International Cohort Analysis of the Antiviral Activities of Zidovudine and Tenofovir in the Presence of the K65R Mutation in Reverse Transcriptase

Philip M. Grant,1* Jonathan Taylor,1 Andrew B. Nevins,1 Vincent Calvez,2 Anne-Geneviève Marcelin,2 Marc Wirden,2 and Andrew R. Zolopa1

Stanford University, Palo Alto, California,1 and Department of Virology, Pitie-Salpetriere Hospital, INSERM U943, Paris, France2

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A K65R mutation in HIV-1 reverse transcriptase can occur with the failure of tenofovir-, didanosine-, abacavir-, and, in some cases, stavudine-containing regimens and leads to reduced phenotypic susceptibility to these drugs and hypersusceptibility to zidovudine, but its clinical impact is poorly described. We identified isolates with the K65R mutation within the Stanford Resistance Database and a French cohort for which subsequent treatment and virological response data were available. The partial genotypic susceptibility score (pGSS) was defined as the genotypic susceptibility score (GSS) excluding the salvage regimen’s nucleoside reverse transcriptase inhibitor (NRTI) component. A three-part virologic response variable was defined (e.g., complete virologic response, partial virologic response, and no virologic response). Univariate, multivariate, and bootstrap analyses evaluated factors associated with the virologic response, focusing on the contributions of zidovudine and tenofovir. Seventy-one of 130 patients (55%) achieved a complete virologic response (defined as an HIV RNA level of <200 copies/ml). In univariate analyses, pGSS and zidovudine use in the salvage regimen were predictors of the virologic response. In a multivariate analysis, pGSS and zidovudine and tenofovir use were associated with the virologic response. Bootstrap analyses showed similar reductions in HIV RNA levels with zidovudine or tenofovir use (0.5 to 0.9 log10). In the presence of K65R, zidovudine and tenofovir are associated with similar reductions in HIV RNA levels. Given its tolerability, tenofovir may be the preferred agent over zidovudine even in the presence of the K65R mutation.

The efficacy of combination antiretroviral therapy can be impaired by several factors, including the development of resistance (30). Although it is common practice to continue nucleoside reverse transcriptase inhibitors (NRTIs) in salvage regimens even for patients with extensive NRTI-related resistance, their role is unclear. A randomized study showed that lamivudine in the presence of the M184V mutation continues to exert an antiviral effect (4). Several nonrandomized studies of patients harboring the M184V mutation also support this result (3, 6, 17). However, apart from the M184V mutation, the residual activity of NRTIs in the presence of NRTI-related resistance is not well-described.

The K65R mutation in HIV-1 reverse transcriptase is the signature mutation associated with tenofovir failure but is also associated with the failure of abacavir, didanosine, zalcitabine (2, 5), and stavudine, the latter especially in HIV-1 subtype C (9). The K65R mutation had been a relatively rare mutation in treated populations, but beginning around 2000, the incidence of the K65R mutation began increasing in treated cohorts from North America and Western Europe. This increase, although small, did correspond with the increasingly widespread use of abacavir and tenofovir. More recently, however, an improved understanding of the drug-drug interaction between tenofovir and didanosine, the reduced use of thymidine analogue-sparing triple-NRTI combinations, and the coformulation of tenofovir with emtricitabine have contributed to the reported declines in the frequency of the K65R mutation seen in Western cohorts (8). Recent reports found a prevalence of the K65R mutation in 2 to 4% of patients failing antiretroviral therapy (7, 20). In the developed world, using optimal regimens, the emergence of the K65R mutation in previously treatment-naïve individuals is uncommon, occurring in 0 to 3% of tenofovir-treated patients (14, 15). In the developing world, the K65R mutation has been reported for 7 to 14% of patients failing stavudine-containing regimens (23, 25).

K65R most commonly associates with M184V, leading to a partial resensitization to tenofovir (13). Thymidine analogue mutations (TAMs) are found infrequently with the K65R mutation (22). The K65R mutation results in a 3- to 4-fold decrease in the phenotypic susceptibility to tenofovir (19) but increases susceptibility to zidovudine (27). However, few clinical studies have addressed the clinical impact of the K65R mutation on the virologic response to NRTIs. In this study, we evaluate the response to antiretrovirals in a multiclinic international cohort of patients harboring the K65R mutation, focusing on the contributions of zidovudine and tenofovir to virologic responses.

MATERIALS AND METHODS

We identified all HIV-1 isolates with the K65R mutation in the Stanford Database from patients monitored at four California clinical programs and within a large French cohort who underwent genotypic resistance testing between 1 July 1997 and 31 December 2005. Study nurses retrospectively reviewed the medical records of patients harboring the K65R mutation. Baseline HIV
RNA levels, antiretroviral history, subsequent treatment regimen, and HIV RNA levels after the isolation of the K65R mutation were recorded by using a standardized data abstraction tool.

Inclusion criteria included a baseline HIV RNA level of \( \geq 500 \) copies/ml, a change of at least one component of the antiretroviral regimen after the detection of the K65R mutation, and the availability of at least one subsequent HIV RNA determination within 12 weeks of initiation of salvage antiretroviral therapy.

Based on the HIV RNA level closest to 12 weeks after the initiation of the salvage regimen, we defined a complete virologic response as achieving an HIV RNA level of \( < 200 \) copies/ml (the lower limit of quantification of the least sensitive viral load assay used). We defined a partial virologic response as a reduction in the baseline HIV RNA level of at least \( 0.5 \log_{10} \) copies/ml but not achieving an HIV RNA level of \( < 200 \) copies/ml.

A genotypic susceptibility score (GSS) was calculated by using the Stanford HIV Resistance Database (http://hivdb.stanford.edu/). For each medication used in the salvage regimen, a score was given based on the interpretation of the genotype (1.0 is susceptible or potential low-level resistance, 0.5 is low-level resistance or intermediate resistance, and 0 is high-level resistance). The GSS was calculated by adding the activity of all the antiretrovirals used in the regimen. The partial GSS (pGSS) was defined as the GSS excluding the NRTI component of the regimen.

Univariate analysis of the association between baseline patient characteristics, baseline genotype, and characteristics of the salvage regimen and the three-part virologic response variable were performed by using a chi-squared test for trend for dichotomous variables and Spearman’s rank correlation coefficient for interval and ordinal variables. We used multivariate stepwise linear regression to assess the association between the three-part virologic response variable and pGSS, the use of zidovudine or tenofovir, and the number of new antiretrovirals in the salvage regimen, controlling for baseline HIV RNA levels. Significance was assessed at an \( \alpha \) level of \( < 0.05 \).

Bootstrap analyses were used to estimate the individual contribution to the change in the HIV RNA level of new (e.g., first time use of the drug), recycled (e.g., drug used previously but not in the most recent regimen), or continued zidovudine and tenofovir in the salvage regimen after controlling for the baseline HIV RNA level, pGSS, and number of new antiretrovirals in the salvage regimen. The 95% confidence intervals (CIs) were constructed by using 1,000 replicates (10).

We also performed subgroup univariate, multivariate, and bootstrap analyses for patients without coincident TAMs detected in their baseline genotype. Statistical analyses were performed by using R, version 2.6.2. Institutional review board approval was obtained from participating centers.

**RESULTS**

Of the 130 patients included, 66% (86/130) were from California and 34% (44/130) from France (Table 1). The mean baseline HIV RNA level was 4.0 \( \log_{10} \) copies/ml. Patients had previously received means of 51, 17, and 29 months of NRTIs, nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs), respectively. Most patients had received nonnucleoside RTIs prior to their inclusion into the cohort, 72% (94/130) had received didanosine, 70% (91/130) had received tenofovir, and 42% (54/130) of patients had received abacavir. A total of 52% of patients (67/130) had received coadministered tenofovir and didanosine.

The most commonly associated NNRTI-related mutation with K65R was M184V, found in 57% (74/130) of isolates (Table 1). The A62V, L74V, and Q151M mutations were present in 15% (19/130), 10% (13/130), and 9% (12/130) of isolates, respectively. At least one TAM (M41L, D67N, K70R, L210W, T215Y/F, or K219Q/E) was present in 28% (37/130) of samples, with 7% (9/130) having three or more TAMs.

Of the 130 patients, zidovudine was included in the salvage regimens of 40 patients (24 patients with new zidovudine and 16 patients with recycled zidovudine). Tenofovir was used in the salvage regimens of 47 patients (9 patients with new tenofovir, 2 patients with recycled tenofovir, and 36 patients with continued tenofovir). Tenofovir was used in combination with zidovudine for 8% (10/130) of patients. Lamivudine (or emtricitabine) was included in 90% (117/130) of salvage regimens. PIs were part of the salvage regimen for 93% (121/130) of patients, the majority with ritonavir boosting (99/130). The most frequently prescribed PIs were lopinavir-ritonavir (48/121) and atazanavir-ritonavir (36/121). The salvage regimens of 25% (32/130) of patients contained an NNRTI. The mean GSS and partial GSS of the salvage regimens were 2.0 and 0.8, respectively.

At a median of 83 days (interquartile range [IQR], 53 to 114 days) after switching to the salvage regimen, 55% (71/130) and 18% (23/130) of patients achieved complete and partial virologic responses, respectively. In univariate analyses, the pres-
ence of coexistent M184V or TAMs detected in the baseline genotype did not predict the virologic response (Table 2). Without correcting for multiple comparisons, the presence of the L74V mutation was significantly associated with the lack of a virologic response ($P = 0.04$). Patients with more active drugs in their salvage regimens (e.g., higher GSS) had significantly improved virologic responses ($P < 0.01$). A higher pGSS also was a predictor of the response ($P = 0.02$). The use of zidovudine or tenofovir led to good virologic responses, with 58% and 64% of patients, respectively, achieving a complete virologic response, but only the use of zidovudine was significantly associated with the three-part virologic response variable in the univariate analysis ($P = 0.04$). Seven of the 10 patients who received both zidovudine and tenofovir achieved a complete virologic response ($P = 0.44$).

In the multivariate analysis after controlling for baseline HIV RNA levels, the partial GSS was strongly associated with the virologic response ($P < 0.01$) (Table 3). Furthermore, the uses of both zidovudine and tenofovir were independently associated with the virologic response ($P < 0.01$ and $P < 0.01$, respectively).

In bootstrap analyses, after controlling for baseline HIV RNA levels, the partial GSS, and the number of new antiretrovirals in the salvage regimen, the use of both zidovudine and tenofovir led to reductions in HIV RNA levels, but only the use of new tenofovir was associated with a significant reduction ($0.9 \log_{10}$ copies/ml; 95% CI, 0.3, 1.6) (Fig. 1). The point estimates for the decrease in HIV RNA levels associated with new zidovudine, recycled zidovudine, and continued tenofovir were 0.6, 0.5, and $0.5 \log_{10}$ copies/ml, but the confidence intervals for these estimates crossed zero. We did not analyze the categories of continued zidovudine or recycled tenofovir given the few patients in these categories (0 and 2 patients, respectively).

In the subgroup of patients without TAMs at baseline, we found that 57% (53/93) and 18% (17/93) of patients achieved a virologic response (Table 2). Without correcting for multiple comparisons, the presence of coexistent M184V or TAMs detected in the baseline genotype did not predict the virologic response (Table 2).

### Table 2. Univariate analysis of predictors of response to salvage antiretroviral therapy in the entire cohort

| Characteristic | All patients | Responders | Partial responders | Nonresponders | $P$ value $^b$ |
|----------------|--------------|------------|--------------------|---------------|----------------|
| Total % of patients (no. of patients/total no. of patients) | 100 (130/130) | 55 (71/130) | 18 (23/130) | 28 (36/126) | NA |
| Baseline characteristics | | | | | |
| Mean no. of NRTIs | 4.0 | 3.9 | 3.7 | 4.4 | 0.06 |
| Mean duration of NRTI regimen (mo) | 51 | 53 | 33 | 60 | 0.59 |
| Mean no. of NNRTIs | 0.8 | 0.8 | 1.0 | 0.9 | 0.50 |
| Mean duration of NNRTI regimen (mo) | 17 | 18 | 14 | 17 | 0.76 |
| Mean no. of PIs | 1.5 | 1.3 | 1.4 | 1.8 | 0.12 |
| Mean duration of PI regimen (mo) | 29 | 28 | 27 | 30 | 0.70 |
| % of patients with baseline resistance mutation (no. of patients/total no. of patients) $^c$ | | | | | |
| M184V | 100 (74/74) | 62 (46/74) | 15 (11/74) | 23 (17/74) | 0.13 |
| A62V | 100 (19/19) | 47 (9/19) | 21 (4/19) | 32 (6/19) | 0.85 |
| L74V | 100 (13/13) | 23 (3/13) | 31 (8/13) | 46 (6/13) | 0.04 |
| Q151M | 100 (12/12) | 58 (7/12) | 0 (0/12) | 42 (5/12) | 0.21 |
| ≥1 TAM | 100 (37/37) | 49 (18/37) | 16 (6/37) | 35 (13/37) | 0.51 |
| ≥3 TAMs | 100 (9/9) | 56 (5/9) | 22 (2/9) | 22 (2/9) | 1.00 |
| Characteristics of salvage regimen | | | | | |
| Total GSS (mean) | 2.0 | 2.2 | 2.1 | 1.6 | <0.01 |
| Partial GSS (mean) | 0.8 | 0.9 | 0.7 | 0.5 | 0.02 |
| % of patients with use of AZT (no. of patients using drug/total no. of patients) | 100 (40/40) | 58 (23/40) | 28 (11/40) | 15 (6/40) | 0.04 |
| % of patients with use of TDF (no. of patients using drug/total no. of patients) | 100 (47/47) | 64 (30/47) | 19 (9/47) | 17 (8/47) | 0.14 |

### Table 3. Multivariate predictors of response controlling for baseline viral load

| Variable | Regression coefficient | Standard error | $P$ value $^a$ |
|----------|------------------------|----------------|---------------|
| Partial GSS | −0.32 | 0.11 | <0.01 |
| Use of AZT | −0.61 | 0.21 | <0.01 |
| Use of TDF | −0.53 | 0.20 | <0.01 |
| No. of new drugs | 0.02 | 0.08 | 0.84 |

$a$ pGSS, partial genotypic susceptibility score; AZT, zidovudine; TDF, tenofovir disoproxil fumarate.

$b$ Significance was assessed by using $t$ tests. Boldface type indicates significance.

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DISCUSSION

In this large cohort of patients harboring the K65R mutation across an international spectrum of clinical settings, 55% of patients were able to achieve a complete virologic response, a finding similar to the 60% of patients harboring the K65R mutation who achieved HIV RNA levels of <50 copies/ml reported in a large Italian cohort (1). As many prospective and retrospective studies have also found, we found that the GSS was associated with the virologic response. The association found between the presence of the L74V mutation and the lack of a virologic response in univariate analyses may have been due only to chance or confounding, as there appears to be no clear virologic explanation for this finding.

We chose to define the pGSS to control for the NNRTI components of the salvage regimen in order to specifically focus on the contribution of zidovudine and tenofovir to the virologic response. After controlling for the pGSS, we found that both zidovudine and tenofovir in the salvage regimen were positively associated with the virologic response in the multivariate analysis. In bootstrap analyses, new or recycled zidovudine and new or continued tenofovir contributed to a 0.5- to 0.9-log₁₀ decrease in HIV RNA levels, although we found only the decline associated with new tenofovir to be significant. Almost all patients in the cohort received lamivudine (or emtricitabine) in their salvage regimens, so the individual antiviral contribution of lamivudine (or emtricitabine) could not be reliably determined. Similar proportions of patients who received zidovudine or tenofovir also received lamivudine (or emtricitabine) in their salvage regimens, so the receipt of lamivudine (or emtricitabine) likely did not bias our findings.

FIG. 1. Bootstrap analyses with 1,000 replicates for change in log₁₀ viral load by medication category, controlling for baseline viral load, partial genotypic susceptibility score, and number of new medications in salvage regimen. (a) Bootstrap analysis for change in log₁₀ viral load for new zidovudine (AZT), controlling for baseline viral load, partial genotypic susceptibility score, and number of new medications in the salvage regimen. (b) Bootstrap analysis for change in log₁₀ viral load for recycled zidovudine, controlling for baseline viral load, partial genotypic susceptibility score, and number of new medications in the salvage regimen. (c) Bootstrap analysis for change in log₁₀ viral load for new tenofovir, controlling for baseline viral load, partial genotypic susceptibility score, and number of new medications in salvage regimen. (d) Bootstrap analysis for change in log₁₀ viral load for continued tenofovir, controlling for baseline viral load, partial genotypic susceptibility score, and number of new medications in salvage regimen. TDF, tenofovir disoproxil fumarate. Point estimates for the change in log₁₀ viral load (95% confidence intervals) for each medication category are as follows: −0.6 (−1.4, 0.1) (a), −0.5 (−1.2, 0.2) (b), −0.9 (−1.6, −0.3) (c), and −0.5 (−1.0, 0.1) (d).
Given the reduced phenotypic susceptibility to tenofovir for patients harboring the K65R mutation, the virologic response that we found with tenofovir was somewhat unexpected. However, the reduction in viral fitness associated with the K65R mutation may explain the retained partial activity of tenofovir despite the reduced phenotypic susceptibility (26). These results are consistent with the lack of HIV RNA rebound among treatment-experienced patients who developed a K65R mutation in clinical trials of tenofovir (21). Additionally, the majority of our isolates also harbored the M184V mutation, which has been shown to increase the susceptibility of the virus to tenofovir. Although not significant, patients in our cohort harboring the M184V mutation did respond more favorably to salvage therapy. The similar magnitudes in the reduction of HIV RNA levels with zidovudine and tenofovir in the bootstrap analyses may be due to the different inherent potencies of zidovudine and tenofovir. In treatment-naive patients, zidovudine monotherapy leads to an approximately 0.5-log10 decrease in HIV RNA levels (11), while tenofovir monotherapy leads to a 1.5-log10 decrease (18). Another retrospective study that evaluated predictors of virologic responses in the setting of the K65R mutation found that the use of zidovudine, but not tenofovir, was associated with the virologic response (1). However, a smaller proportion of patients in their cohort (21%) received tenofovir in their salvage regimens, which may explain why a tenofovir effect was not detected.

Given that our study was retrospective, we were limited to when HIV RNA measurements were available after the initiation of the salvage regimen. Ideally, we would have had HIV RNA assessments at uniform time points for all patients. We chose to focus on the virologic assessment near 12 weeks, a time point for which most patients had virologic assessments available and a near-maximal response to a new antiretroviral regimen can be expected. The relevance of this relatively early time point is supported by recent consensus statements for the management of treatment-resistant populations (12).

Currently, treatment guidelines emphasize the goal of obtaining HIV RNA levels below 50 copies/ml in all patients. We were limited in that the viral load assay used for some samples in this study had a lower limit of quantification of <200 copies/ml, and we defined a complete virologic response using this threshold. Whether the uniform availability of the more sensitive viral load assay and a threshold for a complete virologic response of <50 copies/ml, as was used in a previous study evaluating the virologic response in the presence of the K65R mutation (1), would have changed our results is unknown. However, it seems unlikely to us that the use of the higher cutoff of 200 copies for the viral load assay for defining a complete response systematically biased our results toward zidovudine or tenofovir. Nevertheless, this possibility cannot be fully excluded.

We did not have access to adherence data, and differential adherence between patients on different regimens could have affected our results. Furthermore, given the retrospective design of the study, residual confounding cannot be excluded. We chose to focus specifically on the response to zidovudine and tenofovir, as this seemed to us to be the most relevant choice for NRTIs in the presence of the K65R mutation (likely in combination with lamivudine or emtricitabine).

In the developed world, with the availability of many new, potent, and well-tolerated antiretrovirals, the role for NRTIs in the salvage regimens of patients harboring NRTI-associated resistance is unclear. The OPTIONS study (ACTG 5241) is currently evaluating the role of NRTIs in this setting (www.clinicaltrials.gov/ct2/results?term=ACTG+5241). Our results support the belief that NRTIs have relatively modest activity in the presence of NRTI-associated resistance. This may, however, be an important contribution to the success of an antiretroviral regimen for patients with high levels of resistance. The majority of our patients had prior PI experience but received at least a partially active boosted PI in their salvage regimen. A recent comparison of the potencies of different antiretroviral classes using the inhibitory potential suggests that PIs are inherently more active than NRTIs (24). If one chooses to use NRTIs in salvage therapy for a patient harboring K65R, as may currently be considered the standard of care, given the apparent similarity in responses to zidovudine and tenofovir, it seems reasonable to choose the less toxic and better-tolerated tenofovir. Given the few patients who received both zidovudine and tenofovir in our cohort, it is not clear from our data whether there would be an additional benefit from combining the two agents in salvage therapy.

In the developing world, genotypic resistance testing generally is not available. However, when evaluated, due to the frequently delayed time to detection of virologic failure, the most common mutational pattern after failure with nevirapine, lamivudine, and stavudine (still the most commonly prescribed combination despite recent World Health Organization guidelines) includes multiple TAMs or K65R with M184V and multiple NNRTI mutations (16). For salvage therapy after the failure of a stavudine-containing regimen, recent World Health Organization guidelines recommend a combination of a boosted protease inhibitor plus a combination of tenofovir and lamivudine or emtricitabine (29). Previous guidelines included an option for also including zidovudine (28). Our data, if responses to antiretrovirals with different viral subtypes are similar, suggest that patients can achieve excellent virologic responses with tenofovir-containing regimens in the setting of K65R, and the addition of zidovudine may not be necessary. Future randomized studies should address the question of the optimal choice of NRTIs in salvage therapy.

In summary, patients in our cohort were frequently able to obtain similar high rates of virologic suppression using both zidovudine- and tenofovir-containing regimens. It appears that even for patients harboring the K65R mutation, tenofovir is still able to provide some antiviral activity although reduced compared to its activity in treatment-naive patients without drug-related mutations. This contribution may be important for the success of a salvage regimen and warrants further study. In the meantime, we recommend the use of tenofovir even in the setting of the K65R mutation.

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