Letter to the Editor

Drug Resistance to HIV-1 Integrase Inhibitors among Treatment-naive Patients in Jiangsu, China*

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Integrase strand transfer inhibitors (InSTIs) have been widely used in recent years because of their high genetic barrier to resistance. The World Health Organization (WHO) has recommended dolutegravir (DTG)-containing regimens as the preferred first- and second-line antiretroviral therapy (ART) regimens for people living with human immunodeficiency virus (HIV)[1]. During the long-term treatment process, the appearance of drug resistance mutations to InSTIs is inevitable. A meta-analysis has shown that the resistance rate among InSTI treatment-experienced patients is 3.9% (Raltegravir, RAL), 1.2% (Elvitegravir, EVG), and 0.1% (DTG)[2]. However, resistance to InSTIs has not been reported in treatment-naive populations.

In China, the government highly values the prevention and treatment of HIV/acquired immunodeficiency syndrome (AIDS) and the "Four Free and One Care" policy was announced in and has been implemented since 2003. There are eight kinds of drug on the free list; for example, the standard first-line strategy for treatment-naïve adults and teenagers, tenofovir/zidovudine + lamivudine + efavirenz/nevirapine [two nucleoside reverse-transcriptase inhibitors (NRTIs) + one non-nucleoside reverse-transcriptase inhibitor (NNRTI)], has been used for more than 10 years. Another meta-analysis has shown that, in China, the prevalence of acquired drug resistance (ADR) is 44.7%, which includes 31.4% NRTI, 39.5% NNRTI, and 1.0% protease inhibitor (PI) resistance; that of transmitted drug resistance (TDR) is 3.0%, which includes 0.7% NRTI, 1.4% NNRTI, and 0.5% PI resistance[3]. Considering the high drug resistance rate observed under the current treatment strategy, the InSTI-containing strategy seems to be an alternative choice for HIV treatment. The Chinese Food and Drug Administration has approved the use of RAL, DTG, and fixed-dose combinations. Although no InSTIs have been included in the free drug list in China, InSTI-containing regimens will likely be used to treat patients infected with HIV in the future.

In China, new recommended drugs are usually included in the medical insurance reimbursement catalog. Although providing free InSTIs for HIV/AIDS treatment is not possible, there is the possibility to include InSTIs in the medical insurance reimbursement drug list. In March 2019, Anhui, the neighborhood of Jiangsu province, was the first province in the country to include InSTIs (including DTG and RAL) into provincial medical insurance[4], which greatly reduced the economic burden of patients with HIV/AIDS. However, using InSTIs also increased the economic burden of local government and used a lot of public health resources. Thus, the necessity and effectiveness of using InSTIs should be considered locally.

The *pol* gene of HIV contains three essential enzymes for its replication, reverse transcriptase (RT), protease (PR), and integrase (IN). A previous study has shown that patients with IN-resistant viruses are more likely to have PR-RT mutations than those without an IN-resistant infection[5]. Considering the limited use of InSTIs in China and the association between IN and PR-RT, we believe that IN-resistance mutations are more likely to be observed in samples carrying PR-RT mutations than in those without PR-RT mutations. There has not been large-scale InSTI use in patients with HIV in

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Jiangsu province and the baseline level of InSTI resistance is unclear. To provide scientific suggestions about InSTI use in Jiangsu province, we characterized the current situation of InSTI resistance among treatment-naïve (with PR-PT mutations) individuals in Jiangsu province.

According to the "Workshop on HIV Surveillance and Molecular Epidemiology Research" (http://ncaids.chinacdc.cn/fzyw_10256/gzjz_10269/201804/t20180419_164136.htm) hosted by the National Center for AIDS/STD Control and Prevention, China Center for Disease Control and Prevention (CDC) in 2017, national-scale HIV molecular epidemiology research has begun. Since then, samples of newly diagnosed HIV infections from 13 cities in Jiangsu province have been sent to Jiangsu Provincial CDC. Previously, RT-PR genotypic resistance tests (GRT) were conducted from June 2017 to December 2018 and 252 samples contained RT-PR resistance mutations, according to the Stanford University HIV Drug Resistance Database 8.8 algorithm (Stanford HIVdb). Additionally, the demographic and HIV-related information of each patient was collected at the sample time. This work was approved by the ethical review board of the National Center for AIDS/STD Control and Prevention (Project No. X140617334). Based on this preliminary work, we performed IN GRT on the 252 samples to identify InSTI resistance mutations.

RNA was extracted from 140 μL of plasma using a QIAamp Viral RNA Mini Kit (Qiagen, GmbH, Hilden, Germany). Then, RNA was reverse transcribed into cDNA using SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The process of IN gene sequence amplification was executed following a previously described protocol[6]. The targeted fragments included the first 263 amino acid positions of the *IN* gene (HXB2: 4227-5018). To detect possible contamination, a negative control was set every 15 samples. The positive PCR products were sequenced by Sangon Biotech (Shanghai, China). The quality of IN sequences was assessed using the Quality Control tool (https://www.hiv.lanl.gov/) and the HIV-1 subtypes and circulating recombinant forms (CRFs) were determined using the COMET online tool (http://comet.retrovirology.lu).

All mutations reported in the International AIDS Society list (updated in 2019)[7] and Stanford HIVdb 8.9-1 version were considered as InSTIs mutations. The estimated level of drug resistance was determined using the scores in Stanford HIVdb. The relationship between the total score and level was as follows: (1) 0 to 9, susceptible; (2) 10 to 14, potential low-level resistance; (3) 15 to 29, low-level resistance; (4) 30 to 59, intermediate resistance; and (5) ≥ 60, high-level resistance (https://hivdb.stanford.edu/page/release-notes#resistance.summary). A phylogenetic tree was created using the neighbor-joining method with 1,000 replicates and the international reference strains were downloaded from the Los Alamos HIV Sequence Database (https://www.hiv.lanl.gov/).

In total, 240 IN sequences successfully amplified and sequenced, with a success rate of 95.2% (240/252). The geographic information of 240 patients is shown in Table 1. The mean age of the subjects was 39.0 years, with a median CD4+ T cell count of 268.0 cells/μL. Using the 240 IN sequences,

Table 1. The geographic information of 240 subjects

Items	*n*	%
Sex		
Male	209	87.1
Female	31	12.9
Area		
South Jiangsu	181	75.4
Center Jiangsu	23	9.6
North Jiangsu	36	15.0
Nation		
Han	237	99.2
Others	3	0.8
Education		
Primary education	35	14.6
Junior school	86	35.8
High school	52	21.7
Colleges or Universities	67	27.9
Transmission way		
Heterosexual transmission	130	54.1
Homosexual transmission	107	44.6
Others	3	1.3
Sampling year		
2017	73	30.4
2018	167	69.6
Subtype		
CRF01_AE	84	35.0
CRF07_BC	49	20.4
B	30	12.5
CRF08_BC	25	10.4
CRF68_01B	16	6.7
CRF55_01B	11	4.6
CRF_0107	11	4.6
CRF67_01B	6	2.5
Unknown	3	1.2
C	4	1.7
CRF02_AG	1	0.4

we identified 10 subtypes, including CRF01_AE (35.0%), CRF07_BC (20.4%), B (12.5%), CRF08_BC (10.4%), CRF68_01B (6.7%), CRF55_01B (4.6%), 0107 (4.6%), CRF67_01B (2.5%), C (1.7%), CRF02_AG (0.4%) and 3 unknown sequences.

Drug resistance analysis showed that 30 sequences contained IN-related mutations; 4 of 30 harbored IN major resistance mutations and the drug resistance rate of InSTIs was 1.7% (4/240). Information on these four subjects is listed in Table 2. According to the historical medical records and supplemental medication survey, there was no evidence that the four patients were exposed to any InSTIs. Three IN major resistance mutations (E138A, E138T, and R263K) were identified; E138A and E138T mutations can cause low-level resistance to EVG and RAL and the R263K mutation can cause intermediate resistance to bictegravir, DTG, and EVG and low-level resistance to RAL. Additionally, three IN accessory resistance mutations (A128T, V151I, and E157Q) were detected in the samples. E157Q appears to have little effect on InSTI susceptibility, whereas A128T and V151I have no effect. L74M/I (9.6%, 23/240) were the most frequent mutations observed in our study. Although alone these mutations have a minimal effect, when both are present they reduce susceptibility to each InSTI and cause major InSTI-resistance. The drug resistance mutations and corresponding subtypes were marked in the phylogenetic tree of the IN sequences (Figure 1).

Table 2. The information of samples who were detected with IN major resistance mutations

Sample ID	IN major mutations	Age	Way of spread	Gender	Subtype	CD4	Level of drug resistance			
							BIC	DTG	EVG	RAL
q_nj64	R263K	62	HST	male	B	69	M	M	M	L
r_lyg42	E138A	16	HST	female	B	1,106	S	S	L	L
r_sz140	R263K	33	HST	male	C	160	M	M	M	L
r_yc88	E138T	60	MSM	male	B	828	S	S	L	L

Note. HST: Heterosexual transmission, MSM: the men who have sex with men; S: Susceptible, L: Low-Level Resistance, M: Intermediate Resistance, H: High-Level Resistance; BIC: bictegravir, DTG: dolutegravir, EVG: Elvitegravir, RAL: Raltegravir.

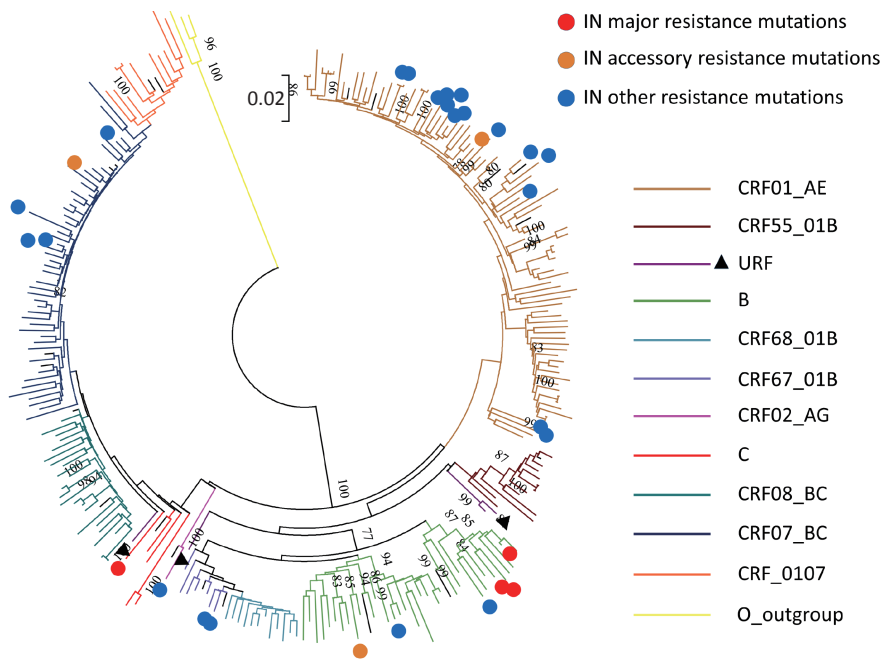

Figure 1. HIV-1 integrase phylogenetic analysis inferred by neighbor-Joining (NJ). There were 240 IN sequences and 28 international reference sequences included in the NJ-tree. Subtype O was outgroup. Bootstrapping was performed with 1,000 replicates; values of more than 70% are shown. Circle marks the sequences with IN-related mutations. Colorful lines distinguish the different subtypes.

Detailed information on drug resistance mutations among the 30 sequences is shown in Supplementary Table S1 (available in www.besjournal.com) and mutations in PR-RT are included. No obvious relationship to drug resistance mutations was observed between IN and PR-RT in this study.

R263K confers very low-level resistance to DTG in site-directed mutagenesis analysis[8] and it is rare in ART-naïve patients. E138A/K reduce DTG/RAL susceptibility in combination with many other mutations[9], whereas they do not reduce InSTI susceptibility alone. The major IN-related resistance mutations detected in this study had little effect on the efficiency of DTG/RAL, which suggested that InSTI-containing treatment strategies have exerted curative effects on HIV in Jiangsu province.

The prevention of, monitoring of, and timely response to population levels of HIV drug resistance are critical to achieving the 90-90-90 targets. However, we do not think that a drug resistance rate of 1.7% to InSTIs justifies baseline drug resistance testing for all patients who initiate InSTI regimens in Jiangsu province. Based on the experience with NNRTIs, the WHO only recommends baseline testing (or switching to different 1st line regimens) when the pretreatment drug resistance is > 10.0%[10]. A previous study reported that drug resistance emerges after at least 3–5 years in IN-TDR cases[11]. Currently, InSTIs have not been used on a large-scale in Jiangsu province. With limited public health resources, more favorable work could be fulfilled, such as including InSTIs in medical insurance.

This study had some limitations. We only tested the IN sequences among samples that had already been tested for resistance mutations in PR-RT gene regions, instead of all treatment-naïve patients in Jiangsu province. Thus, bias existed because the resistance mutations between PR-RT and IN have the same association; samples tested with resistance mutations in the *IN* gene were more likely to be detected with PR-RT resistance. Therefore, the prevalence of InSTIs may be overestimated.

In summary, we identified major IN mutations and other related mutations among treatment-naive patients in Jiangsu, China. The results showed that the mutations had little effect on drug resistance, which indicated the effectiveness and applicability of InSTIs in Jiangsu province. InSTI baseline drug resistance testing should not be recommended until InSTIs have been used on a large-scale in local areas or virological failure emerges. We emphasize that limited public resources should be utilized rationally.

Authors' Contributions LU J, WANG B, and FU G conceived and designed the study. YIN Y, ZHOU Y, and SHI L obtained and administered the database. YIN Y, YUAN D, and CHEN J performed the laboratory work. HU H, ZHANG Z, and XU X performed the analyses and YIN Y and LU J interpreted them. YIN Y drafted the manuscript and all authors critically reviewed it. The final version was approved by all authors.

Conflicts of Interest No competing financial interests exist.

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Supplementary Table S1. Detailed information of drug resistance mutations among 30 sequences

Sequences ID	IN GENE				Protease GENE		Reverse Transcriptase GENE			
	Major mutations	Accessory mutations	Other mutations	resistance to INSTIs	PR	resistance to PIs	NRTI	resistance to NRTI	NNRTI	resistance to NNRTI
q_lyg23			L74I						V106M, V179D	DOR(M), EFV(H), NVP(H)
q_nj179			L74I				T215S	AZT(L), D4T(L)		
q_nj23			L74I				T215A	AZT(L), D4T(L)		
q_nj64	R236K			BIC(L), DTG(L), EVG(L)					V108I	DOR(L), NVP(L)
q_sz245			L74I		K20T	NFV(L)				
q_sz48			L74I				T215S	AZT(L), D4T(L)	K101H, G190A	EFV(M), ETR(L), NVP(H), RPV(L)
q_sz85		V151I							V106I	
q_tz51			L74M						V179VD	EFV(M), NVP(M), RPV(L)
q_wx32		E157Q							E138G, V179E	EFV(M), ETR(M), NVP(M), RPV(M)
q_yc104			L74I				M184I	ABC(L), FTC(H), 3TC(H)	V106M, V179D	DOR(M), EFV(H), NVP(H)
q_yz27		A128T							K103S	EFV(M), NVP(H)
r_lyg39			L74I						L100Deletion, K103Deletion	DOR(L), EFV(H), ETR(M), NVP(H), RPV(H)
r_lyg42	E138A			EVG(L), RAL(L)					K103N	EFV(H), NVP(H)
r_nj223			L74M						V106I	
r_nj245			L74I						V106I	
r_nt27			L74I						K101E	DOR(L), EFV(L), ETR(L), NVP(M)
r_nt302			L74I						E138G	RPV(L)
r_nt310			L74I						Y181C	EFV(M), ETR(M), NVP(H), RPV(M)
r_sz140	R236K			BIC(L), DTG(L), EVG(L)					E138A	RPV(L)
r_wx188			L74M						A98G	DOR(L), EFV(L), NVP(M), RPV(L)
r_wx193			L74I						E138G, V179E	EFV(L), ETR(L), NVP(L), RPV(L)
r_wx60			L74M						K103N	EFV(H), NVP(H)
r_wx64			L74I		Q58E	TPV(L)				
r_yc213			L74M		M46L	NFV(L)	M184P		V179E	
r_yc23			L74I						V106I	
r_yc75			L74I				K70R	AZT(M), D4T(L)	K103N, V108I	DOR(L), EFV(H), NVP(H)
r_yc88	E138T			EVG(L), RAL(L)					V106I	
r_yz161			L74M						V106M, V179D	DOR(M), EFV(H), NVP(H)
r_yz87			L74I		M46I	NFV(M)	A62V			
r_yz91			L74I		M46I	NFV(M)	A62V			

Note. the level of drug resistance extend were marked inside the "()", "L" means low-level, "M" means intermedium-level, and "H" means high level. RT, reverse transcriptase. PR, protease. IN, integrase.