and higher VL, indicating a high susceptibility to further resistance. However, it must be considered NVP is included in the first line of treatment for multiple groups. [19] As a result, this therapy is among the firsts to which HIV exhibits resistance patterns.

Figure 4: TheMajor ART resistance mutations for the latest genotype assay (*N*=82)(2018-2019)

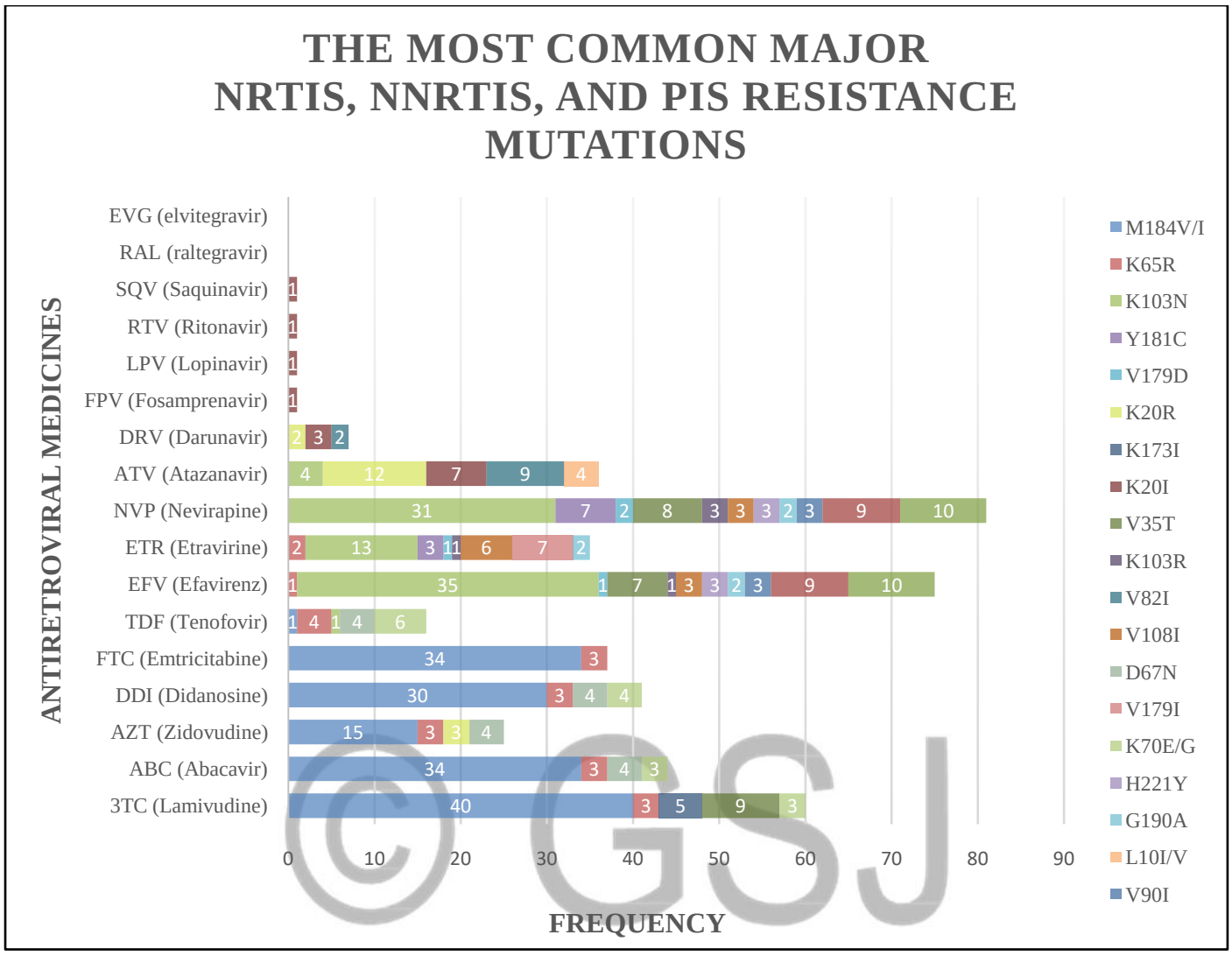


Figure 4shows, the most common major NRTIs, NNRTIs, and PIs resistance mutations for 82 participants [same population displayed in **Figure 3**], where eachcolour represents a differentmutation. NVP had the highest frequency of mutations, around 11 different mutations had appeared to be relevant to NVP resistance. The most frequent mutations in the NVP genotype test were K103N 31 (38.27%) out of 81. In 3TC, M184V/I scored the highest frequency with 40 times equivalent to 66.66% of the total number in 3TC. The total frequency of M184V/I in all ART was to be the highest with a total of 154 times (3TC=40+ABC=34+ AZT= 15+DDI=30+FTC=34+TDF=1). M184V/I had a major impact on NRTIs resistance and K103N in NNRTIs with little impact in PIs (ATV). Results in Figures 4 and 5 show, a fluctuation in the progression of ART as there were such cases that had developed resistance mutation compared to the CD4 and VL results post 6 months treatment.**Table7** below shows the abbreviations and the meaning of all major mutations.

Table 7: Summary of the study mutations and descriptions [amino acid substrates]

Abbreviations [Mutations]	Amino acid	Descriptions
M184V/I	M= Methionine V =Valine 184= Position of the codon	• Selected by 3TC and other NRTIs.
K65R	K= Lysine R= Arginine Position= 65	• Selected by different NRTIs.
Y181C	Y= Tyrosine C= Cysteine Position= 181	• A non-polymorphic mutation. • Selected by NVP, EFV, and ETR.
V179D	V= Valine D= Aspartate Position= 179	• Apolymorphic mutation. • Selected by EFV. • It reduces NVP and EFV susceptibility by 2 to 5-fold. • It reduces ETR and RPV susceptibility by 2 to 3-fold.
K20R	K= Lysine R= Arginine Position= 20	• A non-polymorphic mutation. • Selected by PIs.
K173I	K= Lysine I= Isoleucine Position =173	• Is variant in the STAT1 gene has not been reported previously as a pathogenic variant.
K20I	K= Lysine I= Isoleucine Position =20	• A non-polymorphic mutation. • Selected by PIs.
V35T	V= Valine T=Threonine Position =35	• A polymorphic mutation. • Selected by NNRTIs and NRTIs.
K103R	K= Lysine R= Arginine Position= 103	• A polymorphic mutation • Affects NNRTIs in combination with V179D.
V82I	V= Valine I=Isoleucine Position =82	• A highly polymorphic mutation that is not selected by PIs. • It is the consensus amino acid in subtype G viruses.
V108I	V= Valine I=Isoleucine Position =108	• A relatively non-polymorphic mutation. • Selected by NVP, and EFV.
D67N	D= Aspartate N=Asparagine Position=67	• A non-polymorphic Thymidine Analog Mutations (TAMs). • Associated with low-level resistance to AZT and d4T.
V179I	V= Valine I=Isoleucine Position =179	• A polymorphic mutation. • Selected by EFV.
K70E/G	K=Lysine E= Glutamate G= Glycine Position=70	• Anuncommon non-polymorphic mutation • Selected by d4T, and TDF.

H221Y	H= Histidine Y= Tyrosine Position=221	• A non-polymorphic mutation. • Selected by NNRTI in combination with Y181C
G190A	G=Glycine A= Alanine Position=190	• A non-polymorphic mutation. • Selected by NVP and EFV. • Alone *G190A* reduces NVP susceptibility >50-fold
L101/V	L= Leucine V= Valine Position=101	• Selected in vitro by EFV, and ETR
V90I	V=Valine I= Isoleucine Position=90	• A polymorphic mutation. • It is selected by EFV, and ETR.
P225H	P=Proline H= Histidine Position=225	• A non-polymorphic mutation • Selected by EFV that usually occurs in combination with K103N. • Together K103N and P225H cause >50-fold reduction in susceptibility to NVP and EFV
V179I	V= Valine I = Isoleucine Position=179	• A polymorphic mutation. • Selected by EFV. • It reduces NVP and EFV susceptibility by 2 to 5-fold and ETR

This table retrieved from (Gallant. 2006) [31], (National Library of Medicine. 2019) [32], (Parikh, et al. 2006) [33], (Stanford University. 2019) [34], (Yang .2012) [35], and (Wensing)[37]

4. Discussion

This research aimed to explore patterns of mutation of HIV to ART in Northern Oman in different populations. The study found that mutations leading up to resistance are more common in people undergoing the second-line of treatment by contrast with people in the first-line or third line of treatment. At the same time, this study found that the most common patterns of mutation are seen in people with NVP treatments, and the highest resistance rates are seen in people undergoing 3TC treatment, followed by NVP. Consequently, NNRTI therapy via NVP presents the highest number of mutations while NRTI therapy via 3TC presents the highest incidence of resistance**Figures 3 and 4**. Finally, this study found that while CD_4 counts improve and VL decreases after 6 months the first line of treatment, the use of NNRTIs and NRIs in combination with other NTRTI (nucleotide analog reverse transcriptase), is not sufficient to evade mutation and resistance. While significant improvements are seen in the first-line of treatment in CD_4 and VL counts, the fact that in the second-line of treatment a higher rate of mutations and resistance susceptibility are seen points to the fact that people in this group are more likely to develop resistance in the near future. This may indicate that the second line of treatment may need additional therapies to diminish the likelihood of mutations.[15]

Limitations in interpreting these results should be considered. Firstly, adherence to therapy is a relevant aspect of mutation and resistance.[28]Data on adherence to ART from the sample included in this study were not available. Thus, the implications of adherence to ART for patterns of mutations and resistance susceptibility were not considered. Secondly, ART resistance patterns from transmitted ART resistance could not be considered. This analysis would have required a clear source of infection identification (person) and genotype assays from the infection source. Strengths of the current investigation include the large sample size used, indicative of a high factor of generalisability [20] and the use of data analysis techniques that present a high internal validity factor.[22] Consequently, this study meets the criteria for academic rigour and provides an accurate description of ART resistance patters in the HIV positive population of North Oman.

This study found that patterns of mutation in the studied populations are similar to those reported by other investigations.[5, 6] Similar to these investigations, there is a higher prevalence of HIV infections in the male by contrast to females. This is the first study to report that in the North Oman HIV population, the incidence of HIV is increased with bisexual behaviour. This may be due to the fact that this type of sexual behaviour exposes people to a double risk of infection by engaging with both male and female partners. These findings, combined with rates of homosexual and heterosexual behaviours indicate that in the studied population, HIV is mostly transmitted through unprotected sex. These results are similar to other investigations in HIV transmission patterns in Oman.[5]Another important aspect regarding this mode of transmission is that in this study, the highest rates of transmission have been found in three groups of sexually active populations (15-30, 31-45 and 45-60), with the highest rate being observed in the 31-45 age group. Corroborating data and results obtained in this study indicate that a significant route of preventing new infections is educating HIV carriers and the general public into safe sex practices.

This study also found similar to other reports that the highest rates of resistance are reported for second-line therapies for NRTI and NNRTI. [17] This is because these ARTs are generally used as first-line therapies and people are thus more likely to switch to the second-line when resistance emerges. These results may indicate that genotype assays should be carried out every six months, and medication adjusted accordingly, especially for people with susceptibility to resistance. Data obtained from the paired t-test analysis indicated that with the first-line therapy approach, improvements in CD_4 and VL were seen for the included sample. Nonetheless, considering the mutation patterns observed, these improvements are less likely to be sustained, especially when considering the high number of genomic resistances manifested by patients in the second-line. Other studies [28, 30] on NRTI and NNRTI resistance patters note because these ARTs are used as the main first-line therapy in highly active antiretroviral therapy (HAART), they are becoming more and more ineffective in blocking HIV replication. Additionally, due to the development of resistance to NRTI and NNRTI, new HIV cases in ART naïve patients already present resistance when transmission occurred from an individual infected with ART-resistant HIV. Subsequently, in ART naïve patients, the most common

pattern of resistance is seen for NRTI and NNRTI therapies.[29] This further underlines the need for safe sex education in HIV carriers, as they may spread a resistant virus and thus pose significantly higher treatment management issues for newly infected individuals. At the same time, these findings also point towards the need to develop improved ART therapies to counteract the increasing resistance patterns.

Patterns of resistance detected in the North Oman population thus seem to resemble patterns of resistance to ART from other countries.[3, 4, 25] This study adds to the growing body of evidence mounting towards NRTI and NNRTI development as a response to the increasing prevalence of mutation patterns in this ART line. This study further confirms that in the studied population, genomic assay for detecting resistance to ART even for ART naïve patients may be necessary, given the high rates for NVP resistance. Finally, this study underlines the need for educating patients with HIV and the general public in safe sex practice to avoid new HIV infections, especially with ART-resistant strands. A further recommendation is to initiate a pre-treatment drug resistance (PDR) testing to avoid any chance of transmitted resistance. [16] Also, all HIV-1 medical team including the pharmacist should exert more effort toward improving patient adherence and improving treatment monitoring. [4]

5. Conclusion

An important aspect uncovered by this study and supported by additional investigations is the need for future developments in ART approaches. HIV resistance to antiviral medication targeting the reverse transcriptase stage of the viral replication process is increasing across populations. This trend has also been observed in the population investigated in this study. The national and international guidelines currently recommend the use of combined retroviral therapies albeit most of these recommendations focus on the use of NNRTIs and NRTIs. This may be a significant contributor to increasing patterns of resistance. Current data [18] suggests that using multiple targets in the viral replication process may be more effective in preventing the emergence of resistance conferred through mutations, as these combinations require the virus to produce multiple concomitant mutations. Considering that this is an unlikely scenario, these ART combinations may be more effective in managing HIV replication.

Nevirapine and efavirenz (NNRTIs) could be substituted with alternative drugs. In addition, the use of Zidovudine rather than Tenofovir in the second line. Moreover, high frequency of M184V/I mutation may limit the effectiveness of NRTIs in the second and alternative line of treatments.

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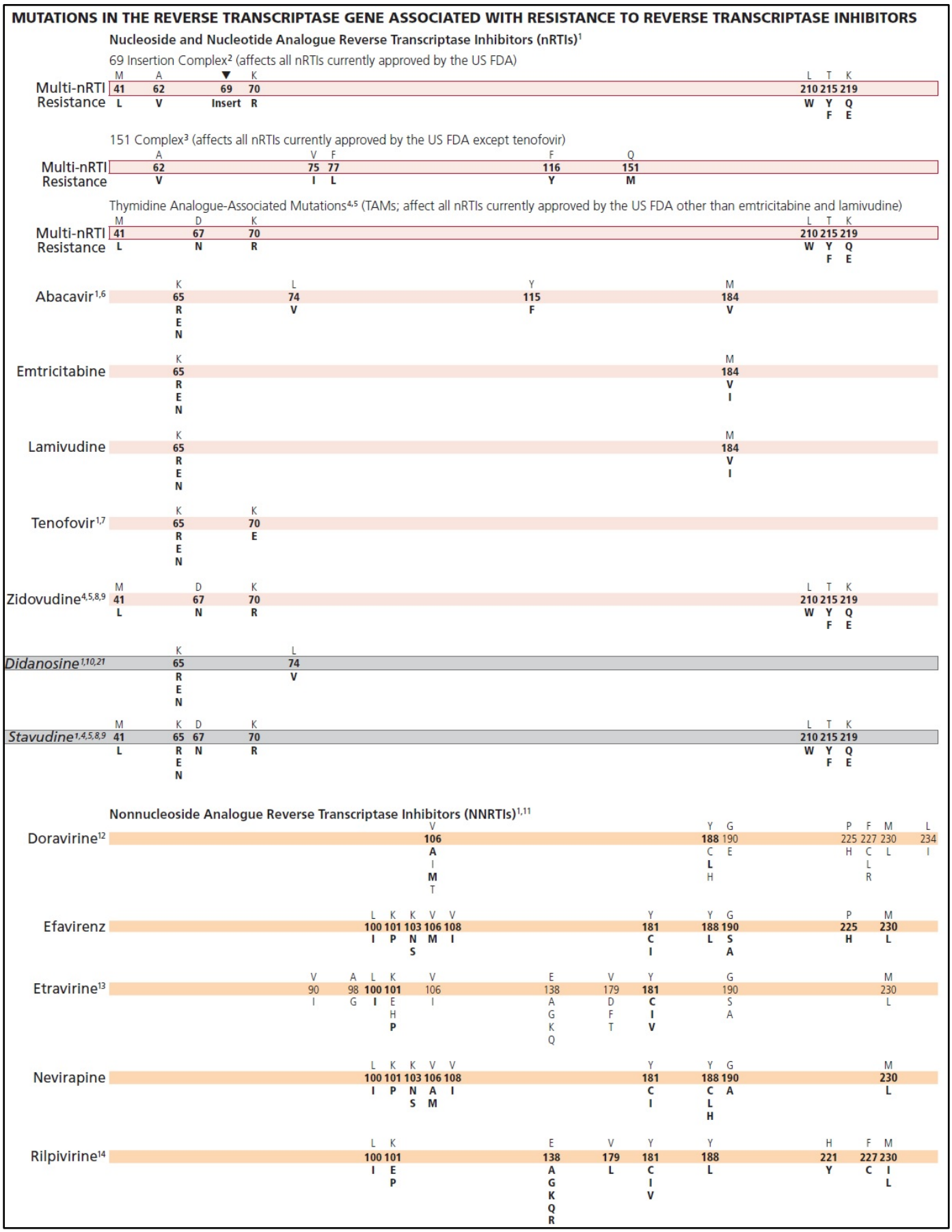
References

1. UNAIDS. *Global HIV & AIDS statistics — 2019 fact sheet* [Internet]. Unaids.org. 2019 [cited 21 September 2019]. Available from: https://www.unaids.org/en/resources/fact-sheet
2. HIVgov. *Global Statistics* [Internet]. HIV.gov. 2019 [cited 21 September 2019]. Available from: https://www.hiv.gov/hiv-basics/overview/data-and-trends/global-statistics
3. Abbas U, Glaubius R, Ding Y, Hood G. Drug resistance from preferred antiretroviral regimens for HIV infection in South Africa: A modeling study. *PLOS ONE*. 2019; 14(7): e0218649.
4. Hosseinipour M, Gupta R, Van Zyl G, Eron J, Nachega J. Emergence of HIV Drug Resistance During First- and Second-Line Antiretroviral Therapy in Resource-Limited Settings. *The Journal of Infectious Diseases*. 2013; 207(suppl_2): S49-S56.
5. Al Awaidy S, Sharanya A. Successes and Challenges of HIV/AIDS Program in Oman: 1984–2015. *Oman Medical Journal*. 2019; 34(1):1-8.
6. UNAIDS. Oman [Internet]. Unaids.org. 2018 [cited 21 September 2019]. Available from: https://www.unaids.org/en/regionscountries/countries/oman
7. Desport M. *Lentiviruses and macrophages*. Norfolk: Caister Academic; 2010.
8. Masemola A, Mashishi T, Khoury G, Mohube P, Mokgotho P, Vardas E et al. Hierarchical Targeting of Subtype C Human Immunodeficiency Virus Type 1 Proteins by CD8+ T Cells: Correlation with Viral Load. *Journal of Virology*. 2004; 78(7):3233-3243.
9. Metodiev K. *Immunodeficiency*. Rijeka: Intech; 2012.
10. Georgiev V. *National Institute of Allergy and Infectious Diseases*, NIH. Totowa, N.J.: Humana; 2009.
11. Goodsell D, Dutta S, Zardecki C, Voigt M, Berman H, Burley S. The RCSB PDB "Molecule of the Month": Inspiring a Molecular View of Biology. *PLOS Biology*. 2015; 13(5): e1002140.
12. Irwin K, Renzette N, Kowalik T, Jensen J. Antiviral drug resistance as an adaptive process. *Virus Evolution*. 2016; 2(1): vew014.
13. Bracq L, Xie M, Benichou S, Bouchet J. Mechanisms for Cell-to-Cell Transmission of HIV-1. *Frontiers in Immunology*. 2018; 9.
14. Vidya Vijayan K, Karthigeyan K, Tripathi S, Hanna L. Pathophysiology of CD4+ T-Cell Depletion in HIV-1 and HIV-2 Infections. *Frontiers in Immunology*. 2017; 8.
15. Yu F, Li W, Wang L, Dai Y, Lu X, Wang Q et al. Combining New Non-Nucleoside Reverse Transcriptase Inhibitors (RTIs) with AZT Results in Strong Synergism against Multi-RTI-Resistant HIV-1 Strains. *Molecules*. 2018; 23(7):1599.
16. Yendewa G, Sahr F, Lakoh S, Ruiz M, Patiño L, Tabernilla A et al. Prevalence of drug resistance mutations among ART-naive and -experienced HIV-infected patients in Sierra Leone. *Journal of Antimicrobial Chemotherapy*. 2019; 74(7):2024-2029.

17. World Health Organisation. *Consolidated guidelines on: The antiretroviral drugs for treating and preventing HIV infection*. 2nd ed. [ebook] Geneva: WHO, 2016, pp.1-480. [Cited 12 Jun. 2019] Available at: https://apps.who.int/iris/bitstream/handle/10665/208825/9789241549684_eng.pdf?sequence=1.
18. Department of Health and Human Services. *FDA-Approved HIV Medicines Understanding HIV/AIDS* [Internet]. AIDSinfo. 2019 [cited 27 September 2019]. Available from: https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/21/58/fda-approved-hiv-medicines
19. Al-Maani A. *HIV Management in Oman* [Internet]. 3rd ed. Muscat: MOH; 2015 [cited 27 September 2019]. Available from: https://www.researchgate.net/publication/299827458.
20. Creswell J. *Research Design: Qualitative, Quantitative, and Mixed Methods Approaches*. 4th ed. London: SAGE; 2014.
21. Yorgure C. *Choosing a Research Method, Scientific Inquiry*. London: Xlibris; 2018.
22. Salkind N. *Encyclopedia of Research Design*. London: SAGE; 2010.
23. Stommel M, Wills C. *Clinical research. Philadelphia*: Lippincott Williams & Wilkins; 2004.
24. Lavrakas, P. *Encyclopaedia of Survey Research Methods*. Los Angeles [u.a.]: SAGE, 2008 [Cited 21 September 2019]. Available at: https://methods.sagepub.com/reference/encyclopedia-of-survey-research-methods.
25. Hamers R, Sigaloff K, Wensing A, Wallis C, Kityo C, Siwale M et al. Patterns of HIV-1 Drug Resistance After First-Line Antiretroviral Therapy (ART) Failure in 6 Sub-Saharan African Countries: Implications for Second-Line ART Strategies. *Clinical Infectious Diseases*. 2012; 54(11):1660-1669.
26. Asahchop E, Wainberg M, Sloan R, Tremblay C. Antiviral Drug Resistance and the Need for Development of New HIV-1 Reverse Transcriptase Inhibitors. *Antimicrobial Agents and Chemotherapy*. 2012; 56(10):5000-5008.
27. Machnowska P, Meixenberger K, Schmidt D, Jessen H, Hillenbrand H, Gunsenheimer-Bartmeyer B et al. Prevalence and persistence of transmitted drug resistance mutations in the German HIV-1 Seroconverter Study Cohort. *PLOS ONE*. 2019; 14(1): e0209605.
28. Chen J, Zhang M, Shang M, Yang W, Wang Z, Shang H. Research on the treatment effects and drug resistances of long-term second-line antiretroviral therapy among HIV-infected patients from Henan Province in China. *BMC Infectious Diseases*. 2018; 18(1):1-10.
29. Gallant, J.E. The M184V mutation: what it does, how to prevent it, and what to do with it when it's there. *PubMed.* 2006, 16(10), pp.556-9.
30. NationalLibraryof Medicine.Genomic variation as it relates to human health. *VCV000451429.2 - ClinVar - NCBI.* [Internet]. 2019 [Cited 26 Sep. 2019]. Available at: https://www.ncbi.nlm.nih.gov/clinvar/variation/451429/

31. Parikh U, Bachelor L, Koontz D, Mellors J. The K65R Mutation in Human Immunodeficiency Virus Type 1 Reverse Transcriptase Exhibits Bidirectional Phenotypic Antagonism with Thymidine Analog Mutations. *Journal of Virology*. 2006; 80(10):4971-4977.
32. Stanford University. *NRTI Resistance Notes - HIV Drug Resistance Database*. [Internet]. 2019 [Cited 26 Sep. 2019]. Available at: https://hivdb.stanford.edu/dr-summary/resistance-notes/NNRTI/
33. Yang C, Liu S, Zhang T, Hou Y, Liu X, Gao Y et al. Transmitted Antiretroviral Drug Resistance and Thumb Subdomain Polymorphisms Among Newly HIV Type 1 Diagnosed Patients Infected with CRF01_AE and CRF07_BC Virus in Guangdong Province, China. AIDS Research and Human Retroviruses. 2012; 28(12):1723-1728.
34. MacFarland T.W and Yates J.M. Wilcoxon matched-pairs Signed- Ranks Test. *Springer Link*, 2016; pp.133-175.
35. Hayes, A. (2019). What a Z-Score Tells Us. [online] Investopedia. Available at: https://www.investopedia.com/terms/z/zscore.asp [Accessed 22 Dec. 2019].
36. Patel, A., Trivedi, S.S, and Patel, P.K. Effect of antiretroviral therapy on clinical and immunologic disease progression in HIV positive children: One year follow up study. *Journal of Family and community medicine,* 2012; 9(3):178-183.
37. Wensing A, Calvez V, Silberstein F, Charpentier C, Günthard H, Paredes R et al. *2019 Update of the Drug Resistance Mutations in HIV-1* [Internet]. Iasusa.org. 2019 [cited 25 December 2019]. Available from:https://www.iasusa.org/wp-content/uploads/2019/07/2019-drug-resistance-mutations-figures.pdf
38. Medeiros M, Arruda É, Guerrant R, Brown C, Hammarskjold M, Rekosh D, et al. Genotype testing and antiretroviral resistance profiles from HIV-1 patients experiencing therapeutic failure in northeast Brazil. *Brazilian Journal of Infectious Diseases*. 2007; 11(4).

Appendix 1: Different mutations in different AR- groups.



MUTATIONS IN THE PROTEASE GENE ASSOCIATED WITH RESISTANCE TO PROTEASE INHIBITORS (PIs)[15,16,17]

	L10	G16	K20	L24	V32	L33	E34	M36	M46	G48	I50	F53	I54	D60	I62	I64	A71	G73	V82	I84	I85	N88	L90	I93
Atazanavir +/- ritonavir[18]	I F V C	E	R M I T V	I	I	I F V	Q	I L V	I L	V	**L**	L Y	L V M T A	E	V	L M V	V I T L	C S T A	A T F I	**V**	V	**S**	M	L M
Darunavir/ ritonavir[19]	V 11 I				V 32 I	L 33 F					I **47 V**		I **50 V**						T 74 P	L **76 V**		I **84 V**		L 89 V	
Lopinavir/ ritonavir[20]	L 10 F I R V		K 20 M R	L 24 I	**32 I**	L 33 F			M 46 I L	I **47 V A**	I **50 V**	F 53 L	I **54 V L A M T S**			L 63 P	A 71 V T	G 73 S	L **76 V**	V **82 A F T S**	I **84 V**			L 90 M	
Tipranavir/ ritonavir	L 10 V					L 33 F		M 36 I L V	K 43 T	M 46 I L	I **47 V**		I 54 A M V	Q **58 E**		H 69 K R		T **74 P**		V **82 L T**	N **83 D**	I **84 V**		L 89 I M V	
Fosamprenavir/ ritonavir[21]	L 10 F I R V				V 32 I				M 46 I L	I 47 V	I **50 V**		I 54 L V M					G 73 S	L 76 V	V 82 A F S T		I **84 V**		L 90 M	
Indinavir/ ritonavir[21]	L 10 I R V		K 20 M R	L 24 I	V 32 I			M 36 I	M **46 I L**				I 54 V				A 71 V T	G 73 S A	L 76 V	V 77 I	V **82 A F T**	I **84 V**		L 90 M	
Nelfinavir[21,22]	L 10 F I				D **30 N**			M 36 I	M 46 I L								A 71 V T			V 77 I	V 82 A F T S	I 84 V	N 88 D S	L **90 M**	
Saquinavir/ ritonavir[21]	L 10 I R V			L 24 I						G **48 V**			I 54 V L		I 62 V		A 71 V T	G 73 S		V 77 I	V 82 A F T S	I 84 V		L **90 M**	

MUTATIONS IN THE ENVELOPE GENE ASSOCIATED WITH RESISTANCE TO ENTRY INHIBITORS

	G36	I37	V38	Q39	Q40	N42	N43
Enfuvirtide[23]	**D S**	**V**	**A M E**	**R**	**H**	**T**	**D**
Maraviroc[24]	See User Note						

MUTATIONS IN THE INTEGRASE GENE ASSOCIATED WITH RESISTANCE TO INTEGRASE STRAND TRANSFER INHIBITORS[25]

	T66	G118	F121	E138	G140	Y143	Q148	S147	S153	N155	E92	T97	L74	R263
Bictegravir[26]		G 118 R		E 138 K	G 140 S		Q **148 H**							R **263 K**
Cabotegravir[27]	T 66 K	G **118 R**		E 138 A K T	G **140** A C **R** S		Q **148 H K R**		S 153 F Y	N 155 H				R **263 K**
Dolutegravir[28]		G **118 R**	F 121 Y	E 138 A K T	G 140 A S		Q **148 H K R**			N 155 H				R **263 K**
Elvitegravir[29]	T **66 I** A K		F **121 Y**				S **147 G** Q **148 H K R**			N **155 H**	E **92 Q** G	T 97 A		R **263 K**
Raltegravir[30]			F **121 Y**	E 138 A K	G 140 A S	Y **143 R H C**	Q **148 H K R**			N **155 H**	E 92 Q	T 97 A	L 74 M	R **263 K**