Effect of concurrent zidovudine use on the resistance pathway selected by abacavir-containing regimens

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Objectives
Abacavir (ABC) selects for four mutations (K65R, L74V, Y115F and M184V) in HIV-1 reverse transcriptase (RT), both \textit{in vitro} and during monotherapy \textit{in vivo}. The aim of this analysis was to compare the selection of these and other nucleoside reverse transcriptase inhibitor (NRTI)-associated mutations by ABC-containing therapies in the presence and absence of concurrent lamivudine (3TC) and/or zidovudine (ZDV) and to assess the effect of these mutations on phenotypic susceptibility to the NRTIs.

Design
This study was a retrospective analysis of the patterns of NRTI-associated mutations selected following virological failure in six multicentre trials conducted during the development of ABC.

Methods
Virological failure was defined as confirmed vRNA above 400 HIV-1 RNA copies/mL. RT genotype and phenotype were determined using standard methods.

Results
K65R was selected infrequently by ABC-containing regimens in the absence of ZDV (13 of 127 patients), while L74V/I was selected more frequently (51 of 127 patients). Selection of both K65R and L74V/I was significantly reduced by co-administration of ZDV with ABC (one of 86 and two of 86 patients, respectively). Y115F was uncommon in the absence (seven of 127 patients) or presence (four of 86 patients) of ZDV. M184V was the most frequently selected mutation by ABC alone (24 of 70 patients) and by ABC plus 3TC (48 of 70 patients). Thymidine analogue mutations were associated with ZDV use. The K65R mutation conferred the broadest phenotypic cross-resistance of the mutations studied.

Conclusions
The resistance pathway selected upon virological failure of ABC-containing regimens is significantly altered by concurrent ZDV use, but not by concurrent 3TC use. These data may have important implications for the efficacy of subsequent lines of NRTI therapies.

Keywords: abacavir, HIV resistance, K65R mutation, L74V mutation, zidovudine

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Introduction
Abacavir (ABC) has been shown to select for four mutations in HIV-1 reverse transcriptase (RT) both \textit{in vitro} and during monotherapy \textit{in vivo} \cite{1–3}. Individually, these mutations (K65R, L74V, Y115F and M184V) decrease susceptibility to ABC, but combinations of these mutations are required for >4.5-fold decreases in susceptibility to ABC, the clinically defined breakpoint for significant loss of ABC activity \textit{in vivo} \cite{4}. These four mutations cause varying levels of cross-resistance to other nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs).

The K65R mutation is associated with broad cross-resistance to the NRTIs, reducing susceptibility to tenofovir (TDF), didanosine (ddI), zalcitabine (ddC), lamivudine (3TC), and emtricitabine (FTC).
stavudine (d4T), emtricitabine (FTC) and amdoxovir (DAPD) in addition to ABC [5–11]. This mutation is associated with increased susceptibility to zidovudine (ZDV) [12]. The L74V mutation causes decreased susceptibility to ddI, ddC and ABC and increased susceptibility to TDF and ZDV [1,13,14]. The Y115F mutation is associated with reduced susceptibility to ABC and may be selected in vitro by TDF [1,15]. The M184V mutation reduces susceptibility to 3TC, FTC, ddC and ddI, but increases susceptibility to ZDV, d4T and TDF [14,16–19].

ABC does not select thymidine-analogue mutations (TAMs), associated with d4T and ZDV, either in vitro or during monotherapy in vivo. However, certain combinations of TAMs, most notably the M41L/L210W/T215Y complex, can cause broad cross-resistance to the NRTI class and have been shown to reduce ABC activity [4,20].

Previous studies have suggested that the pattern of resistance mutations selected by ABC-containing NRTI regimens differs according to the other NRTIs in the regimen. For example, ABC/3TC with or without nelfinavir (NFV) appears to select for the same four mutations seen with ABC monotherapy [21]. In contrast, ABC/ZDV is associated primarily with wild-type virus at rebound [2,21]. The triple therapy regimen ABC/3TC/ZDV selects initially for the M184V mutation, followed by TAMs in some cases [22,23]. The K65R, L74V and Y115F mutations are uncommon with this combination.

We performed a retrospective analysis of the resistance mutations observed in virus samples from patients in whom HIV-1 replication was inadequately suppressed on ABC-containing therapy.

Materials and methods

We analysed virus samples from subjects who experienced virological failure in six studies conducted during the development of ABC. Virological failure genotypes from ABC monotherapy (CNA2001, n = 27; CNAB2002, n = 43) and combination therapies not containing ZDV (PENTA 5, ABC + 3TC ± NFV, n = 8; CNA2007, ABC + EFV+APV, n = 49) were compared with those from ABC + ZDV-containing regimens (CNA2001, ABC monotherapy for 4 weeks, followed by 8 weeks of ABC + ZDV, n = 18; PENTA 5, ZDV + ABC ± NFV, n = 11; CNA3005, ABC + 3TC + ZDV, n = 40; and CNAAB/B3003, ABC + 3TC + ZDV, n = 17). Genotypes reported are the last available while patients were on originally randomized therapy (CNA2001, week 12; CNAB2002, median of week 20; PENTA 5, week 48; CNA2007, median of week 48; CNA3003, week 48; CNA3005, median of week 72).

All of these studies were conducted in antiretroviral therapy (ART)-naive subjects with the exception of CNA2007. Detailed descriptions of these trials have been previously published [2,3,21–26]. Mutations known to be associated with decreased phenotypic sensitivity to ddC, d4T, ddI, 3TC, ZDV, TDF and/or ABC were analysed at HIV-1 RT codons 41, 62, 65, 67, 69, 70, 74, 75, 77, 115, 116, 151, 184, 210, 215 and 219 [27]. The incidence of the ABC-associated mutations K65R, L74V, Y115F and M184V was compared with the incidence of these mutations in ABC regimens without ZDV following virological failure using a $\chi^2$ test with a significance level of $P<0.01$.

Site-directed mutagenesis was carried out using the QuikChange Mutagenesis Kit (Stratagene, La Jolla, CA) according to the manufacturer’s protocol. Assays of phenotypic susceptibility to the marketed NRTIs were conducted by Virologic (South San Francisco, CA, USA) using PhenoSenseTM as described [28].

Results

The incidences of treatment-emergent NRTI mutations after virological failure on ABC-containing regimens with concurrent ZDV (n = 86) and without concurrent ZDV (n = 127) are shown in Table 1. Selection of K65R was infrequent (13 of 127 patients; 10%) following virological failure with ABC monotherapy (CNA2001 and CNA2002) or ABC/3TC ± NFV (PENTA 5) in previously ART-naive patients or ABC/EFV/APV (CNA2007) in ART-experienced patients, while L74V or I was selected more frequently (51 of 127 patients; 40%). In the monotherapy studies

<table>
<thead>
<tr>
<th>Mutation(s)</th>
<th>ABC without ZDV (n = 127)</th>
<th>ABC with ZDV (n = 86)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>61 (48%)</td>
<td>34 (40%)</td>
</tr>
<tr>
<td>K65R alone</td>
<td>2 (2%)</td>
<td>1 (1%)*</td>
</tr>
<tr>
<td>L74V/I alone</td>
<td>23 (18%)</td>
<td>0</td>
</tr>
<tr>
<td>Y115F alone</td>
<td>1 (&lt; 1%)</td>
<td>0</td>
</tr>
<tr>
<td>M184V alone</td>
<td>6 (5%)</td>
<td>29 (34%)</td>
</tr>
<tr>
<td>K65R + M184V</td>
<td>5 (4%)</td>
<td>0</td>
</tr>
<tr>
<td>K65R + L74V/I + M184V</td>
<td>4 (3%)</td>
<td>0</td>
</tr>
<tr>
<td>K65R + 115F + M184V</td>
<td>1 (&lt; 1%)</td>
<td>0</td>
</tr>
<tr>
<td>K65R + L74V/I + 115F + M184V</td>
<td>1 (&lt; 1%)</td>
<td>0</td>
</tr>
<tr>
<td>L74V/I + M184V</td>
<td>19 (15%)</td>
<td>1*</td>
</tr>
<tr>
<td>L74V/I + 115F + M184V</td>
<td>4 (3%)</td>
<td>0</td>
</tr>
<tr>
<td>M184V + ZDV mutations</td>
<td>0</td>
<td>14 (16%)</td>
</tr>
<tr>
<td>M184V + ZDV mutations + L74V/I</td>
<td>0</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>M184V + ZDV mutations + Y115F</td>
<td>0</td>
<td>4 (5%)</td>
</tr>
<tr>
<td>ZDV mutations only</td>
<td>0</td>
<td>3 (3%)</td>
</tr>
</tbody>
</table>

*ZDV added following 4 weeks of ABC monotherapy.
(treatment duration 12–24 weeks), M184V was the most common mutation detected (24 of 70 patients; 34%), followed by L74V (14 of 70 patients; 20%), K65R (10 of 70 patients; 14%) and Y115F (three of 70 patients; 4%). No mutations were detected for 32 of 70 patients (46%) in the monotherapy studies. ABC/3TC ± NFV selected for M184V in eight of eight virological failures in PENTA 5 (treatment duration 48 weeks); in six of these eight failures, L74V was also detected, and in five of these six K65R and/or Y115F (one subject had K65R/L74V/Y115F and M184V) was detected [21]. Notably, K65R was not selected during 48 weeks of treatment for CNA2007, in which most patients (45 of 49; 92%) had baseline virus with TAMs; however, L74V or I emerged for 26 (53%) of these 49 patients and an additional six had L74V or I at baseline in the context of TAMs. Y115F was selected in two of 49 subjects (4%) and M184V in one of 49 subjects (2%) in CNA2007; 55% had M184V at baseline and one patient had Y115F at baseline [24].

Selection of both the K65R and L74V or I mutations was significantly lower (P<0.001) when ZDV was co-administered with ABC. Only three of 86 samples from subjects failing a ZDV/ABC-containing regimen had K65R or L74V and two of these were from CNA2001, where ZDV was added after 4 weeks of ABC monotherapy. Y115F was uncommon in the absence (seven of 127 patients; 6%) or presence (four of 86 patients; 5%) of ZDV. The M184V mutation was observed for one patient on ABC + ZDV in the absence of 3TC in CNA2001, where ZDV was added after 4 weeks of ABC monotherapy. M184V was the most common mutation during ABC monotherapy (24 of 70 patients; 34%) or when 3TC was present (48 of 70 patients; 69%).

Overall, the incidence of M184V in the absence of ZDV was 31% versus 56% in the presence of ZDV treatment. This difference reflects the relative usage of 3TC in each group and selection for M184V by ABC alone. In the group that did not receive ZDV, only 6% (eight of 127 patients) also received 3TC. In the group that did receive ZDV, 66% (57 of 86 patients) also received 3TC. TAMs were associated with ZDV use, but appeared slowly when detected, as previously described for ABC/3TC/ZDV [22,23,29]. No TAMs were detected during 12 weeks of treatment (4 weeks of ABC monotherapy followed by 8 weeks of ABC/ZDV) in the ZDV-containing group of CNA2001 (16 of 18 wild-type) and four of 11 subjects developed TAMs during 48 weeks of treatment in the ABC/ZDV ± NFV group from PENTA 5, with only wild-type virus detected in the other seven patients.

The effects of some of the treatment-emergent mutational patterns on phenotypic susceptibility were evaluated for a series of point mutants in an HXB2 background (Table 2). Changes in phenotypic susceptibility are shown as a ratio of the Virologic WT control (PNL4-3) to the test virus. Fold-changes that result in phenotypic susceptibility above that and are classified as susceptible by Virologic are shown in bold in Table 2, and may indicate reduced activity of the drug for viruses with the mutation in question.

Of the four mutations associated with ABC monotherapy (K65R, L74V, Y115F and M184V), only the K65R mutation conferred broad cross-resistance when present in isolation. None of these mutations alone conferred 4.5-fold decreased susceptibility to ABC, the clinically defined breakpoint. The M184V mutation together with any other ABC-associated mutation was associated with reduced susceptibility to ABC, ddI, 3TC and ddC. As expected from previous studies, M184V and L74V increased phenotypic susceptibility to TDF [14]. The ABC-associated mutations (except Y115F) increased susceptibility to ZDV. The M184V mutation appeared to restore full susceptibility to d4T in the presence of K65R and partially restore susceptibility to TDF.

**Discussion**

These results confirm and extend previous observations that concurrent use of thymidine analogues may reduce the incidence of K65R selection [30–33]. In this study, K65R was detected in only one of 86 samples from subjects on a failing ABC regimen that included ZDV, and that patient had received ABC monotherapy prior to the addition of ZDV (CNA2001). K65R by comparison was detected in 13 of 127 samples from patients failing ABC-containing regimens that did not include ZDV. The incidence of L74V was also significantly reduced by concurrent ZDV treatment (from 40% to 2%; Table 1). It is noteworthy that the therapy-experienced subjects in CNA2007 had a high prevalence of pre-existing TAMs, no selection for K65R in the absence of ZDV, but a very high rate of selection for L74V (53%). This suggests that the TAM/K65R pathways may be antagonistic, but that ZDV may be required to reduce L74V emergence. Selection for M184V on ABC + ZDV in the absence of 3TC was uncommon, being detected in one sample from a subject in CNA2001 where ABC monotherapy preceded addition of ZDV.

Given that M184V, L74V and K65R increase phenotypic susceptibility to ZDV in vitro, individually or in combination (Table 2), it is possible that ZDV is also more effective in vivo against virus with these mutations, reducing the likelihood that they will emerge in the dominant population. The mechanism behind the increased phenotypic susceptibility of HIV with these mutations to ZDV is not entirely clear, but may involve decreased ability of RT with these mutations to remove ZDV following incorporation [12,34,35]. The preponderance of wild-type RT in virological failures on ABC + ZDV ± NFV (23 of 29 patients)
from CNA2001 and PENTA 5 suggests a high genetic barrier to resistance for this combination, consistent with the TAM pathway selected in some patients during virological failure with ABC/3TC/ZDV [22,23,29].

Previous studies have suggested that K65R is negatively associated with thymidine analogue (ZDV and d4T) use [30–33]. However, recent studies have shown that d4T can select for K65R in vitro and that this mutation can cause decreased susceptibility to d4T, consistent with the phenotypic data presented here [9]. Additionally, K65R has emerged following virological failure on several d4T-containing regimens [36,37]. These data suggest that d4T may be less useful as a resistance modulator than ZDV, at least for K65R. Given the rapidly increasing prevalence of K65R (e.g. 8.8% of all samples in 1998 to 3.6% in 2003 for the Virco database [40]), several recent reports of high failure rates in regimens selecting this mutation and the resistance implications, understanding the factors involved in its selection may be clinically important [38–40].

It is important to note that most of these studies involved suboptimal regimens (mono or dual NRTIs) or highly experienced patients (CNA2007) and frequently long periods of detectable viraemia prior to the last genotype (reported here), so these results probably reflect worst case scenarios for mutation selection. Recent studies with ABC/3TC in conjunction with various third agents have found very low rates of selection for K65R or L74V shortly after virological failure [37,41].

The results of this study demonstrate that ZDV is useful as a resistance modulator, at least in combination with ABC, effectively reducing the incidence of selection for K65R and L74V. This may increase the likelihood that agents such as ddI, TDF and others affected by these two mutations will remain efficacious in second-line regimens. The relevance of these findings to combinations including NRTIs other than ABC, 3TC and ZDV remains to be elucidated.

References


Table 2 Fold-change in phenotypic susceptibility for point mutants of HXB2

<table>
<thead>
<tr>
<th>Mutants</th>
<th>ABC</th>
<th>ddI</th>
<th>3TC</th>
<th>d4T</th>
<th>ddC</th>
<th>ZDV</th>
<th>TFV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>0.9</td>
<td>0.8</td>
<td>1.0</td>
<td>0.8</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>K65R</td>
<td>3.0</td>
<td>2.1</td>
<td>9.7</td>
<td>1.8</td>
<td>2.6</td>
<td>0.5</td>
<td>2.1</td>
</tr>
<tr>
<td>L74V</td>
<td>2.1</td>
<td>1.5</td>
<td>1.6</td>
<td>1.1</td>
<td>1.6</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Y115F</td>
<td>2.8</td>
<td>0.8</td>
<td>1.6</td>
<td>0.8</td>
<td>0.6</td>
<td>0.9</td>
<td>1.3</td>
</tr>
<tr>
<td>M184V</td>
<td>3.9</td>
<td>1.4</td>
<td>&gt;120</td>
<td>0.9</td>
<td>2.0</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>K65R/M184V</td>
<td>10.0</td>
<td>3.6</td>
<td>&gt;120</td>
<td>1.0</td>
<td>4.6</td>
<td>0.3</td>
<td>1.2</td>
</tr>
<tr>
<td>L74V/M184V</td>
<td>8.5</td>
<td>2.4</td>
<td>&gt;120</td>
<td>0.9</td>
<td>2.5</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Y115F/M184V</td>
<td>9.8</td>
<td>2.0</td>
<td>&gt;125</td>
<td>0.9</td>
<td>2.0</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>K65R/L74V/M184V</td>
<td>12.0</td>
<td>6.7</td>
<td>&gt;120</td>
<td>1.2</td>
<td>4.4</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>M184V/T215Y</td>
<td>5.4</td>
<td>2.1</td>
<td>&gt;125</td>
<td>1.4</td>
<td>2.7</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>D67N/K70R/T215Y/K219Q</td>
<td>1.5</td>
<td>1.5</td>
<td>3.2</td>
<td>2.0</td>
<td>1.2</td>
<td>30</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Bold text indicates a fold change in phenotype above that classified as sensitive by ViroLogic.
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