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Comparison of susceptibility of HIV-1 variants to antiretroviral drugs by genotypic and recombinant virus phenotypic analyses

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SUMMARY

Objective: This study utilized genotypic and in-house recombinant virus phenotypic assays to examine HIV-1 variant susceptibility to antiretroviral (ARV) drugs; comparisons were made between the analyses.

Methods: A nested PCR was employed to amplify the HIV-1 gag-pol gene, which comprised the entire PR gene (codons 1–99) and the former RT gene (codons 1–312). Genetic resistance was determined by submitting the sequences to the Stanford University Network HIV-1 Database. Phenotypic susceptibilities to six ARV drugs were measured using a high-throughput, multi-cycle, recombinant virus phenotypic assay. Results were expressed in terms of the IC_{50} (half maximal inhibitory concentration) and fold-change values. The relationship between phenotypic drug resistance and genetic polymorphisms was determined.

Results: Nineteen fragment sequences for which recombinant viruses were successfully constructed were translated and compared with the consensus B sequences in the Stanford University Network HIV-1 Database. No recognizable genotypic resistance-associated mutations were noted, except in one sample. Each homologous replication-competent recombinant viral fold-change in the presence of six ARV drugs used widely in China was measured. According to the clinical and statistical criteria, 16 of the 19 samples were susceptible to the six drugs tested. The majority of phenotypic and genotypic results obtained were in agreement, with a concordance rate of 97.4%. Both phenotypic and genotypic assays suggested that sample HN2009001 was resistant to all drugs tested. All phenotypic and genotypic results obtained regarding the susceptibility of the 19 recombinant viruses to nucleoside reverse transcriptase inhibitors (NRTIs) were in agreement. With regard to the genotypic results for the non-nucleoside reverse transcriptase inhibitors (NNRTIs), 7.9% (3/38) were inconsistent with the phenotypic results.

Conclusions: The in-house recombinant virus phenotypic assay was able to provide a straightforward quantitative assessment of resistance. In most cases, the genotypic and novel phenotypic assays yielded similar results. The disparity in HIV-1 susceptibility indicates a need to further investigate the clinical outcomes of antiretroviral therapy in certain individuals.

1. Introduction

The 'Four Frees and One Care' policy was implemented in China in 2003 for AIDS prevention and control. This policy includes free antiretroviral treatment (ART) for AIDS patients. Since the implementation of this policy, the morbidity and mortality rates attributable to HIV infection have declined significantly.[1,2] Unfortunately, many treated patients have developed resistance to one or more drug classes, and this resistance can lead to treatment failure and limitations in alternative treatment regimens.[3] Furthermore, these drug-resistant variants can be transmitted to treatment-naïve patients, thereby becoming a major ART obstacle.[4]

The detection of resistance in treatment-naïve and treatment-experienced patients has become increasingly important for their

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management.[5] There are data that strongly suggest that HIV drug resistance testing is of considerable value in many aspects. The drug resistance assay is essential for the selection of the optimal treatment regimen. Furthermore, HIV drug resistance testing is also of benefit for monitoring the spread of primary drug resistance mutations, as well as the development of new antiretroviral (ARV) drugs.

Two main types of drug resistance assay have been developed and are available: genotypic resistance assays, which can detect specific resistance-related mutations in the target viral genes, and phenotypic resistance assays, which test the capacity of viral replication or enzymatic activity in the presence of a particular drug in vitro. In general, genotypic resistance testing is more widely used than phenotypic resistance testing. This is mainly due to the fact that sequencing is rapid, costs are relatively low, and many genotypic interpretation algorithms are available online, making the genotypic assay a preferred method for clinical resistance testing.[6] However, since many genotypic interpretation algorithms depend on the knowledge acquired from phenotyping, it is difficult to interpret the sequences with unusual mutations or complex combinations of mutation patterns;[7] thus this should be complemented by direct phenotyping.

The phenotypic drug susceptibility assay is able to provide more direct quantitative resistance measurements for each ARV agent, including US Food and Drug Administration (FDA)-approved drugs and compounds under clinical evaluation. In addition, it can be performed without any prior knowledge of mutations. Traditional phenotypic assays that detect drug susceptibility of clinical isolates have several limitations: they require fresh healthy donor peripheral blood mononuclear cells (PBMCs) and are labor-intensive and time-consuming.[8] Recent studies have focused on recombinant virus assays, based on the direct amplification of fragments from patient plasma. Some of these phenotypic methods are based on single-cycle infection assays,[9] whereas others generate infectious viruses.[10]

A novel recombinant phenotypic assay is reported herein, in which the recombinant viruses have the capacity of multiple cycles of replication. In this study, the susceptibility of the constructed recombinant viruses to six ARV drugs was determined, and the relationship between genotypic and recombinant virus phenotypic analyses was evaluated.

2. Materials and methods

2.1. Plasma samples

Plasma specimens were collected from AIDS patients enrolled in the China HIV drug resistance surveillance program. Plasma samples were shipped on dry ice and stored at −80 °C until analyzed. All samples collected in this study were successfully isolated by PBMC co-culture. All patients provided written informed consent and their basic personal and clinical information was acquired by face-to-face interview. This study was approved by the Ethics Committee of the Institutional Review Board of the Academy of Military Medical Sciences.

2.2. Amplification, sequencing, and HIV-1 subtype identification

A nested PCR was employed to amplify the 2039-bp HIV-1 gag-pol gene, which comprised the entire PR gene (codons 1–99) and the former RT gene (codons 1–312). HIV-1 RNA was extracted from 200 μl plasma using the QIAamp Viral RNA Kit (Qiagen) and used as a temple for one-step reverse transcriptase PCR (RT-PCR). The RT-PCR primers were OUT5 5′ TCAGAAGGAGCCACCCCACA 3′ and OUT3 5′ CCCCTGCTTCTGTATTTCTGCTA 3′. The one-step RT-PCR reaction mixture (Takara) contained 5 mM $MgCl_2$, 1 mM dNTP, 10× One Step RNA PCR Buffer (5 μl), 40 U RNase Inhibitor, 5 U AMV RTase XL, 5 U AMV-Optimized Taq, 20 μM (each) primer, and 2 μl of viral RNA, for a final volume of 50 μl. Two microliters of the RT-PCR mixture was used for the nested PCR, with 1.25 U Premix Ex Taq (Takara) and 20 μM (each) primer (IN5: 5′ GCGCATCCAGTG-CATGCAGGGCCTATTG 3′; IN3: 5′ GCGGATGGGTCATAATACACTC-CATGTACCGGTTC 3′, respectively) combined to a total volume of

Table 1
Basic information for 28 HIV-1 samples

Sample ID	Gender	Infection route	Area of origin	Collection year	Treatment
BJ2006001	Male	Heterosexual contact	Beijing	2006	Naïve
BJ2010001	Male	MSM	Beijing	2010	Naïve
BJ2010002	Male	MSM	Beijing	2010	Naïve
BJ2010003	Male	MSM	Beijing	2010	Naïve
GD2005003	Male	Blood transfusion	Guangdong	2005	Treated
GD2005004	Male	Heterosexual contact	Guangdong	2005	Treated
GD2005006	Male	Heterosexual contact	Guangdong	2005	Naïve
GD2005025	Male	IDU	Guangdong	2005	Naïve
GD2005028	Female	Blood transfusion	Guangdong	2005	Naïve
GX2005002	Male	Heterosexual contact	Guangxi	2005	Naïve
GX2005016	Male	Blood transfusion	Guangxi	2005	Naïve
GX2005028	Male	Heterosexual contact	Guangxi	2005	Naïve
HN2002024	Male	Blood transfusion	Henan	2002	Naïve
HN2009001	Male	Blood donation	Henan	2009	Treated
HN2010001	Male	Blood donation	Henan	2010	Naïve
HN2010002	Male	Blood donation	Henan	2010	Naïve
HN2010003	Female	Blood transfusion	Henan	2010	Naïve
HN2010004	Female	Blood donation	Henan	2010	Naïve
HN2010005	Female	Blood donation	Henan	2010	Naïve
NX2005012	Male	Blood transfusion	Ningxia	2005	Naïve
SC2009001	Male	Occupational exposure	Sichuan	2009	Treated
SD2010001	Male	MSM	Shandong	2010	Naïve
SD2013001	Male	MSM	Shandong	2013	Naïve
SD2013005	Male	MSM	Shandong	2013	Naïve
SD2013008	Male	MSM	Shandong	2013	Naïve
SH2007052	Male	Heterosexual contact	Shanghai	2007	Naïve
SX2010001	Male	Blood transfusion	Shanxi	2010	Treated
XJ2010001	Male	MSM	Xinjiang	2010	Naïve

MSM, men who have sex with men; IDU, injection drug user.

50 μl. Both the one-step RT-PCR and nested PCR amplifications were carried out in a Biometra Thermocycler (Biometra).

The patient-derived PR-RT PCR products were electrophoresed on a 1% (w/v) agarose gel, purified with the Wizard SV Gel and PCR Clean-Up System (Promega), and directly sequenced. Subtypes were determined by phylogenetic tree analysis of the PR–RT fragments. The sequences were edited using ContigExpress software and aligned using the BioEdit program. Alignments also included the reference sequences representing HIV-1 genetic circulating recombinant forms obtained from the HIV database.[11] The phylogenetic tree was constructed using the maximum-likelihood method by MEGA (Molecular Evolutionary Genetic Analysis Software, version 5.1), and genetic distance reliability was evaluated using the bootstrap test (1000 bootstrap replicates). REGA[12] and jpHMM (jumping profile Hidden Markov Model) were used to identify sequence subtypes that could not be determined with MEGA. For each patient, drug resistance mutations were determined by submitting the sequences to the Stanford University Network HIV-1 Database.[13] Polymorphisms, which were defined as any change from the subtype B consensus sequence,[14,15] were obtained and analyzed.

2.3. Generation and titration of recombinant viruses

Recombinant viruses were generated with a pNL4.3 vector, as described previously.[16] Briefly, both the purified PCR products derived from patients and the plasmids pNL4.3 were digested with restriction enzymes SphI and AgeI (New England BioLabs),

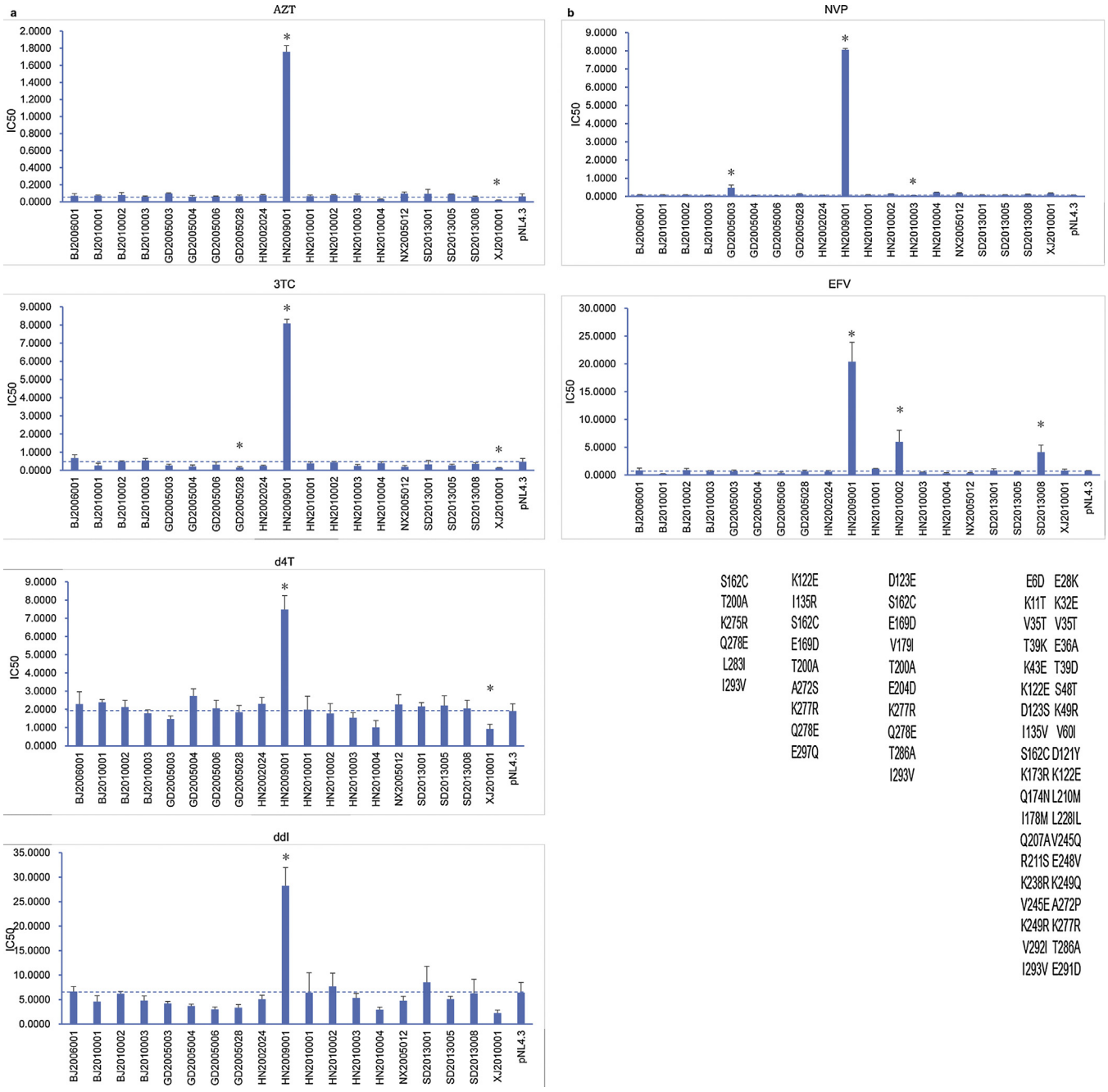

Figure 1. Phenotypic and genetic drug susceptibility of 19 patient-drive recombinant viruses and reference virus to six antiviral drugs. The columns represent the various samples and the column height indicates the IC_{50} value. *Virus met the criteria of resistance or hyper-susceptibility proposed in this article. Under the diagrams are various polymorphisms corresponding to the resistant or hyper-susceptible samples.

respectively. The digested target segments were sub-cloned into a pNL4.3-Δ(SphI-AgeI) vector, with PR (codons 1–99) and RT (codons 1–312) deletions obtained via ligation reactions (New England BioLabs) at 16 °C overnight and an insert-to-vector molar ratio of 5:1. The 2.0-kb patient-derived PCR products replaced the 2.0-kb SphI–AgeI fragment of the pNL4.3 vector. *Escherichia coli* DH5α competent cells (Tiangen) were transformed with the ligation products, grown in liquid culture (Luria–Bertani (LB) medium), and spread onto LB agar plates containing 100 μg/ml ampicillin (TransGen Biotech). Positive clones were identified by DNA sequencing and SphI and AgeI digestion. Exponentially growing HEK293T cells (at a density of 4×10^5 cells/ml) were seeded into 6-well plates containing 2 ml of growth medium and incubated at 37 °C, 5% CO_2 overnight. The next day, 293T cells were transfected with the constructed plasmids using Lipofectamine 2000 (Life Technologies Corporation) according to the manufacturer's instructions. Six hours post-transfection, 3 ml of fresh DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS (Fetal Bovine Serum) was added. After 48 h, culture supernatants containing the recombination viruses were harvested and stored at −80 °C until further use. Finally, sequencing confirmed that HIV-1 gag-pol sequences from the original plasma samples and the corresponding recombinant viruses were nearly identical. In order to obtain a higher recombinant viral titer, viruses were propagated in MT-2 cells in RPMI 1640 medium supplemented with 10% FBS. The recombination viruses were serially diluted 3-fold in 96-well plates and used to infect TZM-bl cells (10^4 cells/well) in the presence of DMEM supplemented with 10% FBS. Viral replication was quantified 48 h post-infection by measuring luciferase activity (relative luminescence units (RLU)) using the Bright-Glo Luciferase Assay System (Promega) in a multi-well plate reader (Wallik 1420; Perkin Elmer). The Reed–Muench method was used to calculate the 50% tissue culture infectious dose ($TCID_{50}$) of the constructed recombinant viruses.

2.4. Antiretroviral drugs

The ARV drugs used in this study and their sources were as follows: zidovudine (ZDV, AZT) and lamivudine (3TC) from Sigma-Aldrich Co.; didanosine (ddI), stavudine (d4T), nevirapine (NVP), and efavirenz (EFV) from Shanghai Desano Chemical Pharmaceutical Development Co., Ltd.

2.5. Drug susceptibility based on a multiple-cycle replication assay

Briefly, triple serial dilutions spanning empirically determined ranges for each drug were added to 384-well plates. Two hundred recombinant viral $TCID_{50}$s were used to infect TZM-bl cells (10^4 cells/well) containing pre-plated ARV drugs. At 48 h post-infection, luciferase reporter gene expression was measured and the percentage of inhibition was calculated according to the following equation: [1 − (luciferase activity in the presence of the drug/luciferase activity in the absence of the drug)] × 100. Drug concentrations required to inhibit viral replication by 50% (IC_{50}) were calculated by (1) plotting the percentage inhibition of luciferase activity versus the $\log_{10}$ drug concentration, and (2) fitting the inhibition curves to the data using non-linear regression analysis and gaining a four parametric sigmoid dose–response equation (GraphPad Prism, version 6.02). Three replicate determinations were performed in duplicate plates for each concentration of ARV drug. Mean IC_{50}s were calculated using all replicates for each virus and were expressed as the mean ± standard deviation. The results were expressed as fold-changes (FC), which reflect the fold-change in the IC_{50} of a particular drug when tested with a patient-derived recombinant virus relative to the IC_{50} of the same drug when tested with a wild-type reference virus ($HIV_{pNL4.3}$).

Cut-off values for the different ARV drugs were used to establish result classifications to include susceptibility, low–intermediate resistance, and high resistance. For AZT, 3TC, EFV, and NVP, <3.0-fold resistance indicated susceptibility, 3.0- to 25-fold indicated low–intermediate resistance, and >25-fold indicated high resistance. For d4T and ddI, a fold resistance of <1.5 was considered susceptible, 1.5–3.0 was considered low–intermediate resistance, and >3.0 was considered high resistance.[17,18] Hyper-susceptibility was defined as a fold-change value <0.4.[19] The virus was defined as resistant or hyper-susceptible if it met two criteria simultaneously: IC_{50} value significantly higher or less than the wild-type ($p < 0.05$) and a fold-change value outside the cut-off range.

2.6. Statistical analyses

To analyze the statistical significance of differences in IC_{50} values compared with the reference virus among the different samples, multiple comparisons (least significant difference test, LSD) were completed with SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA). All analyses were performed using a two-tailed test; *p*-values of <0.05 for the difference of IC_{50} compared with the wild-type reference virus ($HIV_{pNL4.3}$) were defined as statistically significant.

3. Results

3.1. Patient characteristics and genetic subtypes

Samples were obtained from 28 AIDS patients whose personal information was examined carefully. These patients lived in multiple districts and had become infected in various ways; all had complete ART histories. They were from Beijing (n = 4), Guangdong (n = 5), Guangxi (n = 3), Henan (n = 7), Ningxia (n = 1), Shandong (n = 4), Shanghai (n = 1), Shanxi (n = 1), Sichuan (n = 1), and Xinjiang (n = 1). The routes of HIV infection included men who have sex with men (MSM; n = 8), heterosexual contact (n = 6), paid blood

Table 2
Fold-changes (FC) of 19 recombinant viruses compared with $HIV_{pNL4.3}$

Sample ID	Drugs (FC)[a]					
	AZT 3.0, 25[b]	3TC 3.0, 25	d4T 1.5, 3	ddI 1.5, 3	NVP 3.0, 25	EFV 3.0, 25
BJ2006001	1.08	1.42[c]	1.20	1.03	1.31	1.10
BJ2010001	1.11	0.56[c]	1.25	0.72	1.16	0.25
BJ2010002	1.24	1.04	1.11	0.96	1.29	1.15
BJ2010003	0.96	1.16	0.93	0.75	1.03	0.84
GD2005003	1.53	0.58[c]	0.77	0.66	7.39[c,d]	0.85
GD2005004	0.91	0.46[c]	1.43[c]	0.57	0.83	0.42
GD2005006	0.98	0.66	1.07	0.47[c]	0.49	0.45
GD2005028	1.06	0.33[c,d]	0.96	0.52[c]	2.05	0.90
HN2002024	1.20	0.51[c]	1.20	0.78	1.03	0.83
HN2010001	1.05	0.82	1.04	0.99	1.36	1.50
HN2010002	1.19	0.94	0.93	1.19	2.09	7.84[c,d]
HN2010003	1.16	0.52[c]	0.80	0.83	0.93	0.65
HN2010004	0.48	0.83	0.53[c]	0.46[c]	3.41	0.39
HN2009001	27.34[c,d]	17.08[c,d]	3.92[c,d]	4.38[c,d]	126.75[c,d]	26.73[c,d]
NX2005012	1.50	0.41[c]	1.19	0.74	2.80	0.45
SD2013001	1.45	0.68	1.13	1.32	1.10	1.09
SD2013005	1.36	0.58[c]	1.15	0.79	1.29	0.74
SD2013008	0.87	0.75	1.07	0.97	1.79	5.39[c,d]
XJ2010001	0.27[c,d]	0.28[c,d]	0.48[c]	0.35[c,d]	2.74	1.02
pNL4.3	1.00	1.00	1.00	1.00	1.00	1.00

AZT, zidovudine; 3TC, lamivudine; d4T, stavudine; ddI, didanosine; NVP, nevirapine; EFV, efavirenz.

[a] The FC of recombinant viruses compared with $HIV_{pNL4.3}$.

[b] Cut-off values.

[c] $p < 0.05$ when compared to the wild-type via least significant difference (LSD) test.

[d] The virus met the criteria of resistance or hyper-susceptibility proposed in this article.

donation or transfusion (n = 12), occupational exposure (n = 1), and intravenous drug use (IDU) (n = 1). Of these patients, five were ART-experienced (Table 1). Twenty-five (25/28) of the samples were amplified and sequenced successfully. Nineteen gag-pol genetic fragments originating from the corresponding plasma samples were sub-cloned into the pNL4.3 vector, with homologous replication-competent viruses obtained successfully; the identified viral genetic subtypes included B/B′ (n = 11), CRF01_AE (n = 5), and CRF07_BC (n = 3) (GenBank accession numbers **KR030073** to **KR030089**).

3.2. Drug resistance mutations and genetic polymorphisms

All 19 of the fragment sequences for which recombinant viruses were successfully constructed were translated and compared with the consensus B sequences in the Stanford University Network HIV-1 Database.[13] Major drug resistance mutations including M41L, L210W, T215Y, K103N, and K238T were identified in HN2009001, which was interpreted as having high-level resistance to AZT, d4T, ddI, EFV, and NVP and low-level resistance to 3TC. No primary or secondary drug resistance mutations were found in the other 18 patients. However, amino acid substitutions including V35T/I, T39K/D/R, K122E, D123S/E, I135 V/L/R/T/M, S162C/Y, T200A/E, Q207A/E/K/S, R211S/KR, and V245E/Q/I/T were found, with thumb sub-domain amino acid substitutions A272P, K277R, K281R, T286A, E291D, V292I, and I293 V also noted (Figure 1).

3.3. Drug susceptibility testing of recombinant viruses

Nineteen recombinant viruses were constructed successfully, as described above, with a titration of 10^4–10^5 $TCID_{50}$/ml. Fold-changes of the 19 recombinant viruses relative to the

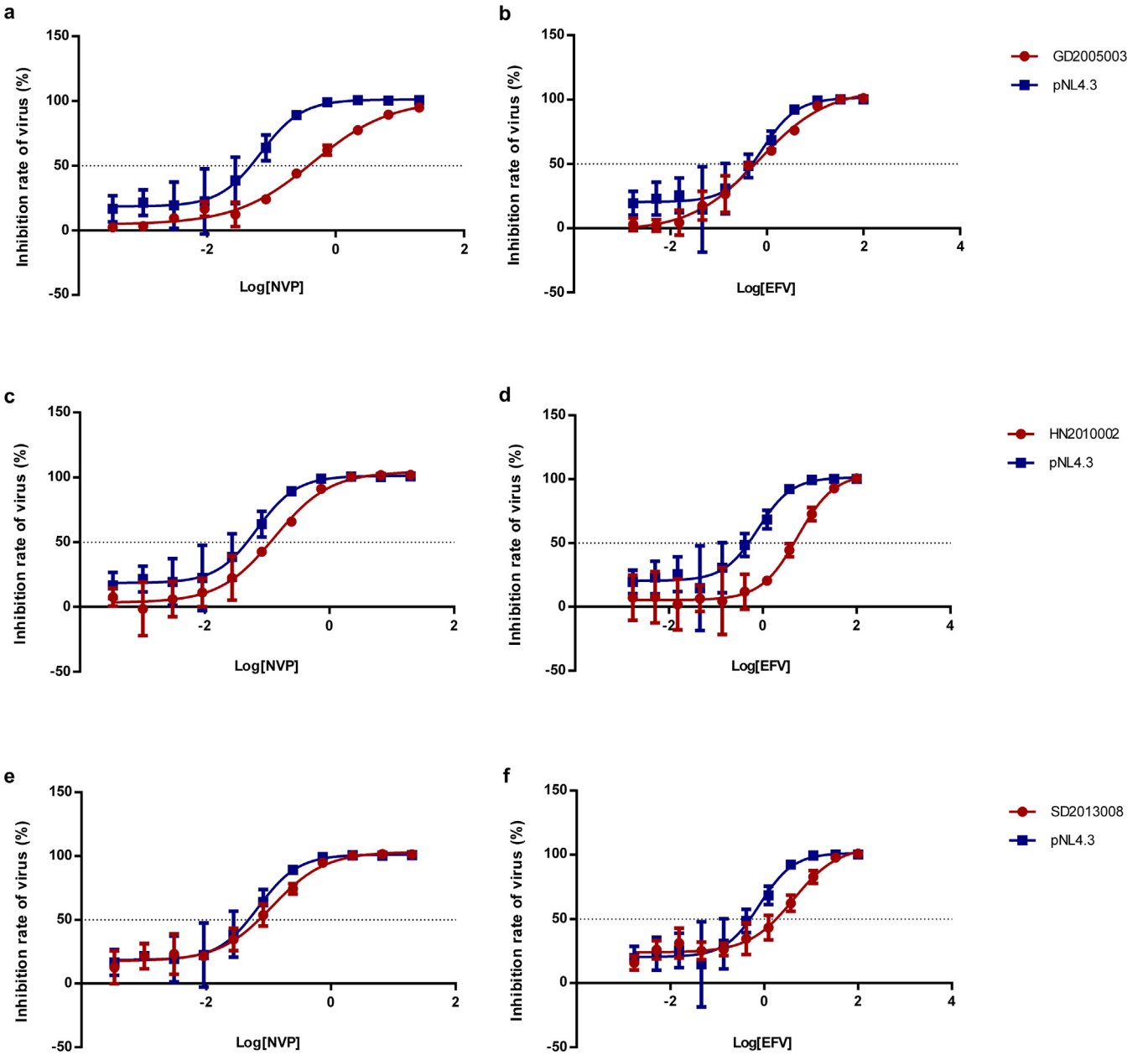

Figure 2. Phenotypic drug susceptibility profiles from three patient-derived primary viral isolates. Dose–response curves corresponding to the susceptible HIV-1pNL4.3 reference viral strain (blue) and the sample virus (red) are shown. The susceptibilities of (a, b) GD2005003, (c, d) HN2010002, and (e, f) SD2013008 to two NNRTIs (NVP and EFV). IC_{50} values were calculated by fitting data to a sigmoid dose–response curve with variable slope, and fold-change values were calculated as the IC_{50} ratio for each drug between the sample and the reference.

reference HIV-$1_{pNL4.3}$ in the presence of the six ARV drugs at IC_{50} were calculated (Table 2). The recombinant viruses displayed a variance in drug susceptibility for all six drugs tested.

In particular, sample HN2009001 was resistant to all drugs tested. IC_{50} values (mean ± standard deviation) for each drug were as follows: AZT, 1.76 ± 0.07 μM; 3TC, 8.08 ± 0.24 μM; ddI, 28.23 ± 3.74 μM; d4T, 7.48 ± 0.77 μM; NVP, 8.06 ± 0.07 μM; EFV, 20.40 ± 3.49 nM. In comparison to the reference virus tested in parallel, the inhibition curves were shifted toward higher drug concentrations for the recombinant virus derived from HN2009001, with larger reductions in susceptibilities to AZT (27.34-fold), 3TC (17.08-fold), NVP (126.75-fold), and EFV (26.73-fold) exhibited. Less pronounced reductions in susceptibilities to d4T (3.92-fold) and ddI (4.38-fold) were also observed.

For the other samples, according to the criteria proposed above, six of 18 viruses were resistant or hyper-susceptible to at least one drug relative to the reference HIV-1 pNL4.3 strain. In detail, the drug sensitivity of GD2005003, GD2005028, HN2010002, and SD2013008 had changed significantly to one kind of drug. Among these samples, HN2010002 and SD2013008 exhibited a large reduction in susceptibility to EFV (7.84- and 5.39-fold, respectively), GD2005003 showed an intermediate resistance level to NVP (7.39-fold, Figure 2), and a less pronounced elevation in GD2005028 susceptibility to 3TC (3.0-fold) was also observed. The susceptibility of XJ2010001 to the three nucleoside reverse transcriptase inhibitors (NRTIs) AZT, 3TC, and ddI increased to 3.7-, 3.6-, and 2.9-fold, respectively (Figure 3).

3.4. Comparison of phenotypic and genotypic results

The phenotypic and genotypic results of the 19 recombinant viruses in relation to the ARV drugs basically corresponded with one another. In total, 114 drug resistance phenotypic results were obtained and 97.4% agreed with the genotypic results. No recognizable genotypic resistance-associated mutations were noted, except in one sample. According to the phenotypic assay, 16 of the 19 samples were susceptible to the six drugs tested. For the sample HN2009001 with major drug resistance mutations, both the phenotypic and genotypic assays suggested that it was resistant to all drugs tested. For the other samples, all phenotypic and genotypic results obtained in relation to the NRTIs were in agreement and all were susceptible to AZT, 3TC, d4T, and ddI, with fold-changes of less than 3-, 3-, 1.5-, and 1.5-fold, respectively (the cut-off value). When examining the phenotypic susceptibility results to the non-nucleoside reverse transcriptase inhibitor (NNRTIs), 7.9% (3/38) were inconsistent with the genotypic results.

4. Discussion

In the present study, recombinant viruses containing patient-amplified PCR fragments encompassing entire protease and former RT (positions 1 to 312) regions in wild-type pNL4.3 vectors were constructed and harvested. Recombinant viral fold-changes in the presence of six ARV drugs used widely in China were measured and the relationships between phenotypic drug resistance and genetic polymorphisms were evaluated.

No recognized genotypic resistance-associated mutations were noted, except in the sample HN2009001, which had major drug resistance mutations including M41L, L210W, T215Y, K103N, and K238T. According to the clinical and statistical criteria, 16 of the 19 samples were susceptible to the six drugs tested. The phenotype and genotype results basically corresponded with one another, with a concordance rate of 97.4%.

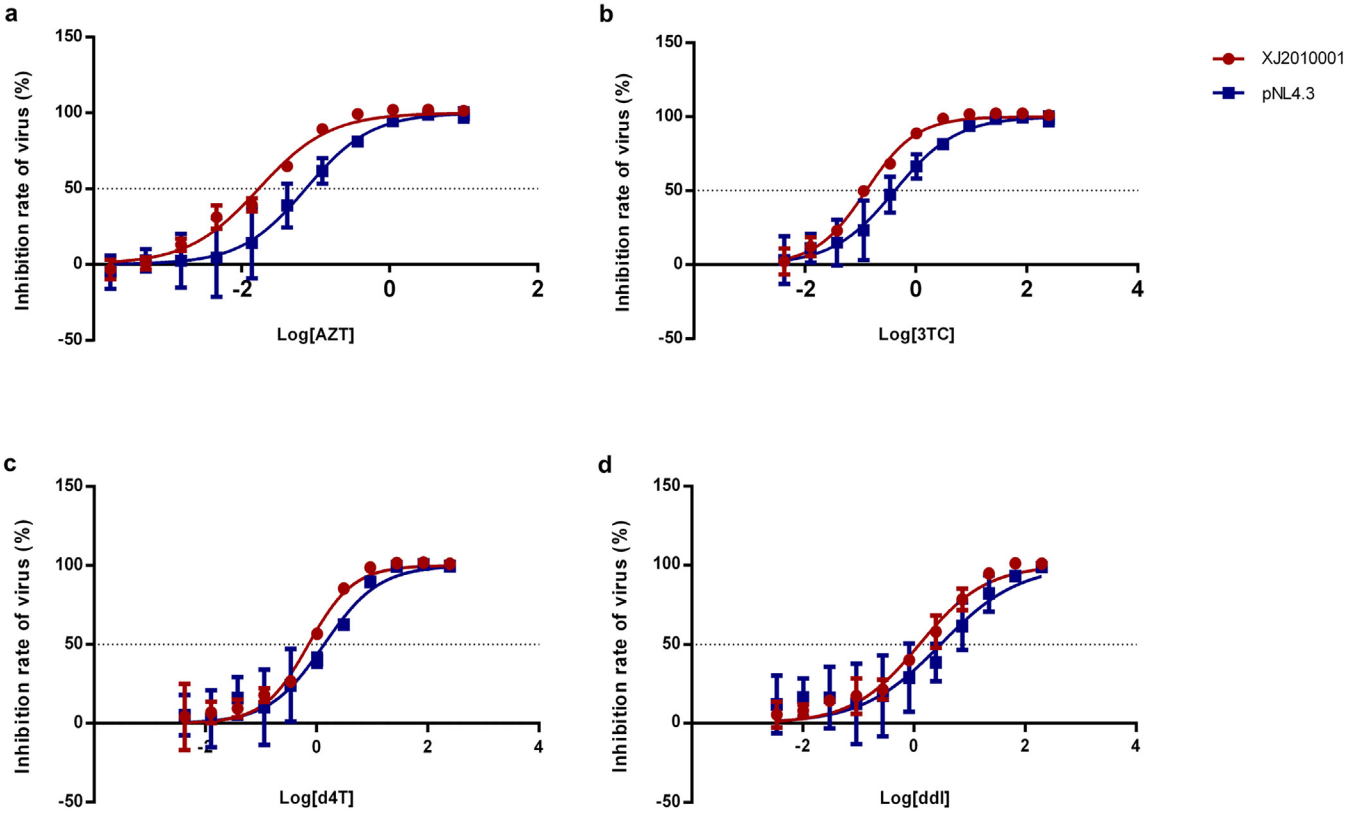

Figure 3. Phenotypic drug susceptibility profiles of XJ2010001. Dose–response curves corresponding to the susceptible HIV-1pNL4.3 reference viral strain (blue) and the sample virus (red) are shown. XJ2010001 susceptibility to four NRTIs: (a) AZT, (b) 3TC, (c) d4T, and (d) ddI. IC_{50} values were calculated by fitting data to a sigmoid dose–response curve with variable slope, and fold-change values were calculated as the IC_{50} ratio for each drug between the sample and the reference.

While the phenotypic results agreed with the genotypic results for the most part, there were some discrepancies between these assays. According to the phenotypic assay, six of the 19 viruses had significant susceptibility alterations to at least one drug compared with the reference HIV-1 pNL4.3 strain, while the genotypic assay identified some amino acid substitutions. It has been widely reported that HIV-1 genetic variability may influence ART efficacy. A polymorphism is defined as a mutation that occurs frequently in a virus not exposed to selective drug pressure. Drug resistance mutations should be commonly recognized as causing or contributing to resistance, should be non-polymorphic in untreated persons, and should be applicable to all HIV-1 subtypes.[20] It has been reported that naturally occurring polymorphisms have a great influence on HIV-1 drug susceptibility. Furthermore, polymorphisms can lead to ART failure in treatment-naïve patients and limit drug regimen efficacy.[21] The Stanford University Network HIV-1 Database[13] contains data from more than 420 published papers concerning mutations generated by viral passage or site-directed mutagenesis.[22] These additional mutations are generally ignored, as they are often not shown in genotyping reports. Substantial polymorphisms were observed in the present study. No explicit explanations regarding the polymorphisms were noted in the Stanford HIV-1 drug resistance database.[13] None of the mutations listed above was included in the other two HIV-1 drug resistance databases (ANRS and Rege).[23,24] Larger phenotypic studies are required to evaluate the impact of these polymorphisms on ART susceptibility and viral fitness in order to improve current interpretation systems.

While some discrepancies were noted regarding the influences of complicated amino acid substitutions, others may have been a result of inappropriate cut-off values. Resistance is defined on the basis of assay-specific biological cut-off values originating from the variation in susceptibility of treatment-naïve individuals to drugs.[25,26] Harrigan et al. defined the biological cut-off as the mean fold-change plus two standard deviations among a population of viruses from 1000 drug-naïve patients.[25] These papers have shown that there is a large disparity in baseline values and that the cut-off interval may be extended to some extent. The disparity in HIV-1 susceptibility indicates a need to further investigate the clinical effectiveness of ART in certain individuals.

In conclusion, this study presents a new and useful tool for assessing drug susceptibility to ARV drugs, permitting the quantitation of the level of resistance. In comparison to traditional phenotypic assays, it is an affordable and rapid in-house assay for the evaluation of susceptibility to ARV drugs of clinical viruses and the mutation profile of antiviral agents that are promising for further development.

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Conflict of interest: The authors declare no conflict of interest.

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