J Antimicrob Chemother 2013; **68**: 419–423
doi:10.1093/jac/dks380 Advance Access publication 30 September 2012

Journal of Antimicrobial Chemotherapy

Decreasing population selection rates of resistance mutation K65R over time in HIV-1 patients receiving combination therapy including tenofovir

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Received 27 April 2012; returned 27 June 2012; revised 21 August 2012; accepted 25 August 2012

Objectives: The use of tenofovir is highly associated with the emergence of mutation K65R, which confers broad resistance to nucleoside/nucleotide analogue reverse transcriptase inhibitors (NRTIs), especially when tenofovir is combined with other NRTIs also selecting for K65R. Although recent HIV-1 treatment guidelines discouraging these combinations resulted in reduced K65R selection with tenofovir, updated information on the impact of currently recommended regimens on the population selection rate of K65R is presently lacking.

Methods: In this study, we evaluated changes over time in the selection rate of resistance mutation K65R in a large population of 2736 HIV-1-infected patients failing combination antiretroviral treatment between 2002 and 2010.

Results: The K65R resistance mutation was detected in 144 patients, a prevalence of 5.3%. A large majority of observed K65R cases were explained by the use of tenofovir, reflecting its wide use in clinical practice. However, changing patterns over time in NRTIs accompanying tenofovir resulted in a persistent decreasing probability of K65R selection by tenofovir-based therapy. The currently recommended NRTI combination tenofovir/emtricitabine was associated with a low probability of K65R emergence. For any given dual NRTI combination including tenofovir, higher selection rates of K65R were consistently observed with a non-nucleoside reverse transcriptase inhibitor than with a protease inhibitor as the third agent.

Discussion: Our finding of a stable time trend of K65R despite elevated use of tenofovir illustrates increased potency of current HIV-1 therapy including tenofovir.

Keywords: virology, trend, treatment, guidelines, epidemiology

Introduction

The lysine-to-arginine mutation at position 65 (K65R) in HIV-1 reverse transcriptase confers broad cross-resistance to nucleoside/nucleotide analogue reverse transcriptase inhibitors (NRTIs). K65R is primarily selected by tenofovir and to a lesser extent by abacavir and didanosine. An initial rise in K65R population prevalence was observed following the increased clinical use of tenofovir dating to 2001.[1,2] However, we previously reported a strong decline in K65R incidence by 2005 despite a continuously increasing number of patients treated and subsequently failing with tenofovir.[3] The highest rate of K65R selection was observed when tenofovir was co-administered with didanosine, and the K65R incidence trend was significantly correlated over time with the number of failures including this drug combination, which is no longer recommended. Although changes in HIV-1 treatment guidelines strongly reduced the probability of K65R selection, updated information on the impact of currently recommended co-formulations of tenofovir/emtricitabine and abacavir/lamivudine on K65R selection at population level is presently lacking.[4,5] This information is imperative to identify drug combinations that can further minimize K65R emergence.

Methods

This study determined the population rate of the K65R resistance mutation and factors associated with observed changes in K65R selection by

Table 1. K65R selection by NRTI combinations including tenofovir, abacavir or didanosine is shown for 131 of 144 K65R patients.

	Failures	K65R	SR (%)	CR (%)	*P*	OR (CI)
TDF	1267	110	8.7	76.4	<0.001	5.6 (3.8–8.1)
TDF/ddI	101	28	27.7	19.4	<0.001	10.05 (6.33–16.04)
+NNRTI	47	20	42.6	13.9	<0.001	17.27 (9.67–30.88)
+PI	33	2	6.1	1.4	0.640	1.46 (0.35–6.15)
+3TC or FTC	12	5	41.7	3.5	<0.001	18.82 (5.91–60.15)
+3TC or FTC+PI	7	1	14.3	0.7	0.293	3.2 (0.40–25.80)
TDF/3TC	186	24	12.9	16.7	<0.001	3.79 (2.38–6.03)
+NNRTI	95	21	22.1	14.6	<0.001	7.67 (4.59–12.81)
+PI	80	1	1.3	0.7	0.264	0.27 (0.03–1.97)
TDF/FTC	561	43	7.7	29.9	<0.001	2.17 (1.46–3.16)
+NNRTI	250	37	14.8	25.7	<0.001	5.01 (3.27–7.54)
+PI	297	6	2.0	4.2	0.027	0.41 (0.15–0.93)
TDF/ABC	103	10	9.7	6.9	0.014	2.42 (1.11–4.76)
+NNRTI	11	6	54.5	4.2	<0.001	31.82 (7.98–133.40)
+PI	50	2	4.0	1.4	0.650	0.87 (0.10–3.38)
+3TC/FTC	2	0	0.0	0.0	0.760	0 (-)
+3TC/FTC+PI	28	1	3.6	0.7	0.872	0.75 (0.02–4.54)
+3TC/FTC+NNRTI	6	1	16.7	0.7	0.206	5.12 (0.10–46.27)
ddI/d4T (no TDF)	260	14	5.4	9.7	0.340	1.29 (0.67–2.28)
+NNRTI	109	9	8.3	6.3	0.043	2.18 (0.95–4.42)
+PI	97	3	3.1	2.1	0.800	0.67 (0.14–2.07)
ABC/3TC (no TDF)	138	7	5.1	4.9	0.522	1.21 (0.47–2.64)
+NNRTI	51	5	9.8	3.5	0.051	2.66 (0.81–6.79)
+PI	84	2	2.4	1.4	0.586	0.53 (0.06–2.01)

TDF, tenofovir; ddI, didanosine; 3TC, lamivudine; FTC, emtricitabine; ABC, abacavir; d4T, stavudine.
For each regimen, the number of therapy failures and K65R cases among the 2736 study patients are reported. The selection rate (SR), defined as the number of K65R cases selected by a regimen over the number of failures with this regimen, and the contribution rate (CR), defined as the number of K65R cases selected by a regimen over the total of 144 K65R cases observed, are calculated. *P* values are calculated to test for an association of the regimen with K65R presence (2×2 contingency table), and ORs with CIs indicate the magnitude of the association.

evaluating K65R emergence over time in a Portuguese HIV-infected drug-resistant cohort between 2002 and 2010.[3] We collected 3820 viral isolates that were obtained from genotypic resistance testing of 2736 HIV-1-infected patients failing combination antiretroviral treatment (cART) containing an NRTI. For each patient, the first occurrence of K65R was considered, excluding subsequent resistance and treatment information from the analysis. First, an association with drug regimens including tenofovir, didanosine or abacavir was identified by determining their distribution in the number of observed K65R cases. The analysis focused on dual NRTI regimens with a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) as a third agent, and with lamivudine or emtricitabine in case of tenofovir/abacavir and tenofovir/didanosine. Second, to capture their impact on K65R selection at the population level, we calculated for each regimen the selection rate, defined as the number of regimen failures with K65R over the number of regimen failures, and the failing rate, defined as the number of regimen failures over the total number of cART failures. Temporal changes in parameters were plotted using a sliding window approach with a window size of 12 months that shifted 1 month in time. The Mann–Whitney *U*-test was performed for statistical comparison between K65R presence and continuous variables and Fisher's exact test for categorical variables. ORs quantified direction and magnitude of the association. The level of statistical significance was set at 5%.

Results

The mutation K65R was detected in 144 patients (5.3%; CI 4.5%–6.2%), and a large majority of these patients (110 patients; 76%) received tenofovir at the time of detection (Table 1). Other NRTIs were lamivudine (34%), emtricitabine (32%), didanosine (31%), abacavir (14%), stavudine (12%) and zidovudine (8%). K65R patients were primarily treated with dual NRTI combinations (130 patients; 90%), which were more often accompanied by an NNRTI (84%) than by a PI (15%) ($P<0.01$), and less frequently with triple NRTI cART (7.6%). Table 1 shows the most prevalent NRTI combinations among K65R patients, accounting for 131 observations (91%). When only tenofovir regimens were considered, tenofovir was mostly administered as dual NRTI pair (99 patients; 90%) with an NNRTI (88%) or a PI (13%) ($P<0.01$) as the third agent, but the distribution of the accompanying NRTI

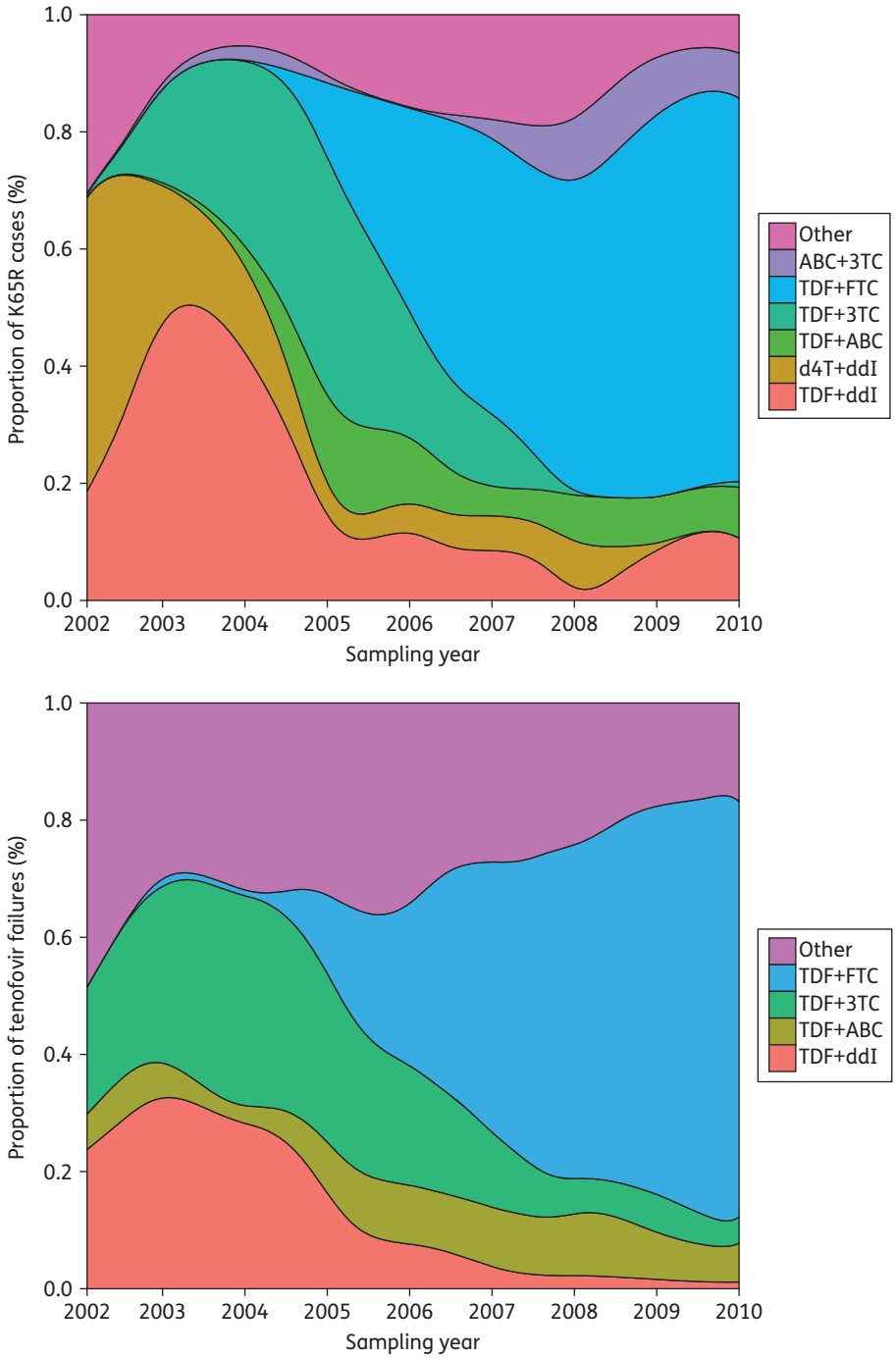

Figure 1. Two density plots of distributions are shown using a sliding window of 12 months with 1 month shifts in time. Calendar year shown spans the time period January to December of that calendar year. Top: different NRTI regimens present among the 144 K65R patients. Bottom: accompanying backbone NRTI among patients failing cART including tenofovir. These trends most likely illustrate changes in treatment guidelines. The predominant prevalence of emtricitabine resulted from the wide use of this effective regimen, recommended for first-line cART. ABC, abacavir; 3TC, lamivudine; TDF, tenofovir; FTC, emtricitabine; d4T, stavudine; ddI, didanosine. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

substantially changed over time (Figure 1). Initially, K65R selection was largely explained by the use of tenofovir/didanosine, accounting for 28 cases (19%, peaking at 55% in 2003), and tenofovir/lamivudine, accounting for 24 cases (17%, peaking at 53% in 2005). However, their contribution to observed K65R decreased dramatically over time, mainly in favour of tenofovir/emtricitabine. This regimen explained 43 K65R cases (30%) and contributed to more than 65% of K65R in 2010.

Furthermore, K65R was detected in 34 (24%) patients while receiving non-tenofovir cART, only three of whom had previous tenofovir experience. Stavudine/didanosine (14 patients; 41%) initially prevailed but was replaced by abacavir/lamivudine (seven patients; 21%) in more recent years. No time trends were observed in NNRTI versus PI distributions.

Next, we calculated the impact of these drug combinations on K65R selection at population level and the prevalence among therapy failures (Table 1). Tenofovir use was high (41%) among the 2736 patients, primarily as part of an NRTI pair (81%) and more often with a PI (65%) than with an NNRTI (35%) as the third agent. The lowest selection rates were observed for tenofovir combined with emtricitabine or abacavir, while non-tenofovir regimens displayed comparable selection rates. Selection rates of dual NRTI regimens were higher with the inclusion of an NNRTI (8.1%) than with a PI (1.2%) ($P<0.01$). This discrepancy between NNRTIs (18.1%) and PIs (2.2%) ($P<0.01$) strongly increased when only dual NRTI regimens including tenofovir were considered. In line with the recommended use of tenofovir for first-line cART,[4] the proportion of failing therapies including tenofovir continuously increased over time. The failing rate of tenofovir-including regimens changed from 16.2% in 2002–04, through 38.1% in 2005–07 to 59.9% in 2008–10 (Figure S1, available as Supplementary data at *JAC* Online). However, the previously observed fall in K65R incidence in 2005 was followed by a fluctuating but stable incidence trend (Figure S2, available as Supplementary data at *JAC* Online), indicating that, over time, the tenofovir-based cART selected less for the K65R mutation. The resulting selection rate of tenofovir-including regimens was 15.8% in 2002–04, 8.8% in 2005–07 and 5.7% in 2008–10 (Figure S1, available as Supplementary data at *JAC* Online). When the number of K65R cases was normalized for the observed time on tenofovir therapy, we observed a similar reduction in selection by tenofovir of, respectively, 17.7, 6.2 and 2.9 cases per 100 patient-years for the three time periods. Among therapy failures, tenofovir was in recent years increasingly co-administered in regimens displaying lower K65R selection rates, more specifically tenofovir/emtricitabine, thereby replacing regimens with high selection rates, in particular combinations of tenofovir with didanosine or lamivudine (Table 1 and Figure 1).

Finally, observed changing patterns in regimens also affected characteristics of K65R patients. Resistance mutations in reverse transcriptase were previously associated with the presence of K65R.[3] As tenofovir was increasingly administered in the presence of emtricitabine (47%) or lamivudine (27%), co-occurrence of K65R with mutation M184V increased up to 77% in 2010. Nucleoside analogue mutations (A62V, F77L, F116Y or Q151M) in K65R patients prevailed at a low level (21%) and decreased with time. NNRTI mutations (Y181C or K103N) occurred in 67% of K65R cases with no clear time trends (data not shown). Zidovudine was rarely included among tenofovir regimens (15%) but this increased over time, reaching a maximum of 22% in 2008. Consistent with the antagonistic effect between K65R and thymidine analogue mutations (TAMs),[3,6,7] K65R incidence inversely correlated with the trend in zidovudine accompanying tenofovir ($P=0.039$, $r=-0.27$). Although our dataset displayed overall decreasing prevalence of zidovudine use or TAMs and mainly first-line use of tenofovir, K65R occurred equally often in first- (36%) or second-line (34%) treatment, with no clear trends over time (data not shown). Regression analyses indicated an increase of 49 days in time on therapy before K65R detection with every additional calendar year ($P=0.002$). With respect to viral genomic diversity,[8] patients were predominantly infected with subtypes B (34.5%) and G (36%) showing equal tenofovir experience (50%). K65R prevalence did not differ between subtype B (2.9%) and subtype G (4.1%) ($P=0.24$).

Discussion

Decreasing population trends of drug resistance in antiretroviral-experienced HIV-1 patients have been attributed to more effective and better tolerated cART.[9,10] Antiretroviral resistance was primarily evaluated at the level of the drug class, but information on population and temporal dynamics of individual drug resistance mutations is warranted as new antiretrovirals enter clinical practice and new regimens become recommended. We have previously reported the existence of strong combination-dependent effects on K65R selection due to inferior tenofovir regimens. In this study, we evaluated population selection rates of K65R by currently recommended drug regimens in a large antiretroviral-experienced patient population. K65R was highly associated with the use of tenofovir, in agreement with our previous analysis.[3] Over time, K65R incidence remained relatively stable despite elevated tenofovir and abacavir use. Our data suggest that this discrepancy is largely explained by a strong and persistent decline in K65R selection rate of tenofovir due to changed treatment guidelines and the availability of tenofovir in co-formulation with emtricitabine. Although tenofovir/emtricitabine selected for most cases, this regimen displayed one of the lowest K65R selection rates and was predominant among therapy failures due to its wide use in current cART. Another regimen with low selection rates for K65R was abacavir/lamivudine, but increased use in recent years resulted in several K65R-related failures in patients naive to tenofovir. Remarkably, the magnitude of K65R selection was significantly higher with the inclusion of an NNRTI than with a PI. In conclusion, our findings illustrate increased potency of current tenofovir regimens for HIV-1 treatment.

Acknowledgements

The analysis described in this manuscript has been presented at the Tenth European Meeting on HIV and Hepatitis—Treatment Strategies and Antiviral Drug Resistance, Barcelona, Spain, 2012 (Abstract P49).

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Kamal Mansinho, Ana Cláudia Miranda, Isabel Aldir, Fernando Ventura, Jaime Nina, Fernando Borges, Emília Valadas, Manuela Doroana, Francisco Antunes, Maria João Aleixo, Maria João Águas, Júlio Botas, Teresa Branco, José Vera, Inês Vaz Pinto, José Poças, Joana Sá, Luis Duque, António Diniz, Ana Mineiro, Flora Gomes, Carlos Santos, Domitília Faria, Paula Fonseca, Paula Proença, Luís Tavares, Cristina Guerreiro, Jorge Narciso, Telo Faria, Eugénio Teófilo, Sofia Pinheiro, Isabel Germano, Umbelina Caixas, Nancy Faria, Ana Paula Reis, Margarida Bentes Jesus, Graça Amaro, Fausto Roxo, Ricardo Abreu and Isabel Neves.

Funding

This work was supported by the Interuniversity Attraction Poles Programme, Belgian State, Belgian Science Policy (IAP-VI P6/41), the European Community's Seventh Framework Programme (FP7/ 2007-2013) under the project 'Collaborative HIV and Anti-HIV Drug Resistance Network' (CHAIN, grant 223131) and the Fonds voor Wetenschappelijk Onderzoek Vlaanderen (grant G.0611.09N). K. T. received funding from Research Fund (PDM) of the KU Leuven and from the Fonds voor Wetenschappelijk Onderzoek (FWO) Flanders. J. S. received funding from Fonds voor Wetenschappelijk Onderzoek (FWO) Flanders. A. B. A. was supported by a post-doc fellowship by the Fundação para a Ciência e Tecnologia (SFRH / BPD / 65605 / 2009).

Transparency declarations

None to declare.

Supplementary data

Figures S1 and S2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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