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Impacts of HIV-1 Subtype Diversity on Long-Term Clinical Outcomes in Antiretroviral Therapy in Guangxi, China

He Jiang, MD,[a,b,c] Guanghua Lan, MD, PhD,[a] Qiuying Zhu, MD,[a] Yi Feng, PhD,[c] Shujia Liang, PhD,[a] Jianjun Li, MD,[a] Xinjuan Zhou, MD,[a,b] Mei Lin, MD, PhD,[a] and Yiming Shao, MD, PhD[a,b,c]

Background: Comprehensively estimating the impacts of HIV-1 subtype diversity on long-term clinical outcomes during antiretroviral therapy (ART) can help inform program recommendations.

Methods: The HIV-1 sequence data and clinical records of 5950 patients from all 14 prefectures in Guangxi, China, during 2008–2020 were included. Evolutional trends of CD4+ T-lymphocyte count and viral load were explored, and the effects of HIV-1 subtypes on clinical outcomes were estimated by the Cox proportional hazards model. The polymorphisms involved in drug resistance mutation were analyzed.

Results: Compared with patients with CRF07_BC, patients with CRF01_AE and CRF08_BC showed poor immunologic and virologic responses to antiretroviral therapy. Although the median expected time from ART initiation to virologic suppression for all patients was approximately 12 months, patients with CRF01_AE and CRF08_BC had a long time to achieve immune recovery and a short time to occur immunologic failure, compared with patients with CRF07_BC. Adjusted analysis showed that both CRF01_AE and CRF08_BC were the negative factors in immune recovery and long-term mortality. In addition, CRF08_BC was a negative factor in virologic suppression and a risk factor of virologic failure. This poor virologic response might result from the high prevalence of drug resistance mutation in CRF08_BC.

Conclusions: Compared with patients with CRF07_BC, patients with CRF01_AE could benefit more from immediate ART, and patients with CRF08_BC are more suitable for PI-based regimens. These data emphasize the importance of routine HIV-1 genotyping before ART, immediate ART, and personalized ART regimens to improve the prognosis for patients undergoing ART.

Key Words: CRF01_AE, CRF07_BC, CRF08_BC, antiretroviral therapy, clinical outcome

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INTRODUCTION

HIV-1 subtype diversity has been proposed to be associated with disease progressions and the responses to antiretroviral therapy (ART).[1] Monitoring the subtype-specific differences in long-term clinical outcomes during ART can help reduce mortality.[2–4] Globally, the most prevalent HIV-1 subtype is C (46.6%), followed by B (12.1%), A (10.3%), CRF02_AG (7.7%), CRF01_AE (5.3%), G (4.6%), and D (2.7%).[5] A previous systematic review conducted in ART-naive patients suggested that HIV-1 subtypes C and D were the most aggressive in disease progression, followed by G, CRF01_AE, and CRF02_AG.[6] A recent study in Sweden suggested that HIV-1 subtype C has a higher risk of virologic failure (VF) in ART than HIV-1 subtype B, which was explained by a lower affinity for protease inhibitors.[7] At present, HIV-1 circulation recombinant forms (CRFs) are found more frequently in China. According to the latest national HIV-1 molecular survey in 2018, CRF07_BC, CRF01_AE, and CRF08_BC are the 3 predominant prevalent HIV-1 subtypes and account for 39.7%, 36.9%, and 9.0% of reported cases, respectively.[8] CRF01_AE was reported to harbor a high prevalence of CXCR4 viruses,[9] which contributed to fast $CD4^+$ T-lymphocyte count (CD4) depletion in natural infection[10,11] and suboptimal CD4 restoration during ART.[12–14] Besides, a recent study revealed that the distributions of drug resistance mutation (DRM) in CRF01_AE, CRF07_BC, and CRF08_BC were inconsistent.[15] Therefore, we hypothesized that long-term clinical outcomes differed between subtypes. Owing to relatively limited clinical data, a previous study in Guangxi between 2014 and 2019 provided insufficient evidence to validate this hypothesis.[10] In this study, we integrated 2 large databases of province-wide surveillance in

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From the [a]Guangxi Key Laboratory of AIDS Prevention and Control and Achievement Transformation, Guangxi Center for Disease Prevention and Control, Nanning, Guangxi, China; [b]School of Public Health, Guangxi Medical University, Nanning, Guangxi, China; and [c]State of Key Laboratory for Infectious Disease Prevention and Control, National Center for AIDS/STD Control and Prevention, Chinese Center for Disease Control and Prevention, China.

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H.J., G.L., M.L., and Y.S. take responsibility for the study design and result interpretation. H.J., Q.Z., Y.F., S.L., J.L., and X.Z. analyzed the data. H.J. drafted the manuscript. All authors reviewed the manuscript and approved the final version for submission.

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H.J., G.L., and Q.Z. contributed equally to this work.

M.L. and Y.S. contributed equally to this work.

Correspondence to: Yiming Shao, MD, PhD, Division of Research on Virology and Immunology, National Center for AIDS/STD Control and Prevention, Chinese Center for Disease Control and Prevention, 155 Changbai Road Changping District, Beijing, 102206, People's Republic of China (e-mail: yshao@bjmu.edu.cn).

China to compare the disparate impacts of HIV-1 subtypes on immunologic responses, virologic responses, and mortality.

METHODS

Study Design and Patients

This cohort study was based on the existing records of the annual HIV-1 molecular epidemiological survey in all 14 prefectures in Guangxi from 2008 to 2020 and the National Free ART database. We included the patients if: (1) they were infected with one of the 3 HIV-1 subtypes, CRF01_AE, CRF07_BC, and CRF08_BC; (2) they were older than 15 years on the date of ART initiation; (3) they initiated ART between January 1, 2008 and December 31, 2020; (4) HIV-1 infectious route was self-reported as sexual contact or injecting drug use; and (5) they were naive to ART when they started a standard triple ART regimen. The following patients were excluded from this study: (1) those without baseline records, (2) those without baseline CD4, and (3) those without any follow-up records or lacking the first follow-up, including drug supply (Fig. 1).

This study was approved by the Ethics Review Board of Guangxi Center for Disease Prevention and Control (Certificate No.: GXIRB2016-0047-1). Each patient signed informed consent at the initiation of ART, allowing the use of the clinical records in future epidemiological researches. No additional informed consent was sought, and all clinical records were deidentified before analysis. We signed a confidentiality agreement and were authorized to use the database for this study.

Phylogenetic Analysis

All available HIV-1 protease and reverse transcriptase sequences (*pol*, HXB2 coordinates: 2253 nt–3554 nt) were extracted from the HIV-1 sequence database. Among them, 1715 sequences were obtained from blood samples collected before ART (pretreatment sequences) and 4235 collected after ART (posttreatment sequences). These sequences were edited by BioEdit v7.1 and aligned by MAFFT v7 (https://mafft.cbrc.jp/alignment/software/), with the major HIV-1 subtypes and CRF reference sequences downloaded from Los Alamos National Laboratory HIV Sequence database

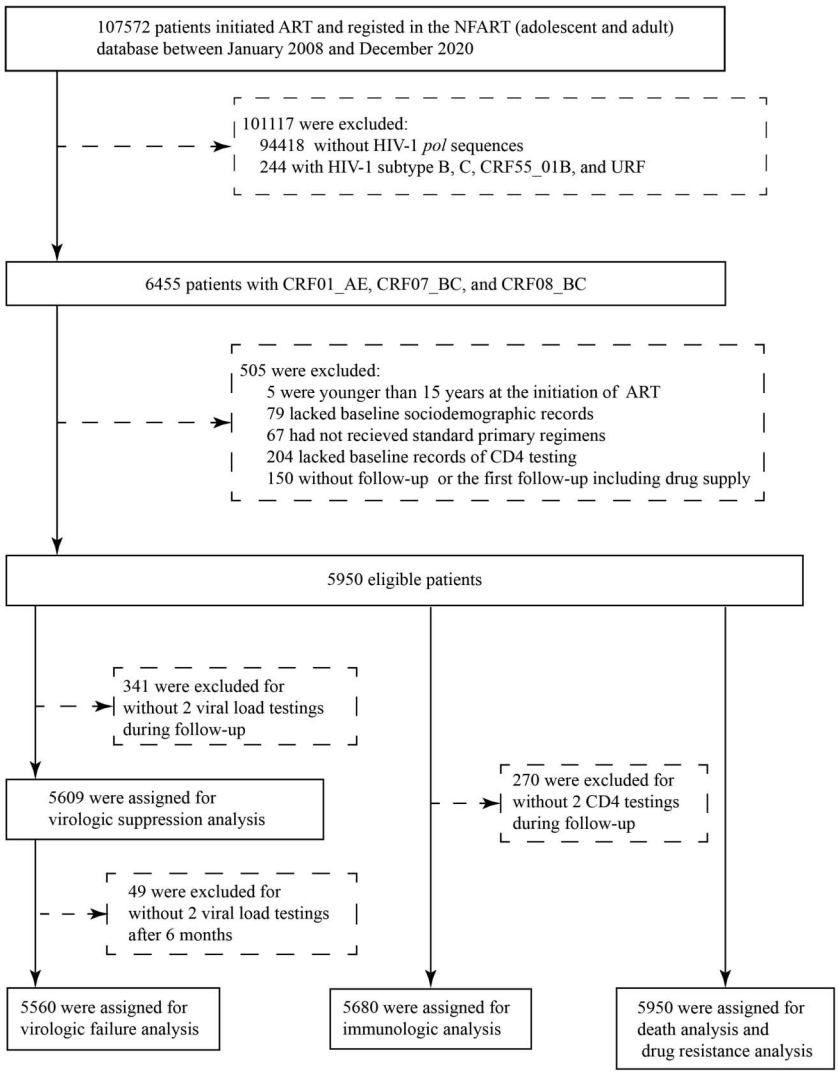

FIGURE 1. Study flowchart. NFART, national free antiretroviral therapy; *pol*, protease and reverse transcriptase sequence; URF, unique recombinant form; CD4, CD4+ T-lymphocyte.

(https://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html). HIV-1 subtypes were determined based on an approximate maximum likelihood phylogenetic tree analysis, which was constructed by FastTree v2.1.8 (https://meta.microbesonline.org/fasttree/) with a general time-reversible model and was visualized by FigTree v1.4.3 (https://tree.bio.ed.ac.uk/software/figtree/). DRM assays were conducted by Stanford University HIV Drug Resistance Database online tool (https://hivdb.stanford.edu/).

Variables and Outcomes Definitions

Baseline CD4 was defined as the last pretreatment testing within 6 months of treatment. If pretreatment CD4 was not available, the earliest testing within 1 month of ART initiation was used for the baseline value. Subsequent CD4 was monitored at least once in every 1–12 months. Viral load testing is not a routine item before ART but is monitored at least once in every 12 months after ART. Follow-up visits were scheduled at 2 weeks, 1, 2, and 3 months, and then every 3 months thereafter. Because all eligible patients should receive the first follow-up, including drug supply, the minimum time of follow-up was 2 weeks after starting ART. Follow-up data were included up to December 31, 2020, stopping ART, lost to follow-up, or events of death. Patients were considered lost to follow-up if they missed scheduled follow-up for more than 90 days. The adherence was defined regarding the proportion of days covered and calculated as 100 multiplied by the total number of days of antiretroviral drug dispensed divided by the total number of days of follow-up.[16] The standard triple ART regimens in China are classified into 2 types: (1) non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen, namely the first-line regimen, refers to 2 NRTIs and 1 non-NRTIs combinations; (2) protease inhibitor (PI)-based regimen, namely the second-line regimen, refers to 2 NRTI and 1 PI combinations. At the initiation of ART, NNRTI-based regimens are recommended for most patients except those who cannot tolerate them. According to the China Area Code, the regions of residence are classified into 2 categories: (1) urban refers to the city areas and (2) rural refers to town, country, and village areas.

We assessed the long-term clinical outcomes by 3 favorable events [virologic suppression (VS), early immune recovery (EIR), and immune recovery (IR)] and 3 unfavorable events [VF, immunologic failure (IF), and death]. These clinical events were defined according to China free antiretroviral therapy manual. VS was defined as the first viral load below the lower limit of detection for the assay (<40 copies/mL). EIR and IR were defined as the first 2 consecutive CD4 of more than 350 cells/μL or 500 cells/μL after starting ART, respectively. VF was defined as the first 2 consecutive viral loads of more than 400 copies/mL after 24 weeks of ART initiation. IF was defined as any one of the following: (1) a posttreatment CD4 falling to or below baseline CD4; (2) a posttreatment CD4 below 50% of the peak CD4; and (3) 2 consecutive CD4 of fewer than 100 cells/μL. Death dates were extracted from the follow-up records and confirmed by local health workers.

Statistical Analysis

We explored the longitudinal changes of viral load and CD4 stratified by HIV-1 subtype. The pairwise χ^2 test and the Dunn Kruskal–Wallis multiple comparisons were used to compare the differences among the 3 subtype groups at the 12-month interval after ART initiation.

We fitted a series of multivariable Cox proportional hazard regression models (Cox model) to estimate the adjusted hazard ratios (aHRs) of occurring clinical events. We initially included all covariables in the Cox models. But there was collinearity between infectious route and adherence. Considering adherence is a strong independent predictor for long-term clinical outcome,[16] we finally included the following covariables in the Cox models: sex, age, baseline CD4, year of ART initiation, adherence, primary regimen type, and region of residence. China is one of the countries with the largest number of HIV-1 subtypes in the world. Although the distribution of subtypes varies greatly among regions, CRF07_BC is the most prevalent HIV-1 subtype in China.[17] For future interregion comparison, we selected the CRF07_BC group as the reference group. The median expected time until 50% of patients achieved the given clinical event was calculated by correcting a covariate-balanced pseudopopulation.[18] To further minimize the potential effects of confounding on mortality, we calculated the overall mortality by selecting all those who died during the follow-up and further calculated the adjusted mortality by restricting the death events occurring after 12 months of ART. The proportional hazard assumptions were checked by the plots of the Schoenfeld residuals, and no violation was found. Finally, we compared the prevalence of DRM sites among HIV-1 subtype groups.

All statistical tests were 2-sided, and $P < 0.05$ was considered statistically significant. Adjusted P value for pairwise comparison was calculated by controlling the false discovery rate.[19] Statistical analyses were performed with R v4.0.3. The rcompanion package and FSA package were used for pairwise comparison. The survival package was used to construct the Cox models, and ggadjustedcurves function was used to calculate the median expected time.

RESULTS

Baseline Characteristics

In total, 5950 eligible patients were included and followed up for a median of 6.1 years; 4690 (78.8%) with CRF01_AE, 635 (10.7%) with CRF07_BC, and 625 (10.5%) with CRF08_BC. The mean age of the CRF01_AE group, the CRF07_BC group, and the CRF08_BC group was 44.8 years (SD =14.1), 40.1 years (SD = 15.6), and 46.6 years (SD = 14.8), respectively. The median baseline CD4 in the 3 groups was 153 cells/μL [interquartile range (IQR), 42–277 cells/μL], 288 cells/μL (IQR, 198–409 cells/μL), and 263 cells/μL (IQR, 151–386 cells/μL), respectively (Table 1).

During the period of follow-up, 5259 (88.4%) patients achieved VS, 3325 (55.9%) achieved EIR, 1969 (33.0%) achieved IR, 673 (11.3%) experienced VF, 1292 (21.7%) experienced IF, and 456 (7.7%) died. Of the 673 patients with VF, 360 (53.5%) received a regimen change and 94 (14.0%) received a regimen change within 90 days after the VF was observed. By comparison, among the 1292 patients with IF, 417 (32.3%) received a regimen change and 98 (7.6%) received a regimen change within 90 days after the IF was observed.

TABLE 1. Baseline Characteristics

Characteristics	CRF01_AE	CRF07_BC	CRF08_BC
Total	4690	635	625
Sex			
Male	3011 (64.2%)	510 (80.3%)	394 (63.0%)
Female	1679 (35.8%)	125 (19.7%)	231 (37.0%)
Age at ART initiation, year, mean (SD)	44.8 (14.1)	40.1 (15.6)	46.6 (14.8)
Infectious route			
Heterosexual	4273 (91.1%)	408 (64.3%)	504 (80.6%)
MSM	154 (3.3%)	189 (29.8%)	7 (1.1%)
IDU	263 (5.6%)	38 (6.0%)	114 (18.2%)
Baseline CD4, cells/μL, median (IQR)	153 (42–277)	288 (198–409)	263 (151–386)
Year of ART initiation			
2016–2020 (the 4th edition of ART manual)	1474 (31.4%)	482 (75.9%)	343 (54.9%)
2012–2015 (The 3rd edition of ART manual)	1977 (42.2%)	133 (20.9%)	204 (32.6%)
2008–2011 (The 2nd edition of ART manual)	1239 (26.4%)	20 (3.1%)	78 (12.5%)
Adherence (PDC), %, median (IQR)	97.8 (89.3–99.5)	98.3 (92.7–99.6)	95.1 (71.1–99.1)
Primary regimen type			
NNRTI-based	4352 (92.8%)	589 (92.8%)	564 (90.2%)
PI-Based	338 (7.2%)	46 (7.2%)	61 (9.8%)
Region of residence			
Urban	2234 (47.6%)	383 (60.3%)	292 (46.7%)
Rural	2456 (52.4%)	252 (39.7%)	333 (53.3%)

MSM, men who have sex with men; IDU, injecting drug use; CD4, $CD4^+$ T-lymphocyte count; PDC, the proportion of days of drug supply covering the whole follow-up. ; NNRTI-based, regimen containing 2 NRTI and 1 NNRTI; PI-based, regimen containing 2 NRTI and 1 PI.

Incomplete Virologic Response was Found in Patients With CRF08_BC

Of the 3 HIV-1 subtype groups, the proportion of detectable viral load, particularly viral load of ≥1000 copies/mL, decreased the slowest in the CRF08_BC group (Fig. 2). At most time points from 12 months after ART initiation, the lowest proportion of undetectable viral load was in the CRF08_BC group (see Table S1, Supplemental Digital Content, http://links.lww.com/QAI/B782).

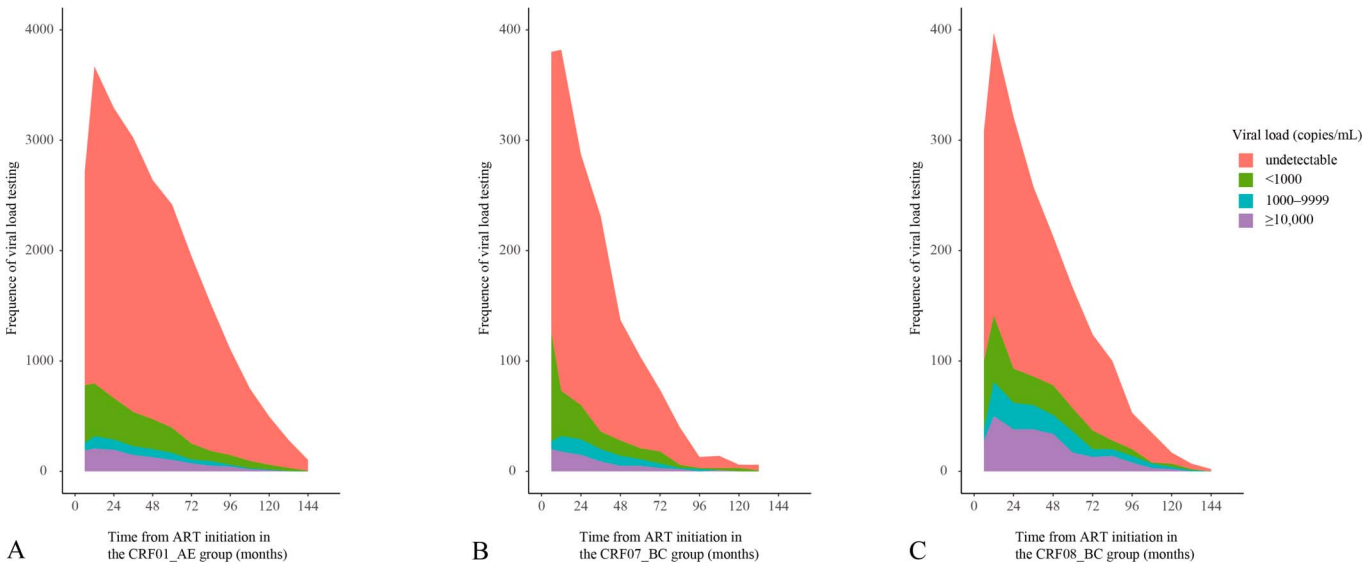

FIGURE 2. Distribution of patients with different levels of viral load after antiretroviral therapy. A, The CRF01_AE group. B, The CRF07_BC group. C, The CRF08_BC group.

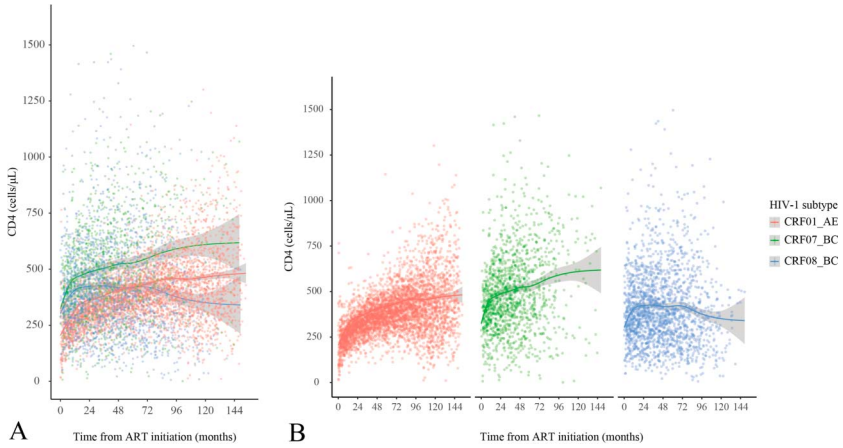

FIGURE 3. CD4 trajectories during follow-up stratified by HIV-1 subtypes.CD4, CD4⁺ T-lymphocyte count. The smooth curves and 95% CI are generated based on all CD4 during follow-up. Each point represents a median CD4 every day. A, The combination of CD4 trajectories for all HIV-1 subtype groups. B, Illustrates CD4 trajectories in the CRF01_AE group, the CRF07_BC group, and the CRF08_BC group.

Suboptimal CD4 Restorations Were Found in Patients With CRF01_AE and CRF08_BC

The lowest median baseline CD4 was in the CRF01_AE group, followed by the CRF08_BC group. Rapidly growing CD4 trajectories were in all 3 groups within approximately 12 months of ART initiation. CD4 trajectories subsequently became less steep in the CRF01_AE group and the CRF07_BC group and declined in the CRF08_BC group (Fig. 3). The median CD4 in the CRF01_AE group exceeded that in the CRF08_BC group at approximately 84 months (see Table S2, Supplemental Digital Content, http://links.lww.com/QAI/B782).

Both CRF01_AE and CRF08_BC Were the Negative Factors of Favorable Events and Risk Factors of Infavorable Events

After adjusting for the covariables, compared with CRF07_BC, CRF01_AE was a negative factor of EIR [aHR, 0.740; 95% confidence interval (CI), 0.652 to 0.841] and IR (aHR, 0.787; 95% CI: 0.670 to 0.924). In addition to showing a negative relation to EIR (aHR, 0.826; 95% CI: 0.703 to 0.970) and IR (aHR, 0.764; 95% CI: 0.620 to 0.943), CRF08_BC is also a negative factor of VS (aHR, 0.772; 95% CI: 0.680 to 0.877) and a risk factor of VF (aHR, 2.028; 95% CI: 1.437 to 2.863) and IF (aHR, 1.441; 95% CI: 1.104 to 1.881). When analyzing mortality, CRF01_AE was a robust risk factor of overall mortality (aHR, 1.528; 95% CI: 1.018 to 2.292) and adjusted mortality (aHR, 1.787; 95% CI: 1.101 to 2.899). However, the significantly higher risk effect of CRF08_BC on mortality when compared with that of CRF07_BC could be found 12 months after ART initiation (aHR, 1.835; 95% CI: 1.080 to 3.119). (Fig. 4 and Table 2).

A short time for CD4 restoration was in patients with CRF01_AE and CRF08_BC when compared with patients with CRF07_BC. In all HIV-1 subtype groups, the median expected time from ART initiation to VS was approximately 12 months. Subsequently, the CRF01_AE group and the CRF08_BC group had a longer median expected time to achieve EIR and IR and a short median expected time to occur IF, compared with the CRF07_BC group (Table 2). The median recovery time from achieving EIR to occurring IF in the CRF07_BC group, the CRF01_AE group, and the CRF08_BC group was 102.1 months, 69.7 months, and 61 months, respectively.

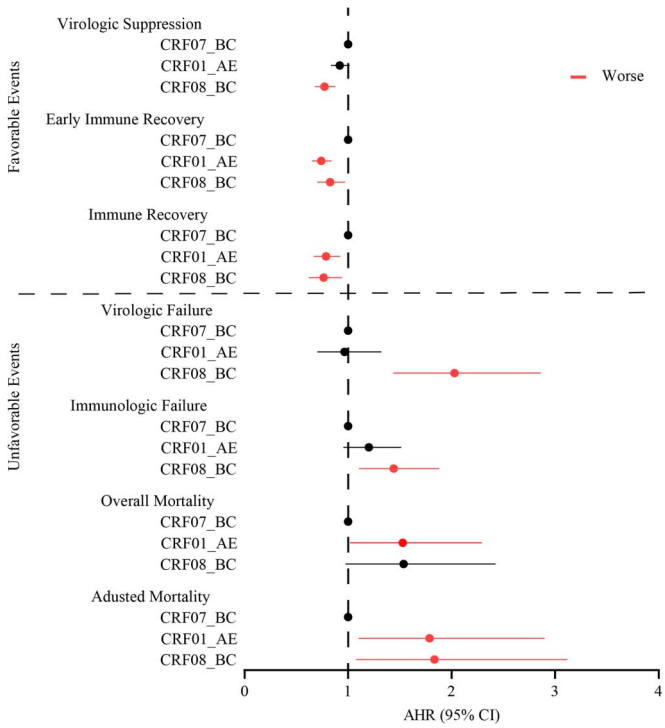

FIGURE 4. aHR (95% CI) for favorable and unfavorable events associated with CRF01_AE, CRF07_BC, and CRF08_BC. CD4, CD4⁺ T-lymphocyte count. The CRF07_BC group is the reference group. aHR is adjusted by sex, age, baseline CD4, the primary regimen type, adherence, year of ART initiation, and region of residence. Red points and error bars represent those patients are less likely to achieve favorable events (virologic suppression, early immune recovery, and immune recovery) and have a high risk of experiencing unfavorable events (virologic failure, immunologic failure, and death). Overall mortality was calculated by selecting all those who died during the follow-up, and the adjusted mortality was calculated by restricting the death events occurring after 12 months of ART.

TABLE 2. Comparison of the Effect of HIV-1 Subtype Diversity on Clinical Events Using the Univariate and Multivariate Cox Proportional Hazard Regression Models

Disease Progression	HR (95% CI)	*P*	aHR (95% CI)	*P*	Median Expected Time (mo)
Virologic suppression					
CRF07_BC	1		1		11.6
CRF01_AE	0.926 (0.844–1.016)	0.103	0.920 (0.834–1.015)	0.097	11.6
CRF08_BC	0.728 (0.642–0.825)	<0.001	0.772 (0.680–0.877)	<0.001	11.6
Early immune recovery					
CRF07_BC	1		1		30.0
CRF01_AE	0.548 (0.487–0.616)	<0.001	0.740 (0.652–0.841)	<0.001	41.4
CRF08_BC	0.712 (0.608–0.833)	<0.001	0.826 (0.703–0.970)	0.020	36.3
Immune recovery					
CRF07_BC	1		1		89.2
CRF01_AE	0.510 (0.439–0.592)	<0.001	0.787 (0.670–0.924)	0.003	114.2
CRF08_BC	0.626 (0.510–0.768)	<0.001	0.764 (0.620–0.943)	0.012	118.4
Virologic failure					
CRF07_BC	1		1		—
CRF01_AE	0.749 (0.553–1.014)	0.062	0.965 (0.704–1.322)	0.823	—
CRF08_BC	2.152 (1.532–3.024)	<0.001	2.028 (1.437–2.863)	<0.001	—
Immunologic failure					
CRF07_BC	1		1		132.1
CRF01_AE	1.276 (1.020–1.596)	0.033	1.201 (0.954–1.513)	0.119	111.1
CRF08_BC	1.729 (1.329–2.250)	<0.001	1.441 (1.104–1.881)	0.007	97.3
Overall mortality					
CRF07_BC	1		1		—
CRF01_AE	0.797 (0.541–1.175)	0.252	1.528 (1.018–2.292)	0.041	—
CRF08_BC	1.550 (0.991–2.424)	0.055	1.537 (0.975–2.424)	0.064	—
Adjusted mortality					
CRF07_BC	1		1		—
CRF01_AE	0.953 (0.599–1.518)	0.841	1.787 (1.101–2.899)	0.019	—
CRF08_BC	1.930 (1.146–3.250)	0.013	1.835 (1.080–3.119)	0.025	—

HR, hazard ratio.

The Cox proportional hazard regression models were fitted by adjusting for sex, age, baseline $CD4^+$ T-lymphocyte count, primary regimen type, adherence, year of ART initiation, and region of residence. The median expected time represents the expected time until 50% of patients achieved events and is calculated considering a covariate-balanced pseudopopulation. Overall mortality is calculated by selecting all those who died during the follow-up. Adjusted mortality is calculated by restricting the death events occurring after 12 months of follow-up.

A High Prevalence of DRM was Found in CRF08_BC

The overall prevalence of DRM was 18.3% (1086/5950), with NNRTI mutation being the most common form (14.7%, 874/5950), followed by NRTI mutation (10.1%; 598/5950) and PI mutation (2.8%; 169/5950). For NNRTI mutation, the most frequent ART drug was nevirapine (13.6%), followed by efavirenz (13.2%), rilpivirine (10.7%), doravirine (9.8%), and etravirine (8.4%). Both in pretreatment sequences and posttreatment sequences, a higher prevalence of NNRTI mutation was in the CRF_08BC group than in the CRF01_AE/CRF07_BC group. (Table 3).

Of the 1715 pretreatment sequences, the most prevalent NNRTI mutations occurred at site 179 (5.5%), followed by sites 106 (2.6%) and 138 (1.7%). Of the 4235 posttreatment sequences, the most prevalent NNRTI mutation was at site 103 (7.1%), followed by sites 179 (5.3%), 106 (4.8%), 190 (4.5%), 230 (4.1%), 181 (3.7%), 101 (2.2%), 138 (1.9%), 225 (1.6%), 108 (1.3%), 188 (1.2%), and 221 (1.2%). CRF08_BC presented a higher prevalence of NNRTI mutation at site 138 in pretreatment sequences and a higher prevalence of mutation at sites 103, 179, 138, 225, and 188 in posttreatment sequences (Fig. 5 and see Table S3, Supplemental Digital Content, http://links.lww.com/QAI/B782).

DISCUSSION

Based on data combined from 2 large databases of province-wide surveillance in China, this study confirmed that HIV-1 subtype diversity had a significant impact on long-term clinical outcomes in ART. Previous investigations have found that the distribution of HIV-1 subtype is correlated with infectious route and region.[2] In China, 47% CRF07_BC was concentrated in men who have sex with men.[20] Of all CRF08_BC, 67% were found in patients infected HIV through heterosexual contact and 23% through injecting drug use.[21] In Guangxi, the proportion of CRF01_AE and CRF08_BC was higher than the overall proportion in China, whereas the proportion of CRF07_BC was lower than the overall proportion in China.[22] The number of men who have

TABLE 3. Prevalence of Drug Resistance Mutation Among Sequences Sampled Before and After ART

Drug Resistance	Pretreatment Sequences Total (n = 1715)	CRF01_AE/CRF07_BC (n = 1418)	CRF08_BC (n = 297)	*P*	Posttreatment Sequences Total (n = 4235)	CRF01_AE/CRF07_BC (n = 3907)	CRF08_BC (n = 328)	*P*
Overall				<0.001				<0.001
No	1602 (93.4%)	1341 (94.6%)	261 (87.9%)		3262 (77.0%)	3052 (78.1%)	210 (64.0%)	
Yes	113 (6.6%)	77 (5.4%)	36 (12.1%)		973 (23.0%)	855 (21.9%)	118 (36.0%)	
PI mutation				0.115				0.286
No	1678 (97.8%)	1391 (98.1%)	287 (96.6%)		4103 (96.9%)	3782 (96.8%)	321 (97.9%)	
Yes	37 (2.2%)	27 (1.9%)	10 (3.4%)		132 (3.1%)	125 (3.2%)	7 (2.1%)	
NRTI mutation				0.387				0.177
No	1697 (99.0%)	1405 (99.1%)	292 (98.3%)		3655 (86.3%)	3380 (86.5%)	275 (83.8%)	
Yes	18 (1.0%)	13 (0.9%)	5 (1.7%)		580 (13.7%)	527 (13.5%)	53 (16.2%)	
NNRTI mutation				<0.001				<0.001
No	1651 (96.3%)	1377 (97.1%)	274 (92.3%)		3425 (80.9%)	3204 (82.0%)	221 (67.4%)	
Yes	64 (3.7%)	41 (2.9%)	23 (7.7%)		810 (19.1%)	703 (18.0%)	107 (32.6%)	

Pretreatment and posttreatment mean sequences were obtained from the samples collected before and after ART, respectively.

sex with men in reported HIV cases increased ranging from 1% to 6% annually between 2010 and 2017.[23] Therefore, the baseline characteristics of the HIV-1 subtype group in our study reflected the epidemic pattern of HIV in Guangxi. When analyzing the aHR of occurring clinical events, we adjusted for the available covariables. Sex, age, baseline CD4, year of ART initiation, adherence, and primary regimen type have been confirmed as the independent predictors of clinical outcomes. In addition, differences in the allocation of medical resources between urban and rural areas in China may affect the clinical outcomes.[24] Therefore, we included these covariables in the Cox models. When selecting the reference group in the Cox models, there was no consensus in the previous studies.[25] Considering CRF07_BC is the most prevalent HIV-1 subtype in China, we selected the CRF07_BC group as the reference group for future interregion comparison. When analyzing the different effects of CRF01_AE and CRF07_BC on EIR and IR, our results were consistent with those of other provinces in China.[12] Therefore, it is reasonable to select the CRF07_BC group as the reference group.

Two important programmatic changes occurred in 2008, namely the criterion of CD4 for starting ART raised to 350 cells/μL, and the second-line regimens were introduced to the National Free ART program.[26] A previous study conducted in Guangxi between 2014 and 2019 showed that patients in different HIV-1 subtype groups exhibited inconsistent baseline CD4 before ART but similar mortality after ART.[10] This study included the surveillance data between 2008 and 2020 and revealed that patients in different HIV-1 subtype groups had not only inconsistent baseline CD4 but also inconsistent subsequent clinical outcomes during ART. We found that the period of the first 12 months of ART initiation was critical for long-term outcomes. During this period, half of the patients were expected to achieve VS, and

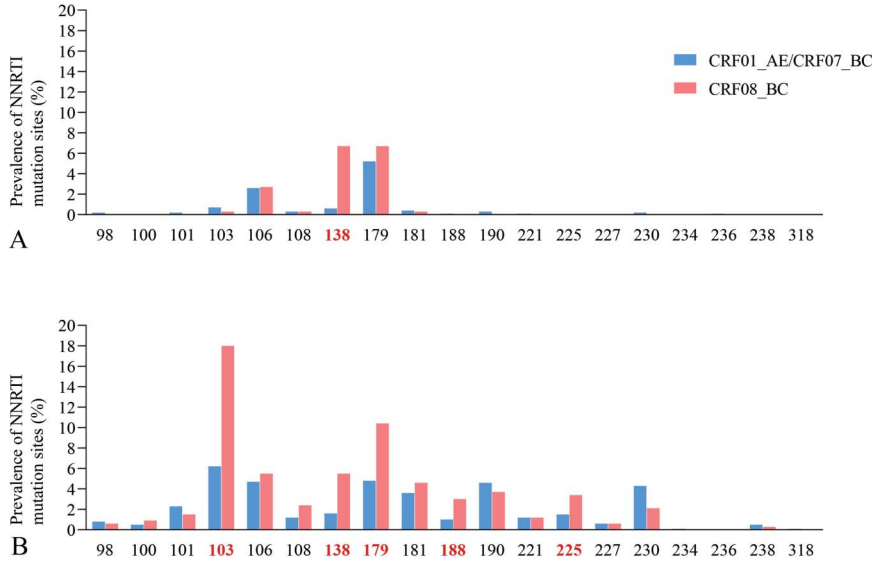

FIGURE 5. Comparison of prevalence of NNRTI mutation sites between the CRF01_AE/CRF07_BC group and the CRF08_BC group. Site numbers in bold and in red indicate that the prevalence of NNRTI mutation sites are significantly different between 2 groups of patients, measured by the χ^2 or Fisher exact test. Pretreatment and posttreatment mean sequences were obtained from the samples collected before and after ART, respectively. A, Comparison among pretreatment sequences (1418 were in the CRF01_AE/CRF07_BC group and 297 were in the CRF08_BC group). B, Comparison among posttreatment sequences (3907 were in the CRF01_AE/CRF07_BC group and 328 were in the CRF08_BC group).

all patients gained a significantly rapid CD4 restoration. After 12 months of ART, the increasing rate of CD4 became less steep in the CRF01_AE group and the CRF07_BC group and declined in the CRF08_BC group. A previous study in the United States revealed that after a significantly rapid increase during the first 1.5 years of ART initiation, CD4 might stabilize or only gradually increase.[27] Therefore, the low baseline CD4 and short recovery time could partly explain why the CRF01_AE group was less likely to achieve IR despite early VS. In addition, we found that the median expected time of occurring IF was earlier than that of achieving IR in the CRF01_AE group, suggesting that IR in the CRF01_AE group was unstable. Those immunological nonresponders account for 10%–40% of, in general, ART-experienced patients[28–30] and were proposed to be associated with complicated mechanisms, including severe immune dysfunctions destroyed by HIV, specific genetic characteristics, ART regimen, and immune reconstitution.[31–35] Our findings suggested that patients with CRF01_AE may benefit more from immediate ART than patients with CRF07_BC.

Furthermore, CRF08_BC was a negative factor in VS and a risk factor in VF in our study. At the population level, the high prevalence of DRM in ART-naive patients with CRF08_BC had been reported in Guangxi and other provinces.[36,37] At the molecular level, an NNRTI mutation of D404N was found in the connection subdomain of the reverse transcriptase of CRF08_BC. This mutation conferred a low-level resistance to NNRTI (nevirapine, efavirenz, and rilpivirine) and NRTI (zidovudine), whereas double mutations of D404 and Y181C and triple mutations of D404N, Y181C, and H221Y presented significantly reduced susceptibility to NNRTI.[38] A series of nationwide surveys from 2004 to 2018 have revealed that the distribution character of HIV-1 subtypes and DRM changed over the years.[8,39,40] First, the proportion of CRF08_BC decreased gradually. Second, although the overall prevalence of DRM among ART-naive patients remained stable over time, NNRTI mutation presented an upward trend. Recent investigations in Guangxi showed that patients with CRF08_BC were more likely to develop DRM and shared DRM in the transmission network, but there was no significant difference in the prevalence of DRM among different infectious route groups.[37,41] These data suggest that it is necessary to modify ART regimens for patients who have a high risk of DRM. PI-based regimens may be more suitable than NNRTI-based regimens for patients with CRF08_BC as the primary regimens.

Considering the cumulative effect caused by continuous clinical events, HIV-1 subtype may ultimately influence mortality. However, the previous study in Guangxi did not observe the subtype-specific differences in long-term mortality.[10] The possible reason is that the acute effects of coinfections and adverse effects outweigh the cumulative effects of poor immunological and virologic responses in mortality in short-term ART.[42,43] Our finding revealed that both CRF01_AE and CRF08_BC showed a higher aHR in the Cox model for mortality after 12 months of ART than that in the Cox model for overall mortality.

The study has some limitations. First, HIV-1 subtype is correlated with infectious route and region, and a sampling bias was inevitable in this study. Second, because HIV-1 genotyping is not routine testing in China, we were able to incorporate only a small proportion of patients with sequence data into the cohort. Patients in the cohort may not be representative of all patients on ART in Guangxi. Third, the distribution of HIV-1 subtype differs between regions. Patients with CRF08_BC are mainly distributed in Guangxi, Yunnan, and Sichuan in southwest China.[21] The application value of our findings should be further validated.

CONCLUSIONS

Our study confirms that HIV-1 subtype is a strong predictor of long-term clinical outcomes in ART. These data may help to develop optimized policies to reduce mortality. Compared with patients with CRF07_BC, patients with CRF01_AE may benefit more from immediate ART, and patients with CRF08_BC are more suitable for PI-based regimens. This study emphasizes the importance of routine HIV-1 genotyping before ART, immediate ART,[26] and personalized ART regimens to improve the prognosis for patients undergoing ART.

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