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HIV drug resistance: analysis of viral genotypes and mutation loci in people living with HIV in Chongqing, China (2016–2023)

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Abstract

Background Large-scale HIV genotype drug resistance study has not been conducted in Chongqing.

Methods A retrospective study was conducted on people living with HIV(PLWH) who received HIV-1 genotype resistance testing at Chongqing Public Health Medical Center from May 2016 to June 2023. The HIV-1pol gene was amplified through RT-PCR and analyzed in terms of genotypic drug resistance.

Results Of the 3015 PLWH tested for HIV-1 drug resistance, 1405 (46.6%) were resistant to at least one antiviral drug. Among non-nucleoside reverse transcriptase inhibitors (NNRTIs), 43.8% were resistant, compared to 29.5% for nucleoside reverse transcriptase inhibitors (NRTIs) and 3.4% for protease inhibitors (PIs). V179D/E and K103N/S were identified as the common mutation sites in the NNRTIs class of drugs, M184V/I and K65R/N were reported as the most common mutation sites in NRTIs, while thymidine analogue mutation (TAM) group was identified in 373 samples. L10FIV was the most common mutation in PIs. The dominant HIV-1 subtype was CRF07_BC.

Conclusions The high prevalence of HIV-1 drug resistance in Chongqing underscores the imperative for rigorous surveillance of the local HIV epidemic. Furthermore, TAMs are associated with HIV-1 multidrug resistance, and timely detection of drug resistance is helpful to reduce the emergence and spread of such drug-resistant strains.

Keywords Genotype, Thymidine analogue mutation, Drug resistance, HIV-1

Introduction

Currently, anti-retroviral therapy (ART) is the most effective treatment for HIV-1 infection and one of the main measures to control viral transmission. Thus far, 26 million HIV-1-infected patients have received ART [1, 2] worldwide. Regimens containing the integrase inhibitors (INSTIs) bictegravir (BIC) or dolutegravir (DTG) are recommended as initial treatment for most individuals. The preferred regimen is two nucleoside reverse transcriptase inhibitors (NRTIs) plus one INSTI or DTG plus lamivudine(3TC) [3]. However, Chinese guideline recommends two NRTIs combined with one non-nucleoside reverse transcriptase inhibitor (NNRTI) as the preferred, standardized, first- line ART regimen and DTG is the second-line regimen because of The National Free Antiretroviral Treatment Program of China [4].

With the widespread implementation of ART therapy, however, antiretroviral drug resistance has become more severe [5]. The extensive application of NRTIs also led to the emergence of the thymidine analogue mutation

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(TAM), and the accumulation of drug-resistant mutations triggers deeper resistance, thus seriously reducing the effectiveness of first-line antiretroviral drug therapy and ultimately leading to an increase in virological treatment failures.

Chongqing is recognized as the largest municipality in China and the economic center of the southwest region. With the development of socioeconomic and the rapid growth of population mobility, HIV-1 populations are frequently on the move and the HIV-1 subtypes are constantly recombining, which may result in a more widespread and complex distribution of HIV-1 genotypes. The transmission drug resistance (TDR) of newly diagnosed HIV patients without treatment was counted at Chongqing from May 2014 to June 2017, and the total positive rate of TDR was found as 9.5%. The rate of NNRTI-, NRTI-, and PI-resistance mutations was9.5%, 2.4% and 0%, respectively [6]. A study of ATR treatment failure in Chongqing found that 95.1%, 99.1%, and 0.9% of patients exhibited resistance to NRTIs, NNRTIs, and PIs, respectively [7]. Thus far, there has not been any large-sample study on HIV genotypic drug resistance in Chongqing. For this reason, the study aimed to retrospectively analyze the genotypic drug resistance detection among PLWH who received ART treatment in Chongqing Public Health Medical Center from May 2016 to June 2023 to gain insights into the HIV-1 drug resistance situation in the region.

Methods

Study design and participants

We collected 3015 PLWH and underwent HIV genetic resistance testing after receiving ART in Chongqing Public Health Medical Center from May 2016 to June 2023. All 3015 PLWH with detectable viral load underwent gene amplification, drug resistance analysis and genetic subtype analysis. Additionally, the results of CD4 T-cell counts from the same period of resistance analysis were collected. The study was approved by the ethics committee of Chongqing Public Health Medical Center (approval number: 2021-014-01-KY; approval date: 18 March, 2021). The study was conducted in accordance with the Declaration of Helsinki.

Table 1 Gene amplification and sequencing primers for HIV-1 *pol*

Procedure	Primers	Position[a]	Length of target fragment	Sequences (5′-3′)
First round	2029 F	2029–2050	1501	TGGAAATGTG-GRAAGGAAGGAC
	3529R	3529–3505		GCTAyy-AAGTCTTTT-GATGGGTCAT
Second round	2249 F	2249–2266	1273	CTTCCCTCARAT-CACTCT
	3521R	3521–3504		GTCTTTTGATGGG TCATA

Positions [a]: Positions of nucleotides within the HIV HXB2 strain (GenBank accession number: K03455)

HIV-1 gene amplification and drug resistance analysis

The viral nucleic acid extraction kit (Shuoshi, Jiangsu, China) was adopted to extract RNA from 200μL of plasma of the study subjects in accordance with the operating instructions. One-step RT-PCR and nested PCR were performed based on a one-step RT-PCR kit (novizan, Nanjing, China) and a PCR kit (novizan, Nanjing, China), respectively. In addition, the amplified fragment was the protease and reverse transcriptase region of the HIV- 1pol gene region, with a fragment length of nearly 1500 bp. Nested PCR was performed on specimens with insufficient one-step RT-PCR amplification products. GeneAmp® 9700 PCR instrument (ABI, USA) was used for both amplification rounds. The target bands were subjected to 1% agarose gel electrophoresis for validation, and the amplified product was sequenced. Sequences were then spliced and edited, and were submitted to the drug resistance database of Stanford University (https://hivdb.stanford.edu/hivdb/by-sequences/) for HIV-1 subtypes and resistance mutation analysis. A list of PCR primers can be found in Table 1.

HIV-1 genetic subtype analysis

HIV-1 genotype analysis was conducted using the Viral Genotyping Tool offered by the National Center for Biotechnology Information (NCBI) website (https://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi). The 2009 RefSet database was selected to drawn online comparison, and the HIV-1 genotypes of the sequences were determined in accordance with the comparison results.

CD4 T-cell count assay

Anticoagulation tubes containing ethylenediaminetetraacetic acid (EDTA) were used to collect 2 mL of venous blood, and the number of CD4 T cells was performed by a flow cytometric assay (BD, USA).

Statistical analysis

Data collection and graphing were performed by GraphPad prism8.0. All analyses were conducted with the statistical software SPSS 22.0. Continuous variables were described as the median with inter-quartile ranges (IQR), whereas categorical variables are shown as percentages. The chi-square (x^2) test was applied to analyze the drug-resistance rate. A statistically significant difference is indicated by $P<0.05$.

Results

Clinical data and HIV-1 genotype distribution of the participants

The HIV-1 RNA protease/reverse transcriptase region in 3015 PLWH was analyzed. Table 2 lists the basic information of participants. The median age of the participants was 49 years with 2258 males (74.9%) and 757 females (25.1%). Most of participants (61.3%) had ART treatment failure. Additionally, the drug resistance rate of ART treatment failure was 43.1% (1297/3015). Among the 3015 PLWH, 18 different HIV-1 genotype were found. The dominant genotype was CRF07_BC (40.4%), followed by CRF01_AE (27.1%), C+B (12.3%), C (2.9%), B (2.6%) and CRF55_01B (1.95%).

Table 2 Characteristics of the participants

Characteristics	Total ($n=3015$)
Age, (years) [median (IQR)]	49 (0.7–90)
Male gender [n/total (%)]	2258/3015 (74.9)
ART treatment failure [n/total (%)]	1904/3015 (61.3)
Drug resistance rate of ART treatment failure [n/total (%)]	1297/3015 (43.1)
Subtype [n/total (%)]	
Non-B	2938/3015 (97.4)
B	77/3015 (2.6)
Distribution of non-B subtypes [n/total (%)]	
CRF07_BC	1217/3015 (40.4)
CRF01_AE	816/3015 (27.1)
CRF08_BC	290/3015 (9.6)
C	89/3015 (2.9)
C+B	373/3015 (12.3)
B+CRF01_AE	23/3015 (0.7)
CRF55-01B	59/3015 (1.95)
CRF52-01B	2/3015 (0.1)
CRF57-BC	4/3015 (0.1)
A	47/3015 (1.6)
CRF64_BC	8/3015 (0.3)
CRF67-01B	2/3015 (0.1)
CRF59-01B	2/3015 (0.1)
CRF15-01B	1/3015 (0.03)
CRF62-BC	2/3015 (0.1)
CRF85-BC	2/3015 (0.1)
D	1/3015 (0.03)

IQR: interquartile range; ART: antiretroviral therapy

Mutations associated with drug resistance in HIV-1

By classifying the presence of resistance mutations (high, moderate and low resistant) to any of the antiviral drugs as resistant, it was found that the overall resistance rate among PLWH treated with antiviral therapy reached 46.6% (1405/3015). The drug resistance rates of NRTI, NNRTI and PI were 29.5% (889/3015), 43.8% (1321/3015) and 3.4% (103/3015), respectively. The percentage of concurrent resistance mutations in NRTIs and NNRTIs only was determined as 26.6% (801/3015), in NRTIs and PIs only 0.9% (27/3015), in NNRTIs and PIs only 1.3% (38/3015), and in all three antivirals 11.4% (343/3015). The drug resistance rates of common NRTIs and NNRTIs drug class are elucidated in Fig. 1. In NRTIs drug class, the high /moderate resistance mutations rates of 3TC and FTC was 26.9%. Tenofovir (TDF), a first-line regimen, achieved a high /moderate resistance rate of 11.5%, and zidovudine (AZT) exhibited a resistance rate of only 3.1% due to a high resistance barrier. Among NNRTIs drug class, efavirenz (EFV) and nevirapine (NVP) resistance arose from non-polymorphic mutations in K103N/S, with the resistance rates of 31.8% and 33.1%, respectively. PIs had a low rate of drug-resistant mutations, and the resistance effects they exerted were mild.

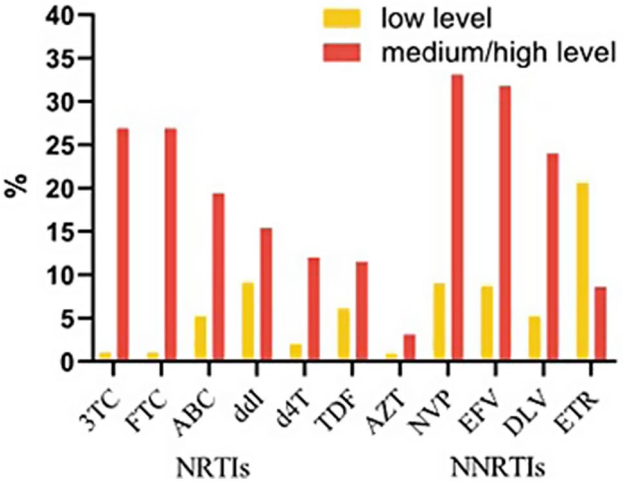

Fig. 1 The proportion and levels of HIV drug resistance to common NRTIs and NNRTIs drugs. NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: nonnucleoside reverse transcriptase inhibitor; 3TC: lamivudine; FTC: emtricitabine; ABC: abacavir; ddI: didanosine; d4T: stavudine; TDF: tenofovir disoproxil fumarate; AZT: zidovudine; NVP: nevirapine; EFV: efavirenz; DLV: delavirdine; ETR: etravirine. NRTIs drugs: 3TC, FTC, ABC, ddI, d4T, TDF, AZT; NNRTIs drugs: NVP, EFV, DLV, ETR

Frequency of HIV-1 drug-resistant mutation loci

Among the 1405 cases which resistance mutations occurred, this study identified 18 NRTI resistance-associated mutation sites, 17 NNRTI resistance associated mutation sites and 14 PI resistance-associated mutation sites. The incidence of M184VI mutation (26.1%; 788/3015) in NRTI-associated mutation sites was more common, followed by K65R/N (13.1%; 396/3015) and K70R (6.5%; 196/3015). Among the NNRTI-associated mutation sites, the highest incidence of V179DE mutations was 15.4% (464/3015), followed by K103N/S (15.1%; 455/3015) and V106MA (11.2%; 338/3015). The most common drug resistance mutation sites in PI were

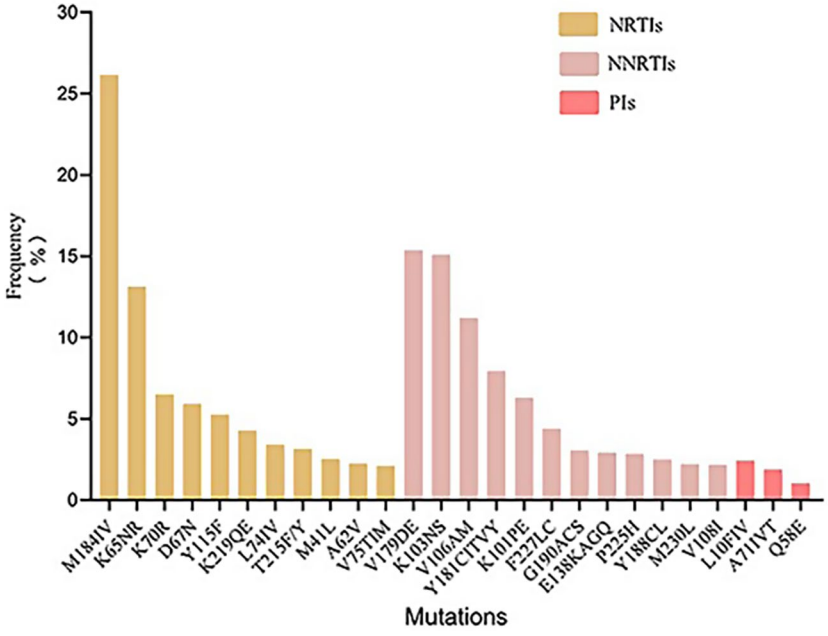

Fig. 2 Frequency of HIV drug resistance mutation loci in NNRTIs, NRTIs and PIs. NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: nonnucleoside reverse transcriptase inhibitor; PIs: protease inhibitors

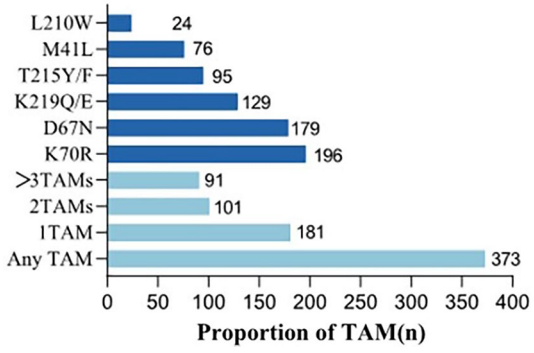

Fig. 3 Distribution of the number of mutations in each of the six mutation loci and the combined mutation status of TAM. TAM: thymidine analogue mutation

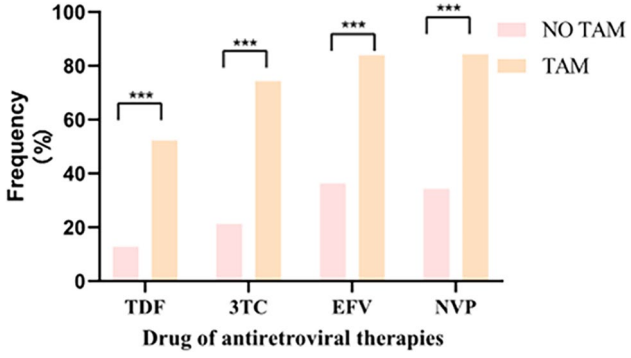

Fig. 4 Comparison of the frequencies of TAM and TAM-free mutations in patients with resistance mutations to TDF, 3TC, EFV, and NVP, respectively (***, $P<0.01$)

L10FIV (2.45%; 74/3015) and A71IVT (1.9%; 57/3015) (Fig. 2).

Prevalence of TAMs in drug-resistant PLWH

TAMs were defined as M41L, D67N, K70R, K219Q/E, L210W and T215Y/F. Among 3015 PLWH, TAMs were identified in 373 PLWH (12.4%), with K70R being the most prevalent in 196 (6.5%). The next most common TAMs consisted of D67N (179 [5.9%] of 3015 PLWH) and K219QE (129 [4.3%] PLWH). 181(6.0%) PLWH had only one TAM, 101 (3.3%) PLWH had two TAMs and 91(3.0%) PLWH had three or more TAMs (Fig. 3).

Compared with participants without TAMs, tenofovir resistance was more common in PLWH with TAMs (195 [52.3%] PLWH with TAMs vs. 335[12.7%] of PLWH without TAMs; $p<0.01$). Similarly, analogous outcomes were yielded in cytosineanalogues (277 [74.3%] PLWH with TAMs vs. 564[21.3%] of PLWH without TAMs; $p<0.01$) and nevirapine or efavirenz (314 [84.2%] or 313[83.9%] of 373 PLWH with TAMs vs. 907[34.3%] or 957 [36.2%] of 2642 without TAMs; $p<0.01$) (Fig. 4).

HIV-1 genotype distribution in drug-resistance PLWH

The main genetic subtypes and their resistance rates in 1405 drug-resistance PLWH were determined as follows. The resistance rate of subtype CRF01-AE was determined as 55.6% (454/816), 37.1% (452/1217) for CRF07-BC, 46.1% (172/373) for B+C, 44.5% (129/290) for CRF08-BC, 55.8% (43/77) for B, 39.3% (35/89) for C, as well as 91.5% (54/59) for CRF55- 01B.

Baseline CD4 T lymphocyte levels

Of the 1405 participants with drug resistance mutations, there were 1124 PLWH (80.8%) with CD4 T-cell counts less than 200cells/L and 281 PLWH (20.0%) more than 200cells/L. Differences in drug-resistant mutations were statistically significant between populations with the different CD4 T-cell counts (χ^2=281.07, P<0.05). The incidence of drug resistance was lower in PLWH with baseline CD4 T-cell counts≥200 cells/L than that in PLWH with <200 cells/L. specific drug resistance profiles were presented in Table 3.

Discussion

In this study, 18 different HIV-1 genotype were found which the prevalence of non-B subtypes was predominant in Chongqing over the last seven years, accounting for 97.4%. Among non-B subtypes, CRF07_BC was the dominate genotype, followed by CRF01_AE and CRF08_BC, which consistent with the result of previous study [8, 9]. The higher proportion of CRF07-BC in Chongqing may be related to its proximity to Yunnan Province. CRF07_BC was originated from Dehong, Yunnan in the early 1990s and consisted of a large number of recombinant strains of the BC subtype, which then spread throughout the country [10, 11]. However, PLWH with CRF01_AE subtype had the highest incidence of drug resistance, which was higher than CRF07_BC, CRF08_BC and B+C subtype. Therefore, a question was raised that whether the differences among subtypes could have an effect on viral drug resistance. Several studies reported that the resistance mutation profiles of different subtypes of strains may differ under drug selection pressure, thus having an effect on viral resistance [12–14].

A previous study showed that the rate of the pretreatment HIV-1 drug resistance in Chongqing was 24.14%, which lower than the percentage observed in Yunnan province with the most severe HIV epidemic in China (34.2%) [8, 15]. However, in this study, we focused the analysis on the drug resistance in PLWH with viral load rebound and clinical suspicion of drug resistance. Therefore, among 3105 participants, the drug-resistance rate was up to 46.6%. There were two reasons accounted for this result. First of all, the site of this research was located in the relatively economically disadvantaged western region of China and PLWH received the first-line free ART medications including two NRTIs combined with one NNRTI. Secondly, the various side effects of free ART drugs led to irregularity and poor adherence with medication, and PLWH were more likely to develop resistance.

Among 1405 drug-resistance participants, the frequency of NNRTI resistance (43.8%) was higher than that of NRTI resistance (29.5%) and PI resistance (3.4%). And the drugs with the most serious HIV-1 resistance in Chongqing consist of three NNRTI class drugs (NVP, EFV, DLV) and two NRTI class drugs (3TC, FTC). Since free ART program recommended two NRTIs in combination with one NNRTI as the first-line ART regimen in which NNRTIs mainly included NVP and EFV. Therefore, in this study, the medium/high resistance level to NVP and EFV were higher than 30%. The high proportion of NNRTI-resistance was primarily attributed to the low genetic barrier to resistance and a long plasmatic half-life [15, 16]. Additionally, TDF and ETR are the representative varieties of new generation NRTI and NNRTI class drugs. Although the prevlance of TDF- and ETR-resistance were significantly lower than that of older generation varieties, the resistance rate of about 10% still has warning significance.

The analysis of drug resistance mutation loci in this study revealed that the most common NRTI-associated mutation loci were M184V/I and K65R/N, with mutation frequencies of 26.1% and 13.1%, respectively. M184V/I mutations resulted in high resistance to 3TC and FTC, and the incidence of M184V/I mutations was significantly higher in those who received 3TC and TDF regimens than in those who received FTC and TDF regimens [17–19]. The thymidine analogue mutation (TAM) group was detected in 373 samples in this study, including M41L, D67N, K70R, K219Q/E, L210W and T215Y/F [20]. The most common mutations in TAMs consisted of K70R (6.5%), D67N (5.9%) and K219QE (4.3%). As revealed by the statistics, PLWH carrying TAMs had the resistance rates of 52.3%, 74.3%, 83.9% and 84.2% to TDF, 3TC, EFV and NVP, respectively. The rates of resistance to TDF, 3TC, EFV and NVP in PLWH without carrying TAMs reached 12.7%, 21.3%, 36.2% and 34.3%, respectively. PLWH carrying TAMs (compared with non-carriers) had significantly more resistance mutations to TDF and NNRTIs (NVP, EFV) and cytidine analogs (lamivudine), and the differences achieved statistical significance (P<0.05). Although accumulation of TAMs loci could cause more widespread resistance, TAMs were transmitted to uninfected individuals, and the above individuals were subsequently at higher risk of ART failure [21]. Common mutation loci in NNRTIs consisted of V179D/E, K103N//S and V106M/A. The frequency of all three mutations was nearly 10-20%. Other major drug-resistant mutant loci consisted of Y181CIV, K101PE, F227L, V108I, L100I, G190ACS, M230L, P225H, E138KAGQ, as well as A98G. The presence of multiple mutant loci alone or in combination can lead to

Table 3 Correlation between baseline CD4 T-cell levels and incidence of drug-resistant mutations in 3015 PLWH

CD4 T-cell count(cells/μL)	< 200	≥ 200	*P*
Number of PLWH	1940	1075	
Number of drug resistance	1245	281	< 0.05

a moderately high level of viral resistance to NVP, DLV, and EFV [22, 23], which could explain the high rate of resistance to NNRTI class drugs in our group of cases. The main resistance loci for PIs included A71IVT and L10FIV mutations. Resistance to PIs was rare, which was correlated with the late introduction of PIs into southwest China, the short duration of clinical application and the high resistance barrier. It is noteworthy that drug resistance is the main cause of virologic failure in PLWH. Drug resistance is dynamic and mutates over time, especially those introduced at later time points, in the form of natural polymorphisms. Drug-resistant strains should be continuously monitored to understand and update the prevalence of major drug-resistant genes in our region.

Additionally, CD4 T-cell counts have commonly served as an indicator of the immune status of PLWH. In this study, the incidence of drug-resistant mutations was significantly lower in PLWH with CD4 T-cell counts≥200/μL than in the <200/μL group since PLWH with high CD4 T-cell counts. A better immune status, adequate disease awareness, and good adherence with medication may be accounted for this result. Therefore, the rate of drug-resistance mutations was lower in PLWH with high CD4 T-cell counts.

There are some limitations in this study. Firstly, some of the cases in this group contained incomplete clinical data, which did not affect the analysis of the main results, whereas it was still the limitation of this study as a retrospective study. secondly, this research focused on the situation of patients who received ART treatment based on The National Free Antiretroviral Treatment Program of China. Therefore, our study did not analyze the drug resistance for integrase inhibitors.

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Conclusions

In brief, the distribution of HIV-1 genotypes in Chongqing is complicated and diverse. The drug resistance mutation loci in this study revealed that PLWH carrying TAMs have significantly increased the probability of resistance mutations to some ART drugs and exhibit a higher rate of resistance to NNRTIs, while resistance to PIs is rare. Accordingly, it is necessary to strengthen drug resistance surveillance and HIV-1 viral load testing to determine the therapeutic schedule and adjust the medication regimen.

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Author contributions
WWG and GZ analyzed the patient data; ML, PSW and JGL collected the patient data; RND wrote and edited the review. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval
Research ethics approval was obtained from the Chongqing Public Health Medical Center Research Ethics Committee (No.2016GWZX001).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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