Research Article ISSN 3065-565X

International Journal of Research in Virology

HIV-1 Co-receptor Tropism Prediction Among Chronic Patients in Kiambu County, Kenya

Gachoki Jackline*, Muturi Margaret and Gachara George

Department of Medical Laboratory Sciences, Kenyatta University, Nairobi, Kenya.

***Correspondence:**
Gachoki Jackline, Department of Medical Laboratory Sciences, Kenyatta University, Nairobi, Kenya.

Received: 10 Jul 2026; **Accepted:** 17 Aug 2026; **Published:** 29 Aug 2026

Citation: Gachoki Jackline, Muturi Margaret, Gachara George. HIV-1 Co-receptor Tropism Prediction Among Chronic Patients in Kiambu County, Kenya. Int J Res Virol. 2026; 3(2): 1-8.

ABSTRACT

Entry of human immunodeficiency virus-1 (HIV-1) into host cells involves an interaction between host cell receptors and the virus envelope glycoproteins, specifically, glycoprotein 120 (gp120). The variable 3 (V3) loop region of HIV-1 gp120 envelope protein plays an essential role in HIV-1 coreceptor binding and is thus a determinant for host cell tropism. Viral tropism is therefore an important consideration during coreceptor-dependent therapy development, necessitating the current study. Venous blood samples were collected and plasma obtained from 110 patients attending HIV Clinics in 3 hospitals in Kiambu County. RNA extraction was done, followed by reverse transcriptase polymerase chain reaction (RT-PCR) and Sanger sequencing. Six sequences (N=6) were generated for downstream analysis, and all were determined to be subtype A1 using phy-logenetic analysis. To validate the study findings due to the few sequences, an additional 62 HIV-1 subtype A1 sequences from the HIV Los Alamos National Library (LANL) database were extracted and analysed with the study sequences for tropism and genetic diversity validation of the V3 loop region. Five of the six study sequences were identified as CXCR4-tropic strains. The V3 region comprising 35 amino acids harboured a conserved N-linked glycosylation site at amino acid position 301 in all the sequences. The V3 crown region was conserved in all the sequences except for the CCR5-tropic strain that harboured mutations. Although HIV-1 subtype A1 showed high CCR5 usage on validation, CXCR4 usage was common among the study sequences. CCR5-using strains were likely to harbour V3 crown mutations in this subtype. Further research to understand the genetic diversity of subtype A1 V3 region is advocated.

Keywords

HIV-1 infections, Viral tropism, Receptor: CXCR4, Receptor: CCR5.

Abbreviations

CCR5: C-C chemokine receptor Type 5, CD4: Cluster of Differentiation 4, CXCR4: C-X-C chemokine receptor Type 4, EDTA: Ethylenediaminetetraacetic acid, SSA: Sub-Saharan Africa, UNAIDS: Joint United Nations Programme on HIV/AIDS, FDA: Food and Drug Administration, HIV-1: Human Immunodeficiency Virus-Type 1, PCR- Polymerase Chain Reaction, RNA: Ribonucleic acid, RT-PCR: Reverse Transcriptase-Polymerase Chain Reaction, V3: Variable region 3.

Background

Human immunodeficiency virus-1 (HIV-1) replication is highly dependent on successful host cell entry. Cell entry is initiated when the viral glycoprotein (gp)160 that comprises of gp120/gp41 attaches to a susceptible host cell receptor. The gp120 binds to a host cell CD4 receptor, inducing several conformational changes in gp120, exposing the third variable (V3) loop region for recognition by chemokine coreceptors CCR5 or CXCR4 [1]. This initiates several refolding events in gp41, which leads to the formation of a trimeric fusion peptide that eventually fuses with the host cell membrane, completing the entry process [1,2]. Based on the coreceptor type used, HIV-1 CCR5-utilizing strains are basically associated with transmission ability, while CXCR4-using strains are associated with disease progression [3]. Viral coreceptor tropism can be determined genotypically based on the examination of the V3 loop sequence of gp120, which is a key determinant for coreceptor tropism, or phenotypically based on syncytium formation by HIV-1 cells on MT-2 cells or viral replication on non-

lymphoid cell lines that stably support CD4 cells and either CCR5 or CXCR4 [4].

The V3 region of HIV-1 has been shown to contain key antigenic sites and neutralizing epitopes that are exposed upon binding to the CD4 receptor. Due to the exposed nature of the HIV-1 V3 loop region, it has a high immune activation ability in comparison to the HIV-2 V3 region, which is less exposed [5]. Therefore, there is substantial genetic variation and amino acid diversity in HIV-1, which influence envelope gene evolution to maintain viral replication fitness and the ability to escape selection pressure from antiretroviral therapy and the immune system [6]. The V3 region remains a key determinant of viral coreceptor tropism for HIV and is therefore a promising target region for the development of new antiviral drugs and vaccines [1]. Continued research on neutralizing antibodies and understanding mechanisms of immune escape shows continued co-evolution in HIV-1 antigenicity and coreceptor usage during infection [7,8]. Polymorphism on HIV-1 coreceptors, CCR5 and CXCR4, influences susceptibility to HIV-1 and disease progression [9]. CCR5- delta 32 deletion and chemokine coreceptor 2- 64I polymorphism (CCR2-64I) confer protection to HIV-1 while some promoter mutations 59356 C/T to CCR5 increase the coreceptor expression accelerating infectivity [10,11].

HIV-1 therapy involves a variety of drugs that target and inhibit various steps during the replication cycle of the virus [12]. There are resistance mutations identified against non-nucleoside reverse transcriptase inhibitors, nucleotide reverse transcriptase inhibitors, integrase strand transfer inhibitors, and protease inhibitors, which are commonly used for patient management [13-15]. According to a drug resistance report of 2024, there is increasing prevalence of dolutegravir resistance in low- and middle-income countries with experienced treatment cohorts compared to treatment-naïve cohorts [16]. This necessitates a need for adding other drug classes like entry inhibitors in the pool of currently used drugs in regions like Sub-Saharan Africa (SSA). This is because SSA continues to bear the highest burden of HIV, with different clades of the virus and thus variability in genetic variants [17]. The CCR5 antagonist, Maraviroc (an entry inhibitor), is an FDA-approved drug that has not been used in Kenya [18,19].

In Kenya, the HIV-1 prevalence has declined in recent years. However, several HIV-1 subtypes remain in circulation with varying intensity and distribution across various geographic regions [20]. This study explores the genomic characterization of the V3 region for coreceptor tropism and stratifies by early or chronic infections among HIV-1 sequences in Kenya in a group of HIV-1 patients who attended 3 health facilities in Kiambu County, Kenya. This was a sub-study within a main study that enrolled patients who were newly diagnosed with HIV-1 or patients who were not yet initiated on dolutegravir in these 3 facilities.

Methods

Study design and setting

This was a cross-sectional study conducted in 3 hospitals: AIC Kijabe Hospital, AIC Kijabe-Marira, and Holy Cross Thigio in Kiambu County. Data collection was done between September 2020 and November 2020. Purposive sampling techniques were employed to select patients who were newly diagnosed or those who were treatment-experienced but had not been started on a dolutegravir-based regimen. These patients were then enrolled into the study after obtaining their written informed consent.

Ethical considerations

Ethical approval was sought and granted from the AIC Kijabe Hospital Institutional Ethics and Research Review Committee, reference number KH-IERC/02718/0084/2020, and a research permit obtained from the National Commission for Science, Technology and Innovation (NACOSTI) under license number NACOSTI/P/20/6034.

Study population, Sample collection and preparation

Treatment-naïve and experienced patients not on a dolutegravir regimen visiting the HIV clinics in the 3 healthcare facilities were selected. After the study was explained to them and written informed consent given, they were enrolled into the study. Following study enrolment, patient demographic data, including sex, age, prior and current treatment therapies used, treatment therapy start date, viral load, and present comorbidities, were collected. Five (5) mL of venous blood sample was then collected in EDTA plasma precipitate tubes from each patient. The blood was thereafter centrifuged for 10 minutes at 1100 rpm and plasma stored at -80°C until further processing.

RNA extraction, PCR and gene sequencing

RNA extraction from the plasma samples was performed using the Qiagen viral RNA mini kit (Qiagen Hilden, Germany) as per manufacturer's instructions and stored at -80°C until subsequent use. A 313 bp portion of the HIV-1 envelope gene was amplified using a nested RT-PCR protocol using previously designed primers E80 and E105 (first round), E110 and E125 (second round) [21]. The first-round PCR was run using 6µl of RNA template and 19 µl of One Step Ahead RT-PCR Qiagen master mix under the following conditions: reverse transcription for 10 min at 50°C, initial denaturation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 30s. The final extension was done at 72°C for 7 min. The second-round PCR was done using 5µl of first-round template and 20µl of One Step Ahead RT-PCR master mix under the following conditions: initial denaturation at 94°C for 3 min followed by 36 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min followed by a final extension at 72°C for 7 min. After PCR, the products were run on a 2% agarose gel at 120 V for 45 minutes to confirm bands of ~313 bp. Sanger sequencing was done using an ABI 3500xL Genetic Analyzer (Applied Biosystems). The resulting HIV-1 nucleotide sequences were manually edited and assembled using BioEdit Ver 7.2.5 software [22]. The assembled nucleotide sequences were then aligned using a reference sequence, HIV-1 HXB2 (reference number: K03455.1), using CLUSTALW in MEGA version 10.05 [23].

Phylogenetic tree construction

The study sequences, together with HIV-1 Subtype reference sequences downloaded from GenBank, were aligned using the HIV-1 HBX2, and a phylogenetic tree was constructed using MEGA version 10.05 utilizing the maximum likelihood method [23]. Bootstrap values were used to assess the topology and robustness of the resulting phylogenetic tree.

Generation of additional HIV-1 sequences

To comprehensively describe the genetic diversity of HIV-1 subtype A1 in Kenya due to the limited number of sequences generated from the study, additional sequences were extracted (Figure1) from the HIV Los Alamos National Library (LANL) database (https://www.hiv.lanl.gov) (accessed 20th March 2026) and used to validate the primary analysis. The sequences were selected based on HIV-1 subtype: A1, Country: Kenya, genomic region: envelope region, stage of infection: early or chronic sequences, preferably sampled until the year 2020. Early infection was defined as a period comprising seroconversion or having a recent infection, and chronic infection as the period post-recent infection [24]. To avoid overestimation of coreceptor usage and genetic diversity, only one sequence per patient was used; the first sequence for patients with multiple sequences was used.

HIV-1 Coreceptor determination and V3 diversity

Aligned sequences were used to analyse coreceptor tropism. Coreceptor tropism was predicted using Geno2pheno [25] at 5% FPR, 10% FPR, and 15% FPR prediction tool. Net charge of the sequences was determined by the formula (V3 net charge=(R+K+H)-(D+E)). Amino acids were extracted against a reference sequence using the online Los Alamos gene cutter tool (https://www.hiv.lanl.gov/content/sequence/GENE_CUTTER/cutter.html). The extracted V3 sequences were then applied to the N-Glycosite tool (https://www.hiv.lanl.gov/content/sequence/GLYCOSITE/glycosite.html) to identify N-linked glycosylation sites in the sequences. To visualize the V3 amino acids of the various sequences and their positions, WebLogo was used [26].

Statistical analysis

Proportions for categorical variables and median values were calculated for continuous variables. Co-receptor usage prediction agreement among the algorithms was calculated using percent agreement due to the smaller number of reads. Statistical analyses were conducted using R version 4.5.2.

Results

Patient Characteristics

The total number of enrolled patients was 110; however, one patient was excluded from the analysis due to missing clinical data. Females accounted for the larger proportion of enrolled patients at 83% (91/109), with 11% (10/91) enrolled in the Prevention of Mother-to-Child Transmission of HIV (PMTCT) program. The age range of the patients was 15 - 69 years, with a median age of 42 (IQR 33-46). The proportion of enrolled patients on a second-line regimen of treatment was 39%. At least 58% of the enrolled patients had been on more than 1 treatment regimen during their years of living with HIV, with the median year of treatment initiation being 2012 (IQR 2008-2016). Patients with a viral load of ≥1000 copies were 12% (12/109), and the viral load of newly diagnosed patients (n=6) was undetermined, as the study depended

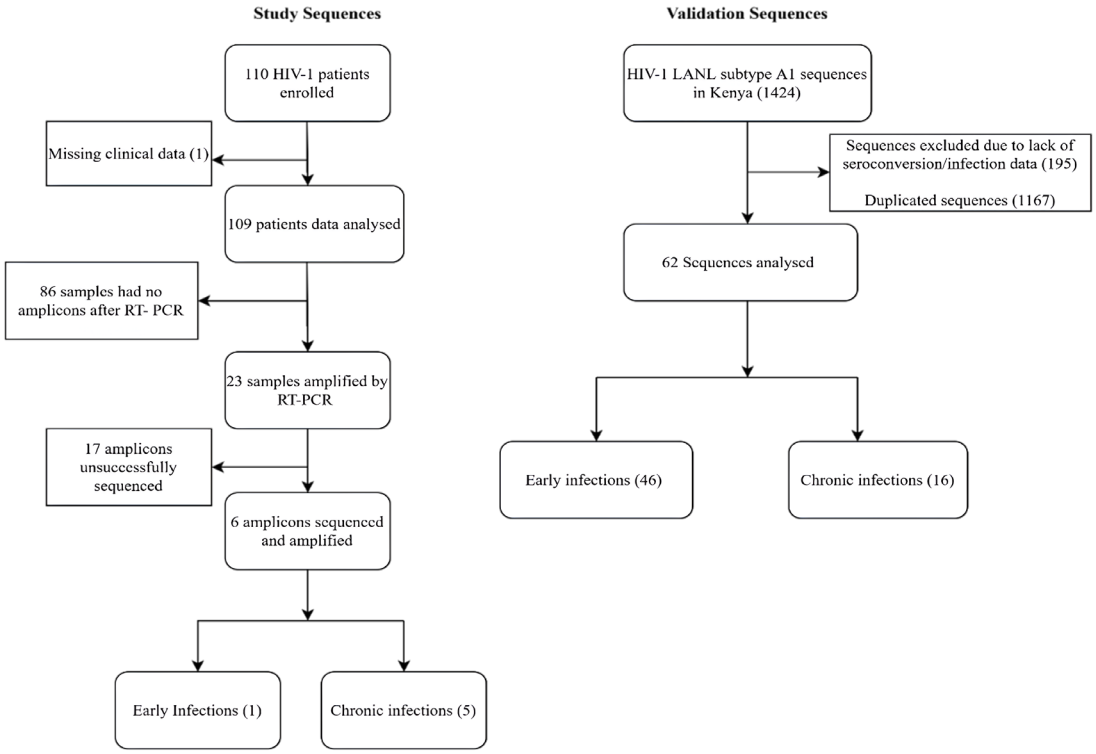

Figure 1: Flowchart showing study and validation sequences distribution among the early and chronic infections arms.

on recorded data. *Mycobacterium tuberculosis* was detected in 3 newly diagnosed patients and in one HIV treatment-experienced patient who had undetectable levels of viral load. This is shown in Table 1.

Table 1: Patient demographics and clinical characteristics of enrolled HIV-1 participants attending 3 hospitals in Kiambu County.

Characteristic	Overall	Male	Female
	N = 109[1]	N = 18[1]	N = 91[1]
Age, years Median (IQR)	42.0 (33.0,46.0)	42.0 (31.0,46.0)	41.0 (33.0,47.0)
(missing)	1	1	0
Treatment class			
First-line	63 (61%)	5 (36%)	58 (65%)
Second-line	40 (39%)	9 (64%)	31(35%)
Newly diagnosed	6	4	2
No. of previous regimens used			
0	46 (42%)	6 (33%)	40 (44%)
1	51 (47%)	12 (67%)	39 (43%)
2	10 (9.2%)	0 (0%)	10 (11%)
3	2 (1.8%)	0 (0%)	2 (2.2%)
Viral load			
<50	80 (78%)	10 (71%)	70 (79%)
<1000	11 (11%)	1 (7.1%)	10 (11%)
>1000	12 (12%)	3 (21%)	9 (10%)
Undetermined	6	4	2
Coinfections			
MTB	4 (3.7%)	2 (11.1%)	2 (2.2%)
Comorbidities			
DCM	1 (0.9%)	0 (0%)	1 (1.1%)
DM	1(0.9%)	1(5.6%)	0 (0%)
Hypertensive	2 (0.9%)	0 (0%)	2 (1.1%)
[1] n / N (%); Median (Q1, Q3)			

DCM- Dilated Cardiomyopathy; DM- Diabetes Mellitus; MTB- Mycobacterium tuberculosis.

Laboratory analysis and characteristics of the sample patients

Out of the 110 samples collected, HIV DNA was successfully amplified by RT-PCR in 23, and 6 yielded good-quality sequences upon sequencing. All 23 amplified samples had detectable viral loads or were newly diagnosed. Among the 6 samples sequenced, four were from patients on second-line treatment, one from a patient on first-line treatment, and one from a newly diagnosed patient-see Table 2.

Table 2: Characteristics of patients with amplified envelope sequences in this study.

Sample ID	Sex	Age in years	Treatment start year	Viral Load	Treatment class
12	Female	44	2017	35029	Second-line
17	Male	42	2017	137	Second-line
19	Female	40	2006	6855	Second-line
32	Female	45	2013	307940	First-line
36	Female	19	2013	533	Second-line
74	Female	51	2020	undetermined	-

Phylogenetic analysis

Maximum likelihood phylogenetic analysis of the six HIV-1 sequences showed that all belonged to subtype A1. The HIV-1 sequences in red clustered together and separately from other subtype A1 reference sequences. This is shown in Figure 2.

V3 loop analysis

Predicted tropism

Geno2pheno 5% FPR had a prediction agreement of 83% with 10% FPR and 15% FPR. Using 10% FPR and 15% FPR, the prediction agreement increased to 100%. Five out of the current six study samples were detected as CXCR4-using strains, as shown in Table 3. Among them was a newly diagnosed patient, while the others were treatment-experienced, with three of these on second-line treatment regimens at the time of data collection.

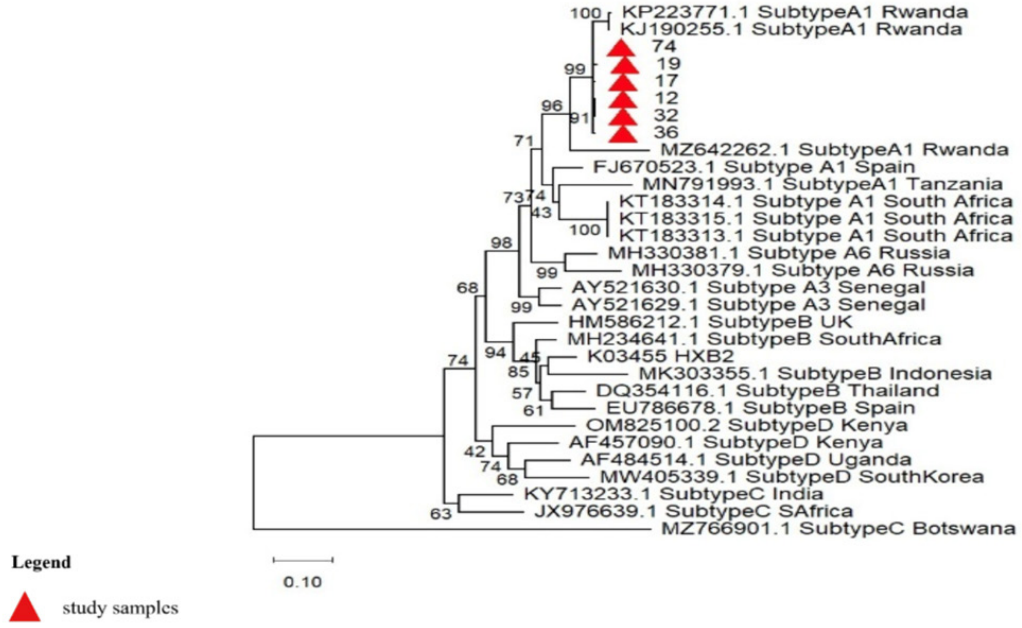

Figure 2: Phylogenetic tree of HIV-1 sequences from the HIV-1 sequences and selected global sequences. The six study sequences clustered around subtype A1. The reference sequences are labelled with their GenBank accession numbers, subtypes, and country of origin.

The treatment initiation for these patients with CXCR4-tropic strains ranged from 2013 to 2020. The one patient with CCR5 usage was on second-line treatment with a treatment start year of 2006. The 11/25 rule predicted only 1/6 as a CXCR4 strain. A mean of +5 positive charge and a net charge of +1 were associated with CXCR4 sequences, while the CCR5 sequence had a positive charge of +6 and a net charge of +3.

Table 3: Tropism prediction results for study HIV-1 sequences.

Sample ID	5% FPR	10.00%	15.00%
		FPR	FPR
12	CXCR4	CXCR4	CXCR4
17	CXCR4	CXCR4	CXCR4
19	CCR5	CCR5	CCR5
32	CCR5	CXCR4	CXCR4
36	CXCR4	CXCR4	CXCR4
74	CXCR4	CXCR4	CXCR4

V3 loop sequence diversity

All V3 regions among the study sequences had 35 amino acids, with most of the sequences having a conserved GPGQ motif except for sample 19, which had substitutions G→V, G→H, and Q→R as seen in Figure 3. The GPGQ motif crown is an important target site for antibody neutralization. The canonical N-linked glycosylation site at amino acid 301 (env numbering relative to HXB2) was conserved in all sequences. All sequences had an N[T] T pattern at the N-linked glycosylation site. These sites have key roles in evasion of host immune response and virus interactions with the target receptor, CD4, and coreceptors for cell entry.

Secondary analysis

Predicted tropism

To assess the consistency of these findings, we repeated the analysis using a combined dataset of the primary study dataset and extracted dataset from HIV LANL, N=68 (see Figure 1). The dataset was stratified into early (n=47) and chronic infections (n=21). CCR5-using strains were the most common, accounting for 76% (52/68) of the dataset. CXCR4 variants accounted for 17% (8/47) of the early infection and 29% (6/21) of the chronic infection sequences. The median sampling year of the additional sequences was 2006 (IQR:1986-2017).

The secondary analysis showed that CCR5 tropism was prevalent in the combined dataset, compared to the study dataset that showed high CXCR4 usage.

V3 loop sequence diversity

Ninety-one percent (62/68) of the HIV-1 strains had a V3 loop with 35 amino acids, with a conserved V3 crown in 56/68 sequences. CCR5-tropic strains showed frequent variant mutations in the V3 crown, with 7/52 sequences having variants compared to 2/14 sequences of the CXCR4 variants. N-linked glycosylation sites were conserved in most of the sequences, except for 3 CXCR4 sequences. A mean positive charge of +6 was associated with both the CCR5 and CXCR4 sequences. A mean net charge of +4 was associated with CCR5 sequences, and +3 was associated with CXCR4 sequences.

The findings of the V3 loop sequence diversity were consistent with our primary findings, whereby most sequences had 35 amino acids, with V3 crown and N- linked glycosylation sites conserved. The net charge of CXCR4 strains was also shown to be lower compared to the CCR5 strains in both analyses.

Discussion

The study characterized the V3 loop region of the HIV-1 gp120, which is crucial in the development of curative and preventative therapeutics. The study observed low variability in the coreceptor tropism across the prediction algorithms used in the current study. Geno2pheno method at 10% and 15% FPR was used to infer the sequence tropism owing to its consistency in prediction agreement. Retrospective analyses of Maraviroc plus optimized Therapy in viremic Antiretroviral Treatment Experienced Patients 1 and 2 (MOTIVATE 1 and 2) and Maraviroc versus Efavirenz Regimens as Initial Therapy (MERIT) study showed that a false positive rate greater than 5.75% was associated with a better discriminatory ability for CCR5-using and non CCR5-using strains leading to favourable response to Maraviroc therapy which was apparent among the 10% and 15% FPR [27,28].

The study sequences showed a conserved GPGQ, V3 crown for all but one sequence, which had a unique variant VPHR motif shown in Figure 3. GPGQ motifs are commonly found in non-subtype

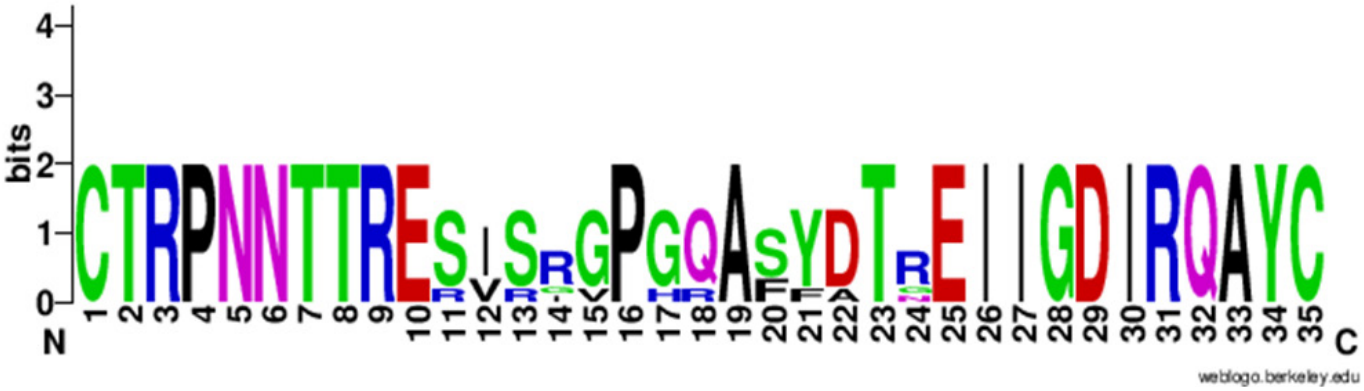

Figure 3: Graphical presentation of the V3 region amino acid variation in the HIV-1 sequences. The Y- axis shows the frequency of amino acids among the sequences at that position, and the X-axis shows the amino acid position on the sequences.

B and especially in Africa [29]. The sequence with an altered V3 crown utilized CCR5 coreceptor and was from a patient who had the longest period of ART usage since 2006. Additional sequences showed a higher frequency of crown motif variants among the CCR5-using strains compared to their CXCR4 counterparts. Some variants have been shown to occlude or dampen the V3 crown antigenic nature and thereby redirect neutralizing antibodies to other antigenic epitopes. Therefore, the virus conserves functional areas of viral entry [5]. V3 crown has generally been shown to be a conserved region, which is why they elicit antibody response [29]. V3 N-linked glycosylation sites were conserved for all study sequences. Glycosylation at this position is crucial for stability of the V3 loop and modulation of coreceptor binding. Mutations at this position are likely to make the virus more susceptible to monoclonal antibodies [30,31].

The study population had many patients with chronic HIV-1 infections, and among the sequenced samples, 5/6 were from chronically infected patients. CXCR4-tropic strains are significantly increased among patients with chronic infections [32,33]. Among the CXCR4 patients, the majority had been infected for ≥3 years with detectable viral load, with only one patient being newly diagnosed. Chronic sequences were fewer in the additional sequences; however, CCR5-using strains were dominant in both early and chronic infections. There are no extensive studies on subtype A1 that have compared coreceptor usage in early and late stages of the infection to determine how the infections vary in comparison with subtype B and C [24]. Wambui et al. had identified frequent CXCR4 usage in treatment-experienced groups; however, the majority of the CXCR4 strains were linked to HIV-1 Subtype D in the study [33]. HIV-1 subtype A1 has been shown to have a higher preference for CCR5 usage in Kenya [34].

Using the combined dataset, CCR5 was predominant with a 76% usage across the sequences, which is congruent with 78.3% by Wambui et al., 69.6% by Mabeya et al., and 75% by Abisi et al.; the majority of these studies included treatment-naïve participants with little or no treatment-experienced study participants [33,35,36]. An increased CXCR4 usage among newly diagnosed patients has also been identified among HIV-1 subtype A1 by Abisi et al. [35]. CXCR4 prevalence in this study may be due to study participants being chronically infected with HIV-1. It may also be a result of sampling bias due to the use of purposive sampling in the study population, as this was a sub-study nested under a main study which studied pretreatment resistance to dolutegravir. It thus mainly targeted newly enrolled and treatment-experienced patients who were yet to be transitioned to a dolutegravir-based regimen. There is evidence associating CXCR4 tropism with viral immune activation that leads to high T-cell turnover and finally CD4 T-cell depletion due to overstimulation. During this period, the virus evades the immune system by using alternative receptor binding sites apart from CCR5 [7]. The newly diagnosed participant had a comorbid infection, *Mycobacterium tuberculosis,* which could have increased the chances of being immunocompromised. This co-infection is associated with immunodeficiency and immune activation, with a likelihood of CXCR4 utilisation.

CXCR4 coreceptor usage among the majority of sequences would limit Maraviroc drug usage in this population. In the MOTIVATE study, patients with CXCR4-binding viruses at the time of therapy initiation were likely to have therapy failure on Maraviroc therapy [37]. However, our findings on Maraviroc usage in the larger population are not generalisable due to our sample size limitation.

The study had several limitations. First, our data were extracted from patients' records, and thus some variables like viral load may not have reflected patients' current state at the time of sample collection. Secondly, the male-to-female ratio favoured females since the study was targeting dolutegravir-naïve patients. At the time of sample collection, the majority of women were hesitant to initiate on a dolutegravir -based regimen due to safety concerns of the drug among pregnant women and women of childbearing age [38]. Lastly, the number of our final sequences was quite small, and this affected our ability to make any substantial conclusions; additional sequences could only be used to validate our analysis.

Conclusion

CXCR4-tropic strains were common among the study-sequenced samples, although HIV-1 Subtype A1 demonstrated dominance of CCR5 usage among the combined dataset. The CCR5-tropic strains were likely to have a mutated V3 crown motif among HIV-1 subtype A1. Future directions for research may include larger cohorts of newly infected and chronically infected patients to understand the genetic evolution and diversity of the V3 region among subtype A1 over time in Kenya and the East African region.

Supplementary Materials

The sequences were deposited in GenBank under accession numbers PX872929-PX872934.

Acknowledgments

We would like to acknowledge the medical personnel at AIC Kijabe Hospital, AIC Kijabe-Marira, and Holy Cross Thigio for their assistance during the data collection period.

References

1. Zhang C, Ruohan Zhu, Qizhi Cao, et al. Discoveries and developments of CXCR4-targeted HIV-1 entry inhibitors. Exp Biol Med. 2020; 245: 477-485.
2. Chen B. Molecular Mechanism of HIV-1 Entry. Trends Microbiol. 2019; 27: 878-891.
3. Connor RI, Sheridan KE, Ceradini D, et al. Change in coreceptor use correlates with disease progression in HIV-1 infected individuals. J Exp Med. 1997; 185: 621-628.
4. Lin NH, Kuritzkes DR. Tropism testing in the clinical management of HIV-1 infection. Curr Opin HIV AIDS. 2009; 4: 481-487.
5. Barroso H, Pedro Borrego, Ines Bartolo, et al. Evolutionary and structural features of the C2, V3 and C3 envelope regions underlying the differences in HIV-1 and HIV-2 biology and infection. PLoS One. 2011; 6: 14548.

6. Dang LVP, Hung Viet Pham, Thanh Thi DINH, et al. Characterization of envelope sequence of HIV virus in children infected with HIV in Vietnam. SAGE Open Med. 2020; 8: 2050312120937198.
7. Connell BJ, Lucas E Hermans, Annemarie MJ Wensing, et al. Immune activation correlates with and predicts CXCR4 co-receptor tropism switch in HIV-1 infection. Scientific Reports. 2020; 10: 15866.
8. Marichannegowda MH, Michelle Zemil, Lindsay Wieczorek, et al. Tracking coreceptor switch of the transmitted/founder HIV-1 identifies co- evolution of HIV-1 antigenicity, coreceptor usage and CD4 subset targeting: the RV217 acute infection cohort study. Ebio Medicine. 2023; 98.
9. Ioannidis JP, Despina G Contopoulos Ioannidis, Philip S Rosenberg, et al. Effects of CCR5-Delta32, CCR2-64I alleles on HIV-1 disease progression: An international meta-analysis of individual-patient data. Ann Intern Med. 2001; 135: 782- 95.
10. John GC, Thomas Bird, Julie Overbaugh, et al. CCR5 promoter polymorphisms in a Kenyan perinatal human immuno-deficiency virus type 1 cohort: association with increased 2-year maternal mortality. J Infect Dis. 2001; 184: 89-92.
11. Smith MW, Dean M, Carrington M, et al. Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), ALIVE Study. Science. 1997; 277: 959-65.
12. Arts EJ, Hazuda DJ. HIV-1 antiretroviral drug therapy. Cold Spring Harb Perspect Med. 2012; 2: 007161.
13. Blassel L, Anna Zhukova, Christian J Villaona Arenas, et al. Drug resistance mutations in HIV: new bioinformatics approaches and challenges. Curr Opin Virol. 2021; 51: 56-64.
14. Inzaule SC, Raph L Hamers, Irene Mukui, et al. Emergence of untreatable, multidrug-resistant HIV-1 in patients failing second-line therapy in Kenya. AIDS. 2017; 31: 1495-1498.
15. Inzaule SC, Raph L Hamers, Meg Doherty, et al. Curbing the rise of HIV drug resistance in low-income and middle-income countries: the role of dolutegravir-containing regimens. Lancet Infect Dis. 2019; 19: 246-252.
16. WHO. HIV drug resistance: brief report 2024. Geneva: World Health Organization. 2024.
17. Giovanetti M, Massimo Ciccozzi, Cristina Parolin, et al. Molecular Epidemiology of HIV-1 in African Countries: A Comprehensive Overview. Pathogens. 2020; 9.
18. Carter NJ, Keating GM. Maraviroc. Drugs. 2007; 67: 2277-2288.
19. Ministry of Health, National AIDS STI Control Program. Kenya HIV Prevention and Treatment Guidelines, 2022 Edition. Nairobi, Kenya. NASCOP. 2022.
20. Nduva GM, Frederick Otieno, Joshua Kimani, et al. Quantifying rates of HIV-1 flow between risk groups and geographic locations in Kenya: A country-wide phylogenetic study. Virus Evol. 2022; 8.
21. Sanders-Buell E, Salminen M, McCutchan F. Sequencing primers for HIV-1. Human retroviruses and AIDS. 1995.
22. Hall TA. Bioedit A User-Friendly Biological Sequence Alignment Editor And Analysis Program For Windows 95/98/NT. Nucleic acids symp. Ser. 1999.
23. Tamura K, Joel Dudley, Masatoshi, et al., MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol. 2007; 24: 1596-1599.
24. Matume ND, Tebit DM, Bessong PO. HIV-1 subtype C predicted co-receptor tropism in Africa: an individual sequence level meta-analysis. AIDS Res Ther. 2020; 17: 5.
25. Lengauer T, Oliver Sander, Saleta Sierra, et al. Bioinformatics prediction of HIV coreceptor usage. Nat Biotechnol. 2007; 25: 1407-1410.
26. Crooks GE, Gary Hon, John Marc Chandonia, et al. WebLogo: a sequence logo generator. Genome Res. 2004; 14: 1188-1190.
27. Harrigan P. Optimization of clinically relevant cut-points for the determination of HIV co-receptor usage to predict maraviroc responses in treatment-experienced (TE) patients using population V3 genotyping: PE3. 4/8. HIV Medicine. 2009; 10: 71.
28. McGovern RA, Alexander Thielen, Simon Portsmouth, et al. Population-Based Sequencing of the V3-loop Can Predict the Virological Response to Maraviroc in Treatment-Naive Patients of the MERIT Trial. J Acquir Immune Defic Syndr. 2012; 61: 279-286.
29. Lopes LR, Antonio Carlos Da Sliva junior, Paulo Bandiera Paiva, et al. Global Variability of V3 Loop Tetrapeptide Motif: a Concern for HIV-1 Neutralizing Antibodies-based Vaccine Design and Antiretroviral Therapy. JOMMID. 2021; 9: 108-115.
30. Wang W, Jianhui Nie, Courtney Prochnow, et al. A systematic study of the N-glycosylation sites of HIV-1 envelope protein on infectivity and antibody-mediated neutralization. Retrovirology. 2013; 10: 14.
31. Kitawi RC, Carlo W Hunja, Rashid Aman, et al. Partial HIV C2V3 envelope sequence analysis reveals association of coreceptor tropism, envelope glycosylation and viral genotypic variability among Kenyan patients on HAART Viro J. 2017; 14: 29.
32. Matume ND, Denis M Tebit, Laurie R Gray, et al. Next generation sequencing reveals a high frequency of CXCR4 utilizing viruses in HIV- 1 chronically infected drug experienced individuals in South Africa. J Clin Virol. 2018; 103: 81-87.
33. Wambui V, Michael Kiptoo, Joyceline Kinyua, et al. Predicted HIV-1 coreceptor usage among Kenya patients shows a high tendency for subtype d to be cxcr4 tropic. AIDS Res Ther. 2012; 9: 22.
34. Nyamache AK, Anne W T Muigai, Zipporah Nganga, et al. Profile of HIV Type 1 Coreceptor Tropism Among Kenyan Patients from 2009 to 2010. AIDS Res Hum Retroviruses. 2013; 29: 1105-1109.

35. Abisi HK, Leon E Otieno, Erastus Irungu, et al. Net charge and position 22 of the V3 loop are associated with HIV-1 tropism in recently infected female sex workers in Nairobi, Kenya. Medicine (Baltimore). 2022; 101: 32024.

36. Mabeya S, Jomo Kenyatta, Anthony Kebira Nyamache, et al. Predominance of CCR5 tropism in non-b HIV-1 subtypes circulating in Kisii County, Kenya. East African Medical Journal. 2018; 95: 1889-1898.

37. Fätkenheuer G, Mark Nelson, Adriano Lazzarin, et al. Subgroup Analyses of Maraviroc in Previously Treated R5 HIV-1 Infection. N Engl J Med. 2008; 359: 1442-1455.

38. Zash R, Denise L Jacobson, Modiegi Diseko, et al. Comparative safety of dolutegravir-based or efavirenz-based antiretroviral treatment started during pregnancy in Botswana: an observational study. Lancet Glob Health. 2018; 6: 804-810.