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ORIGINAL RESEARCH

Patterns of emergent resistance-associated mutations after initiation of non-nucleoside reverse-transcriptase inhibitor-containing antiretroviral regimens in Taiwan: a multicenter cohort study

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For the Taiwan HIV Study Group

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Background: Increasing trends of resistance-associated mutations (RAMs) to non-nucleoside reverse-transcriptase inhibitors (nNRTIs) have raised concerns about the effectiveness of the regimens in the national HIV treatment programs in resource-limited countries. We aimed to retrospectively investigate the incidence and patterns of emergent RAMs of HIV-1 in HIV-positive adults experiencing virological failure to first-line nNRTI-containing combination antiretroviral therapy (cART) in Taiwan.

Patients and methods: Between June 2012 and March 2016, 1138 antiretroviral-naïve HIV-positive adults without baseline RAMs who initiated nNRTI-containing regimens were included for analysis. Virological failure was defined as plasma viral load (PVL) ≥200 copies/mL after 6 months of cART or confirmed PVL ≥200 copies/mL after achieving PVL <50 copies/mL. Population sequencing was retrospectively performed to detect baseline and emergent RAMs. RAMs were interpreted using the International AIDS Society-USA 2016 mutations list.

Results: Seventy-one patients (6.2%) developed virological failure, which occurred in 14.8% (43/291), 3.9% (26/675), and 1.2% (2/172) of patients receiving 2 nucleoside reverse-transcriptase inhibitors (NRTIs) plus nevirapine, efavirenz, and rilpivirine, respectively. Among those, 53 (74.6%) had emergent RAMs identified, which included 43 (81.1%), 53 (100.0%), and 1 (1.9%) with RAMs to NRTIs, nNRTIs, and protease inhibitors, respectively; and 43 (81.1%) had multi-drug resistance. The most common emergent RAMs to NRTIs were M184V/I (42.3%) and K65R (28.2%), and those to nNRTIs were Y181C (42.3%), K103N (15.5%), G190A/E/Q (12.7%), V179D/E (12.7%), and V108I (9.9%).

Conclusion: While the rates of virological failure varied with the nNRTI used, the rate of emergent RAMs of HIV-1 to NRTIs and nNRTIs among the antiretroviral-naïve patients who failed nNRTI-containing cART remained low.

Keywords: antiretroviral therapy, treatment guidelines, virological failure, genotypic resistance, population sequencing, nNRTIs, RAM

Introduction

Combination antiretroviral therapy (cART) consisting of 2 nucleoside reverse-transcriptase inhibitors (NRTIs) and 1 non-nucleoside reverse-transcriptase inhibitor (nNRTI) remains the recommended first-line regimen in the World Health Organization (WHO) treatment guidelines for adults with HIV-1 infection in low- and middle-income countries, with boosted protease inhibitor (PI)-containing regimens as the

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second-line therapy for patients who fail the first-line cART.[1] While the scale-up of antiretroviral therapy has significantly reduced morbidity, mortality, mother-to-child transmission, and incidence rate of HIV-1 infection,[1,2] the emergence of drug-resistant viral strains, because of either poor adherence or use of suboptimal regimens, has raised concerns about the effectiveness of nNRTI-containing regimens, onward transmission of drug-resistant strains, and adverse outcomes in patients with delayed detection of virological failure,[3,4] especially in resource-limited settings.[5]

The first-generation nNRTIs have a relatively low genetic barrier to development of resistance, and therefore, failure of nNRTI-containing regimens as the first-line cART often results in the emergence of reverse transcriptase resistance-associated mutations (RAMs), which may jeopardize the opportunities for subsequent selection of effective and better-tolerated antiretroviral regimens to achieve sustained viral suppression.[6–8] In previous studies, the incidence or prevalence of RAMs in patients failing first-line cART ranged from 53% to 95%, and 38–64% of these patients would have HIV-1 harboring dual-class resistance, depending on the patients' adherence to antiretroviral therapy, the baseline prevalence of transmitted drug resistance, and the frequency of monitoring of virological responses.[9–12]

Drug resistance testing that is performed at baseline and when virological failures occur is essential to construct effective antiretroviral regimens in antiretroviral-naïve patients before initiation of cART or in antiretroviral-experienced patients with virological failure. However, routine drug resistance testing before initiation or switching cART may not be available to HIV-treating clinicians in resource-limited settings, where drug resistance testing is mostly performed retrospectively as part of public health surveillance programs to monitor the spread of drug-resistant HIV-1. In this multicenter study, we aimed to retrospectively investigate the incidence and patterns of emergent RAMs of HIV-1 to antiretroviral agents in HIV-positive adults who had no baseline information on RAMs, but developed virological failure to first-line nNRTI-containing cART in Taiwan between 2012 and 2016.

Patients and methods

Study setting

By the end of December 2016, 33,428 indigenous cases of HIV infection had been diagnosed and reported to Taiwan Centers for Disease Control (CDC) since the first case of HIV-1 infection was diagnosed in Taiwan in 1984, and about 79% of the patients who survived were receiving cART in 2015 (Taiwan CDC, unpublished data). cART has been provided free of charge at the designated hospitals for HIV care around Taiwan since its introduction in April 1997; and monitoring of glucose, lipids, liver and renal function, plasma viral load (PVL), and CD4 counts is reimbursed by the National Health Insurance or a special budget from the Taiwan CDC. The national HIV treatment guidelines recommended coformulated zidovudine/lamivudine (GlaxoSmithKline, Brentford, Middlesex, UK), abacavir/lamivudine (GlaxoSmithKline), or tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC) (Gilead Sciences, Foster City, CA, USA), or TDF (Gilead Sciences) plus lamivudine (GlaxoSmithKline) in combination with either efavirenz (Bristol-Myers Squibb, Princeton, NJ, USA) or nevirapine (Boehringer Ingelheim, Ingelheim, Germany); or zidovudine/lamivudine plus rilpivirine (Janssen, Beersel, Belgium) for patients with baseline HIV RNA <100,000 copies/mL as the preferred first-line regimens since June 2012, before the introduction of 3 coformulated, single-tablet regimens (TDF/FTC/efavirenz [Gilead Sciences], TDF/FTC/rilpivirine [Gilead Sciences], and abacavir/lamivudine/dolutegravir [GlaxoSmithKline]) on June 1, 2016.

During the study period, genotypic resistance testing of HIV-1 was not routinely performed in antiretroviral-naïve patients before initiation of cART; however, in the participating hospitals in this study, baseline genotypic resistance testing was performed at the same time or within 4 weeks of cART initiation; and changes to antiretroviral therapy would be made when the report that was available about 4–8 weeks later revealed the presence of transmitted drug resistance of HIV-1. In patients with unsatisfactory virological response or viral rebound after achieving viral suppression with cART, genotypic resistance testing was performed before choices of second-line or salvage regimens were to be made. According to recent surveillance of the trends of transmitted drug resistance of HIV-1 between 2006 and 2014, 11.1% of HIV-1 from antiretroviral-naïve patients harbored at least 1 RAM; while the overall prevalence of transmitted drug resistance appeared to have stabilized and a decline in antiretroviral resistance mutations to NRTIs and PIs was observed, the prevalence of resistance mutations to nNRTIs remained higher than 5%.[13,14]

Study population

In this retrospective study, we included 1138 HIV-positive adults aged 18 years or greater who initiated cART of 2 NRTIs plus 1 nNRTI at 3 major designated hospitals (National Taiwan University Hospital, Taipei; Far Eastern Memorial Hospital, New Taipei City; and Taoyuan General Hospital, Taoyuan) with access to genotypic resistance testing in northern Taiwan between June 2012 and March 2016. Patients without genotypic resistance data and those with RAMs to nNRTIs or NRTIs at baseline were excluded from analysis (Figure 1).

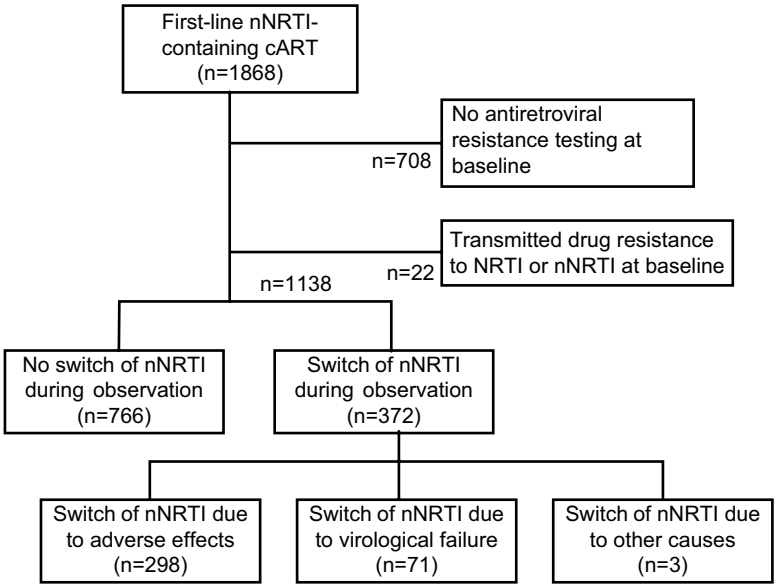

Figure 1 Flow chart of the study.
Abbreviations: cART, combination antiretroviral therapy; nNRTI, non-nucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor.

We used a standardized case record form to collect information on the demographics, clinical characteristics, and virological and immunological parameters at baseline and during follow-up after cART was initiated. After initiation of cART, the patients were seen by HIV-treating infectious diseases clinicians and case managers to inquire about the tolerability and adverse effects and to counsel for adherence for the first 2–4 weeks, and subsequently every 4–12 weeks.

The study was approved by the Research Ethics Committee or institutional review board at the 3 participating hospitals (National Taiwan University Hospital, Far Eastern Memorial Hospital, and Taoyuan General Hospital). Verbal or written informed consent was waived because of the retrospective study design. The confidentiality of the included patients was protected by adhering to the guidelines of good clinical practice.

Plasma HIV-1 viral load measurement and genotyping

According to the national HIV treatment guidelines, determinations of PVL and CD4 counts are performed 4–6 weeks after initiation of cART, and subsequently every 12–16 weeks within the first year and every 24 weeks thereafter in patients who have achieved viral suppression. PVL was measured using Cobas Amplicor HIV-1 Monitor™ Test, version 1.5, (Roche Diagnostics Corporation, Indianapolis, USA) with a detection limit of 20 copies/mL. The genotypic resistance assay was performed using an automatic sequencer (3100 Avant Genetic Analyzer; Applied Biosystems, Foster City, CA, USA) to determine the sequences of a 1.2 kb fragment covering the protease and the first 240 amino acids of the reverse transcriptase (RT) gene, as described previously.[13,14] The reverse transcription and polymerase chain reaction amplifications were carried out with appropriate negative controls to detect any possible contamination during the experiments. The laboratory personnel who performed sequencing had participated in the Quality Control for Molecular Diagnostics program since 2016. Antiretroviral resistance mutations were identified using the HIVdb program of the Stanford University HIV Drug Resistance Database,[15] in accordance with the drug resistance mutation list of the International AIDS Society-USA Consensus Guidelines.[16]

Virological failure was defined as having PVL ≥200 copies/mL at or after 6 months of cART initiation; or PVL ≥200 copies/mL after ever achieving virological suppression (PVL <50 copies/mL). Changes of NRTIs or nNRTIs when intolerance or adverse effects occurred without viral rebound were not considered virological failure in this study.

Statistical analysis

Variables were summarized as proportions for categorical variables and the mean values and SDs for continuous variables. Categorical data were analyzed using χ^2 or Fisher's exact tests, as appropriate, and continuous variables were compared using the Mann–Whitney *U*-test. The 95% CIs

were computed using a binomial distribution. All variables with $p<0.1$ in univariate analysis were selected for subsequent multivariate analysis. Multivariate analysis was performed using logistic regression. All tests were two tailed and a p value <0.05 was considered significant. Data were analyzed using SPSS 19.0 software (SPSS, Chicago, IL, USA).

Results

Study population

During the 3.5-year study period, 1868 antiretroviral-naïve patients initiated nNRTI-containing regimens at the 3 participating hospitals according the national HIV treatment guidelines, and 1138 patients (60.9%) whose specimens were sent for genotypic resistance testing and were confirmed as not having transmitted drug resistance were included in the study (Figure 1). The baseline characteristics of the included patients are shown in Table 1. The mean age of the patients was 32.4±9.0 years, and 98.1% were men. Men who have sex with men (91.1%) were the major HIV transmission risk group in this cohort, followed by injecting drug users (5.1%). Hepatitis B surface antigen and anti-hepatitis C virus antibody were detected in 10.4% and 7.4% of the patients, respectively. The mean baseline CD4 count was 296±225 cells/μL, PVL was 4.86±0.7 $\log_{10}$ copies/mL, and 447 patients (38.5%) had PVL ≥5 $\log_{10}$ copies/mL at baseline. Subtype B was the most common HIV-1 subtype, accounting for 87.6%.

First-line nNRTI-containing regimens

Of the NRTIs as the backbone of first-line cART prescribed in 1138 patients, TDF plus lamivudine (n=478, 42.0%) and TDF/FTC (n=156, 13.7%) were the most common, followed by zidovudine/lamivudine (n=460, 40.4%) and abacavir/lamivudine (n=44, 3.9%). Of the nNRTIs prescribed in combination with NRTI backbones, efavirenz, nevirapine, and rilpivirine accounted for 59.3% (n=675), 25.6% (n=291), and 15.1% (n=172), respectively. In total, 372 patients (32.7%) had to switch their first-line nNRTI-containing cART because of adverse effects or intolerance (n=298, 26.2%), subsequent virological failure (n=71, 6.2%) and other causes (n=3, 0.3%) (Figure 1). The mean interval from initiation to switch of cART owing to adverse effects or intolerance was 69±134 days, and that to switch because of virological failure was 175±134 days (Table 1). The mean PVL at baseline was 5.26±0.68 $\log_{10}$ copies/mL in the 71 patients with virological failure. For those experiencing virologic failure, the mean PVL was 4.57 ± 0.84 $\log_{10}$ copies/mL, with 63 patients (88.7%) having PVL >1,000 copies/mL.

Table 1 Characteristics of patients receiving first-line regimens containing nNRTIs

Variables	Number of patients (N=1138)
Male gender	1110 (98.1)
Age (years)	32.4±9.0
Mode of exposure	
Male-to-male sex contact	1037 (91.1)
Heterosexual contact	38 (3.3)
IDU	58 (5.1)
Other/unknown	5 (0.5)
Co-infections at baseline	
Positive HBsAg	120/1130 (10.6)
Positive anti-HCV	84/1135 (7.4)
Baseline CD4 count (cells/μL)	296±225
Baseline PVL ($\log_{10}$ copies/mL)	4.86±0.7
Baseline PVL >5 $\log_{10}$ copies/mL	447 (38.5)
HIV subtype	
B	997 (87.6)
CRF01_AE	53 (4.7)
CRF07_BC	67 (5.9)
Others	21 (1.8)
Backbone agent	
TDF plus 3TC	478 (42.0)
TDF/FTC	156 (13.7)
ABC/3TC	44 (3.9)
AZT/3TC	460 (40.4)
nNRTI agent	
EFV	675 (59.3)
NVP	291 (25.6)
RPV	172 (15.1)
nNRTI change	372 (32.7)
Interval to change (days)	90±141
Reasons for changing nNRTI	
Adverse effects	298 (26.2)
Duration to change (days)	69±134
Viral rebound/poor responses	71 (6.2)
Duration to change (days)	175±134

Note: Data are shown as n (%), mean±SD, or n/N (%).
Abbreviations: 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; EFV, efavirenz; FTC, emtricitabine; HBsAg, surface antigen of the hepatitis B virus; HCV, hepatitis C virus; IDU, injecting drug user; nNRTI, non-nucleoside reverse-transcriptase inhibitor; NVP, nevirapine; PVL, plasma viral load; RPV, rilpivirine; TDF, tenofovir disoproxil fumarate.

Emergence of RAMs

The overall incidence of virological failure varied among the patients receiving different nNRTI-containing regimens and those with PVL ≥5 $\log_{10}$ copies/mL versus those with PVL <5 $\log_{10}$ copies/mL (Figure S1). The rate of virological failure for NRTIs plus nevirapine, efavirenz, and rilpivirine was 14.8% (43/291), 3.9% (26/675), and 1.2% (2/172), respectively. In subgroup analysis in patients with PVL ≥5 $\log_{10}$ copies/mL at baseline, the rate of virological failure to regimens containing 2 NRTIs plus nevirapine and 2 NRTIs plus efavirenz was 25.0% (33/132) and 5.4% (16/295), respectively ($p<0.001$)

(OR 2.26, 95% CI 1.51–3.39) (Figure S1). For patients with PVL <5 $\log_{10}$ copies/mL at baseline, the rate of virological failure to 2 NRTIs plus nevirapine was significantly higher than that to 2 NRTIs plus efavirenz (6.3% [10/159] vs 2.6% [10/380], p=0.04) as well as 2 NRTIs plus rilpivirine (6.3% vs 1.2% [2/172], p=0.013); in contrast, there was no statistically significant difference between 2 NRTIs plus efavirenz and 2 NRTIs plus rilpivirine in terms of virological failure (2.6% vs 1.2%, p=0.273) (Figure S1).

Of the 71 patients (6.2%) who experienced virological failure, emergent RAMs were identified in 53 patients (74.6%), no RAMs were identified in 13 patients (18.3%), and amplification for genotypic resistance testing failed in 5 patients (7.1%) owing to low viral loads. Overall, 43 patients (81.1%) had emergent RAMs to NRTIs, 53 (100.0%) to nNRTIs, and 1 (1.9%) to PIs; and 43 (81.1%) had RAMs to 2 or more classes of antiretroviral agent (multi-drug resistance). Of the emergent RAMs to NRTIs, M184V/I (42.3%) and K65R (28.2%) were the most common; and of those RAMs to nNRTIs, Y181C (42.3%) and K103N (15.5%) were the most common, followed by G190A/E/Q (12.7%) and V179D/E (12.7%), and V108I (9.9%) (Figure 2). The emergence of M184V/I mutations was less common than RAMs to nNRTI in patients with virological failure who received 2 NRTIs plus nevirapine (51.2% vs 83.7%) as well as in those who received 2 NRTIs plus efavirenz (26.9% vs

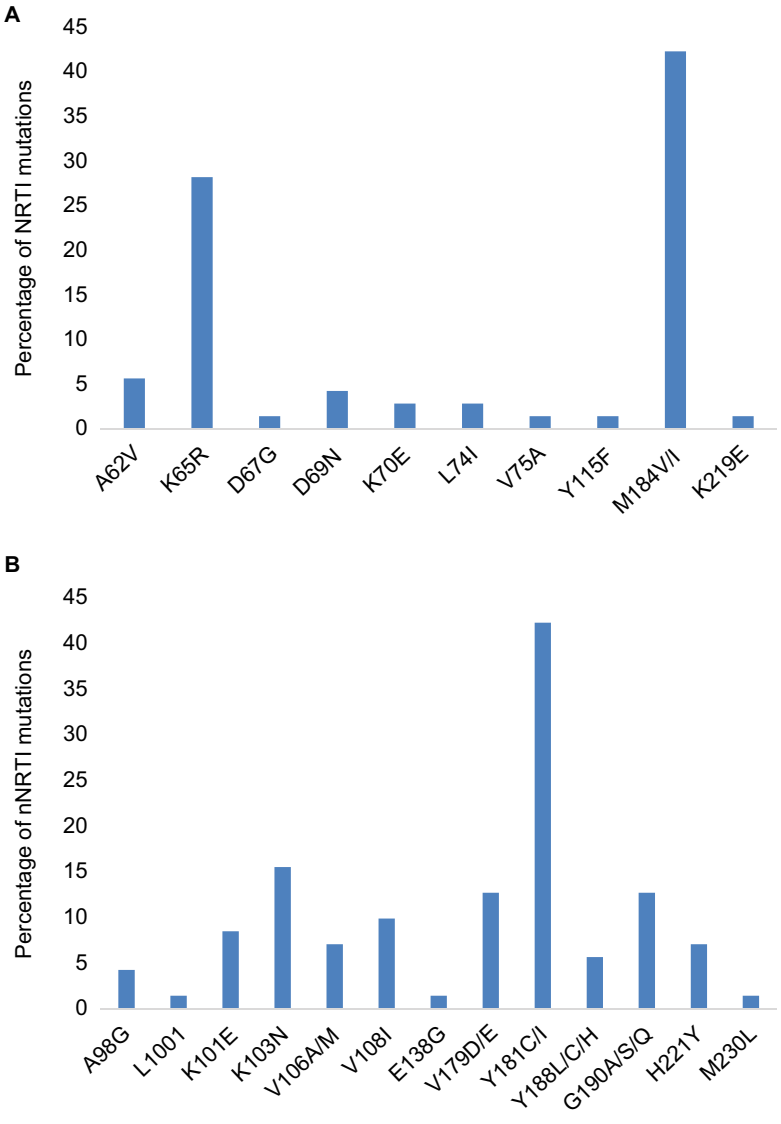

Figure 2 Resistance-associated mutations to **(A)** NRTIs and **(B)** nNRTIs in patients experiencing virological failure with nNRTI-containing first-line regimens.
Abbreviations: nNRTI, non-nucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor.

61.5%) (Figure 3). Of the 43 patients who received 2 NRTIs plus nevirapine, 19 (44.2%) had resistance to K65R and nNRTI with or without M184V/I. Of the 26 patients who received 2 NRTIs plus efavirenz, only 1 patient (3.8%) had resistance to K65R and nNRTIs (Figure 3).

Associated factors with emergent RAMs

As K65R, Y181C/I, and K103N were the most common RAMs in our patients receiving nNRTI-containing regimens with virological failure, univariate and multivariate logistic regression analyses were performed to identify the factors associated with these emergent RAMs. For the patients experiencing virological failure with emergent K65R, regimens containing TDF/FTC or TDF plus lamivudine and nevirapine were more likely to be associated with emergence of K65R compared with other regimens (adjusted odds ratio [AOR] 6.02; 95% CI 1.36–27.03) (Table 2A). In multivariate analysis, use of regimens of TDF/FTC or TDF plus lamivudine and nevirapine was statistically significantly associated with emergence of Y181C/I (AOR 31.25; 95% CI 6.21–166.67) (Table 2B). The use of zidovudine/lamivudine plus efavirenz (AOR 38.46; 95% CI 2.11–1000) was statistically significantly associated with the presence of K103N mutation (Table 2C).

Second-line regimens and the virological outcome

For the individuals who experienced virological failure (n=71) with a mean follow-up of 124±53 days, second-line regimens were selected based on previous antiretroviral regimens used and the results of genotypic resistance testing. Zidovudine/lamivudine plus PI with or without ritonavir (n=37, 52.1%) and TDF/FTC or TDF plus lamivudine and PI with or without ritonavir (n=23, 32.4%) were the most commonly used second-line regimens, followed by raltegravir plus 2 NRTIs or boosted PI (n=6, 8.5%) and rilpivirine plus 2 NRTIs (n=4, 5.6%). At 24 weeks, 43 patients (60.6%) had suppressed PVL <50 copies/mL and 57 (80.3%) had PVL <200 copies/mL; and at 48 weeks, 62 patients (87.3%) had PVL <200 copies/mL.

Discussion

In this 3.5-year study period observational study, we found that, given the background rate of transmitted drug resistance to nNRTIs at 6–11.8% in Taiwan,[13,17] 6.2% of the patients experienced virological failure to nNRTI-containing regimens in the short-term follow-up, after excluding the patients who discontinued first-line nNRTI-containing regimens early owing to adverse effects and intolerance, as well as those without baseline resistance testing. Apart from the common emergent RAMs observed in patients with first-line treatment failure in previous studies, such as M184V/I (42.3%), Y181C (42.3%), and K103N (15.5%), 28.2% of our patients with virological failure harbored K65R mutation, which was 6 times more likely to occur in those individuals receiving regimens containing TDF/FTC or TDF plus lamivudine and nevirapine.

The rate of virological failure (6.2%) in our patients appears to be higher than that (2.2%) reported among the patients on nNRTI-containing regimens in the Swiss HIV Cohort Study.[18] Other than study population and design, the difference might be also related to the backbone NRTIs and the definition used for virological failure. A meta-analysis

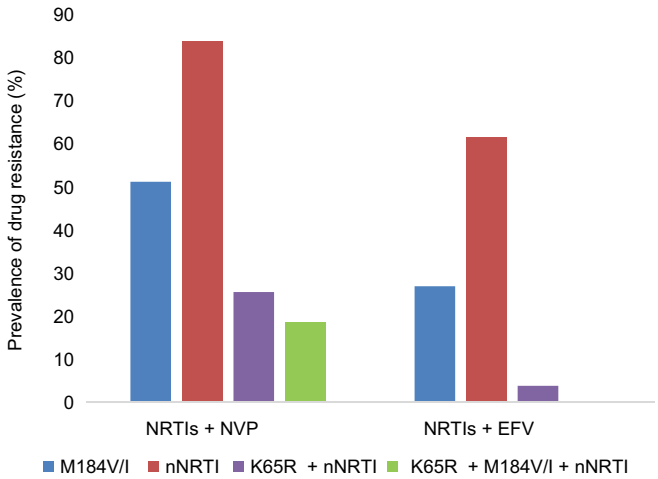

Figure 3 Prevalence of drug resistance by mutations and by first-line regimen received. The mutation patterns in patients with virological failure who received 2 NRTIs plus NVP (n=43) and 2 NRTIs plus EFV (n=26) are shown.
Abbreviations: EFV, efavirenz; NRTIs, nucleoside reverse-transcriptase inhibitors; nNRTI, non-nucleoside reverse-transcriptase inhibitor; NVP, nevirapine.

showed that a lower pill burden was associated with a lower risk of virological failure and drug resistance compared with multiple-tablet regimens.[19,20] In the Swiss HIV Cohort Study, TDF/FTC was the major NRTI backbone (47.5%). In our study, only 13.7% of the patients were prescribed TDF/FTC as the backbone because of the late introduction of the coformulated regimen. The failure rate in our patients taking TDF/FTC-containing regimens was 3.2% (5/156), while the rate was 8.4% (45/478) in those taking regimens containing TDF plus lamivudine (data not shown). Moreover, virological failure was defined as PVL ≥400 copies/mL after 180 days of uninterrupted treatment in the Swiss HIV Cohort Study, while

Table 2 Analysis of associated factors with emergent major RAMs of K65R among patients experiencing virological failure (**A**). Analysis of associated factors with emergent major RAMs of Y181C/I among patients experiencing virological failure (**B**). Analysis of associated factors with emergent major RAMs of K103N among patients experiencing virological failure (**C**).

A

Characteristics	K65R* (n=20)	No K65R* (n=51)	Univariate analysis OR (95% CI)	*p*	Multivariate analysis AOR (95% CI)	*p*
Age (years)	30.3±6.9	31.3±7.7	0.98 (0.91–1.06)	0.625		
Male gender	20 (100)	49 (96.1)	1.41 (1.21–1.64)	0.369		
MSM	19 (95.0)	45 (88.2)	2.53 (0.29–22.50)	0.39		
CD4 (cells/μL)	152±143	175±170	0.999 (0.996–1.002)	0.593		
CD4 <100 cells/μL	10 (50.0)	22 (43.1)	1.32 (0.47–3.72)	0.601	1.24 (0.35–4.41)	0.736
PVL (log_{10} copies/mL)	5.23±0.57	5.30±0.72	0.914 (0.423–1.972)	0.821		
PVL >5 log_{10} copies/mL	12 (60.0)	37 (72.5)	0.57 (0.19–1.68)	0.304	0.36 (0.91–1.42)	0.144
M184V/I	9 (45.0)	12 (43.1)	1.08 (0.38–3.05)	0.887	0.71 (0.20–2.57)	0.608
cART containing						
TDF	16 (80.0)	32 (62.7)	2.38 (0.69–8.16)	0.162		
AZT	4 (20.0)	16 (31.3)	0.55 (0.16–1.90)	0.338		
NVP	19 (95.0)	24 (47.1)	21.38 (2.66–171.9)	<0.001		
EFV	1 (5.0)	25 (49.0)	0.06 (0.007–0.44)	0.001		
cART regimen						
TDF/FTC or 3TC+NVP	15 (75%)	16 (31.4%)	6.56 (2.03–21.2)	0.001	6.02 (1.36–27.03)	0.018
TDF/FTC or 3TC+EFV	1 (5%)	16 (31.4%)	0.12 (0.14–0.94)	0.019	0.29 (0.03–3.18)	0.31
AZT/3TC+NVP	4 (20%)	7 (13.7%)	1.57 (0.41–6.09)	0.511		
AZT/3TC+EFV	0 (0%)	7 (13.7%)	0.69 (0.58–0.81)	0.081		

B

Characteristics	Y181C/I* (n=30)	No Y181C/I* (n=41)	Univariate analysis OR (95% CI)	*p*	Multivariate analysis AOR (95% CI)	*p*
Age (years)	30.8±7.0	31.1±7.9	0.995 (0.93–1.06)	0.874		
Male gender	30 (100)	39 (95.1)	1.77 (1.44–2.18)	0.22		
MSM	27 (90.0)	37 (72.5)	1.02 (0.41–2.50)	0.973		
CD4 (cells/μL)	137±122	192±184	0.998 (0.995–1.001)	0.162		
CD4 <100 cells/μL	16 (53.3)	16 (39.0)	1.28 (0.84–1.95)	0.231	1.19 (0.27–5.32)	0.842
PVL (log_{10} copies/mL)	5.35±0.51	5.19±0.78	1.42 (0.69–2.92)	0.346		
PVL >5 log_{10} copies/mL	23 (76.7)	26 (63.4)	1.29 (0.87–1.90)	0.233	1.06 (0.21–5.41)	0.944
M184V/I	18 (60.0)	13 (31.7)	1.67 (1.05–2.65)	0.018	2.14 (0.48–9.62)	0.32
cART containing						
TDF	27 (90.0)	21 (51.2)	1.99 (1.39–2.84)	0.001		
AZT	3 (10.0)	17 (41.5)	0.55 (0.39–0.78)	0.004		
NVP	29 (96.7)	14 (34.1)	2.96 (1.92–4.58)	<0.001		
EFV	1 (3.3)	25 (61.0)	0.37 (0.25–0.5)	<0.001		
cART regimen						
TDF/FTC or 3TC+NVP	26 (86.7)	5 (12.2)	5.58 (2.48–12.54)	<0.001	31.25 (6.21–166.67)	<0.001
TDF/FTC or 3TC+EFV	1 (3.3)	16 (39.0)	0.49 (0.36–0.67)	<0.001	0.44 (0.04–5.15)	0.512
AZT/3TC+NVP	3 (10.0)	8 (19.5)	0.76 (0.49–1.16)	0.274		
AZT/3TC+EFV	0 (0)	7 (17.1)	0.53 (0.42–0.67)	0.017		

C

Characteristics	K103N* (n=11)	No K103N* (n=60)	Univariate analysis OR (95% CI)	p	Multivariate analysis AOR (95% CI)	p
Age (years)	31.2±4.5	31.0±7.9	1.004 (0.922–1.093)	0.931		
Male gender	11 (100)	60 (98.4)	1.19 (1.07–1.32)	0.539		
MSM	10 (90.9)	54 (90.0)	1.02 (0.74–1.40)	0.926		
CD4 (cells/μL)	153±152	172±165	0.999 (0.995–1.003)	0.72		
CD4 <100 cells/μL	6 (54.5)	26 (43.3)	1.07 (0.87–1.32)	0.492	3.61 (0.54–24.39)	0.187
PVL (log_{10} copies/mL)	5.34±0.82	5.24±0.65	1.24 (0.47–3.27)	0.67		
PVL >5 log_{10} copies/mL	7 (63.6)	42 (70.0)	0.96 (0.76–1.20)	0.675	0.7 (0.12–4.22)	0.7
M184V/I	5 (45.5)	26 (43.3)	1.01 (0.83–1.24)	0.896	3.45 (0.54–22.22)	0.191
cART containing						
TDF	6 (54.5)	42 (70.0)	0.89 (0.70–1.14)	0.314		
AZT	4 (36.4)	16 (26.7)	1.08 (0.84–1.38)	0.511		
NVP	1 (9.1)	42 (70.0)	0.66 (0.50–0.87)	<0.001		
EFV	10 (90.9)	16 (26.7)	1.59 (1.17–2.16)	<0.001		
cART regimen						
TDF/FTC or 3TC+NVP	1 (9.1)	30 (50.0)	0.78 (0.64–0.94)	0.012	0.28 (0.01–5.49)	0.4
TDF/FTC or 3TC+EFV	5 (45.5)	12 (20.0)	1.26 (0.91–1.74)	0.069	6.49 (0.53–76.92)	0.142
AZT/3TC+NVP	0 (0)	11 (18.3)	0.82 (0.72–0.92)	0.122		
AZT/3TC+EFV	4 (36.4)	3 (5.0)	2.08 (0.89–4.91)	0.001	38.46 (2.11–1000)	0.014

Note: *Data are shown as n (%) or mean±SD.

Abbreviations: 3TC, lamivudine; AOR, adjusted odds ratio; AZT, zidovudine; cART, combination antiretroviral therapy; EFV, efavirenz; FTC, emtricitabine; MSM, men who have sex with men; NVP, nevirapine; PVL, plasma viral load; RAM, resistance-associated mutation; TDF, tenofovir disoproxil fumarate.

we defined virological failure as HIV-1 PVL ≥200 copies/mL after 6 months of cART initiation.

Antiviral potency may be different among the three different nNRTIs used in this study. Efavirenz has been shown to have more potent binding affinity to the reverse transcriptase.[21,22] The difference in clinical effectiveness was also demonstrated in clinical observational studies. In the Dutch ATHENA cohort, virological failure was noted in 10.8% of the patients who were prescribed TDF plus lamivudine and efavirenz, and 27% of those prescribed TDF plus lamivudine and nevirapine.[23] The results of our study are in line with those of the Dutch Athena cohort because the higher virological failure (14.8%) occurred among the patients who was prescribed nevirapine-containing regimens, especially for those with baseline PVL >5 log_{10} copies/mL (25.0%).

The WHO has recommended TDF to replace thymidine analogs, such as zidovudine and stavudine, as part of the NRTI backbone in the first-line regimens. Therefore, the high prevalence of K65R mutation in our patients who failed the first-line regimen could provide some information on the factors associated with the emergence of RAMs to TDF. First, the coadministered antiretrovirals in the first-line regimens could influence the emergence of K65R. The prevalence of K65R mutation was significantly higher in our patients receiving 2 NRTIs plus nevirapine than that in those receiving efavirenz-containing regimens (44.2% vs 3.8%, $p<0.001$), with an AOR of 6.02 (Figure 3; Table 2), an observation similar to that reported by the TenoRes Study Group.[24] Nevertheless, we could not demonstrate the benefit of combination with FTC compared to lamivudine in the emergent resistance to TDF owing to the limited number of patients who received TDF/FTC, nor could we examine the potential impact of the coformulated NRTIs. Second, in our patients with virological failure with resistance to TDF, only 16.7% (8/48) had resistance to both coadministered antiretrovirals (Figure 3), which was significantly different from the proportion (65%) observed in the TenoRes Study Group.[24] In our study, single K65R mutation and resistance to TDF and coadministered drugs were only observed in patients receiving 2 NRTIs plus nevirapine (Figure 3). Because of the small sample size, we could not determine the factors that might contribute to the differences. Finally, in our study, the contribution of low baseline CD4 count (<100 cells/μL) and high PVL (>5 log_{10} copies/mL) to the emergence of resistance to TDF was not observed (Table 2). The average PVL at treatment failure was 4.42±0.92 log_{10} copies/mL and 4.91±0.68 log_{10} copies/mL in patients with K65R mutations (n=12) and those with K65R/M184V mutations (n=8), respectively.

The WHO treatment guidelines recommend that second-line therapy should consist of 2 NRTIs plus a ritonavir-boosted PI in those who fail to respond to the recommended first-line regimens. If failure occurs on a TDF plus lamivudine (or FTC)-based first-line regimen with emergent K65R, zidovudine plus lamivudine should be used as the NRTI

backbone in the second-line regimens before the genotypic resistance report is available. In contrast, for patients who fail to respond to a zidovudine plus lamivudine-based first-line regimen, TDF/FTC or TDF plus lamivudine is recommended.[25] In our cohort, 84.5% of the patients who experienced virological failure were changed to NRTIs plus PI with or without ritonavir, and 90.1% achieved viral suppression (<50 copies/mL) in 24 weeks. Poor adherence, however, may remain a major determinant of virological response in individuals on second-line cART,[26] because wild-type HIV strains were detected in 25.4% of the patients (n=18) despite virological failure.

There are several limitations to our study. First, information on adherence to cART was not available for the included patients, and, therefore, it is not possible to determine the confounding effect of adherence on the emergence of RAMs to NRTIs or nNRTIs. Second, TDF/FTC has been recommended for the first-line NRTI backbone since early 2016 in Taiwan; hence, we could not demonstrate in those who experienced virological failure that TDF plus lamivudine was associated with a higher odds of emergent resistance to TDF than TDF/FTC.[24] Third, about one-fourth of the patients (n=298, 26.2%) had to switch first-line cART because of adverse effects or intolerance, and emergent RAMs were not determined in this group. Since the majority of these patients (40.4%) received the first-line regimens containing zidovudine, its influence on the detection of RAMs or Q151M complex can be neglected.[27–29]

Conclusion

A substantial proportion of the patients discontinued first-line nNRTI-containing regimens owing to adverse effects, yet the rate of short-term virological failure to nNRTI-containing regimens remained low for patients who were able to tolerate the regimens in Taiwan. The most common RAMs detected in those with virological failure were related to exposure to TDF, lamivudine or FTC, nevirapine, and efavirenz. Boosted PIs containing second-line regimens were used in 94.4% of those patients, with 60.6% being able to achieve virological responses.

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Disclosure

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Supplementary material

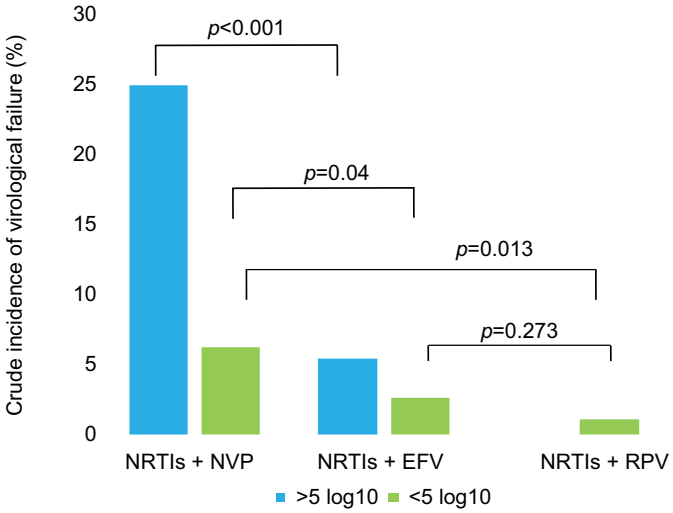

Figure S1 Crude incidence of virological failure to 2 NRTIs plus NVP, EFV, or RPV in patients with plasma HIV RNA load >5 $\log_{10}$ copies/mL or <5 $\log_{10}$ copies/mL at baseline.
Abbreviations: EFV, efavirenz; NRTIs, nucleoside reverse-transcriptase inhibitors; NVP, nevirapine; RPV, rilpivirine.