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In Vitro Cross-Resistance Profiles of Rilpivirine, Dapivirine, and MIV-150, Nonnucleoside Reverse Transcriptase Inhibitor Microbicides in Clinical Development for the Prevention of HIV-1 Infection

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ABSTRACT Rilpivirine (RPV), dapivirine (DPV), and MIV-150 are in development as microbicides. It is not known whether they will block infection of circulating nonnucleoside reverse transcriptase inhibitor (NNRTI)-resistant human immunodeficiency virus type 1 (HIV-1) variants. Here, we demonstrate that the activity of DPV and MIV-150 is compromised by many resistant viruses containing single or double substitutions. High DPV genital tract concentrations from DPV ring use may block replication of resistant viruses. However, MIV-150 genital tract concentrations may be insufficient to inhibit many resistant viruses, including those harboring K103N or Y181C.

KEYWORDS HIV-1, MIV-150, NNRTI, antiretroviral resistance, dapivirine, prevention

Nonnucleoside reverse transcriptase (RT) inhibitors (NNRTIs) comprise a group of small amphiphilic compounds with diverse chemical structures that inhibit human immunodeficiency virus type 1 (HIV-1) (but not HIV-2) replication. They interact with HIV-1 RT by binding to a single site, termed the NNRTI-binding pocket, on the p66 subunit of the p66/p51 heterodimeric enzyme (1). In many low- and middle-income countries (LMIC), particularly in sub-Saharan Africa, NNRTIs are used in both HIV-1 treatment and prevention strategies (2). Specifically, the NNRTIs nevirapine, efavirenz, and rilpivirine (RPV) are used in first-line antiretroviral therapies, whereas etravirine is reserved for salvage therapy. For prevention of HIV-1 infection, the following drugs are used or being tested. Nevirapine is used to block mother-to-child transmission. A dapivirine (DPV)-containing ring provided moderate efficacy in HIV-1-negative female participants, particularly in compliant women over 25 years of age (3, 4). A microbicide gel formulation (PC-1005) containing the phenylethylthiazolylthiourea derivative MIV-150 is in phase I clinical studies (5), and an injectable long-acting RPV formulation was evaluated in the clinical study HPTN 076 for preexposure prophylaxis (6, 7).

Due to their extensive use in LMIC, there have been significant increases in acquired NNRTI drug resistance, and consequently, the proportion of newly infected patients with transmitted drug resistance has also increased (8, 9). In this regard, four NNRTI resistance mutations—K101E, K103N, Y181C, and G190A—account for >80% of NNRTI-associated transmitted drug resistance in all regions and subtypes (10). Currently, it is unknown whether the NNRTIs used in prevention strategies (e.g., DPV, RPV, or MIV-150) will prevent infection of circulating NNRTI-resistant HIV-1 variants. Importantly, and relevant to this study, there is also a paucity of information in regard to the resistance and cross-resistance profiles of DPV and MIV-150. To address these important knowledge gaps, we constructed by site-directed mutagenesis 28 subtype B HIV-1^{LAI} infectious viruses containing single NNRTI resistance mutations spanning 17 different

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TABLE 1 Susceptibility of HIV-1 containing single or double NNRTI resistance mutations to RPV, DPV, and MIV-150

Virus	RPV		DPV		MIV-150	
	EC_{50} (nM)[a]	Fold R[b] (*P* value)	EC_{50} (nM)	Fold R (*P* value)	EC_{50} (nM)	Fold R (*P* value)
WT	0.33 ± 0.13		0.64 ± 0.13		0.68 ± 0.09	
V90I	0.59 ± 0.13	1.8	1.09 ± 0.47	1.7	0.98 ± 0.14	1.4
L100I	0.36 ± 0.13	1.1	9.36 ± 1.37	14.7 (<0.01)	5.03 ± 1.35	7.4 (<0.01)
L100V	0.27 ± 0.13	0.8	6.15 ± 1.79	9.6 (<0.01)	5.06 ± 2.42	7.5 (0.02)
K101E	1.12 ± 0.24	3.5 (<0.01)	2.29 ± 0.68	3.6 (<0.01)	3.25 ± 0.75	4.8 (<0.01)
K101P	13.10 ± 1.70	40.1 (<0.01)	≥62.5	≥100 (<0.01)	≥62.5	≥100 (<0.01)
K103N	0.53 ± 0.11	1.6	3.03 ± 0.47	4.8 (<0.01)	23.40 ± 2.81	34.6 (<0.01)
K103S	0.49 ± 0.11	1.5	4.61 ± 0.43	7.2 (<0.01)	15.30 ± 2.03	22.7 (<0.01)
V106I	0.48 ± 0.12	1.5	0.94 ± 0.14	1.5	0.78 ± 0.11	1.20
V108I	0.38 ± 0.01	1.2	0.96 ± 0.20	1.5	0.89 ± 0.15	1.3
E138A	0.80 ± 0.41	2.5 (0.06)	1.29 ± 0.33	2.0 (0.02)	1.32 ± 0.23	2.0 (0.01)
E138K	0.96 ± 0.18	2.9 (<0.01)	2.79 ± 0.70	4.4 (<0.01)	1.89 ± 0.17	2.8 (<0.01)
V179D	0.39 ± 0.15	1.2	0.81 ± 0.33	1.3	0.73 ± 0.35	1.1
V179F	0.003 ± 0.002	0.01 (>0.05)	0.20 ± 0.01	0.3 (<0.01)	0.03 ± 0.01	0.1
G190A	0.42 ± 0.03	1.3	0.77 ± 0.15	1.2	0.40 ± 0.09	0.6
G190S	0.25 ± 0.17	0.8	0.84 ± 0.01	1.3	0.09 ± 0.01	0.1
Y181C	0.60 ± 0.25	1.8	5.06 ± 1.50	7.9 (<0.01)	10.20 ± 1.60	15.1 (<0.01)
Y181I	7.68 ± 0.78	23.5 (<0.01)	≥62.5	≥100 (<0.01)	≥62.5	≥100 (<0.01)
Y181V	7.36 ± 1.20	22.6 (<0.01)	≥62.5	≥100 (<0.01)	39.60 ± 4.10	58.6 (<0.01)
Y188C	0.08 ± 0.04	0.3 (>0.05)	0.39 ± 0.20	0.6	0.21 ± 0.05	0.3 (>0.05)
Y188H	0.11 ± 0.04	0.3 (>0.05)	0.69 ± 0.33	1.1	1.16 ± 0.48	1.7
Y188L	1.69 ± 0.23	5.2 (<0.01)	55.30 ± 6.53	86.7 (<0.01)	≥62.5	≥100 (<0.01)
H221Y	0.45 ± 0.20	1.37	0.85 ± 0.20	1.3	0.91 ± 0.19	1.3
P225H	0.29 ± 0.17	0.9	0.69 ± 0.25	1.1	0.76 ± 0.32	1.1
F227C	1.11 ± 0.27	3.40 (0.01)	4.37 ± 0.78	6.9 (<0.01)	10.01 ± 3.16	14.8 (<0.01)
F227L	0.28 ± 0.26	0.9	0.61 ± 0.19	1.0	0.88 ± 0.31	1.3
M230L	2.58 ± 1.13	7.9 (0.01)	10.10 ± 0.38	15.8 (<0.01)	15.30 ± 2.87	22.6 (<0.01)
P236L	0.55 ± 0.10	1.7	1.00 ± 0.18	1.6	0.63 ± 0.07	0.9
N348I	0.50 ± 0.13	1.5	1.07 ± 0.47	1.7	0.99 ± 0.34	1.5
K101E/G190A	1.42 ± 0.22	4.4 (<0.01)	4.22 ± 0.31	6.6 (<0.01)	1.45 ± 0.21	2.2 (<0.01)
K101E/K103N	0.50 ± 0.07	1.5	12.60 ± 1.84	19.7 (<0.01)	38.10 ± 7.38	56.3 (<0.01)
K101E/Y181C	3.12 ± 0.48	9.5 (<0.01)	40.70 ± 9.86	63.8 (<0.01)	35.50 ± 13.80	52.5 (<0.01)
K103N/G190A	0.02 ± 0.01	0.06 (<0.01)	0.98 ± 0.17	1.5	1.07 ± 0.25	1.6
K103N/Y181C	1.87 ± 0.34	5.7 (<0.01)	56.20 ± 3.06	88.0 (<0.01)	182.00 ± 51.20	≥250 (<0.01)
Y181C/G190A	0.70 ± 0.10	2.1 (<0.01)	7.22 ± 2.27	11.3 (<0.01)	1.26 ± 0.39	1.9

[a]The concentrations of drug required to inhibit viral replication by 50% (EC_{50}s) from three independent experiments. Data reported as means ± standard deviations from at least three independent experiments.

[b]Mean fold change in the EC_{50} of mutant virus versus WT virus. EC_{50}s were compared for statistically significant differences (*P* value of <0.05) using a nonpaired, two-sample equal-variance (homoscedastic) test.

codons (V90I, L100I/V, K101E/P, K103N/S, V106I, V108I, E138A/K, V179D/F, G190A/S, I181C/I/V, Y188C/H/L, H221Y, P225H, F227C/L, M230L, P236L, and N348I). We also constructed six subtype B HIV-1^{LAI} infectious viruses containing two NNRTI resistance mutations (K101E and G190A [K101E/G190A], K101E/K103N, K101E/Y181C, K103N/G190A, K103N/Y181C, and Y181C/G190A). Drug susceptibility in a single cycle assay using TZM-bl cells was determined for RPV (Selleckchem, TX, USA), DPV (Selleckchem, TX, USA), and MIV-150 (Cayman Chemical Company, MI, USA) as described previously (11, 12). Low-, intermediate-, and high-level resistance was defined as 2- to 8-fold, 8- to 20-fold, and >20-fold changes in drug susceptibility, respectively, compared to the wild-type (WT) virus. Of the three NNRTIs studied, RPV exhibited the best antiviral activity across the panel of mutant viruses tested and retained full sensitivity against 19 of 28 variants containing a single substitution and 2 of 6 variants containing double substitutions (Table 1 and Fig. 1). The E138A/K, F227C, K101E, Y188L, M230L, K101E/G190A, and K103N/Y181C substitutions conferred low-level RPV resistance, while the Y181I/V and K101P substitutions conferred high-level resistance. The RPV resistance profile reported in this study is consistent with those previously published (13, 14). In contrast to RPV, DPV retained activity against only 15 of the 28 viruses containing a single substitution and 1 of 6 viruses containing double substitutions (Table 1 and Fig. 1). The K101E, E138K, K103N/S, F227C, Y181C, and K101E/G190A substitutions con-

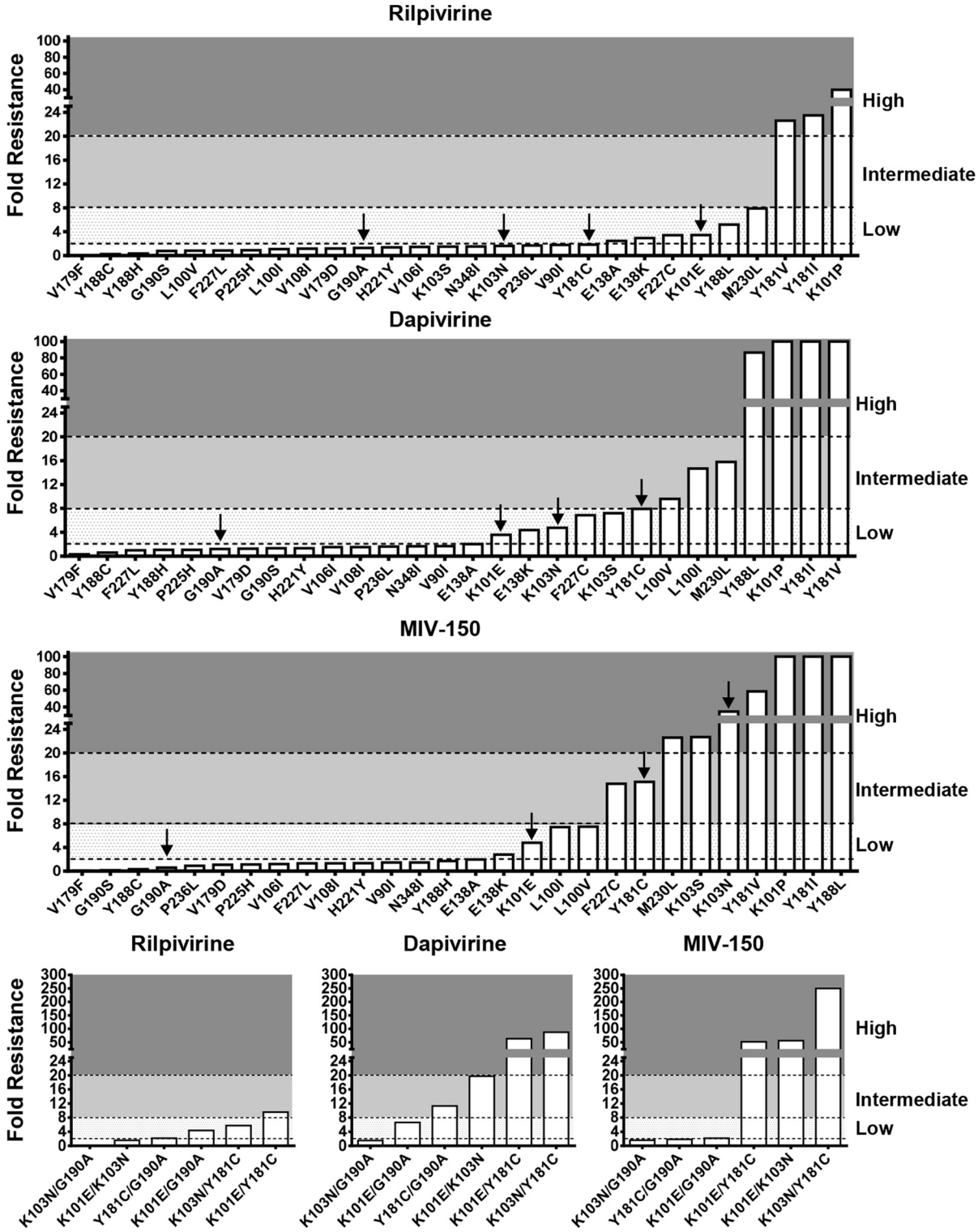

FIG 1 NNRTI cross-resistance profiles for RPV, DPV, and MIV-150. Low-, intermediate- and high-level resistance was defined as 2- to 8-fold, 8- to 20-fold, and >20-fold changes in drug susceptibility compared to the WT virus. The black arrows indicate the four most commonly transmitted drug resistance mutations, G190A, K101E, Y181C, and K103N.

ferred low-level resistance to DPV, whereas the L100I/V, M230L, K101E/K103N, and Y181C/G190A substitutions and the Y188L, K101P, Y181I/V, K101E/Y181C, and K103N/Y181C substitutions were found to confer intermediate and high-level resistance, respectively. The DPV cross-resistance profile reported in our study is consistent with

TABLE 2 Susceptibility of recombinant viruses containing full-length patient-derived WT subtype C RT sequences with and without E138A to RPV, DPV, and MIV-150

Virus accession no.[a]	E138A present	RPV		DPV		MIV-150	
		EC_{50} (μM)[b]	Fold R[c] (*P* value)	EC_{50} (μM)	Fold R (*P* value)	EC_{50} (μM)	Fold R (*P* value)
AF361879	No	0.30 ± 0.03		0.68 ± 0.10		0.56 ± 0.10	
AY043176	No	0.24 ± 0.04		0.49 ± 0.02		0.36 ± 0.05	
Avg[d]	No	0.27 ± 0.04		0.58 ± 0.13		0.46 ± 0.13	
DQ351238	Yes	0.40 ± 0.12	1.5	1.20 ± 0.33	2.1 (<0.01)	1.40 ± 0.59	3.1 (<0.01)
AY901981	Yes	0.64 ± 0.14	2.4 (<0.01)	1.80 ± 0.51	3.0 (<0.01)	1.50 ± 0.23	3.2 (<0.01)
AF443097	Yes	0.41 ± 0.12	1.5	1.30 ± 0.50	2.3 (<0.01)	0.86 ± 0.16	1.9 (<0.01)
AY253303	Yes	0.23 ± 0.04	0.9	0.83 ± 0.13	1.4	0.60 ± 0.05	1.3
AY734559	Yes	0.36 ± 0.14	1.3	0.77 ± 0.26	1.3	0.63 ± 0.15	1.4
FJ199637	Yes	0.54 ± 0.07	2.0 (<0.01)	2.70 ± 0.19	4.7 (<0.01)	1.60 ± 0.31	3.4 (<0.01)

[a]The GenBank accession number or sequence identifier for the full-length subtype C RT gene for the virus.
[b]The concentrations of drug required to inhibit viral replication by 50% (EC_{50}s) are reported as means ± standard deviations from at least three independent experiments.
[c]Mean fold change in the EC_{50} of WT virus with the E138A substitution vs WT virus. EC_{50}s from three independent experiments were compared for statistically significant differences (*P* value of <0.05) using a nonpaired, two-sample equal-variance (homoscedastic) test.
[d]The median EC_{50}s for the two viruses (with GenBank accession numbers AF361897 and AY043176) that did not harbor E138A were used as the WT reference for determination of the fold R value.

prior *in vitro* studies of DPV resistance selection and cross-resistance profiling (15–17). Additionally, Penrose et al. recently reported that there was frequent cross-resistance to DPV in subtype C-infected individuals after first-line therapy failure and reported that the L100I and K103N substitutions were significantly more frequent in samples with >500-fold resistance to DPV compared to samples with ≤500-fold resistance (18). However, the limitation of this study (18) is that each clinical isolate contained on average three NNRTI resistance mutations, making it difficult to identify the genetic determinants for resistance. Similar to DPV, MIV-150 was also found to be active against only 15 of the HIV-1 variants containing single NNRTI substitutions tested and 2 of 6 variants containing two substitutions tested. However, high-level resistance was more frequently observed for MIV-150 than for either DPV or RPV (Table 1 and Fig. 1). Notably, the M230L, K103S, K103N, Y181V, K101P, Y181I, Y188L, K101E/K103N, K101E/Y181C, and K103N/Y181C substitutions all conferred high-level resistance. The F227C and Y181C substitutions and the L100L/V, K101E, and K101E/G190A substitutions were found to confer intermediate- and low-level MIV-150 resistance, respectively (Table 1 and Fig. 1). To our knowledge, this is the first study to define in detail the cross-resistance profile for MIV-150, although one prior study identified different combinations of E138K, Y181I, Y181C, K103N, L100I, or K101E in simian immunodeficiency viruses expressing HIV reverse transcriptase (SHIV-RT viruses) exposed to MIV-150 in rhesus macaques, although no phenotypic data were provided (19). Additionally, prior studies have reported on the resistance profiles of the MIV-150 analogs, namely, MIV-160 and MIV-170 (16, 17).

Recently, we reported that an E138A substitution occurs more frequently in subtype C sequences (range, 5.9 to 7.5%) than subtype B sequences (range, 0 to 2.3%) from treatment-naive individuals ($P < 0.01$) (11). Because E138A in subtype C HIV-1 decreases RPV susceptibility, we previously proposed that this polymorphism may impact prevention (and treatment) strategies that include RPV in geographic areas where subtype C infection is prevalent (11). Accordingly, in this study, we synthesized (GenScript, NJ, USA) and cloned into our HIV-1^{LAI} viral vector (as described previously [12]) full-length subtype C RT sequences from two antiretroviral-naive individuals that did not harbor E138A and from six antiretroviral-naive individuals that contained E138A. Phenotypic analyses revealed that 2 of the recombinant viruses that contained E138A conferred low-level resistance (2.4- and 2.0-fold, respectively) to RPV (Table 2). In contrast, four of the six recombinant viruses that contained E138A conferred decreased susceptibility to DPV (range, 2.1- to 4.7-fold) and MIV-150 (range, 1.9- to 3.4-fold)

(Table 2). These data highlight that the RT genetic backbone influences, at least to some extent, the ability of E138A to decrease NNRTI susceptibility and suggest that the low-level resistance conferred by E138A is unlikely to impact RPV, DPV, or MIV-150 activity.

In summary, this study provides the first detailed insights into the antiviral activity of RPV, DPV, and MIV-150 against a broad panel of recombinant viruses containing substitutions that are known to decrease NNRTI susceptibility. We also evaluated their activity against WT subtype C RTs that contained E138A. The pharmacokinetics of the long-acting RPV formulation has been investigated in healthy individuals in two different studies (20, 21). In cervicovaginal fluid (CVL), RPV concentrations at day 28 postadministration were 12, 15, and 98 ng/ml (68, 107, and 232 nM, respectively) following injected doses of 300, 600, and 1,200 mg, respectively. In the rectal fluid (RF), RPV concentrations at day 28 postadministration were 11.9 ng/ml (32 nM), following a 600- mg injection. The RPV concentrations in the CVL and RF exceed the concentrations of drug required to inhibit viral replication by 50% (EC_{50}s) for all of the NNRTI-resistant variants listed in Table 1, suggesting that RPV may prevent infection from transmitted NNRTI-resistant viruses. With regard to DPV, pharmacokinetic studies have shown that the vaginal fluid concentration on day 28 of DPV ring use ranged from 14.9 to 65 μg/ml (45 to 198 μM) (22, 23). These concentrations far exceed the reported EC_{50}s for the WT and mutant HIV-1 in Tables 1 and 2, suggesting that the ring would effectively inhibit replication of all the resistant viruses tested. (Note that exact EC_{50}s for DPV for the K101P and Y181I/V HIV-1 viruses could not be determined, as they exceeded the highest concentration of drug used in the assay.) In contrast, pharmacokinetic studies of PC-1005 (MIV-150 and zinc acetate in a carrageenan gel) yielded concentrations of MIV-150 in cervicovaginal lavage fluid samples ranging from ~100 to 170 nM (5). In this regard, it is questionable whether these concentrations will effectively block the mutant viruses which exhibited high-level MIV-150 resistance (K101P, K103N/S, Y181C/I/V, F227C, M230L, K101E/K103N, K101E/Y181C, and K103N/Y181C) and for which EC_{50}s range from 10 to 100 nM. Importantly, both K103N and Y181C, which are frequently associated with transmitted NNRTI resistance, fall into this category.

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We declare that we have no conflicts of interest.

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