

Low Frequency of Integrase Inhibitor Resistance Mutations Among Therapy-Naïve HIV Patients in Southeast China

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Background: With the widespread use of integrase strand transfer inhibitors (INSTIs) in the clinical setting, transmission of INSTIs-resistance mutations may increase. Data regarding transmitted drug resistance mutations (TDRM) to INSTIs in Chinese HIV patients are limited. The aim of this study was to summarize the INSTIs TDRM, including the frequency of protease inhibitors (PIs) and reverse transcriptase (RT) inhibitors (RTIs) mutations in treatment-naïve patients in Southeast China.

Methods: HIV-1 positive patients were retrospectively selected between April 2018 and October 2020 from the Mengchao Hepatobiliary Hospital of Fujian Medical University, the largest designated HIV/AIDS care hospital in Southeast China. Individuals who were antiretroviral therapy-naïve and received antiretroviral drug resistance testing at baseline were included. Clinical data including demographic data, CD4 counts, HIV-RNA loads, and drug resistance mutations were collected.

Results: A total of 147 patients were enrolled. INSTIs TDRM was rare, with only one primary integrase mutation E138K observed in one sample and one secondary mutation E157Q detected in another sample. The overall prevalence of INSTIs TDRM was 1.36%. A substantial proportion of patients harbored common INSTIs-associated polymorphic variants. Two samples harbored the T215S, M184V and K70E mutations related to nucleoside RTIs (NRTIs). Twelve patients carried nonnucleoside RTIs (NNRTIs)-resistance mutations. Two individuals harbored PIs-resistance mutations: Q58E in one patient and M46I, I54V, V82A, L10F, and Q58E mutations in another patient. The total TDRM rate for RTIs and PIs was 10.20% (15/147), but only 0.68% (1/147) was according to the WHO recommendations on TDRM.

Conclusion: The rate of INSTIs TDRM was low among therapy-naïve HIV patients in Southeast China. INSTIs as a first-line regimen are suitable for untreated HIV-1 patients in Southeast China. But special attention must be still paid to INSTIs TDRM in clinical practice.

Keywords: HIV, transmitted drug resistance mutations, integrase strand transfer inhibitors, Southeast China

Introduction

Over the past decades, the extensive use of antiretroviral therapy (ART) for HIV patients has increased the incidence of TDRM.¹ TDRM may result in treatment failure, disease progression, and mortality among newly infected HIV patients. Resistance against RTIs and PIs has frequently been determined in HIV patients.¹⁻⁵

Attention to TDRM to INSTIs has gradually claimed increased interest after the widespread application of INSTIs. INSTIs are recommended as the first-line treatment regimens for HIV-1 patients, due to their high efficacy and good tolerability,^{6,7}

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and have been increasingly used in treatment-naïve patients with HIV since their introduction in China in 2009. In recent years, several studies on drug resistance to INSTIs have been declared in Mainland China and Taiwan.^{8–10} However, data on resistance to INSTIs in the ART-naïve population is insufficient in China. Furthermore, the prevalence of TDRM to INSTIs may vary across different regions due to different geographic and socio-economic conditions.

At present, no data related to INSTIs-resistance mutants have been reported in Southeast China. A better understanding of drug resistance against INSTIs is crucial for their efficient use in treatment regimens. Thus, our objective was to summarize INSTIs-resistance patterns including PIs and RTs mutations in treatment-naïve HIV patients in Southeast China.

Materials and Methods

Ethical Consideration

The study was approved by the ethics committee of Mengchao Hepatobiliary Hospital of Fujian Medical University (The Ethics reference number: 2020–035-01). Existing clinical information and laboratory data were anonymously used and were abstracted from electronic medical records. Thus, the need for writing informed consent was waived.

Study Population

HIV-1 patients were retrospectively selected between April 2018 and October 2020 from those attending the Mengchao Hepatobiliary Hospital of Fujian Medical University, the largest designated HIV/AIDS care hospital in Southeast China. Individuals who were ART-naïve and had initial antiretroviral drug resistance screening test were included. Patients with HIV-RNA < 250 IU/mL, HIV-2 infection, incomplete data, or previous exposure to ART were excluded.

Data Collection

Demographic information including sex, age, occupation, educational background, and transmission route was collected from medical records for each patient. Laboratory variables such as HIV-RNA loads, CD4 counts, and drug-resistance data were further collected. All information was carefully checked after abstraction. CD4 counts were determined using the BD FACSCount system (Becton Dickenson, California, USA). Plasma HIV-RNA levels

were quantitatively tested with the Ampliform HIV-1 Monitor Test, version 1.5 (Roche, Basel, Switzerland). The detection limit threshold was < 20 IU/mL.

HIV drug-resistance test was carried out as follows: HIV-RNA was extracted from plasma samples using the QIAamp Viral RNA Mini Kit (Qiagen, Duesseldorf, Germany). The HIV gene was amplified using RT-PCR Kit (TaKaRa Biotechnology, Dalian, China). The obtained cDNA was amplified in a second round of nested PCR. After purification, the PCR products were sequenced by TSINGKE Biological Technology Co. Ltd (Beijing, China), and The sequences of amplified specific region were uploaded to GenBank.

Drug-resistant mutations were determined by interrogating the Stanford University HIV Drug Resistance Database (<https://hivdb.stanford.edu/>). The database defines DRM following four criteria including polymorphism frequency, treatment prevalence, in vitro phenotypes and association with VF. It provides an estimated level of resistance to a drug according to the penalty scores for the four criteria. There are five level of estimated levels: Susceptible, Potential low-level resistance, Low-level resistance, Intermediate resistance and High-level resistance.

Results

Among the 214 treatment-naïve patients, a total of 147 patients were evaluated in this study. Most were male patients (95.92%) and the median age was 34 years (range, 18 to 83 years). The majority of patients acquired HIV by sexual transmission (48.98% male/male, 31.29% male/female sexual contact). The median CD4 counts was 166 cells/ μ L (range: 2–1004 cells/ μ L). HIV-RNA loads ranged from 5.2×10^4 IU/mL to 3.7×10^7 IU/mL. The most common subtypes were CRF01_AE and CRF07_BC (45.58% and 42.18%). Subtype B HIV-1 strains were determined in 4.76%, subtype C in 2.72%, and CRF08_BC in 2.04%. CRF55_01B and CRF33_01B each accounted for 1.36% of the subjects (Table 1).

The rate of INSTIs TDRM was 1.36%. Only one major INSTIs-resistance mutation (E138K) was detected in one sample. It suggested low-level resistance to raltegravir (RAL) and elvitegravir (EVG) and potential resistance to bictegravir (BIC) and dolutegravir (DTG). One accessory INSTIs-resistance mutation (E157Q) was observed in another sample, which can contribute to resistance if present along with other major resistance mutations resistance to RAL and EVG. The vast majority of samples harbored common INSTIs-associated polymorphic variants. The

Table 1 Clinical Characteristics of Patients at Enrolment

Variables	Patients (No, %)
Age (years)	
18–50	110 (74.83)
51–83	37 (25.17)
Sex	
Male	141 (95.92)
Female	6 (4.08)
Transmission route	
MSM	72 (48.98)
Heterosexual	46 (31.29)
Injection drug	1 (0.68)
Unknown	28 (19.05)
Marital status	
Married	78 (53.06)
Unmarried	69 (46.94)
Ethnicity	
Han	147 (100)
CD4 count(cells/ μ L)	
<200	78 (53.06)
\geq 200	69 (46.94)
HIV RNA (IU/mL)	
< 1×10^5	55 (37.41)
$\geq 1 \times 10^5$	92 (62.59)
Gene type	
CRF_01AE	67 (45.58)
CRF_07BC	62 (42.18)
B	7 (4.76)
C	4 (2.72)
CRF08_BC	3 (2.04)
CRF55_01B	2 (1.36)
CRF33_01B	2 (1.36)

Abbreviation: MSM, men who have sex with men.

most frequently observed polymorphic variants were T124A, T125A and S283G, followed by L101I, T112V, I135L, and K136Q.

In one participant, two NRTIs-resistance mutations (M184V, K70E) were observed. The M184V mutation can contribute to resistance if present along with other major resistance mutations high level resistance to lamivudine (3TC) and emtricitabine, and low-level resistance to didanosine and abacavir. NNRTIs-resistance mutations were most frequently identified. A total of 12 samples contained 13 NNRTIs-resistance mutations: V106I in 4 samples, V179E, V179D mutations each in 3 samples, E138A mutations in 2 samples and V106M in 1 sample. These resistance mutations can contribute to resistance if

present along with other major resistance mutations potential low-level resistance to NNRTIs (Table 2).

Two patients harbored PIs-resistance mutations: Q58E mutation in one patient, 3 major mutations (M46I, I54V, V82A) and 2 accessory mutations (L10F, Q58E) in another individual (Table 2).

The total TDRM rate to RTIs and PIs was 10.20% (15/147) with 0.68% (1/147) the World Health Organization (WHO) recommendation on TDRM. Only one patient had two-class drug resistance mutations. For the remaining 14 patients, one-class drug resistance mutations were detected.

Discussion

Studies regarding TDRM to INSTIs are not common, especially in China. We analyzed INSTIs-resistance patterns including mutations to RTIs and PIs widely used in HIV therapy-naïve patients in Southeast China.

In our study, we identified the frequency of TDRM and polymorphisms in the HIV-1 integrase gene in 147 naïve-treatment patients from Southeast China. One major (E138K) and one accessory (E157Q) INSTIs-resistance mutation were detected in two different samples. A large proportion of ordinary polymorphic variants were detected. Most HIV-1 strains showed full susceptibility to the four most currently used INSTIs drugs. These findings were consistent with a previous study in China indicating that major INSTIs-selected TDRM are absent in INSTIs-treatment naïve adult patients surveyed at the Tiantan Hospital.¹⁰ Several studies have also reported similar rates of INSTIs TDRM in treatment-naïve patients from other countries. In Italy, 0.2% of patients harbored INSTIs TDRM among 455 ART-naïve patients from 2006 to 2016.¹¹ In South Korea, major INSTIs TDRM were identified in 3.4% of naïve patients.¹² In Morocco, 5.2% of the study sample contained secondary mutations (L74IM, T97A), while no primary INSTIs-resistance mutations were detected among 77 ART-naïve patients.¹³ The prevalence of INSTIs-associated resistance mutations was 2.3% in Austrian patients recently diagnosed with HIV.¹⁴ In the United Kingdom, no major mutation for INSTIs was detected in treatment-naïve patients.¹⁵

We report for the first time the identification of the integrase E138K mutation associated with INSTIs-resistance in HIV patients in China. This mutant confers low-level resistance to RAL and EVG, but sensitivity to DTG and BIC according to the Stanford University algorithm. Usually, E138K is a nonpolymorphic mutation

Table 2 Distribution of Drug Resistance Mutations in Patients

Patients Code	Subtypes	Mutation Type				Drug Resistance
		INSTIs	NRTIs	NNRTIs	PIs	
8	CRF01_AE		K70E, M184V		M46I,I54V,V82A L10F, Q58E	FTC,3TC:H;DDI,D4T,TDF:L;ABC:I
13	CRF01_AE			VI06I		DOR:L; ETR,NVP,RPV:P
17	CRF55-01B			VI79E		EFV,NVP,ETR,RPV:P
23	CRF01_AE			VI79D		EFV,NVP:I; RPV:L; ETR:P
36	CRF08_BC			EI38A		ETR:P; RPV:L
37	C	EI57Q				EVG,RAL:P
38	CRF07_BC			EI38A		ETR:P; RPV:L
46	CRF01_AE			VI06I		DOR,ETR,NVP,RPV:P
50	CRF07_BC				Q58E	NFV/r:P; TPV/r:L
51	CRF01_AE			VI79D		EFV,NVP,ETR,RPV:P
55	CRF55_01B			VI79E		EFV,ETR,NVP,RPV:P
61	CRF01_AE			VI06I		DOR,ETR,NVP,RPV:P
69	B	EI38K				BIC,DTG:P; EVG,RAL:L
74	CRF01_AE			VI79D		EFV,NVP,RPV,ETR: P
120	B			VI06I		DOR,ETR,NVP,RPV:P
145	B			VI06M, VI79E		DOR,ETR,NVP,RPV:P
147	CRF01_AE		T215S			FTC,3TC:H;DDI,D4T,TDF:L;ABC:I

Abbreviations: INSTIs, integrase strand transfer inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; H, high-level resistance; I, intermediate resistance; L, low-level resistance; P, potential resistance; FTC, emtricitabine; 3TC, lamivudine; DDI, didanosine; D4T, stavudine; TDF, tenofovir disoproxil fumarate; ABC, abacavir; DOR, doravirine; ETR, etravirine; NVP, nevirapine; RPV, rilpivirine; EVG, elvitegravir; RAL, raltegravir; NFV/r, nelfinavir/r; TPV/r, tipranavir/r; BIC, bictegravir; DTG, dolutegravir.

emerging in patients with virological treatment failure after receiving RAL, EVG, or DTG.¹⁶ Nevertheless, it also constitutes part of the natural genetic polymorphism of the IN gene.¹⁷ This mutation alone has not been reported to reduce susceptibility INSTIs treatment significantly, but may exhibit a great reduction in EVG and RAL susceptibility and intermediate-level resistance to BIC and DTG when it occurs in combination with other major INSTIs DRM.¹⁸

Another INSTIs-resistance mutation observed in our study was E157Q, which confers potential resistance to RAL and EVG, but sensitivity to DTG and BIC. Similar to the study conducted by Liu et al¹⁰, who first reported the presence of the E157Q mutation among Chinese ART-naïve patients in 2019, the frequency of the integrase E157Q mutation was very low in China. E157Q is a common polymorphic mutation selected by RAL treatment in HIV patients¹⁹ and by exposure to EVG in vitro. The prevalence of the E157Q polymorphic mutation was 2.7% in INSTIs-naïve patients from 17 French clinical centers,²⁰ and 2.4% in ART-naïve patients in Italy.²¹ These studies have demonstrated that the E157Q mutation itself has minimal effects on RAL and DTG activity and viral infectivity. E157Q has been shown to act as a compensatory mutation due to its ability to restore

damaged enzymatic activity caused by N155H or R263K substitution.^{20–22}

As displayed in our result, the frequency of TDRM to RTIs and PIs was also low. Two individuals were detected with resistance mutation to NRTIs. And PIs-resistance mutations were identified in two patients. However, 12 patients (8.16%) harbored resistance to NNRTIs. The total TDRM rate to RTIs and PIs was 10.20% (15/147), including 0.68% (1/147) WHO recommendation DRM. These results were in accordance with previous reports indicating that TDRM to RTIs and PIs are not common in treatment-naïve patients living in China.^{23,24}

In summary our study showed that the overall TDRM rate was low, and the frequency of INSTIs-resistance mutations was absent in our patient sample. However, the frequency of INSTIs-resistance mutations may increase with the extensive use of ART, since E138K, a major INSTIs-resistance mutation, was also detected in our study cohort. Thus, it is necessary to pay attention to the presence of TDRM, including those suggestive of INSTIs-resistance during baseline evaluations of patients during the routine clinical work-up.

Our study had some limitations. The small sample size may have resulted in a selection bias. Furthermore, the

retrospective nature of the study limited the interpretation of the rate to TDRM. A large prospective study should be conducted to explore the frequency of TDRM in therapy-naïve patients in Southeast China.

Conclusion

Our study demonstrated that the frequency of TDRM was low in our HIV patient cohort. However, TDRM associated with INSTIs-treatment resistance, including E138K and E517Q were also detected in therapy-naïve HIV patients in Southeast China. Thus, TDRM test should be conducted in untreated HIV patients in Southeast China before initiating ART.

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Disclosure

The authors declare that they have no competing interests.

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