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Susceptibility to Lenacapavir Among Newly Diagnosed HIV-Positive Patients Followed Up in Mozambique That Presented With Primary Antiretroviral Resistance to Other Classes

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ABSTRACT

Multidrug-resistant HIV patients have limited ART options. Lenacapavir (LEN) is a capsid inhibitor that exhibits substantial antiviral activity in patients with therapeutic failure but is also proposed for PrEP. Herein, we assessed LEN susceptibility among ART-naive HIV patients with drug resistance in Mozambique. In this study, 63 patients with DRM against PIs, NRTIs, NNRTIs, and INSTIs were included. The gag (p24) and env fragments were amplified with a low-cost in-house protocol and sequenced with nanopore. HIVDR database from Stanford University was used to assess LEN resistance and geno2pheno to assess viral tropism and protease/maturation inhibitor-associated mutations. A total of 59 patients were successfully sequenced. About 29% had DRMs to PIs, 5% to NRTI, 83% to NNRTI, and 2% to INSTI. No DRMs to LEN were detected. Additionally, 42% of the sequences presented protease/maturation inhibitor-associated mutations. A relationship was observed between the E138A/G mutation and protease/maturation inhibitors ($p = 0.004$). We identified changes at the first codon position of position 56 of the p24 *gag* gene, which represents a key site for resistance to LEN. Also, codon 66 was highly conserved. Our results support the potential effectiveness of lenacapavir as a PrEP regimen or rescue therapy for patients with at least one drug-resistance mutation.

These first two authors contributed equally to this study.

1 | Introduction

The availability of antiretroviral (ARV) drugs for people living with HIV (PLWH) is currently extensive through global distribution [1]. Nevertheless, the emergency of drug resistance mutations (DRMs) capable of compromising ARVs susceptibility, might significantly raise the likelihood of treatment failure for a patient which could jeopardize WHO strategies to end HIV/AIDS as a global public health problem by 2030 [2, 3].

Currently, it is estimated that more than 6 million individuals have initiated pre-exposure prophylaxis (PrEP) medication, which comprises oral daily and on-demand tenofovir-based regimens, long-acting injections of cabotegravir, and a 1-month dapivirine vaginal ring [4, 5]. However, there are gaps in terms of uptake, adherence, and persistence in PrEP use among cisgender women, beyond disparities in access to PrEP [6]. Therefore, the option of long-acting injectable pre-exposure prophylaxis (LAI-PrEP) could transform HIV prevention for individuals struggling with oral PrEP medication adherence and significantly reduce the global HIV epidemic [7–9]. Indeed, according to the PURPOSE 1 trial, in which 5338 women were prophylactically treated and divided into three groups: the first received lenacapavir, the second TAF + FTC, and the third the standard PrEP regimen with TDF + FTC, no participants, including women and adolescent girls in South Africa and Uganda, who received twice-yearly lenacapavir injections as PrEP for HIV, acquired HIV infection at 1 year, demonstrating the safety and efficacy of lenacapavir for PrEP in HIV-negative cisgender women [10]. Also, PURPOSE 2 showed that HIV incidence with twice-yearly lenacapavir was significantly lower than both the background incidence and the incidence with F/TDF [11]. Another study showed that 88% of multidrug-resistant patients who received lenacapavir had a decrease of at least 0.5 $\log_{10}$ copies per milliliter in the viral load by day 15 with the administration of lenacapavir [12]. However, early detection of DRMs or polymorphisms that could compromise the efficacy of lenacapavir is crucial and may require the development of genomic surveillance systems which can be easily implemented in resource-limited settings, such as the low- and middle-income countries (LMICs), to maximize the track HIV-1 resistant strains and global response to HIV/AIDS pandemic. The genetic pressure exerted by lenacapavir on viral evolution led to the identification of Q67H, N74D, and Q67H substitutions as the primary resistance-associated mutations [13].

To the best of our knowledge, there are no published studies on lenacapavir susceptibility levels for a key portion of the most at-risk populations such as women, young individuals from 15 to 24 years, men who have sex with men (MSM), and transgender women, in Portuguese-speaking African countries (PALOP). In this study, we assessed lenacapavir sensitivity in ART-naive patients and those with pretreatment drug resistance against ARVs that constitute the first-line ART regimen in Mozambique, a Sub-Saharan African country with a high prevalence of transmitted drug resistance [14]. Additionally, we investigated seroconversion, protease/maturation inhibitor-associated mutations (RAMs) as well as their relationship with the main mutations present in PR, RT, and INSTI to identify other possible polymorphisms capable of compromising ARVs from the maturation inhibitor class.

2 | Materials and Methods

2.1 | Study Design and Setting

This is part of a cross-sectional study conducted with 63 newly diagnosed HIV-positive patients who were ART-naive in Manhiça district, a rural area located 80 kilometers north of Maputo, the capital city of Mozambique. All patients underwent resistance testing targeting the PR (PIs), RT (NRTIs and NNRTIs), and IN (INSTIs) genes. Resistance profile was assessed considering the the list published by Tzou et al., 2020 [15] and HIVdb Program, which is available online in the Stanford University HIV Drug Resistance Database (https://hivdb.stanford.edu/). All patients enrolled presented at least one major DRM to PR, RT, or IN. The project was approved by the ethics committee of Mozambique (protocol number 368/CNBS/23, dated July 10, 2023) and all participants received and signed informed consent before being included in the study.

2.2 | RNA Extraction and PCR Conditions

Total RNA previously extracted and stored at −80°C was thawed and used for further analyses. For sequencing purposes, amino acid positions 1 to 231 in the *gag* gene (HXB2 position: 1186 to 1878 bp), were amplified from 5 μL of extracted RNA in a 20 μL final master mix containing 10x PCR Buffer, 50 mM $MgSO_4$, 10 mM dNTP, 0.1 M DTT, Inhibitor RNAse, RT superscript III (200 U/μL), Taq Platinum High Fidelity enzyme, and primers. All reagents were supplied by Life Technologies. One-step PCR was conducted for each specimen followed by a nested PCR, using an *in-house* protocol with previously published primer sets by Locateli et al., 2008 [16]. The one-step RT-PCR cycling conditions were as follows: (i) 30 min at 50°C and 3 min at 94°C for cDNA synthesis; (ii) 35 cycles of denaturation (30 s at 94°C), annealing (30 s at 55°C), and elongation (2.5 min at 68°C), and (iii) a final elongation step for 10 min at 68°C with subsequent cooling to 4°C. The nested PCR was performed using 5 μL of amplicon with Go Taq Green Master Mix (Promega, USA). The nested-PCR cycling conditions were as follows: (i) 3 min at 94°C; (ii) 35 cycles of denaturation (30 s at 94°C), annealing (30 s at 55°C), and elongation (1.5 min at 72°C), and (iii) a final elongation step for 10 min at 72°C with subsequent cooling to 4°C. The final PCR products with an estimate of 1800 pb were revealed through electrophoresis using 1% of the agarose gel.

2.3 | HIV-1 Sequencing and Drug Resistance Assessment

All the final PCR products were purified using 1xAMPure XP beads (Beckman Coulter, Brea, CA, USA), followed by normalization to an initial input of 80 ng/μL. DNA library preparation was conducted using the Ligation Sequencing kit (SQK-NBD114.24, Oxford Nanopore Technologies) and Native Barcoding Expansion 1–96 kit (R10.4.1, Oxford Nanopore Technologies), following the reaction conditions as previously described [17]. Sequencing was performed for up to 12 h on a MinION Mk1C Sequencing Device. Reads were mapped and

aligned against sample-specific reference sequences constructed for the *Gag* genomic region using the Geneious Prime v2024.0.5 (https://www.geneious.com/features/prime) and the assembled FASTQ files used to generate the consensus FASTA file. The consensus FASTA file sequences were submitted to drug resistance analysis in the HIV drug resistance database for Capsid from Stanford University (https://hivdb.stanford.edu/hivdb-capsid/by-reads/) to assess LEN resistance and also submitted to geno2pheno to assess viral tropism (https://coreceptor.geno2pheno.org/) and protease/maturation inhibitor-associated mutations (https://gag.geno2pheno.org/).

2.4 | Statistical Analysis

Statistical data analysis was conducted using the R version 3.4.1 (R Foundation, Vienna, Austria). Quantitative data were presented with descriptive statistics, including sample size (N) and median with interquartile range (IQR; represented as 25th and 75th percentiles). Categorical data were expressed as proportions and frequencies, with comparisons made using the $\chi 2$ test. In cases of small sample sizes (such as expected observation values < 5.0 in more than one cell for four-field tables, or over 20% for contingency tables), $\chi 2$ with Yates correction or a two-tailed Fisher's exact test was employed. Differences in all tests were considered significant at p-value < 0.05.

3 | Results

3.1 | Demographic, Behavioral, and Clinical Characteristics of HIV-1 ART-Naive Patients

The sociodemographic, behavioral, and clinical description as well as the seroconversion profile of the 63 HIV-1 patients enrolled in this study are presented in Table 1. All patients presented at least one major drug resistance mutation, either for PIs, NRTIs, NNRTIs, or INSTIs. The median age of these patients was 36.0 (IQR: 29.0–43.0) years. Patients in the age group of 35–44 years (36.5%, 23/63), male (52.4%, 33/63), who completed 5–8 grades of education (38.1%, 24/63), employed (85.7%, 54/63), with a monthly income between $55–$125, and infected in Mozambique, were the most prevalent demographic characteristics. Regarding behavioral characteristics, we observed that patients with less than 2 sexual partners (71.4%, 45/63), who were unaware of their partner's HIV status (73.3%, 44/63), alcohol consumers (50.8%, 32/63), and without tattoos (73%, 46/63) were predominant. Regarding the clinical profile, patients without comorbidities (82.5%, 52/63) and who were unaware of whether their partner was on antiretroviral treatment (74.6%, 47/63) were predominant. In addition, 14.3% had undergone colonoscopy, 4.8% had had a blood transfusion, and 34.9% had undergone some traditional surgery. Infection with the CCR5 coreceptor (68.9%, 31/63) was predominant, compared with infections with the CXCR4 coreceptor (31.1%, 14/63).

No mutations affecting lenacapavir susceptibility have been identified. Overall, 13 (34.2%) of the patients had seroconversion in less than 1 year, 13 (34.2%) had between 1 and 3 years and 12 (31.6%) had seroconversion in more than 3 years. Infection by the CCR5 co-receptor was prevalent in all these groups, being 63% in patients with less than 1 year of seroconversion, 56% in patients between 1 and 3 years of seroconversion and 77% for patients with more than 3 years of seroconversion (Figure 1). The regions of infection showed a statistically significant relationship ($p = 0.029$), while no statistically significant relationship was observed between the time of seroconversion with age group, sex, educational level, occupation, marital status, and monthly income ($p > 0.05$). Regarding behavioral characteristics, a statistically significant relationship was observed between the time of seroconversion and having a tattoo ($p = 0.019$), while the number of sexual partners, knowledge of the patient's serological status, and use of any substance before sexual intercourse did not present any statistically significant relationship ($p > 0.05$). Similarly, clinical determinants, including colonoscopy, blood transfusion, traditional surgery, comorbidities, partner use of ARVs, and viral tropism, did not show any statistically significant relationship with time to seroconversion ($p > 0.05$) (Table 2).

3.2 | Relationship Between Protease/Maturation Inhibitor-Associated Mutations With Major Mutations of ARV Drug Classes

In these studied population, 28.8% (17/59) had mutations in PI (M46L and N88K/T), 5.1% (3/59) had mutations in NRTI (K219N, S68G and T215TS), 83.1% had mutations in NNRTI (E138A/G, F227L/P/S, V179D/E, G190A, K101EP, V108I, Y181C, K103N, L100I, P225H, V106IM, and Y188L) and 1.7% (1/59) had a mutation against INSTI (S147G). Overall, 42.1% (24/59) of the sequences presented some protease/maturation inhibitor-associated mutations. No statistical significance was observed between the classes of mutations grouped into PI, NRTI, NNRTI, and INSTI ($p > 0.05$). Nonetheless, the E138A/G mutation (23.7%) present in only 7.7% of sequences with protease/maturation inhibitor-associated mutations was statistically significant ($p = 0.004$) (Table 2).

3.3 | Polymorphisms Associated With Resistance to Capsid Inhibitors

We identified considerable variability at the nucleotide level at positions associated with causing major or accessory mutations against capsid inhibitors (Figure 2). Amino acid position 56 (major mutation) showed the greatest genetic variability (5 polymorphisms (CTC, TTR, TTA, CTG, and TTG) different from the HXB2 (CTA) prototype) but without any effect or alteration in the phenotype. Furthermore, positions 67, 70, and 74 in the major mutations showed low genetic variability without any alteration in the phenotype or amino acid. Interestingly, position 66, related to the main mutation, was the only one that did not present any polymorphism, showing it to be a highly conserved region. Accessory mutations 57 and 105 presented polymorphisms without any alteration of the phenotype, while in position 107, two polymorphisms were observed (AGT and TCT) with alteration in the first and second nucleotide that led to the alteration of the amino acid threonine (T) to the amino acid serine (S).

TABLE 1 | Sociodemographic characteristics of HIV-1 ART-naive patients with any DRM and susceptible to Lenacapavir at the time of seroconversion.

Independent variables	*N* (%)	Seroconversion time			*p*-value
		< 1 year	1–3 years	> 3 years	
Overall	63 (100)	13 (34.2)	13 (34.2)	12 (31.6)	
Demographic characteristic					
Age in years, Median (IQR)	36.0 (29.0–43.0)	29.0 (26.6–43.5)	37.0 (28.0–44.0)	39.0 (33.2–44.0)	0.275
Age distribution					
18–24 years	6 (9.5)	2 (66.7)	1 (33.3)	0 (0.0)	0.617
25–34 years	21 (33.3)	6 (46.2)	3 (23.1)	4 (30.1)	
35–44 years	23 (36.5)	3 (21.4)	6 (42.9)	5 (35.7)	
45–54 years	12 (19.0)	2 (28.6)	2 (28.6)	3 (42.9)	
≥ 55 years	1 (1.6)	0 (0.0)	1 (100)	0 (0.0)	
Sex, %					
Female	30 (47.6)	8 (34.8)	6 (26.1)	9 (39.1)	0.336
Male	33 (52.4)	5 (33.3)	7 (46.7)	3 (20.0)	
Educational level					
No education	17 (27.0)	2 (18.2)	5 (45.5)	4 (36.4)	0.129
Completed 1–4th grade	4 (6.3)	1 (100)	0 (0.0)	0 (0.0)	
Completed 5–8th grade	24 (38.1)	3 (20.0)	5 (33.3)	7 (46.7)	
Completed high school	17 (27.0)	7 (63.6)	3 (27.3)	1 (9.1)	
Any high education	1 (1.6)	13 (34.2)	13 (34.2)	12 (31.6)	
Occupation					
Unemployed	9 (14.3)	2 (28.6)	3 (42.9)	2 (28.6)	0.864
Employed	54 (85.7)	1 (35.5)	10 (32.3)	10 (32.3)	
Marital status					
Unmarried	18 (28.6)	5 (41.7)	2 (16.7)	5 (41.7)	0.297
Married	45 (71.4)	8 (30.8)	11 (42.3)	7 (26.9)	
Monthly income, $					
< 55	27 (42.9)	5 (27.8)	8 (44.4)	5 (27.8)	0.358
55–125	29 (46.0)	7 (43.8)	3 (18.8)	6 (37.5)	
126–250	6 (9.5)	1 (33.3)	2 (66.7)	0 (0.0)	
> 250	1 (1.6)	0 (0.0)	0 (0.0)	1 (100)	
HIV infection region					
Mozambique	55 (87.3)	11 (33.3)	13 (39.4)	9 (27.3)	0.029*
Other (South Africa)	5 (7.9)	0 (0.0)	0 (0.0)	3 (100)	
Unknown	3 (4.8)	2 (100)	0 (0.0)	0 (0.0)	
Behavioral characteristic					
No. sexual partners in the past 12 months					
< 2 sexual partners	45 (71.4)	9 (30.0)	11 (36.7)	10 (33.3)	0.569
≥ 2 sexual partners	18 (28.6)	4 (50.0)	2 (25.0)	2 (25.0)	
HIV-positive partner					
No	5 (8.3)	2 (67.7)	1 (33.3)	0 (0.0)	0.128
Yes	11 (18.3)	4 (66.7)	0 (0.0)	2 (33.3)	
Unknown	44 (73.3)	7 (24.1)	12 (41.4)	10 (34.5)	

(Continues)

TABLE 1 | (Continued)

Independent variables	*N* (%)	Seroconversion time			*p*-value
		< 1 year	1–3 years	> 3 years	
Substances used before sex					
None	26 (41.3)	6 (35.3)	6 (35.3)	5 (29.4)	0.323
Alcohol	32 (50.8)	4 (22.2)	7 (38.9)	7 (38.9)	
Alcohol + cigarettes	3 (4.8)	2 (100)	0 (0.0)	0 (0.0)	
Cocaine/crack	1 (1.6)	1 (100)	0 (0.0)	0 (0.0)	
Tattoo					
No	46 (73.0)	8 (25.0)	12 (37.5)	12 (37.5)	0.019*
Yes	17 (27.0)	5 (83.3)	1 (16.7)	0 (0.0)	
Clinical characteristic					
Medical procedures					
Colonoscopy	9 (14.3)	4 (66.7)	0 (0.0)	2 (33.3)	0.098
Blood transfusion	3 (4.8)	0 (0.0)	0 (0.0)	1 (100)	0.343
Traditional surgery	22 (34.9)	4 (33.3)	4 (33.3)	4 (33.3)	0.988
STIs comorbidities					
No	52 (82.5)	10 (30.3)	12 (36.4)	11 (33.3)	0.427
Yes	11 (17.5)	3 (60.0)	1 (20.0)	1 (20.0)	
Partner on ART					
No	6 (9.5)	2 (66.7)	1 (33.3)	0 (0.0)	0.213
Yes	10 (15.9)	3 (50.0)	0 (0.0)	3 (50.0)	
Unknown	47 (74.6)	8 (27.6)	12 (41.4)	9 (31.0)	
HIV-1 viral tropism					
CCR5	31 (68.9)	10 (50.0)	5 (25.0)	5 (25.0)	0.555
CXCR4	14 (31.1)	3 (30.0)	4 (40.0)	3 (30.0)	

*The results were statistically significant ($p < 0.05$)

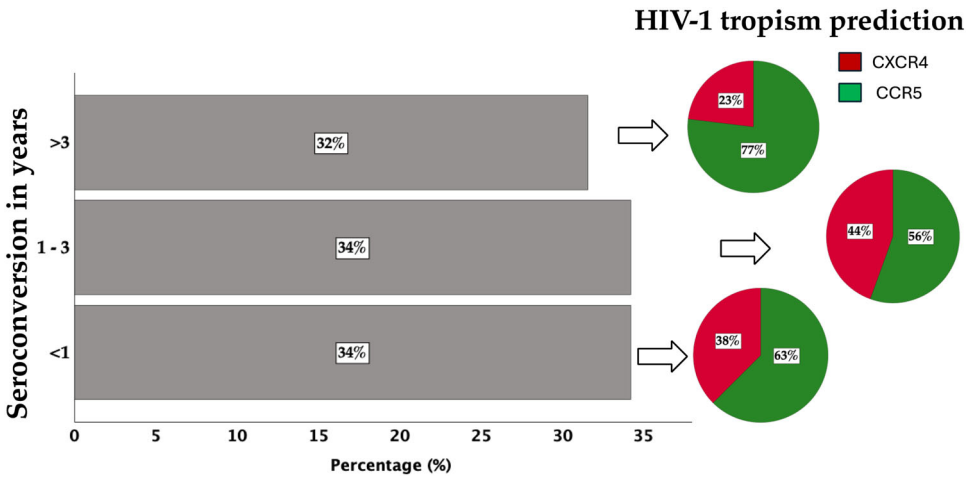

FIGURE 1 | Distribution of HIV patients with DRM according to seroconversion time and viral tropism. The time to seroconversion was calculated by determining the number of months between the date of the last reported negative HIV test and the date of their confirmed positive HIV test. The geno2pheno website, available at https://coreceptor.geno2pheno.org/, was used to predict viral tropism.

4 | Discussion

To the best of our knowledge, this was the first study conducted in sub-Saharan Africa focused on the surveillance of resistance mutations to injectable lenacapavir, a promising new ARV drug. Our genomic analysis of the gag gene, in particular p24, did not identify primary or accessory resistance mutations to lenacapavir, which was expected, given that this drug is not yet

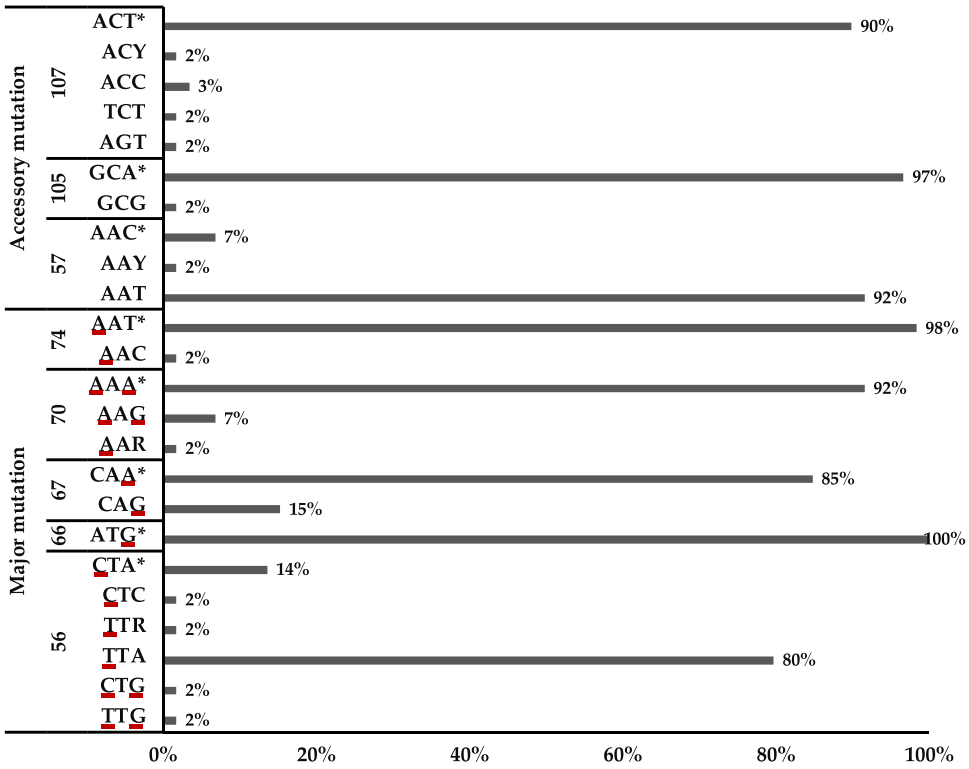

FIGURE 2 | Polymorphisms identified at positions associated with resistance to capsid inhibitors (p24). We highlighted possible mutations at the nucleotide level conferring changes in the viral phenotype (resistant). Mutations at positions 56, 70, and 74 preferably occur at the first nucleotide level, while 67 at the last nucleotide level. All the nucleotide changes refer to transversion mutations (purine ↔ pyrimidine), which are less likely to occur. It is worth mentioning that codon 66, despite having no genetic variability, any mutation in the last nucleotide of the codon (transition) will compromise therapeutic efficacy. Note: The underlined nucleotides correspond to the possible positions capable of altering the sensitive to resistant phenotype. *Wild-type HXB2 codon.

widely administered due to its high cost (up to $44 819 per person per year) and its injectable form of application, which requires specialized infrastructure for administration [18].

This study represents a milestone in the field of HIV/AIDS in Mozambique, a sub-Saharan African country, a priority region for the WHO to eradicate HIV/AIDS by 2030, with special emphasis on young women, considered a key population. Additionally, this study aligns with the recent results of the PURPOSE trial [10]. The present study is fundamental by assessing primary drug resistance in a population of HIV-1 patients from Mozambique, indicating a lack of primary drug resistance mutations to this drug and therefore emphasizing the potential of lenacapavir as a promising PrEP regimen.

Specific polymorphisms in the *gag* gene are critical targets of viral protease action, which can confer resistance to PIs and influence the viral maturation process [19]. Among the main polymorphisms associated with resistance to PIs are mutations 128 V, 132 F, 200I, 390 A, and 401 V, while mutations 362 M/F and 370 A are related to viral maturation [20]. Indeed, we identified these polymorphisms in ART-naive patients in Mozambique. Furthermore, the E138K mutation in NNRTIs demonstrated a correlation with resistance to PIs and maturation, suggesting the presence of epistasis impacting antiretroviral drug resistance. Similarly, in the context of the 3'PPT region of the *nef* gene, a possible influence on resistance to second-generation INSTI, such as dolutegravir-DTG, was observed [21–23]. Although previous studies indicate that mutations such as Q69K in gag co-evolve with gp120 and minor mutations in the protease, which highlights the complex relationship between viral genes [24, 25]. These findings suggest that mutations in the *gag* gene may play a relevant role in the assembly and maturation of the viral particle, directly affecting the effectiveness of protease inhibitors and the evolution of viral resistance (Table 2).

In vitro resistance studies of lenacapavir identified seven mutations in the HIV capsid (L56I, M66I, Q67H, K70N, N74D, N74S, and T107N) associated with reduced drug sensitivity, with a variation ranging from 4 to 1000 times compared to the wild-type virus [26]. However, most of these mutations are associated with a significant reduction in viral infectivity, except for the Q67H mutation. This mutation maintained infectivity comparable to the wild-type virus, although it showed a five-fold reduction in sensitivity to lenacapavir [26]. A recent study analyzed 1500 HIV sequences from treated and untreated HIV patients, regardless of previous failure with PI, and did not detect any of the seven mutations mentioned [27]. This finding suggests no pre-existing genotypic resistance to lenacapavir exists in this substantial cohort of patients. Our results are consistent with this study, as we also did not identify any primary or accessory mutations associated with lenacapavir resistance in ART-naive patients. These data are significant as they indicate the absence of prior resistance to lenacapavir in patients without a history of using this drug, further supporting

TABLE 2 | Relationship between protease/maturation inhibitor-associated mutations (RAMs) with major mutations of ARV drug classes.

Major mutations of ARV drug classes		Protease/maturation inhibitor-associated mutations (RAMs)[a]		
	N (%)	*N* (%)	Yes (%)	*p*-value
Overall	59 (100)	33 (57.9)	24 (42.1)	
PI major	17 (28.8)	10 (62.5)	6 (37.5)	0.660
M46L	1 (1.7)	1 (100)	0 (0.0)	0.390
N88K/T	16 (27.1)	9 (60.0)	6 (40.0)	0.847
NRTI major	3 (5.1)	1 (33.3)	2 (66.7)	0.567
K219N	1 (1.7)	0 (0.0)	1 (100)	0.237
S68G	1 (1.7)	1 (100)	0 (0.0)	1.000
T215TS	1 (1.7)	0 (0.0)	1 (100)	0.421
NNRTI major	49 (83.1)	28 (59.6)	19 (40.4)	0.578
E138A/G	14 (23.7)	12 (92.3)	1 (7.7)	0.004*
F227L/P/S	7 (11.9)	2 (33.3)	4 (66.7)	0.227
V179D/E	6 (10.2)	3 (50.0)	3 (50.0)	0.689
G190A	4 (6.8)	1 (25.0)	3 (75.0)	0.300
K101EP	3 (5.1)	1 (33.3)	2 (66.7)	0.567
V108I	2 (3.4)	0 (0.0)	2 (100)	0.173
Y181C	1 (1.7)	0 (0.0)	1 (100)	0.421
K103N	14 (23.7)	7 (50.0)	7 (50.0)	0.544
L100I	1 (1.7)	1 (100)	0 (0.0)	1.000
P225H	1 (1.7)	1 (100)	0 (0.0)	1.000
V106IM	3 (5.1)	2 (66.7)	1 (33.3)	1.000
Y188L	2 (3.4)	1 (50.0)	1 (50.0)	1.000
INSTI major				
S147G	1 (1.7)	1 (100)	0 (0.0)	1.000

*The results were statistically significant ($p < 0.05$)
[a]Protease associate mutation (128IV, 132 F, 200I, 390 A, and 401 V); maturation associate mutation (362IMV and 370AE).

the potential efficacy of lenacapavir as a promising PrEP regimen.

Previous studies, based on a large data set of over 23 000 sequences covering all HIV-1 subtypes, suggest that the p24 protein of the gag gene is highly conserved, with more than 89% conservation at the amino acid level [28]. Non-synonymous mutations in this region may lead to a significant reduction in viral fitness due to the complexity of the p24 structure and the viral assembly process [29]. However, in our study, we observed molecular variations at the nucleotide level which, although not resulting in phenotypic changes (amino acids) when compared to the HXB2 prototype, showed significantly different codon usage. Notably, we identified changes at the first codon position corresponding to position 56 of the p24 gene, a key site for resistance to lenacapavir. Two hypotheses could explain these findings: (i) the genetic barrier of HIV-1C may be lower compared to non-C subtypes, such as sub-subtype A6, which has shown polymorphisms that could impact the therapeutic efficacy of first-line ART regimens; (ii) the differential use of codons may act as a gene expression regulatory mechanism. These observations underscore the importance of investigating genetic variations in the context of viral resistance and the response to antiviral treatment. However, additional studies are needed to understand which nucleotide sequence is considered wild-type in the majority of HIV-1C sequences globally, to detect the most frequent alterations at the nucleotide level and their impact on therapeutic response. Codon 66 in the *gag* gene (p24) was highly conserved since it encodes Methionine (Figure 2). This position is likely to be subject to a high rate of negative selection, which does not allow for a change in the phenotype, since any change, even in the last base of the codon (AUG), reflects a change in the phenotype, which may lead to a change to isoleucine, which reduces susceptibility to lenacapavir by up to 1000 times. This finding raises an important debate about the genetic barrier of lenacapavir, which, in addition to its high cost, can easily select resistant variants on a large scale.

Our study has some limitations. First, the main limitation of this study lies in the small sample size, in a context of high incidence and prevalence of HIV-1 in Mozambique. Second, the present study enrolled only newly diagnosed and ART-naive HIV patients, excluding those with a history of ARV treatment, even though the rate of acquired drug resistance represents a significant burden in sub-Saharan Africa. Finally, the sample coverage was restricted to the Manhiça region, which limits the generalization of the results to the reality of the entire territory of Mozambique. These limitations suggest the need for further

studies with more comprehensive samples, including patients with a history of ART, to provide a more complete picture of the dynamics of viral resistance and therapeutic response in African settings.

In conclusion, our findings reinforce the potential efficacy of lenacapavir as a PrEP regimen or rescue therapy in patients harboring HIV-1 drug resistance, aligning with previous studies that did not detect primary resistance mutations.

Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

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