The Risks and Incidence of K65R and L74V Mutations and Subsequent Virologic Responses

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The L74V and K65R mutations confer resistance to several nucleoside analogues, and the impact on subsequent regimens is unclear. The risk of developing L74V or K65R mutation in the era of highly active antiretroviral therapy (HAART) was 4.5 and 2.8 cases per 100 person-years, respectively; concomitant receipt of boosted protease inhibitors protected against K65R. High rates of virologic suppression in the presence of either mutation were observed if the next regimen contained at least 2 active agents. If suboptimal HAART was used, patients with K65R experienced significantly higher rates of virologic suppression than did those with L74V (P = .01).

In the current era, therapy-naive individuals who commence antiretroviral treatment have a high probability of durable virologic suppression [1]. The L74V and K65R reverse-transcriptase resistance mutations, both of which are single–amino acid substitutions, confer reduced susceptibility to many nucleoside reverse-transcriptase inhibitors (NRTIs). After a steady increase in the prevalence of these mutations during 2000–2004 secondary to the increasing use of abacavir and tenofovir, both of which select for K65R, the incidence of K65R mutations began to decrease in recent years [2], probably as a result of reduced use of nonthymidine triple-nucleoside combinations. L74V, selected for by didanosine and abacavir, remains rare if a thymidine analogue is included in the treatment regimen [3]. In the presence of M184V, L74V confers reduced susceptibility to abacavir and didanosine [4] and appears to be associated with an inferior virologic response to tenofovir-based regimens [5]. One study, which used single-genome sequencing, demonstrated that the L74V mutation may be associated with a “hidden” K65R mutation that is not detected by standard genotyping [6]. A phenotypic in vitro study suggested that the K65R mutation may be associated with decreased antiretroviral susceptibility, compared with the L74V mutation [4], in terms of resistance to NRTIs, although it is important to note that this finding has yet to be confirmed in prospective trials [6–11].

A subanalysis of the Gilead 903 study demonstrated high rates of virologic success among a small number of individuals with a K65R mutation who experienced treatment failure and who switched to a HAART regimen based on a protease inhibitor (PI) boosted with ritonavir (PI/r) (Gilead data on file). To further investigate the success of subsequent antiretroviral therapy in the presence of K65R and/or L74V mutations, we analyzed virologic responses among individuals in our cohort. We also compared the risk of development of mutations after receipt of different antiretroviral combinations with the degree of protection conferred by the use of a PI.

Methods. The Chelsea and Westminster HIV cohort, the largest single clinic cohort in Europe, prospectively collects data on antiretroviral history, genotype resistance test results, and virologic and immunological parameters for all individuals. Through this cohort’s database, we identified individuals who developed a K65R and/or L74V mutation since January 2000, when both abacavir and tenofovir became routinely available and when genotype resistance testing became part of routine clinical care. We first reviewed the antiretroviral regimens used before the appearance of either mutation. Individuals were classified on the basis of the components of the NRTI backbone before first appearance of either mutation. Individuals were further divided according to whether the regimen contained a PI or a nonnucleoside reverse-transcriptase inhibitor (NNRTI) or consisted of NRTIs only.

Next, we calculated the risk of developing either mutation per 100 patient-years of exposure for each defined combination. Denominator data were based on all antiretroviral exposures after 1 January 2000. Data were analyzed using SAS statistical software, version 8.02 (SAS Institute). Duration of use of the combination regimen was calculated using univariate procedures in SAS software and was stratified on the basis of the class of drugs described in 3 groups. The numbers of patients presented in the stratified analyses are not mutually exclusive, because individuals may have fallen into >1 category during the study period.
We analyzed the same cohort to evaluate the success of HAART regimens after the acquisition of these mutations. Patients with <6 months of follow-up data, who had no viral load data at 6 months, or who did not undergo a change in therapy were excluded from the study. The study’s end point was predefined as a 6-month plasma viral load <50 copies/mL, and patients who had missing data or who did not switch treatments were excluded from the on-treatment analysis. The number of active drugs in the subsequent regimen was estimated by reviewing the Virco virtual phenotypes on the basis of both the results of the resistance test performed before the switch and all of the results of all resistance tests ever performed (to include any archived mutations). Median values and interquartile ranges were calculated for CD4 cell counts and viral loads at the time of the treatment switch, and the Mann-Whitney U test was used to calculate P values for the differences between the 2 groups in terms of CD4 cell count and viral load at the time of the switch and the number of active drugs. The rates of viral suppression, according to the number of active agents (≥2 vs. <2), were calculated and compared between the L74V and K65R groups.

Results and discussion. A total of 129 patients with an L74V mutation and 81 patients with a K65R mutation from the cohort were eligible. The risk of developing an L74V or K65R mutation with antiretroviral combinations containing tenofovir, didanosine, or abacavir are presented in table 1, with the results subdivided according to PI or NNRTI use. The overall risk of developing either mutation was 4.49 and 2.82 cases per 100 person-years for L74V and K65R, respectively.

L74V appeared most frequently among recipients of regimens with a tenofovir-didanosine or abacavir-didanosine backbone. Regimens including tenofovir, didanosine, and an NNRTI (risk, 16.41 cases per 100 person-years) or abacavir, didanosine, and an NRTI (risk, 15.28 cases per 100 person-years) were associated with the highest rates of L74V mutation. This concurs with data demonstrating high rates of virologic failure associated with tenofovir-didanosine-NNRTI–based regimens [12–14]. The L74V mutation was uncommon among recipients of nucleoside-alone regimens that did not include didanosine, abacavir, or tenofovir (risk, 0.45 cases per 100 person-years).

The K65R mutation occurred most frequently after receipt of a triple-nucleoside regimen of abacavir, tenofovir, and didanosine (risk, 41.48 cases per 100 person-years). The K65R mutation remained common among recipients of regimens including abacavir, tenofovir, didanosine, and an NNRTI (risk, 14.13 cases per 100 person-years) or tenofovir, didanosine, and an NNRTI (risk, 19.40 cases per 100 person-years), but the use of a boosted-PI appeared to be protective (risk, 1.78 and 0.81 cases per 100 person-years, respectively).

Several studies have reported high rates of treatment failure

### Table 1. Calculated risk of developing L74V or K65R mutations.

<table>
<thead>
<tr>
<th>Mutation, NRTI backbone</th>
<th>Regimen</th>
<th>NNRTIs</th>
<th>Risk per 100 patient-years of exposure</th>
<th>No. of patients</th>
<th>PIs with or without NNRTIs</th>
<th>Risk per 100 patient-years of exposure</th>
<th>No. of patients</th>
<th>NRTIs only</th>
<th>Risk per 100 patient-years of exposure</th>
<th>No. of patients</th>
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<tbody>
<tr>
<td>L74V mutation</td>
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<tr>
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<td>1.03</td>
<td>2</td>
<td>1.31</td>
<td>2</td>
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<tr>
<td>Didanosine (no tenofovir or abacavir)</td>
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<td>20</td>
<td>3.26</td>
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<tr>
<td>Abacavir (no tenofovir or didanosine)</td>
<td>1.79</td>
<td>10</td>
<td>3.36</td>
<td>9</td>
<td></td>
<td>1.10</td>
<td>4</td>
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<tr>
<td>Tenofovir and didanosine (no abacavir)</td>
<td>16.41</td>
<td>11</td>
<td>4.03</td>
<td>5</td>
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<tr>
<td>Didanosine and abacavir (no tenofovir)</td>
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<td>15</td>
<td>9.01</td>
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<td>15.28</td>
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<td>Tenofovir, didanosine, and abacavir</td>
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<tr>
<td>No tenofovir, didanosine, or abacavir</td>
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<td>8</td>
<td>1.01</td>
<td>9</td>
<td></td>
<td>4.36</td>
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<td>2</td>
<td>0.37</td>
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<td>0.27</td>
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<td>13</td>
<td>0.61</td>
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<tr>
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<td>1.12</td>
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<td>2.64</td>
<td>5</td>
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<tr>
<td>Didanosine and abacavir (no tenofovir)</td>
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<td>4</td>
<td>...</td>
<td>...</td>
<td></td>
<td>5.09</td>
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<tr>
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<td>5</td>
<td>1.78</td>
<td>1</td>
<td></td>
<td>41.48</td>
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<tr>
<td>No tenofovir, didanosine, or abacavir</td>
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<td>...</td>
<td>0.11</td>
<td>1</td>
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**NOTE.** NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor.
and resistance in recipients of thymidine-sparing triple-NRTI combinations [15–18]. It is clear that some triple-nucleoside regimens lack potency, including regimens including tenofovir, abacavir, and lamivudine [15, 16, 18] and tenofovir, lamivudine, and didanosine [17]; these regimens are associated with resistance, which was also seen in our cohort, and with unacceptable levels of virologic failure. Inclusion of a thymidine analogue in quadruple-nucleoside [19–21] or triple-nucleoside [22] combinations may improve efficacy. The combination regimen of abacavir, lamivudine, and zidovudine may lack potency as initial therapy [23, 24], but it appears to be effective as part of induction-maintenance strategies [25, 26].

Ninety subjects with an L74V mutation and 51 subjects with a K65R mutation were eligible for subsequent analysis. The median CD4 cell counts were 219.5 cells/mm$^3$ (interquartile range, 88–335 cells/mm$^3$) and 195 cells/mm$^3$ (interquartile range, 122–301 cells/mm$^3$) in the L74V and K65R groups, respectively ($P = .723$). There was no statistically significant difference between the 2 groups in the viral load at the time of switch (median viral load, 15,835 and 20,330 copies/mL, respectively; $P = .749$); rates of viral suppression are presented in figure 1. The components of the next regimen for the individuals who did not achieve virologic suppression were analyzed to assess whether there were any differences between the 2 groups in terms of containing a PI/r, an NNRTI, or neither. There were no statistically significant differences; of the patients who did not experience virologic suppression in the K65R and L74V groups, 14% and 24%, respectively, were receiving an NRTI-only combination ($P = .974$), 44% and 22% were receiving an NNRTI ($P = .459$), 28% and 52% were receiving boosted PI therapy ($P = .503$), and 14% and 4% were receiving unboosted PI-based HAART ($P = .912$).

There was a trend toward greater virologic success among individuals with a K65R mutation than among those with an L74V mutation, although more subjects with a K65R mutation were not receiving a boosted PI-based regimen. A total of 39 (76.5%) of 51 subjects with a K65R mutation achieved a viral load $<50$ copies/mL, compared with 50 (55.5%) of 90 subjects with an L74V mutation. The differences between the 2 groups could not be accounted for by differences in baseline immunologic or virologic parameters or in the number of active drugs. When $\geqslant$2 active drugs (with activity determined on the basis of all resistance test data, including archived mutations) were included in the next regimen, there was no difference between the K65R and L74V groups in terms of subsequent viral suppression. When $<2$ active drugs were used, then the K65R mutation was associated with significantly higher rates of virologic success than an L74V mutation (70% vs. 26%; $P = .012$). However, K65R mutations are frequently accompanied by the M184V mutation in subjects for whom a tenofovir-based regimen fails [27], and M184V mutations enhance susceptibility to tenofovir; this may, in part, explain our results.

PI-based therapy conferred protection against the development of K65R mutations but not L74V mutations in our cohort. In terms of development of the K65R mutation, this protective effect is in keeping with the finding that, although combination treatment with tenofovir, didanosine, and an NNRTI is associated with virologic failure in treatment-naïve subjects, a regimen of tenofovir, didanosine, and a boosted PI may be more robust. The earliest evidence of virologic failure during treatment with tenofovir, didanosine, and an NNRTI in therapy-naïve subjects came from a small, retrospective analysis [13]; prospective studies have since confirmed this finding [12, 14]. In contrast, receipt of tenofovir, didanosine, and a boosted PI does not appear to be associated with increased rates of virologic failure. In the BMS 045 Study, there was no reduction in the efficacy of a regimen of tenofovir, didanosine, and boosted atazanavir, compared with other backbones, in treatment-experienced subjects (BMS data on file). Cohort analyses support the use of tenofovir, didanosine, and a boosted PI in experienced subjects [28, 29].

Limitations of our analysis include the small numbers of patients in each group, despite the relatively large cohort size, and because we confined our analysis to the immediately preceding regimen, the influence of previous therapy is not included. In addition, by selecting only subjects with 6-month viral load data available, we excluded subjects who may have discontinued therapy before this point because of an inadequate response; this may have yielded incorrectly high response rates. However, we selected this restriction because, for many subjects,
the reasons for discontinuation of a regimen before this point were unclear. In summary, rates of L74V and K65R mutations were low in our cohort, and use of a boosted PI protected against the development of K65R mutations. High rates of virologic suppression in the presence of either mutation were observed among subjects with ≥2 active agents in their next regimen. However, among patients with limited treatment options (i.e., <2 active drugs), those with the K65R mutation experienced significantly higher rates of virologic suppression than did those with the L74V mutation.

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References


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