AIDS Rev 2002;4:224-232

Might the M184V Substitution in HIV-1 RT confer Clinical Benefit?

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Abstract

The M184V substitution in HIV-1 RT develops rapidly following initiation of therapy with 3TC and confers high-level phenotypic resistance to this drug both *in vitro* and *in vivo*. Interestingly, the presence of M184V is also associated with alteration of several mechanisms relating to RT function that include decreased RT processivity, reduced nucleotide-dependent primer unblocking, increased fidelity, hypersensitization to other NRTIs, impaired viral fitness, and delayed appearance of mutations in RT that are responsible for resistance to thymidine analogues (i.e. thymidine-associated mutations or TAMs). Collectively, these factors might explain the residual antiviral effect and clinical benefit observed with continued use of 3TC in combination therapy regimens following the emergence of M184V. Indeed, the results of numerous controlled as well as observational clinical studies are suggestive of improved therapeutic outcome associated with continued usage of 3TC and maintenance of the M184V mutation. However, several of these trials did not possess adequate statistical power to resolve whether or not continued use of 3TC provided actual benefit, nor were they specifically designed to test the M184V benefit hypothesis in prospective fashion. There is a need for randomized clinical trials of this type in order to validate the potential benefit of maintenance of M184V and whether continued use of 3TC is the only means of attaining this objective.

Key words

HIV-1. Resistance. Reverse Transcriptase. 3TC. M184V. Benefit. Fitness. Processivity. Fidelity.

Introduction

The emergence of drug-resistant HIV-1 is both a consequence and limitation of antiretroviral therapy and has been shown to significantly diminish the effectiveness and duration of benefit associated with combination therapy regimens for the treatment of HIV/AIDS[1-5]. Although resistance-conferring mutations in both the HIV-1 reverse transcriptase (RT) and protease (PR) genes may often precede the initiation of therapy, due to both spontaneous mutagenesis and the spread of resistant viruses by sexual and other means of transmission, it is generally believed that multiple drug mutations to any single or combination of antiretroviral agents (ARVs) are selected during continued viral replication in the presence of incompletely suppressive drug regimens[6-8]. For the protease inhibitors (PIs)[9-11], and most nucleoside analogue reverse transcriptase inhibitors (NRTIs), the development of progressive high-level phenotypic drug

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resistance follows the accumulation of primary resistance-conferring mutations in the HIV-1 PR and RT genes, respectively[12-14]. However, in the case of the non-nucleoside reverse transcriptase inhibitors (NNRTIs), which have lower genetic barriers for the development of drug resistance, a single primary drug resistance mutation is generally sufficient to abrogate antiviral activity and produce extensive cross-resistance within this class of ARVs[15,16]. Similarly, a single resistance-conferring mutation encoding a methionine to valine amino acid substitution at position 184 (i.e. M184V) in the RT enzyme also rapidly results in high-level resistance (i.e. 100 to 1000 fold increase in IC_{50}) to the nucleoside analogue lamivudine ([-]-2', 3'-dideoxy-3'-thiacytidine, 3TC) both *in vitro* and *in vivo*[17-21]. Unlike the situation with the NNRTIs, there is also considerable evidence at this time suggesting that lamivudine may, in fact, continue to contribute to the effectiveness of antiretroviral combination therapy regimens, even after the appearance of the M184V mutation and development of high-level phenotypic drug resistance to 3TC as confirmed by *in vitro* drug susceptibility assays[22-25].

In this review, recent laboratory findings on the effects of the M184V mutation on RT function and viral replication kinetics will be discussed in relation to clinical studies in which the presence of the M184V mutation has been associated with a positive treatment outcome. The clinical implications of HIV-1 drug resistance are significant and illustrate the need for continued research in this area. In order to confront HIV-1 drug resistance, the continued optimization of antiretroviral therapy constitutes an important goal that needs to be pursued in tandem with new drug discovery. Approaches based on the maintenance of the M184V substitution in HIV-1 RT through the use of sufficiently selective antiretroviral regimens may represent a viable intervention that should be considered alongside other therapeutic options.

(Research performed by Marco Petrella was in partial fulfillment of the Ph.D. degree, Faculty of Graduate Studies and Research, McGill University, Montreal, Canada).

Development of M184V in HIV-1 RT

Lamivudine ([-]-2', 3'-dideoxy-3'-thiacytidine, 3TC) is a potent and highly selective nucleoside analogue inhibitor of wild-type HIV-1 RT[26-29]. As with other members of this class of antiretroviral drugs, 3TC is phosphorylated to its active triphosphosphate form (3TCTP) by host cellular kinases. 3TCTP lacks a 3'-hydroxyl group on the nucleoside pentose ring that is required for DNA polymerization and, hence, the antiviral activity of 3TC and other NRTIs is based on the ability of these compounds to prematurely terminate viral DNA strand elongation[30-34]. Resistance to 3TC is rapidly selected in tissue culture following serial passage of wild-type HIV-1 in the presence of increasing concentrations of drug. In addition, 3TC-resistant HIV-1 can be isolated from patients who experience virological failure as early as eight weeks following initiation of a 3TC-containing regimen[35-37]. Resistance to 3TC follows the development of a single primary mutation in the HIV-1 RT gene that encodes a methionine to valine amino acid substitution at position 184 (i.e. M184V) in both the p66 and p51 subunits of HIV-1 RT. The appearance of this mutation is usually preceded by another more transient mutation, in which the methionine residue at position 184 is replaced with isoleucine (i.e. M184I)[38].

Use of the limited dilution method to quantify the relative proportions of HIV-1 variants that are selected by 3TC *in vitro* has determined that the frequency of M184I (56%) is initially more than 4 times greater than that observed for M184V (12.5%)[38]. These findings indicate that HIV-1 RT has a mutational bias for the M184I substitution which explains the earlier appearance of this variant over M184V following initiation of treatment with 3TC[38,39]. Both the M184I and M184V substitutions each only require a single nucleotide change or mutation in the HIV-1 genetic sequence. However, HIV-1 variants harboring M184I are less fit than their M184V counterparts and are therefore rapidly out-competed both *in vitro* and *in vivo* by the latter[12,38,40,41].

The proximity of the methionine amino acid residue at position 184 in relation to the active site of HIV-1 RT is important for RT enzyme function[42-44]. M184V is a discriminatory mutation[45] that significantly reduces the affinity of HIV-1 RT for some NRTIs in comparison with naturally occurring deoxynucleoside triphosphates (dNTPs) as preferential substrates for the mutated enzyme[46,47]. This altered selectivity of M184V RT is responsible for high-level phenotypic drug resistance to 3TC *in vitro* and has been shown to increase the concentration of drug needed to inhibit viral replication by 50 percent (i.e. IC_{50}) for M184V HIV-1 from 100 to 1000 times over levels observed for wild-type virus[35-37,48,49].

M184V does not confer significant cross-resistance to other NRTIs

The M184V mutation can be selected by structurally unrelated NRTIs such as abacavir (ABC)[50-52] and less frequently by didanosine (ddI) or zalcitabine (ddC)[18,53,54]. M184V is, in fact, the first resistance mutation that emerges following *in vitro* or *in vivo* exposure to ABC and, contrary to the situation with 3TC, confers only low-level resistance (i.e. 2 to 4 fold increases in IC_{50}) to ABC. Indeed, the latter represents a high genetic barrier compound in regard to development of drug resistance, and requires the accumulation of several nucleoside analogue mutations (NAMs) in RT (e.g. M41L, K65R and Y115F in addition to M184V) before significant loss of antiviral activity (i.e. > 10 fold increase in IC_{50}) is observed *in vitro*[55,56]. Similar attenuation of antiviral drug susceptibility with M184V has been reported for both ddI and ddC only in the presence of additional mutations[18]. These laboratory findings are of clinical relevance and predict that the emergence of the M184V mutation should not be associated with broad cross-resistance to most NRTIs including zidovudine (ZDV), ddI, ddC and ABC; this has been confirmed by observational and controlled clinical trials[57,58].

In the CNA3003 study, for example, antiretroviral *naïve* patients initially randomized to a dual NRTI regimen consisting of 3TC and ZDV were eligible to receive ABC with or without additional ARVs following the sixteen-week

double-blind phase of this study. Despite the presence of the M184V mutation in more than 70 percent of patients, the intensification of therapy with ABC produced further suppression of viral replication and 65 percent of patients attained < 400 copies/ml plasma HIV-1 RNA after 48 weeks of therapy. Thymidine analogue mutations (TAMs) were also observed infrequently in this group of patients[59]. Low selection rates for TAMs or for the Q151M multi-dideoxynucleoside resistance mutation in the presence of M184V have been described in other studies in which patients were treated for up to 48 weeks with a stavudine (d4T)/3TC dual NRTI regimen[60]. Taken together, the results from these and related protocols strongly suggest that the presence of the M184V mutation does not, by itself, limit treatment-sequencing options available with most other NRTIs or compromise the clinical effectiveness of either ABC- or ddI-containing regimens.

Reversal of Resistance to AZT and Synergistic Antiviral Activity with Other Drugs

The M184V substitution in HIV-1 RT may also have a role in the reversal of phenotypic susceptibility to ZDV in HIV-1 variants that have already acquired ZDV resistance mutations[14,61-63]. For example, in the DELTA roll-over study, selection of the M184V mutation was associated with a transient resensitization to ZDV during a one-year follow-up period in 20 of 29 patients in whom baseline HIV-1 isolates were phenotypically resistant to ZDV[64]. Restoration of antiviral susceptibility to ZDV observed during concomitant treatment with 3TC is thought to be mediated primarily by impaired rescue of dideoxy-terminated primers by HIV-1 RT containing the M184V resistance mutation[65]. Two related mechanisms, notably enhanced pyrophosphorolysis and nucleotide-dependent primer unblocking, have been identified as the underlying cause of resistance to ZDV and d4T[13,45,66,67]. Rescue of viral DNA synthesis by either mechanism requires the excision of ZDV 5'-monophosphate (ZDVMP) from the 3' terminus of the polymerizing c-DNA strand and is facilitated by pyrophosphate (PPi), or alternatively, by ATP which is believed to be the principal PPi donor *in vivo*. Furthermore, ATP binding and consequently, uncoupling of ZDVMP-terminated primers, have been reported to increase concomitantly with the development of TAMs[68-70].

In addition to reports of a potential benefit regarding reversal of ZDV resistance, the M184V mutation may also enable a synergistic interaction between 3TC and ZDV that temporarily boosts the *in vitro* antiviral activity of ZDV[71,72]. Similarly, the IC_{50} values for two related nucleotide analogue inhibitors of HIV-1 RT, i.e. adefovir (PMEA) and tenofovir (PMPA or TDF), are approximately two-fold lower for M184V-containing HIV-1 in comparison to wild-type virus and appear to be unaffected by the presence of ZDV resistance-conferring mutations in RT[73-76]. Reduced nucleotide-dependent primer unblocking and reduced levels of pyrophosphorolysis have been documented in HIV-1 RT that contains the M184V mutation[13,65], and this provides a possible mechanism to explain the resensitization that occurs when viruses that are initially resistant to ZDV regain susceptibility to this drug. Notably, the incorporation of ZDV triphosphate into a growing viral DNA chain may not be as easily reversed in the case of viruses and RT enzymes containing the M184V substitution. Hence, in this situation, DNA chain termination will still be expected to occur to some extent at least. Furthermore, since even wild-type RT possesses some degree of nucleotide primer unblocking activity, it also follows that drugs such as d4T, PMEA and PMPA might also display heightened antiviral activity against M184V-containing viruses for the same reason. It is only the later accumulation of other mutations in RT, such as E118I, that may play a negative compensatory role in regard to M184V, which may reverse these effects[21,77,78].

Indeed, the possibility that viruses containing M184V may remain minimally sensitive to 3TC for these same reasons should not be discounted. It is also correct that M184V discriminates against incorporation of 3TC triphosphate at levels between 50-200 fold, depending on how these measurements are performed; nonetheless, once a single molecule of 3TC-TP is incorporated into viral DNA, the likelihood of its excision is reduced compared to wild-type RT because of the M184V effect on pyrophosphorolysis/nucleotide primer unblocking. In this context, it has been shown that modest concentrations of 3TC-TP can exert chain termination effects against M184V-containing RT in biochemical assays[20,71]. However, reduced pyrophosphorolysis/nucleotide primer unblocking by HIV-1 RT containing the M184V substitution has not been consistently demonstrated in all situations and, therefore, may not represent the sole mechanism responsible for heightened antiviral susceptibility to other compounds[79].

Improved HIV-1 RT Fidelity with M184V and Delayed Emergence of TAMs

Resistance mutations to ARVs arise spontaneously as a result of the error-prone replication of HIV-1 and, in addition, are selected both *in vitro* and *in vivo* by pharmacological pressure[80-82]. The high rate of spontaneous mutation in HIV-1 has been largely attributed to the absence of a 3'->5'exonuclease proof-reading mechanism. Sequence analyses of HIV-1 DNA have detected several types of mutations including base substitutions, additions and deletions[80]. The frequency of spontaneous mutation for HIV-1 varies considerably as a result of differences among viral strains studied *in vitro*[39]. Overall mutation rates for wild-type laboratory strains of HIV-1 have been reported to range from $97x10^{-4}$ to $200x10^{-4}$ per nucleotide for HXB2 to as high as $800x10^{-4}$ per nucleotide for the HIV-1 NY5 strain[39,80].

In addition to the low fidelity of DNA synthesis by HIV-1 RT, other interdependent factors that affect rates of HIV mutagenesis include RT processivity, fitness, viral pool size, and availability of target cells for infection[83-86]. It follows that an alteration in any single one or combination of these factors might influence the development of HIV drug resistance. Of relevance is the positive effect of the M184V substitution on HIV-1 RT fidelity[87-89]. Furthermore, the presence of the M184V substitution in both HIV-1 and in simian immunodeficiency virus (SIV), containing large genomic deletions, results in a relative

inability to regain replication competency due to compensatory mutations and reversions compared to matched wild-type variants lacking M184V[90]. Clinical benefit due to M184V is not evident for all classes of ARVs, and may be limited to the delayed emergence of TAMs[91,92]. In the ALBI trial[93], for example, the T215Y mutation developed in a significantly higher proportion of patients randomized to treatment with ddI/d4T (62%) compared to those who received ZDV/3TC (10%)[94]. The Q151M multi-nucleoside resistance mutation was also observed less frequently in patients treated with 3TC[94].

Similar results have also been obtained following a retrospective analysis of the effect of the M184V substitution in RT on the incidence of TAMs and fold differences in phenotypic resistance to ZDV and d4T among baseline HIV-1 clinical isolates from NRTI-experienced patients enrolled in the CNAB 3002 study. Patients previously treated with 3TC prior to initiating a new regimen with 3TC, ABC and ZDV were observed to have a significantly lower proportion of isolates that contained 3 or more TAMs (9%) in comparison to 3TC-naïve patients (36%). In addition, the frequency of viral isolates containing D67N, L210W and T215Y/F was also lower in 3TC-experienced patients.This reduction in proportion of TAMs was independent of levels of plasma HIV-1 RNA and duration of prior treatment with ARVs. Levels of phenotypic resistance to ZDV and d4T were also reduced in patients in whom M184V was selected as a result of previous exposure to 3TC, compared to cases in which this mutation was not present[95].

The development of ZDV resistance was also evaluated in patients experiencing virological failure with 3TC-containing regimens in the AVANTI 2 and 3 clinical studies. In these trials, antiretroviral therapy-*naïve* patients with HIV infection were randomly assigned to treatment with 3TC/ZDV or 3TC/ZDV/IDV for 52 weeks in AVANTI 2 or with 3TC/ZDV and nelfinavir (NFV) for 28 weeks in the case of AVANTI 3[96,97]. Using combined data from both trials, genotypic analysis revealed ZDV resistance-conferring mutations in 27% of patients from the 3TC/ZDV arm of AVANTI 2, whereas these mutations were absent in patients from both arms of AVANTI 3, as well as in patients who received 3TC/ZDV/IDV in AVANTI 2[98]. The M184V mutation, in these studies, was present in viral isolates from most patients who were treated with 3TC/ZDV. Overall, these results compare favorably to those from the CNA3003 study of ABC intensification therapy, in which selection rates for TAMs and Q151M were also reduced following appearance of M184V[59]. In contrast to these findings, it has been demonstrated that the presence of either the M184I or M184V substitutions in RT did not significantly restrict the kinetics or extent of mutagenesis in the PR gene of HIV-1 compared to wild-type virus during tissue culture selection with protease inhibitors[92]. In other experiments, the development of drug resistance to the NNRTIs nevirapine and loviride was not delayed in M184V-containing HIV-1 compared with wild-type HIV-1 strain IIIB[91]. Hence, the potential protective effects of M184V against selection of resistance-conferring mutations may be limited to delayed emergence of TAMs and further research on this topic is required. Of course, the multiple alterations in RT enzyme function associated with M184V may contribute to these effects.

In fact, a number of clinical trials with triple drug combination therapy regimens have been performed in which the first and most prevalent mutation to have arisen in the context of an initial regimen was M184V (Table 1). The finding that the ocurrence of M184V is so extensive suggests that this substitution should be considered to be a marker of ongoing viral replication in the face of drug pressure as much as a determinant of resistance to 3TC. The various clinical trials in Table 1 represent situations in which the development of M184V was not necessarily accompanied by a sharp rebound in viral load, as long as the other two drugs in the regimen continued to maintain antiviral effect. This is also reflected by the observation that patients in each case had a significantly reduced likelihood of developing TAMs or mutations associated with protease inhibitor resistance. Thus, while the occurrence of M184V may sometimes be predictive of treatment failure, this is not always the case. Moreover, viruses containing M184V remain susceptible to all other approved antiviral drugs.

Diminished HIV-1 RT processivity and impaired viral fitness with M184V

The processivity of the HIV-1 RT enzyme may be affected by the presence of several NAMs. These mutations which include L74V and M184V reduce the proces-

Table 1. *Incidence of various resistance-conferring mutations in patients on initial triple combination therapy regimens*

Study	Treatment	No.	% Patients M184V	% Patients with TAMs	% Patients Protease inhibitor resistance
Start I & II	3TC/ZDV/IDV	34	59	0	6
AVANTI 2	3TC/ZDV/IDV	11	45	0	0
AVANTI 3	3TC/ZDV/NFV	7	43	0	14
CNA3005	3TC/ZDV/IDV	29	70	0	5
NZTA4002	3TC/ZDV/NFV	33	61	3	52
ACTG 347	3TC/ZDV/APV	7	57	0	14
ACTG 343	3TC/ZDV/IDV	17	82	0	0

*Sampling for genotypic analysis was performed on clinical isolates from patients whose viral load had rebounded to >400 copies HIV-1 RNA/ml.

sivity of RT, while it is unclear what role the ZDV resistance-conferring mutations (i.e. D67N, K70R and T215Y/F) may play in this regard[98-101]. Furthermore, certain combinations of M184V in the presence of TAMs, in particular the T215Y/F mutation, have been shown to interact additively or synergistically to inhibit RT processivity to a higher degree than produced by M184V alone[103]. The acquisition of a compensatory mutation at position 219 in RT together with T215Y/F may result in higher RT processivity than is observed for wild-type virus[104,105].

HIV-1 RT processivity may be a major determinant of viral replication capacity or fitness[79,101,102,104]. It has been shown that HIV-1 harboring drug resistance mutations to nucleoside analogues have a measurable replication disadvantage in comparison to wild-type virus. However, it has also been reported that the extent of the impairment of HIV-1 fitness, associated with RT mutations, is less than that produced by primary PR drug resistance mutations[106-112]. Although estimates for the fitness of M184V HIV-1 mutants vary considerably depending on laboratory methodology and the viral strain utilized, the replication efficiency of these viruses appears to be reduced by about 3 to 10 percent in comparison to wild-type HIV-1[25,41,101]. In a recent study that examined the fitness of multi-class resistant HIV-1 acquired during primary HIV infection (PHI), it was observed that plasma HIV-1 RNA levels in two cases were initially suppressed but increased to levels comparable to those for PHI patients without these mutations following disappearance of the M184V mutation. Remarkably, a third PHI case infected with M184V virus maintained consistently low levels of plasma HIV-1 RNA for up to five years from the estimated time of seroconversion. Furthermore, virus from another individual could only be isolated for growth competition experiments following the loss of the M184V mutation[113]. Of relevance to these observations, the M184V mutation has also been reported to produce a slight impairment of SIV fitness, although this did not affect disease outcome in a macaque study of SIV infection[114].

As stated previously, in either SIV or HIV-1 variants containing large deletions in the viral genome, the simultaneous presence of M184V in RT has been shown to severely restrict the ability of these initially attenuated viruses to regain viral replication competence as a result of compensatory mutagenesis[90]. HIV-1 variants containing M184V have also been reported to show slower escape from neutralizing antibodies as consequence of mutations in the envelope (*env*) gene, than did wild-type virus[22,23,115].

Diminished fitness may also be advantageous in helping to improve HIV-1-related disease outcome, as inefficient viral replication has been shown to be associated with reduced plasma viremia, delayed emergence of resistance mutations, and improved immunological responses to antiretroviral therapy. With respect to the latter, immunological and virological discordance, in which CD4 cell counts are stabilized or increase despite detectable plasma HIV-1 RNA, it has been partially attributed to the emergence of fitness-reducing resistance mutations such as D30N in the HIV-1 PR gene in patients continuing treatment with failing PR inhibitor-based regimens[116-119].

Similarly, impaired fitness associated with M184V may explain residual antiviral activity reported for 3TC following the development of high-level resistance to this drug. In the NUCA3001 study, 366 patients with baseline CD4 cell counts between 200 to 500 cells/mm^3 and less than 4 weeks of prior exposure to ZDV were randomized to receive treatment with 3TC monotherapy (300 mg every 12 h), ZDV monotherapy (200 mg every 8 h) or combination therapy with 3TC (150 or 300 mg every 12 h) with ZDV for up to 52 weeks[120,121]. In this study, plasma HIV-1 RNA in the 3TC monotherapy arm attained a nadir of –1.2 $\log_{10}$ by week 4 after initiation of treatment before rebound occurred concomitant with the appearance of M184V; however, viral load levels consistently remained below baseline (0.6 to 0.3 log 10 viral load reduction), and were significantly lower than those resulting from treatment with ZDV alone, for the trial's 52-week duration[122]. The development of the M184V mutation in 3TC/ZDV-treated patients enrolled in the AVANTI 2 and 3 trials was also associated with significant reduction of baseline plasma HIV-1 RNA that was, in fact, greater than would be expected with ZDV monotherapy[98]. Collectively, these results from the NUCA3001 and AVANTI trials provide further evidence for a residual antiviral effect with 3TC following the emergence of M184V.

Coincidently, lamivudine (3TC)-resistant hepatitis B virus (HBV) variants have been selected in patients following prolonged treatment with this drug. As with HIV-1, this resistance results from either isoleucine (I) or valine (V) substitutions in place of methionine (M) within the C domain of the highly conserved tyrosine-methionine-aspartate-aspartate (i.e. YMDD) motif of the HBV DNA polymerase. In most patients with chronic HBV infection, serum HBV-DNA remains suppressed below baseline so long as treatment with 3TC is continued even after emergence of M184V[123-125]. HBV variants that contain M184V are thought to have decreased replication capacity compared to wild-type virus, which helps to explain the sustained antiviral activity of 3TC in this circumstance[123,126,127].

The Trilège trial was designed to evaluate virological outcomes with induction antiretroviral therapy followed by maintenance therapy with a less potent regimen. A total of 378 antiretroviral-*naïve* patients with HIV-1 infection received treatment during the induction phase of the trial with 3TC/ZDV/IDV for a 12-week period. Of these patients, 279 attained the virologic endpoint for the induction phase which required a reduction of plasma HIV-1 RNA to <500 copies/ml and were randomly assigned to the maintenance phase to continue treatment with 3TC/ZDV/IDV or, alternatively, with 3TC/ZDV or ZDV/IDV[126]. The effectiveness of either dual combination regimen to maintain plasma HIV-1 RNA below 500 c/ml or to produce further suppression to below 50 c/ml was diminished in comparison to that noted with 3TC/ZDV/IDV. Furthermore, despite reduced antiviral potency, maintenance therapy with 3TC/ZDV or ZDV/IDV in the Trilège trial did not compromise the virological benefit conferred by subsequent treatment with either the original induction regimen or other antiretroviral combinations[129]. Removal of 3TC from the triple-drug induction regimen was associated with rapid rebound of HIV-1 RNA that increased from –1.66 $\log_{10}$ at the time of virological failure to near pre-treatment levels (i.e. –0.31 $\log_{10}$) six weeks later. In contrast, plasma HIV-1 RNA in the 3TC/ZDV group did not rebound as sharply as was

the case in patients treated with ZDV/IDV, and remained suppressed at a level of –1.38 $\log_{10}$ below baseline for the six-week period following removal of IDV from the induction regimen[130].

Others have also reported differential kinetics of plasma HIV-RNA rebound in patients experiencing virological failure on triple antiretroviral therapy regimens. In these studies, it was noted that the slope of plasma HIV-RNA for virus escaping with the M184V 3TC-resistance mutation was lower and did not attain as high levels compared to those cases in which virological failure followed the emergence of HIV-1 containing NNRTI resistance-conferring mutations (e.g. Y181C or K103N)[131].

Effect of M184V on HIV disease outcomes and need for additional clinical trials

Further clinical evidence regarding continued use of 3TC in the face of the M184V mutation is provided by the CAESAR trial. Briefly, patients with HIV-1 infection were randomized to receive either placebo, 3TC or, alternatively, a combination of 3TC and loviride, an NNRTI, added onto a ZDV-based regimen for up to 52 weeks. The results on 1.080 patients revealed that treatment with 3TC resulted in significantly less HIV disease progression and death compared to the placebo arm[132,133]. However, the clinical benefit conferred by 3TC in this study was of limited duration, most likely due to accumulation of other resistance-conferring mutations.

To date, clinical benefits resulting from selection of M184V in 3TC-containing regimens have been largely inferred from mechanistic studies of RT function and the results of *post-hoc* and meta-analyses from numerous clinical trials. However, discordant findings that interrogate the utility of continuing treatment with 3TC after the development of high-level resistance have also been published. A notable example is ACTG 370 in which suppression of baseline plasma HIV-1 RNA levels to $\leq$ 200 copies/ml was reported to be superior after 24 weeks of therapy when 3TC was replaced by delavirdine (DLV) (73% response for DLV vs 58% response for 3TC maintenance) in NRTI-experienced patients also treated with IDV and ZDV[133]. Although differences in virologic outcome between both treatment groups in this study were not statistically significant ($p = 0.29$), these results nevertheless reflect the need for other randomized clinical trials that will be sufficiently powered to validate the M184V benefit hypothesis. In this regard, the COLATE trial, a large multi-center European study initiated by the Copenhagen HIV Programme (CHIP), may help to address this important objective. In this study, 160 patients experiencing viral load rebound (plasma HIV-1 RNA $\geq$ 1000 copies/ml) on an initial 3TC-containing regimen will be randomized to one of two treatment groups in which 3TC is either continued or substituted by another drug in individualized second-line combination therapy regimens. This study also involves new restrictions to be placed in regard to use of other ARVs.

Conclusion

Several mechanisms including decreased RT processivity, reduced nucleotide-dependent primer unblocking, increased fidelity, hypersensitization to other NRTIs, and impaired viral fitness have been invoked to explain the clinical benefits associated with continued 3TC therapy following emergence of the M184V substitution in RT. However, the importance of each of these factors in regard to therapeutic outcome may be difficult to ascertain, and, indeed, it is increasingly clear that M184V can have multiple simultaneous effects based, in large part, on its strategic location close to the active catalytic site of RT. Thus, multiple mechanisms may, in fact, be responsible, including reduced RT processivity and impairment of viral fitness. In designing future clinical trials to test the M184V benefit hypothesis, as is the case with COLATE, consideration of these mechanisms alongside the potential for augmentation of the antiviral activity of other drugs will be important factors to help guide the selection of antiretroviral drugs to be used in combination with 3TC.

3TC was one of the first ARVs to result in reductions in HIV/AIDS-related morbidity and mortality and remains a cornerstone of current antiretroviral therapy. Hopefully, continued research to further study the potential benefits of M184V will lead to optimized therapy with available drugs and provide insight into future optimization of combination regimens. It should be noted, as well, that continued 3TC usage may not be the only means of preserving M184V and that alternative ways of attaining this goal could be explored. These could include a variety of measures that would keep pressure on M184V including the use of ABC and/or low doses of 3TC. These and related concepts could likewise constitute the basis of future clinical trials although, to be sure, continued 3TC usage is the only clinically proven means of preserving the M184V mutation at this time.

Finally, none of the points made in this paper in regard to potential benefits of M184V would justify, as some have suggested, that this substitution be deliberately selected by 3TC as part of a therapeutic strategy. Antiviral drugs should ideally be used for their intended purpose which is to arrest viral replication and reduce viral load. The arguments raised here pertain only to the wisdom of whether to maintain M184V once it has already been selected.

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