

Factors Associated with the Emergence of K65R in Patients with HIV-1 Infection Treated with Combination Antiretroviral Therapy Containing Tenofovir

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Background. The human immunodeficiency virus type 1 reverse-transcriptase mutation K65R is a single-point mutation that has become more frequent after increased use of tenofovir disoproxil fumarate (TDF). We aimed to identify predictors for the emergence of K65R, using clinical data and genotypic resistance tests from the Swiss HIV Cohort Study.

Methods. A total of 222 patients with genotypic resistance tests performed while receiving treatment with TDF-containing regimens were stratified by detectability of K65R (K65R group, 42 patients; undetected K65R group, 180 patients). Patient characteristics at start of that treatment were analyzed.

Results. In an adjusted logistic regression, TDF treatment with nonnucleoside reverse-transcriptase inhibitors and/or didanosine was associated with the emergence of K65R, whereas the presence of any of the thymidine analogue mutations D67N, K70R, T215F, or K219E/Q was protective. The previously undescribed mutational pattern K65R/G190S/Y181C was observed in 6 of 21 patients treated with efavirenz and TDF. Salvage therapy after TDF treatment was started for 36 patients with K65R and for 118 patients from the wild-type group. Proportions of patients attaining human immunodeficiency virus type 1 loads <50 copies/mL after 24 weeks of continuous treatment were similar for the K65R group (44.1%; 95% confidence interval, 27.2%–62.1%) and the wild-type group (51.9%; 95% confidence interval, 42.0%–61.6%).

Conclusions. In settings where thymidine analogue mutations are less likely to be present, such as at start of first-line therapy or after extended treatment interruptions, combinations of TDF with other K65R-inducing components or with efavirenz or nevirapine may carry an enhanced risk of the emergence of K65R. The finding of a distinct mutational pattern selected by treatment with TDF and efavirenz suggests a potential fitness interaction between K65R and nonnucleoside reverse-transcriptase inhibitor-induced mutations.

The nucleoside reverse-transcriptase inhibitor (NRTI) tenofovir disoproxil fumarate (TDF) has become an important component of HIV combination therapy in

Switzerland because of its potency and once-daily dosing [1, 2]. However, emergence of resistance and viral breakthrough can occur quickly, such as when TDF is used in combination with didanosine (ddI) and efavirenz (EFV) [3–5] or with abacavir (ABC) and lamivudine (3TC) [6]. The key mutation for resistance

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against TDF is a lysine→arginine switch at position 65 in the reverse-transcriptase (RT) gene (i.e., K65R), which requires only 1 nucleotide base change [7, 8]. But contrary to other single-point mutations inducing HIV drug resistance, such as the RT mutation M184V, the prevalence of K65R in TDF-exposed individuals is limited, rarely >2%, despite the widespread use of TDF and other drugs, such as ABC and ddI [9, 10], that also select for K65R. Some increases in the prevalence of K65R may, however, have occurred in recent years [11, 12].

Thymidine analogue mutations (TAMs) selected by zidovudine or stavudine counteract the selection of the K65R mutation, as shown both in vitro [13] and in patients [10, 12, 14, 15]. Parikh et al. [16] elucidated the biochemical mechanisms and further demonstrated that TAMs and K65R do not appear on the same viral genome because of competing mutational pathways [17]. In contrast, inclusion of TDF in first-line therapy [2, 18, 19] or combination therapy with TDF and ddI [14, 20] promotes the emergence of K65R. Our aim was to confirm and extend current knowledge about baseline predictors for the K65R mutation and to identify mutational correlates.

METHODS

Data and patient selection. Our analysis included clinical and genotypic data collected until July 2007. The Swiss HIV Cohort Study (SHCS) is a nationwide, clinic-based cohort study with continuous enrollment and semiannual study visits [21]. The SHCS has been approved by ethical committees of all participating institutions, and written informed consent was obtained from participants. The SHCS resistance database contains all genotypic HIV resistance tests performed by the 4 authorized laboratories in Switzerland, stored in SmartGene's (Zug, Switzerland) Integrated Database Network System (IDNS, version 3.4.0) [22].

The database was screened for resistance tests performed from January 2002 through July 2007 for patients receiving treatment with TDF or ≥ 30 days after end of treatment. Because tests were obtained under various circumstances (e.g., at therapy initiation or in salvage settings), we further restricted selection to reduce confounding. First, we excluded samples from patients who had previously experienced a virological failure during treatment with TDF, ABC, or ddI without resistance testing, because the K65R mutation may have already emerged in those patients. Moreover, we included only resistance tests that had been performed after ample exposure to TDF, to allow for selection of the K65R mutation, which can occur as early as after 12 weeks of treatment [3, 6, 23]. Thus, we considered only tests that were performed after ≥ 90 days of continuous therapy with TDF or, in cases in which patients already had exposure to TDF, tests done after 30 days of continuous treatment with the current regimen and ≥ 90 days of

prior cumulative treatment with TDF. Only the first test per patient fulfilling all inclusion criteria was considered.

Throughout this project, virological failure was defined as an on-treatment HIV RNA level >500 copies/mL after ≥ 180 days of continuous treatment. Moreover, the study baseline was set at the start of the TDF-containing regimen for which a genotypic test was available, which did not necessarily correspond with the initiation of TDF.

Furthermore, we retrieved all available resistance tests conducted before the study baseline for included patients. The resistance database was complemented by retrospective sequencing of the virus from frozen plasma samples in the SHCS repository (full protease gene and codons 29–225 of the RT gene) [24]. For this, plasma specimens with a viral load >250 copies/mL were selected according to a predefined algorithm. Initially, we searched for specimens obtained while the participant was receiving treatment with TDF, ddI, or ABC. If none were available, we further considered plasma samples taken near the time of the latest virological failure events before the study baseline. For the remaining patients, we obtained pretreatment specimens.

Analysis. Patients were grouped according to the presence or absence of the K65R mutation. With use of the Mann-Whitney *U* test for continuous variables and Fisher's exact test for categorical variables, as well as univariable and multivariable logistic regression models, the following factors at the start of the TDF treatment were compared between the 2 groups: sociodemographic characteristics; presence of TAMs, M184V, protease inhibitor (PI) mutations, or nonnucleoside reverse-transcriptase inhibitor in (NNRTI) mutations; HIV-1 subtype; previous exposure to ddI, ABC, or TDF; number of previous regimens; number of previous virological failures; and current treatment with ddI, ABC, NNRTI, PI, or thymidine analogues. In a secondary analysis, we further included viral factors potentially linked to the presence or absence of TAMs (RT mutations 214L and 83K) [25–27].

Associations of K65R with other RT mutations from on-treatment tests were assessed using Fisher's exact test, with adjustments for multiple testing (0.05 false-discovery rate, by the Benjamini-Hochberg method) [28]. Mutations selected for analysis were based on the 2006 International AIDS Society–USA drug mutation list [29]. TAMs were stratified into TAMs group 1 (M41L, L210W, and T215Y) and TAMs group 2 (D67N, K70R, T215F, K219E, and K219Q).

We compared treatment response to the first therapy (after treatment with TDF) between the K65R and the wild-type groups by calculating the group-wise proportion of individuals attaining an HIV RNA level <50 copies/mL at week 12 or week 24. If such salvage treatment lasted <12 weeks, the patient was included in the week 12 analysis but was excluded from the week 24 analysis.

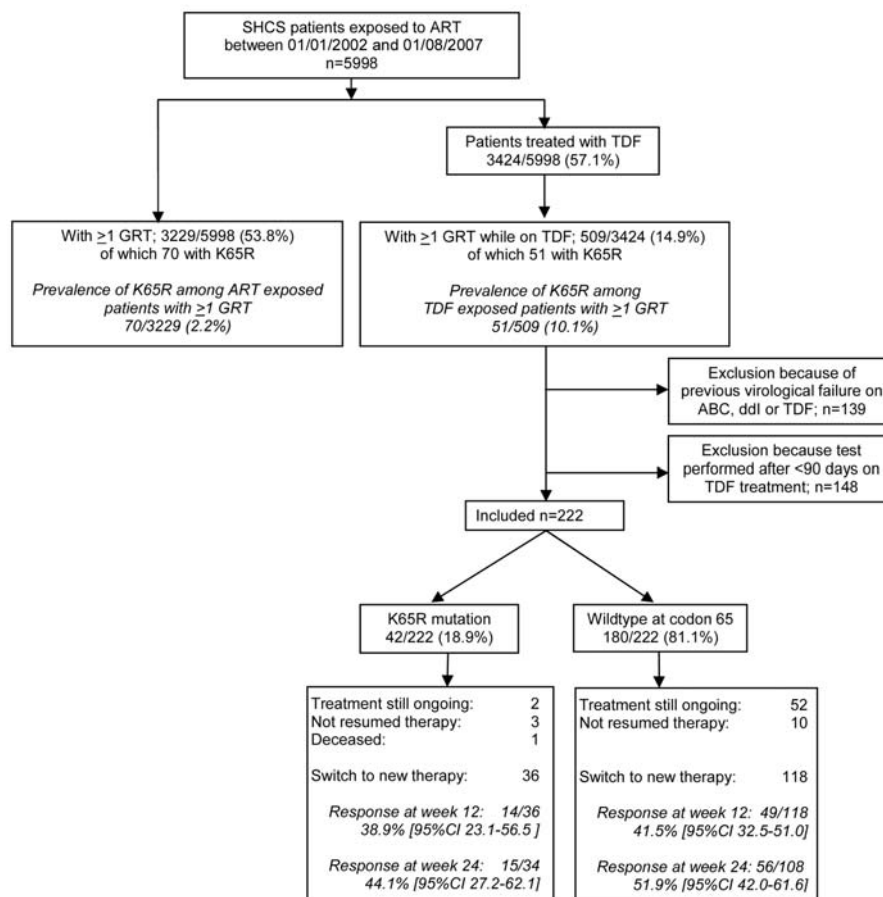


Figure 1. Flow chart of patient selection and calculation of prevalence of K65R. ABC, abacavir; ART, antiretroviral therapy; GRT, genotypic resistance test, TDF, tenofovir; ddl, didanosine.

Statistical analyses were performed with Stata 10 SE software (StataCorp). All tests of significance were 2 sided, and P values $<.05$ were considered to be statistically significant.

RESULTS

Prevalence of K65R. By July 2007, the SHCS drug resistance database contained samples from 70 patients with the K65R mutation, corresponding to a cumulative prevalence of 2.2% among all SHCS participants with at least 1 genotypic resistance test (figure 1). We found no time trend for the prevalence of K65R for the period 2002–2007 ($P = .154$, by Cochran-Armitage test; data not shown), although we noted an increase in prevalence, from 0.7% in 2002 to 2.0% in 2003, that coincided with the registration of TDF in Switzerland. Among patients with a resistance test performed on TDF, the prevalence of K65R was 10.1%.

Clinical and genotypic correlates at baseline with K65R.

In this analysis, we included 222 patients (42 in the K65R group and 180 in the wild-type group). For 32 (14.4%) of those 222 patients, the treatment under consideration was their first antiretroviral therapy (table 1). A total of 71 patients (32.0%) had

already been exposed to TDF during a previous treatment period without virological failure (median exposure time, 5.7 months [interquartile range, 2.9–11.3 months]). Genotypic resistance tests performed before the start of the TDF-containing regimen were available for 186 of 222 patients (in the K65R group, 36 [85.7%]; in the wild-type group, 152 [84.4%]). No K65R mutation was detected in those samples.

Among 36 patients in the K65R group with a genotypic resistance test before the start of TDF, 1 (2.8%) harbored viruses with TAMs group 2, compared with 30 (19.7%) in the wild-type group (table 1). No such difference was observed for TAMs group 1, which were detected in 4 patients (11.1%) from the K65R group and 25 patients (16.4%) from the wild-type group. Moreover, patients of the K65R group were more frequently receiving first-line therapy (28.6%) than were patients in the wild-type group (11.1%), and a higher proportion was receiving combination therapy containing ddI (59.5% vs. 37.8% in wild-type group). Of note, no instance of K65R was observed in 25 patients who received zidovudine or stavudine with TDF. Therapies are detailed in table 2. We identified strong associations of K65R with the additional drug class included in combination

Table 1. Characteristics at the start of the tenofovir-containing regimen (baseline).

Characteristic	Mutation type		P
	K65R (n = 42)	Wild type (n = 180)	
Female sex	16 (38.1)	58 (32.2)	.472
Age, median years (IQR)	40.5 (37–47)	41 (37–46.5)	.759
Mode of HIV acquisition			
Heterosexual intercourse	16 (38.1)	74 (41.1)	.871
Injection drug use	10 (23.8)	40 (22.2)	
Male homosexual intercourse	14 (33.3)	61 (33.9)	
Other	2 (4.8)	5 (2.8)	
Ethnicity			
White	33 (78.6)	137 (76.1)	.656
Black	6 (14.3)	34 (18.9)	
Other	3 (7.1)	9 (5)	
HIV subtype			
B	33 (78.6)	134 (74.4)	.124
CRF01_AE	2 (4.8)	1 (0.6)	
C	2 (4.8)	8 (4.4)	
Other	5 (11.9)	37 (20.6)	
Nadir CD4 cell count, median (IQR) ^a	132 (60–241)	142 (50–220)	.980
Baseline CD4 cell count, median (IQR) ^a	191 (90–288)	266 (153–407)	.016
Baseline log ₁₀ HIV RNA level, median (IQR) ^a	4.6 (2.3–5.3)	3.8 (1.2–5.2)	.095
Previous CDC C event	30 (71.4)	108 (60)	.216
Baseline mutation			
Baseline test available	36 (85.7)	152 (84.4)	1.000
TAMs (any)	5 (13.9)	45 (29.6)	.061
TAMs group 1 ^b	4 (11.1) ^b	25 (16.4)	.608
TAMs group 2 ^c	1 (2.8) ^c	30 (19.7)	.011
NNRTI mutations	9 (25)	30 (19.7)	.497
PI mutations	4 (11.1)	31 (20.4)	.241
RT184V/I	11 (30.6)	54 (35.5)	.698
RT214L	7 (19.4)	36 (23.7)	.665
RT83K	10 (27.8)	31 (20.4)	.371
On first-line antiretroviral therapy	12 (28.6)	20 (11.1)	.007
Current treatment, combined with tenofovir			
NNRTI	38 (90.5)	56 (31.1)	.000
PI	0 (0)	108 (60)	.000
Didanosine	25 (59.5)	68 (37.8)	.014
Lamivudine or emtricitabine	17 (40.5)	99 (55)	.122
Abacavir	3 (7.1)	18 (10)	.772
Zidovudine or stavudine	0 (0)	25 (13.9)	.006
>95% Adherent ^d	13 (41.9)	82 (50)	.439
Treatment history ^e			
Previous regimens, median no. (IQR)	4.5 (3.0–7.0)	5.0 (3.0–7.0)	.794
Previous exposure to didanosine or abacavir	17 (56.7)	93 (58.1)	.882
Previous exposure to tenofovir	9 (30.0)	62 (38.8)	.363
Experienced previous virological failure(s)			
0 failure	16 (53.4)	81 (50.6)	.745
1 failure	10 (33.3)	56 (35.0)	
≥2 failures	4 (13.3)	23 (14.4)	
Virological failure with treatment of zidovudine or stavudine	13 (43.3)	80 (50.0)	.503

(continued)

Table 1. (Continued.)

Characteristic	Mutation type		P
	K65R (n = 42)	Wild type (n = 180)	
Duration of previous exposure to zidovudine or stavudine, median years (IQR)	2.7 (1.1–5.3)	3.6 (1.0–6.3)	.364
Virological failures on NNRTI	2 (6.7)	15 (9.4)	.633
Virological failures on lamivudine	11 (36.7)	73 (45.6)	.365

NOTE. Data represent no. (%) of patients, unless otherwise indicated. CDC, Centers for Disease Control and Prevention; IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RT, reverse transcriptase; TAMs, thymidine analogue mutations.

^a Baseline laboratory parameters available for 34 and 179 of the K65R and the wild-type groups, respectively.

^b TAMs group 1, any RT gene mutation of the following: 41L, 210W, or 215Y; 3 of 4 present as mixture in K65R group.

^c TAMs group 2, any RT gene mutation of the following: 67N, 70R, 215F, 219E, or 219Q; present as mixture in K65R group.

^d Adherence measure available only for 31 and 164 of the K65R and the wild-type groups, respectively.

^e Comparison only for patients not receiving first-line therapy (30 in the K65R group and 160 in the wild-type group).

therapy other than NRTIs. No instance of K65R emergence was observed for treatments including PI. Conversely, 91% of patients in the K65R group were receiving a combination therapy with NNRTIs. Accordingly, use of NNRTI arose as the most predictive factor associated with the emergence of K65R in a multivariable logistic regression analysis (OR, 23.6; 95% CI, 7.3–76.3) (table 3). Other associations observed in this model were treatment with first-line therapy (OR, 3.6; 95% CI, 1.1 to 12.2) or treatment with combination therapy containing ddI (OR, 3.6; 95% CI, 1.3–9.9). No association of K65R with HIV subtype C was observed [30, 31].

Other RT mutations associated with K65R. We performed 2 analyses for the identification of mutational associations of RT mutations with K65R. First, we considered only the 222

genotypic tests performed while patients were receiving treatment with TDF (cross-sectional approach); later, we also considered all preceding resistance tests, if available, assuming that all mutations ever detected before the start of the TDF-containing regimen would still be present at time of resistance testing during treatment with TDF (cumulative approach; data not shown). A comparison of these 2 approaches allowed us to draw conclusions about the viral evolution of HIV-1 within patients.

On the basis of unadjusted *P* values <.05 from the cross-sectional analysis, we identified 4 NNRTI mutations (L100I, K103N, G190S, and Y181C) that were more frequently observed in the K65R group and 3 TAMs (M41L, D67N, and T215Y) that were much rarer or absent in the K65R group (table 4).

Table 2. Antiretroviral therapy combinations with tenofovir.

Treatment combinations including tenofovir	No. (%) of patients			
	First-line regimens		Later regimens	
	K65R	Wild type	K65R	Wild type
Efavirenz and lamivudine-emtricitabine	5 (36)	9 (64)	2 (17)	10 (83)
Efavirenz and didanosine	0	0	13 (46)	15 (54)
Efavirenz and abacavir	0	0	1 (100)	0
Nevirapine and lamivudine-emtricitabine	4 (100)	0	3 (60)	2 (40)
Nevirapine and didanosine	3 (100)	0	7 (54)	6 (46)
Boosted atazanavir and lamivudine-emtricitabine	0	0	0	18 (100)
Lopinavir and lamivudine-emtricitabine	0	6 (100)	0	10 (100)
Lopinavir and didanosine	0	1 (100)	0	12 (100)
Other NNRTI and NRTI	0	0	0	7 (100)
Other boosted PI and NRTI	0	0	0	36 (100)
Other unboosted PI and NRTI	0	0	0	13 (100)
3-Class combination (PI, NNRTI, and NRTI)	0	0	0	4 (100)
Single-class NRTI	0	4 (100)	4 (17)	20 (83)
Other	0	0	0	7 (100)

NOTE. Percentages were calculated on the basis of the number of patients on a specific regimen, stratified by the 2 treatment groups (patients receiving first-line therapy and patients receiving later treatments). NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor.

Table 3. Factors associated with the presence of K65R in univariable and multivariable logistic regression analyses (n = 222).

Variable(s)	OR ^a (95% CI)	
	Univariable	Multivariable
Baseline mutations		
TAMs group 1 present ^b	0.63 (0.21–1.95)	1.11 (0.20–6.03)
No TAMs group 1 present	1 (Reference)	1 (Reference)
No baseline resistance test	0.85 (0.32–2.23)	0.79 (0.19–3.33)
TAMs group 2 present ^c	0.12 (0.02–0.92)	0.07 (0.01–0.64)
No TAMs group 1 present	1 (Reference)	1 (Reference)
No baseline resistance test	0.75 (0.29–1.96)	Not done ^d
NNRTI mutations present	1.36 (0.58–3.18)	...
No NNRTI mutations present	1 (Reference)	...
No baseline resistance test	0.97 (0.37–2.57)	...
PI mutations present	0.49 (0.16–1.48)	...
No PI mutations present	1 (Reference)	...
No baseline resistance test	0.81 (0.31–2.12)	...
Female sex	1.29 (0.64–2.60)	2.43 (0.71–8.33)
Age, years, per year increase	1.00 (0.97–1.04)	1.02 (0.97–1.07)
Mode of HIV acquisition		
Heterosexual contact	1 (Reference)	1 (Reference)
Injection drug use	1.16 (0.48–2.78)	3.08 (0.80–11.89)
Homosexual bisexual contact	1.06 (0.48–2.35)	2.30 (0.60–8.74)
Other	1.85 (0.33–10.40)	0.66 (0.06–7.25)
Previous CDC stage C event	1.67 (0.80–3.47)	2.87 (1.05–7.84)
Baseline CD4 cell count, per 10-cell increase	0.85 (0.71–1.02)	...
Baseline log ₁₀ HIV RNA level, per log increase	1.14 (0.97–1.35)	...
Ethnicity		
White	1 (Reference)	1 (Reference)
Black	0.73 (0.28–1.89)	0.96 (0.15–6.18)
Other	1.38 (0.35–5.40)	0.74 (0.08–7.12)
HIV subtype		
B	1 (Reference)	...
CRF01_AE	8.12 (0.71–92.29)	27.08 (0.57–1,282)
C	1.02 (0.21–5.01)	0.94 (0.09–9.45)
Other	0.55 (0.20–1.50)	0.64 (0.10–4.15)
Adherence		
<95%	1.38 (0.64–3.01)	...
≥95%	1 (Reference)	...
No information available	4.34 (1.65–11.39)	...
On first-line antiretroviral therapy	3.20 (1.42–7.23)	3.64 (1.08–12.24)
Current treatment, in combination with tenofovir		
Zidovudine or stavudine ^e	0.11 (0–0.62)	Not done
Lamivudine	0.56 (0.28–1.10)	...
Abacavir	0.69 (0.19–2.47)	...
Didanosine	2.42 (1.22–4.81)	3.62 (1.32–9.94)
NNRTI	21.04 (7.16–61.79)	23.59 (7.29–76.28)
PI ^e	0.01 (0–0.06)	Not done
Previous exposure to didanosine or abacavir	0.64 (0.32–1.26)	...
Previous exposure to tenofovir	0.52 (0.23–1.15)	...
Previous failure event(s)		
No previous virological failure	1 (Reference)	...
1 Previous virological failure	0.64 (0.29–1.42)	...
≥2 Previous virological failures	0.63 (0.20–1.96)	...

(continued)

Table 3. (Continued.)

Variable(s)	OR ^a (95% CI)	
	Univariable	Multivariable
Previous virological failure on lamivudine	0.52 (0.25–1.10)	...
Previous virological failure on NNRTI	0.55 (0.12–2.50)	...
Previous virological failure on zidovudine or stavudine	0.56 (0.27–1.15)	...

NOTE. CDC, Centers for Disease Control and Prevention; NNRTI, nonnucleoside reverse-transcriptase inhibitor; PI, protease inhibitor; RT, reverse transcriptase; TAMs, thymidine analogue mutations.

^a By logistic regression analysis.

^b TAMs group 1, any RT gene mutation of the following: 41L, 210W, or 215Y.

^c TAMs group 2, any RT gene mutation of the following: 67N, 70R, 215F, 219E, or 219Q.

^d Not included in multivariable model because of collinearity.

^e OR from exact logistic regression. For computational reasons, only univariable estimates could be obtained.

After adjustment for multiple testing, only T215Y, G190S, and Y181C reached statistical significance (table 4). The latter 2 mutations, together with K65R, were identified as a distinct mutational pattern in 6 patients treated with EFV and TDF. The cumulative approach confirmed that G190S and Y181C were not present at study baseline and must have been coselected with K65R (data not shown).

In a secondary analysis, we investigated associations of grouped TAMs with K65R (TAMs group 1 or TAMs group 2), again using the cross-sectional and the cumulative methods. In the cross-sectional analysis, TAMs group 1 were found in 2 patients from the K65R group and in 41 patients from the wild-type group. In contrast, the cumulative approach showed that 5 patients in the K65R and 44 patients in the wild-type group harbored viruses with TAMs group 1. TAMs group 2 were detected in 1 and 45 patients from the K65R and the wild-type group, respectively, with the cross-sectional method and 2 and 53 patients, respectively, with the cumulative approach. Thus, viruses of 4 patients with K65R had lost TAMs between the baseline sample and the detection of the K65R mutation (3 with TAMs group 1 and 1 with TAMs group 2). Three of these patients had extended treatment breaks, with a range of 1.5–4.8 years, before beginning the TDF-containing regimen. The fourth patient had a 1-year respite from therapy but resumed treatment with stavudine, ddI, and nevirapine and achieved viral suppression before switching to therapy with TDF. Taken together, virus populations these 4 patients demonstrated shifts toward wild-type status once selection pressure by antiretroviral drugs was removed.

Clinical outcomes of TDF-containing regimens. We further studied treatment outcomes of the TDF-containing regimens and of continuation of drug histories (figure 1). As of the database closure for this analysis, 168 patients had stopped the TDF-containing regimen. For 112 patients—that is, 33 patients (78.6%) from the K65R group and 79 patients (43.9%) from the wild-type group—immunologic or virologic failure was cited as the reason for stopping. Antiretroviral therapy–

related toxicities were reported as being the cause for stopping the TDF-containing regimen in 16 patients (8.9%) of the wild-type group, and 1 patient of the K65R group died while being treated with TDF. All other stop reasons (for 6 patients in the K65R group and for 33 patients in the wild-type group) were either unknown or not clearly specified.

In total, 154 patients switched to a new therapy (from the K65R group, 36 patients; from the wild-type group, 118 patients). In the K65R group, 31 patients switched to a PI, of whom 26 patients received a ritonavir boosted regimen and 23 had a regimen with a combination of zidovudine or stavudine. Moreover, 4 patients switched to a single-class NRTI therapy with ABC, and 1 patient continued with NNRTI treatment but replaced TDF with a different NRTI. At week twelve, 38.9% of the K65R group and 41.5% of the wild-type group showed a virological response to those new treatments ($P = .848$, by Fisher's exact test) (figure 1). At week 24, virological response was 44.1% for patients with K65R and 51.9% in the wild-type group ($P = .556$, by Fisher's exact test). An intent-to-treat approach yielded similar results (data not shown). We repeated these analyses with logistic regression models adjusted for baseline HIV RNA level, the inclusion of enfuvirtide, and the number of active drugs in the new regimen with a genotypic sensitivity score <15, as calculated by the Stanford algorithm on the basis of cumulative drug resistance information [32]. We found no evidence that patients harboring viruses with the K65R mutation had a worse treatment outcome at week 12 (OR, 1.2; 95% CI, 0.50–2.70) and week 24 (OR, 0.92, 95% CI, 0.40–2.12), when compared with the wild-type group.

DISCUSSION

Of 222 patients receiving a TDF-containing antiretroviral treatment, combinations of TDF with NNRTIs and/or ddI were highly associated with the emergence of the K65R mutation. In contrast, not a single patient receiving TDF combined with PI or thymidine analogues harbored viruses with the K65R

Table 4. Reverse-transcriptase mutations associated with K65R in genotypic resistance tests performed on combination therapy with tenofovir.

Mutation	Proportion of patients with mutation (%)		<i>P</i> ^a	Critical <i>P</i> ^b	Significant
	K65R	Wild type			
NNRTI					
100I	6/40 (15)	5/137 (3.6)	.018	.009	
103N	16/40 (40)	31/137 (22.6)	.041	.014	
106M	2/40 (5)	2/137 (1.5)	.220	.027	
106A	1/40 (2.5)	0/137 (0)	.226	.029	
108I	3/40 (7.5)	7/137 (5.1)	.696	.042	
181C ^c	19/40 (47.5)	7/137 (5.1)	.000	.002	Yes
188L	1/40 (2.5)	4/137 (2.9)	1	.044	
188C	2/40 (5)	1/137 (0.7)	.128	.018	
188H	0/40 (0)	2/137 (1.5)	1	.045	
190A	6/40 (15)	8/137 (5.8)	.090	.015	
190S	8/40 (20)	2/137 (1.5)	<.001	.003	Yes
190E	1/40 (2.5)	1/137 (0.7)	.402	.030	
225H	1/40 (2.5)	1/137 (0.7)	.402	.032	
TAMs group 1					
41L	1/30 (3.3)	32/155 (20.6)	.020	.011	
210W	0/30 (0)	15/155 (9.7)	.136	.020	
215Y	0/30 (0)	29/155 (18.7)	.005	.006	Yes
Any	1/30 (3.3)	37/155 (23.9)	.012	.008	
TAMs group 2					
67N	1/30 (3.3)	31/155 (20)	.032	.012	
70R	1/30 (3.3)	20/155 (12.9)	.207	.023	
215F	0/30 (0)	12/155 (7.7)	.220	.024	
219Q	0/30 (0)	12/155 (7.7)	.220	.026	
219E	0/30 (0)	6/155 (3.9)	.592	.035	
Any	1/30 (3.3)	42/155 (27.1)	.004	.005	Yes
NRTI (other than TAMs)					
115F	2/42 (4.8)	1/180 (0.6)	.093	.017	
116Y	0/42 (0)	1/180 (0.6)	1	.047	
70E ^d	2/42 (4.8)	7/180 (3.9)	.680	.039	
151M	0/42 (0)	1/180 (0.6)	1	.048	
62V	2/42 (4.8)	2/180 (1.1)	.163	.021	
74V	2/42 (4.8)	7/180 (3.9)	.680	.041	
184V/I	14/40 (35)	59/168 (35.1)	1	.050	
Other (not drug-resistance related)					
214L	7/42 (16.7)	38/180 (21.1)	.671	.038	
68G	2/42 (4.8)	5/180 (2.8)	.619	.036	
83K	9/42 (21.4)	30/180 (16.7)	.501	.033	

NOTE. Percentages were calculated on the basis of the number of patients in each group who were ever exposed to the respective drug class (e.g., NNRTI, thymidine analogues, or NRTIs without thymidine analogues). NNRTI, non-nucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; RT, reverse transcriptase; TAMs, thymidine analogue mutations.

^a By Fisher's exact test.

^b Benjamini-Hochberg critical value, with the assumption of a false-discovery rate of 0.05.

^c For 6 patients treated with efavirenz, a distinct mutational pattern consisting of G190S and Y181C was observed, which always appeared in combination with K65R. Those patients are all infected with subtype B viruses.

^d In the 2 patients from the K65R group, K65R and K70E were present as mixtures.

mutation. The presence of 1 or several mutations of the TAMs group 2 (D67N, K70R, 215F, 219Q, and 219E) at the start of the TDF-containing regimen appeared to have a protective effect against the emergence of K65R. Among 4 patients who had lost TAMs because of extended therapy interruptions, we noted that K65R could still be selected, despite the likely presence of TAMs in minor viral populations. However, in none of these patients did the TAMs reemerge, which further supports the hypothesis that TAMs and the K65R mutation cannot exist on the same genome [17].

We further observed a previously undescribed pattern of NNRTI mutations (G190S and Y181C) and K65R in EFV-treated patients. Those NNRTI-specific mutations were not present at the start of combination therapy with TDF and EFV and must have been coselected with K65R.

Moreover, we investigated therapy success of the subsequent treatment among patients who had stopped the TDF-containing regimen and who switched to a new regimen. We did not observe statistically significant differences between the K65R group and the wild-type group in the proportion of patients with HIV RNA levels <50 copies/mL after 12 weeks and 24 weeks of continuous treatment. At week 24, approximately one-half of the patients reached plasma viremia levels <50 copies/mL, a result that is in line with or even better than salvage trials before tipranavir, darunavir, and raltegravir became widely available [33–37]. Taking due account of small sample size and short follow-up period, these results suggest that patients who harbor viruses with the K65R mutation are as likely to attain viral loads <50 copies/mL as are patients with K65WT.

This study provides support for a protective effect of TAMs—and in particular TAMs group 2—against the emergence of K65R. Thus, it is not surprising that patients who initiate their first antiretroviral therapy with combination therapy that includes TDF are at a higher risk of acquiring K65R if their antiretroviral therapy fails, given the fact that the prevalence of transmitted resistance and TAMs remains ~10% in Switzerland [38]. In contrast, patients with extended treatment histories and previous virological failure are more likely to harbor viruses carrying TAMs [V.v.W. and H.F.G., unpublished data]. Why were there no TAMs at study baseline in the pretreated patients from the K65R group? Extended treatment interruptions might be one answer, as case reviews from 4 patients indicated. Furthermore, we investigated viral factors (RT mutations 214L and 83K), which have recently been linked with the absence of TAMs in pretreated patients [25, 26], but the analysis results were not conclusive.

We noted that the additional drugs other than TDF that were included in combination therapy might play an important role in the emergence of K65R. Combinations of TDF and ddI appear to be problematic, because ddI and TDF share the same mutational pathway for selection of K65R.

Moreover, whereas K65R was absent in patients treated with PI, the use of EFV or nevirapine was highly associated with the emergence of K65R. This may be due to synergistic fitness effects with NNRTI-induced mutations on the reverse transcriptase, as our observation of the previously unreported K65R/G190S/Y181C mutational pattern in 6 EFV-treated patients suggests. Because G190S is associated with a high fitness cost relative to the wild type [39], K65R and/or Y181C may compensate for this. Such fitness synergies have been described between the NRTI mutation L74V and the NNRTI mutations K103N and L100I [40].

Alternatively, K65R may occur preferentially with treatment with EFV or nevirapine compared with PI regimens because only 1 mutation is required to confer full resistance against those NNRTIs, whereas PIs often retain residual activity against HIV-1 despite the presence of PI mutations. Thus, in combination therapy with TDF and NNRTI, insufficient intracellular concentrations of combination therapy with TDF can quickly lead to viral breakthrough and full NNRTI resistance, followed by the emergence of additional mutations [3, 23]. In line with this argument, patients with no previous virological failure who were treated with an NNRTI and TDF ($n = 53$) generally had viruses with more resistance mutations (median, 3 mutations; interquartile range, 1–4 mutations) than did patients who received therapy with a combination of TDF and a ritonavir-boosted PI ($n = 44$) (median, 0 mutations; interquartile range, 0–1 mutation; data not shown).

Because these data stem from a representative cohort study reflecting current clinical practice, we consider the conclusions to be clinically relevant. The study has limitations, however. We have compared patients with highly diverse treatment histories and who were selected with no randomization; therefore, we cannot exclude the possibility that there were unmeasured confounding factors. Patients receiving tenofovir had to have a genotypic drug resistance test available, implying a selection bias. However, during this study, genotypic resistance testing in patients with failing antiretroviral treatment was already clinically routine [33]. The time point of genotypic testing might also have confounded our results, in particular for the baseline resistance testing. Predefined stringent selection criteria likely have minimized the impact on confounding. We also noted no systematic effect of timing of resistance testing on our results (data not shown).

Our findings suggest that optimal future treatment regimens should avoid combining EFV or nevirapine with TDF and additional NRTI drugs that favor the selection of the K65R mutation, such as ddI or ABC. Furthermore, the highly protective effect of boosted PIs and the observed antagonistic effects of TAMs on the emergence of K65R suggest a potentially pivotal role of combining thymidine analogues, boosted PIs, and te-

nofovir in salvage situations. This strategy should be explored in prospective studies.

THE SWISS HIV COHORT STUDY

The members of the SHCS are M. Battegay, E. Bernasconi, J. Böni, H. C. Bucher, P. Bürgisser, A. Calmy, S. Cattacin, M. Cavassini, R. Dubs, M. Egger, L. Elzi, P. Erb, M. Fischer, M. Flepp, A. Fontana, P. Francioli (president of the SHCS, Centre Hospitalier Universitaire Vaudois, Lausanne), H. Furrer (chairman of the Clinical and Laboratory Committee), C. Fux, M. Gorgievski, H. Günthard (chairman of the Scientific Board), H. Hirsch, B. Hirschel, I. Hösli, C. Kahlert, L. Kaiser, U. Karrer, C. Kind, T. Klimkait, B. Ledergerber, G. Martinetti, B. Martinez, N. Müller, D. Nadal, M. Opravil, F. Paccaud, G. Pantaleo, A. Rauch, S. Regenass, M. Rickenbach (head of the Data Center), C. Rudin (chairman of the Mother & Child Substudy), P. Schmid, D. Schultze, J. Schüpbach, R. Speck, P. Taffé, P. Tarr, A. Telenti, A. Trkola, P. Vernazza, R. Weber, and S. Yerly.

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