Relationships among various nucleoside resistance-conferring mutations in the reverse transcriptase of HIV-1

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Highly active antiretroviral therapy has significantly improved HIV-related morbidity and mortality, and nucleoside reverse transcriptase inhibitors remain an essential component of treatment. However, the emergence of HIV-1 mutated strains that are resistant to one or more antiretroviral drugs is a leading cause of treatment failure among patients living with HIV/AIDS. These resistant strains may often suffer from a replication disadvantage in comparison with wild-type viruses when grown in the absence of drug pressure and a potential benefit in this regard has been shown for lamivudine-resistant viruses that contain a M184V mutation in reverse transcriptase, as well as for several other drug-resistant viral variants. Interactions between different mutations may complicate the understanding of HIV drug resistance with regard to the likelihood of therapeutic success.

Keywords: HIV, NRTIs, resistance, mutations, antiretroviral drugs, nucleosides, diminished sensitivity, viral fitness

Introduction

The introduction of nucleoside reverse transcriptase inhibitors (NRTIs), followed by highly active antiretroviral therapy (HAART) in the mid-1990s, significantly improved HIV-related morbidity and mortality.1 However, the use of anti-HIV drugs has been accompanied by the emergence of drug-specific resistance, potentially leading to virological and clinical failure. The problem of drug resistance is offset to some extent by findings that resistant viruses may have a measurable replication disadvantage in comparison with wild-type strains in the absence of drug pressure.2 This diminution in viral replication or viral fitness is the result of changes in the reverse transcriptase (RT) and protease (PR) enzymes of HIV that affect their function. These changes are, of course, a consequence of mutations in the RT and PR coding sequences and the selection of mutated viruses by antiviral drug pressure.

During treatment failure, numerous compensatory mutations may also accumulate that may help to restore viral replicative capacity.3 A better understanding of the origins and mechanisms of antiretroviral (ARV) resistance may lead to improved clinical strategies that will maximize the usefulness of antiviral drugs. For example, therapeutic regimens containing lamivudine have been shown to be highly effective in the treatment of HIV-infected patients, despite the fact that a single mutation at position 184, involving a transition from a methionine to valine (M184V), confers a loss of susceptibility of 100- to 1000-fold to this drug.4,6 Moreover, lamivudine selects rapidly for this mutation compared with the development of resistance to most other drugs.6,8

It is important that the rate of emergence of M184V may vary as a consequence of the antiviral regimen used in treatment. For example, a randomized clinical trial showed that patients who received a regimen of stavudine, lamivudine and nelfinavir were far more likely to develop the M184V mutation than were individuals who received the same two nucleoside compounds plus ritonavir-boosted lopinavir. This difference was attributed to a superior ability of the lopinavir-containing regimen to suppress viral replication and, consequently, to prevent the emergence of the M184V mutation.10 These results highlight the fact that the M184V mutation is likely to quickly emerge if the use of lamivudine in therapy is not accompanied by other potent drugs that can efficiently suppress viral replication. Statistically significant differences were not present between the two arms with regard to genotype or HIV viral load at baseline, and levels of adherence were similar in the two arms; however, other effects including those related to drug pharmacokinetics cannot be ruled out.

Interestingly, in addition, lamivudine may actually be able to continue to contribute to the effectiveness of ARV combination therapy regimens despite high level resistance,11 as will be explained below. Emtricitabine is another cytosine analogue, structurally related to lamivudine, which has shown more potent antiviral activity than lamivudine in certain cell lines.12 This high potency may result from a greater binding affinity of the triphosphate to HIV-1 RT and more efficient incorporation into viral DNA during synthesis by this enzyme.13 Indeed, limited clinical studies have reported better antiviral activity of emtricitabine (200 mg/day) compared with lamivudine (150 mg twice daily).14 With regard to resistance, emtricitabine also selects for M184V, which confers high-level resistance to itself as well as to lamivudine, although clinical results suggest that M184V may be selected less readily by the former than the latter drug.14–17 The latter observation may be due, in part, to higher rates of patient adherence to emtricitabine, which was given once daily, than to lamivudine.
vudine, which was dosed twice daily, in these studies. Useful information would be provided by studies that directly compared both of these drugs in once daily regimens with regard to the presence of the M184V substitution.

Some in vitro data have shown that the M184V mutation may also be selected by abacavir and didanosine, but confers only low-level resistance to these drugs.\textsuperscript{18,19} Such low-level resistance in the absence of additional mutations that confer diminished sensitivity to other NRTIs may not have clinical significance. The fact that M184V confers diminished replication fitness has been shown both in vitro\textsuperscript{20} and in clinical studies.\textsuperscript{21–24} Biochemical studies have shown that the M184V mutation confers diminished processivity to RT, meaning that the enzyme is less efficient than its wild-type counterpart at transcribing viral RNA into DNA.\textsuperscript{20}

In the NUCA 3001 study, patients who had received fewer than 4 weeks of therapy with zidovudine were randomized to receive either lamivudine monotherapy, zidovudine monotherapy or combination therapy with lamivudine/zidovudine for up to 52 weeks.\textsuperscript{21,22} It was shown that viraemia in the lamivudine monotherapy arm attained a nadir of 1.2 log\textsubscript{10} RNA copies/mL by week 4 after initiation of therapy prior to rebound, and that this occurred concomitant with the appearance of M184V; however, viraemia remained below baseline and was consistently lower than that resulting from treatment with zidovudine alone.

Two studies, AVANTI 2 and AVANTI 3, compared the efficacy of zidovudine/lamivudine combined with a protease inhibitor (PI), i.e. indinavir or nelfinavir.\textsuperscript{23} The development of the M184V mutation in lamivudine/zidovudine-treated patients in both of these studies was associated with a partial plasma viral load rebound in those experiencing virological failure. In contrast, the presence of lamivudine-associated mutations in the absence of M184V had a significantly negative effect on viral load suppression.

The Triage trial evaluated virological outcome after induction with lamivudine/zidovudine and indinavir, followed by maintenance therapy with either lamivudine/zidovudine or zidovudine/indinavir. Removal of lamivudine from the triple drug regimen was associated with higher viral load rebound compared with maintenance on lamivudine/zidovudine.\textsuperscript{24} Moreover, the majority of patients on lamivudine/zidovudine who failed therapy harboured M184V.\textsuperscript{24} These studies suggest that maintenance of the M184V mutation may confer clinical benefit.

The potential benefit of maintaining M184V in heavily treated patients as a strategy for salvage therapy is still a topic of debate. One recent study on 20 patients with multidrug resistance evaluated a strategy of partial interruption of either the PI or NRTI component of a combination regimen. Among 15 patients who stopped PIs and continued on NRTIs, viraemia remained stable in 13 of them. In contrast, the five patients who discontinued NRTI all demonstrated increased levels of viraemia. In three of the latter individuals, a delayed loss of M184V was associated with a rise in plasma viral load.\textsuperscript{25} Additional evidence has come from a study in which lamivudine was removed from the antiretroviral regimen of five heavily experienced patients on failing regimens, all of whom had developed the M184V mutation. These individuals had maintained stable but detectable viral loads for some time prior to the discontinuation of lamivudine, and were otherwise maintained on the same drugs. In each instance, the withholding of lamivudine led to an increase in plasma viraemia, despite the fact that the M184V mutation was still present on the basis of genotyping.\textsuperscript{25} Tissue culture data also support the notion that lamivudine can continue to exert a modest antiviral effect despite the presence of the M184V substitution.\textsuperscript{26} Despite these findings, it is obvious that the potential benefit of maintaining lamivudine therapy or the presence of M184V in this context should be evaluated in randomized clinical trials.

In addition, M184V can result in hypersensitization of HIV to several NRTIs including both zidovudine and stavudine, as well as to the NRTI tenofovir. This is related to the ability of RT to cleave incorporated NRTIs, a step termed nucleotide excision,\textsuperscript{26,29} and to the fact that this reaction is rendered inefficient if the M184V mutation is present.

Resistance-conferring mutations for NRTIs (Table 1) can be classified in relation to different drugs. Thymidine analogue mutations (TAMs) are specific for zidovudine and stavudine, but, when present together with other mutations, can result in increased levels of resistance to such drugs as abacavir and didanosine. Multinucleoside resistance mutations, e.g. Q151M, can also be selected for by HAART. The impact of M184V on various mutations associated with resistance to NRTIs is complex, as reviewed below.

### TAMs

As stated, TAMs, which include M41L, D67N, K70R, L210W, T215Y/F and K219Q, are selected primarily by zidovudine\textsuperscript{30–33} and stavudine.\textsuperscript{34} These mutations accumulate in a stepwise manner to cause increasing levels of resistance. The presence of the M184V mutation is associated with a lower incidence of TAMs in patients who are multiple-drug experienced, as well as lower levels of resistance to both zidovudine and stavudine than those that would be present if only TAMs were present.\textsuperscript{35} TAMs may sometimes also be selected by didanosine\textsuperscript{36} and can confer low-level resistance level to abacavir.\textsuperscript{37} The additional presence of M184V can then increase resistance to abacavir by 10-fold.\textsuperscript{19,38} With regard to lamivudine, the combination of TAMs and M184V did not increase levels of resistance in cell culture.\textsuperscript{27}

#### L74V

L74V is the most common resistance mutation associated with didanosine and is sometimes also associated with resistance to abacavir.\textsuperscript{39} L74V confers a low level of resistance to abacavir on its own, but such levels are increased when any of M184V, K65R and Y115F\textsuperscript{19} are also present. As shown for M184V, the L74V mutation may also lower viral replicative capacity\textsuperscript{40} and RT processivity.\textsuperscript{20} In one tissue culture study, the association of M184V/L74V was shown to confer lower viral replicative capacity than that associated with either mutation alone.\textsuperscript{41} L74V may also be able to partially reverse the effects of T215Y with regard to resistance to zidovudine or stavudine.\textsuperscript{42} In addition, both M184V and L74V confer modest hypersusceptibility to tenofovir.\textsuperscript{43}

#### V75T

V75T is selected by stavudine \textit{in vitro}\textsuperscript{44} and confers cross-resistance to didanosine and zalcitabine. It occurs in \textasciitilde 4% of patients failing stavudine.\textsuperscript{45}

#### K65R

K65R confers resistance to zalcitabine, didanosine and abacavir, and is the only mutation that has been shown to be selected for by tenofovir \textit{in vitro}.\textsuperscript{46,47} As stated, the M184V mutation can heighten sensitivity to tenofovir. In contrast, M184V, when associated with K65R, resulted in even higher resistance to lamivudine than that associated with M184V alone.\textsuperscript{48}
Multinucleoside drug resistance can be divided into three patterns: the 69 insertion complex, the Q151M complex and NRTI-associated mutations (NAMs).

### The 69 insertion complex (T69D/A/S)

This rare pattern is associated with resistance to all approved NRTIs, including tenofovir. It confers high-level resistance when associated with TAMs. In one study, the presence of M184V in association with T69D/A/S reversed levels of resistance to tenofovir from 20- to six-fold.

### The Q151M complex

These mutations include A62V, V75I, F77L, F116Y and Q151M, and confer high levels of resistance to virtually all NRTIs. The first four mutations can rapidly accumulate after the emergence of Q151M, which, on its own, confers only a low level of resistance. The accumulation of the other four mutations results in increased levels of resistance to all NRTIs except tenofovir, as well as improved viral replicative capacity over the diminished fitness associated with Q151M. Fortunately, the Q151M complex of mutations is rare, even in multidrug-experienced patients (<5%).

### Other NAMs

Multidrug resistance involving NAMs is generally associated with TAMs as well as two other mutations, E44D/A and V118I. The latter two mutations were first described as part of an alternative pathway toward development of resistance to lamivudine. Clinical isolates, these mutations are often present in the presence of TAMs, but not M184V. Site-directed mutagenesis studies showed that E44D and V118I yielded low to moderate levels of resistance to lamivudine. However, lamivudine does not alone effectively select for these mutations, and two large databases from Italy and Canada have shown that E44D and V118I are associated with treatment histories involving stavudine and didanosine. Multidrug resistance involving NAMs is also caused resistance to stavudine (three- to 7.5-fold). A major study evaluated the prevalence of E44D/A and V118I in 83 drug-naïve and 261 treated patients. The latter individuals were stratified based on moderate drug experience (one to five drugs) versus extensive treatment (more than five regimens). The prevalence of these mutations was statistically higher in the heavily treated subjects. A second independent study yielded similar results in that the prevalence of the 44/118 mutations increased in proportion to the number of drug regimens that had been used and reached >40% in patients who had been on four or more regimens. The 44/118 mutations were observed in patients who had been treated with didanosine and stavudine, but not with other NRTIs.

It has also been shown by site-directed mutagenesis that the addition of E44D/V118I to a background of TAMs (41/210/211/215) did not significantly increase levels of resistance to abacavir (3.8- to 4.7-fold), whereas the addition of M184V increased resistance by eight-fold, regardless of whether or not E44D/V118I were present. This study also documented that diminished sensitivity to zidovudine was not reversed by the addition of E44D/V118I (>100-fold), in contrast to

### Table 1: Effect of various NRTI-associated mutations (NAMs) on drug resistance and viral replicative capacity (VRC)

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Mutations observed in the pattern</th>
<th>Primary drug affected</th>
<th>Fold resistance (IC₅₀)</th>
<th>Other drugs affected</th>
<th>Impact on VRC</th>
<th>Hypersusceptibility</th>
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<tbody>
<tr>
<td>Single mutation NRTI resistance</td>
<td>M184V</td>
<td>TAMs</td>
<td>L74V, K65R, Y115F</td>
<td>ddI &lt;10</td>
<td>ABC, ddC</td>
<td>NT</td>
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<td>Multiple NRTI resistance</td>
<td>TAMs</td>
<td>K70R, L210W, K219Q</td>
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the addition of M184V, which reduced levels of resistance to zidovudine both in the presence and absence of E44D/V118I. Thus, the E44D/V118I mutations appear to be compensatory, a phenomenon that until recently has been described only for protease inhibitors. A cross-sectional study, which evaluated the impact of M184V on viral load in individuals harbouring V118I, showed significantly higher viral loads in patients harbouring M184V with V118I compared with patients harbouring M184V alone. M184V can also be rapidly deselected in the absence of drug pressure because of its diminished fitness. In contrast, the E44D/V118I mutations appear to be stable. Among 19 patients who had discontinued therapy for 2–6 months, a reversion to wild-type codons at these positions was observed in only two patients. This may be important in regard to the horizontal transmission of V118I in the context of multidrug resistance, since viral genotypes associated with drug resistance may persist over a long period of time.

Conclusions

Lamivudine was one of the first drugs shown to be associated with diminished HIV morbidity and mortality. Its benefit may be exerted even after emergence of M184V, a mutation that confers a high level of resistance to this drug. Further clinical trials and in vitro studies are required to provide additional understanding of the complexities that govern interactions among the mutations in RT that are associated with drug resistance.

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References

9. Reference deleted.
38. Opravil, M., Yerly, S., Staszewski, S. et al. (2000). Prior treatment with mono or dual NRTIs before HAART as predictor of virological failure in simplified abacavir-based triple NRTI regimens: results from the simplified maintenance trial (SMT) and CAN 30017. Antiviral Therapy 5, Suppl. 3, 96–106.
45. Ross, L. L., Fisher, R., Scaiesella, A. et al. (2000). Patients failing on stavudine-based therapies that have developed thymidine analogue mutations; multidrug resistance or V75T mutations have reduced phenotypic susceptibility to stavudine. Antiviral Therapy 5, Suppl. 3, 38–9.
47. Wainberg, M. A., Miller, M. D., Quan, Y. et al. (1999). In vitro selection and characterization of HIV-1 with reduced susceptibility to PMPA. Antiviral Therapy 4, 87–94.