

The development of resistance of HIV-1 to zalcitabine

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Introduction

Antiviral therapies against HIV infection are known to select for variants with reduced sensitivity (resistance) to the antiviral agent. The development of high-level viral resistance has been temporally associated with the loss of favourable effects on activity markers (e.g., CD4+ cell count and plasma viral RNA levels) with a number of antiretroviral agents. For example, the emergence of resistance mediated through the Met¹⁸⁴Leu mutation during lamivudine (3TC, 2'3'-dideoxy-3'-thiacytidine) therapy is found to correlate with at least partial loss of antiviral effect of this agent [1–3]. Selection of resistant virus, therefore, represents a major cause of failure of antiviral therapy. Furthermore, a relationship between the development of resistance to zidovudine (ZDV, 3'-azido-2'3'-dideoxythymidine) and increased risk of clinical progression or death has been reported, raising concerns that appearance of resistant virus may have negative consequences for patients [4–7].

Resistance to ZDV has been reported in 10–15% of persons presenting with primary HIV-1 infection in urban areas [8,9], underlining the importance of starting antiretroviral therapy with more than one agent. Rationales for combination therapy also include (i) the potential for antiviral synergy, as has been reported *in vitro* with a range of agents both from the same class, for example, ZDV and zalcitabine (ddC, 2'3'-dideoxycytidine) [10], and different classes, for example, ZDV or ddC or both with proteinase inhibitor [11]; and (ii) the potential to delay or prevent resistance to one or more agents in the combination. The benefits of combination therapy on disease progression and survival

have been reported recently with ZDV–ddC or ZDV–didanosine (ddI, 2'3'-dideoxyinosine) therapy compared with ZDV monotherapy as initial therapy [12–14], and ddC–saquinavir (SQV) compared with either agent alone in ZDV-experienced patients [15].

There remains an urgent need not only for new antiviral drugs, but also for new regimens of drug combinations. Despite a number of combination regimens achieving reductions in viral load over 1-year periods to below the level of detection of currently available assays, these regimens are likely to fail eventually in the majority of patients due to the appearance of resistant virus, which arises from residual viral replication in less well penetrated 'sanctuary sites' (such as the central nervous system or genital tract) secondary to episodic compliance failure, or possibly from acquisition of drug-resistant virus from another individual. Long-term management of persons with HIV infection is therefore likely to involve the sequencing of combination regimens. Given the limited number of antiretroviral agents currently available, we must consider whether therapies can be optimally sequenced to attain the maximal magnitude and duration of reductions in viral load achievable with each regimen. This can only be possible by reserving those antiviral compounds which induce the greatest levels of resistance and cross-resistance for later use.

Currently available antiretroviral therapies involve the inhibition of the viral enzymes reverse transcriptase (RT) or proteinase. The nucleoside analogue ZDV is the longest established and most widely used anti-HIV RT agent [16]. Other nucleoside analogue RT inhibitors include ddC, ddI, stavudine (d4T, 2'3'-didehydro-2'3'-dideoxythymidine) and 3TC. Two of

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these compounds, ddC and 3TC, share a common nucleotide base, but differ in their sugar moieties and stereochemistry (Fig. 1). Both are approved for use in combination with ZDV.

This review summarizes some of the mechanisms of resistance and examines the resistance profile of ddC *in vitro* and in clinical trials.

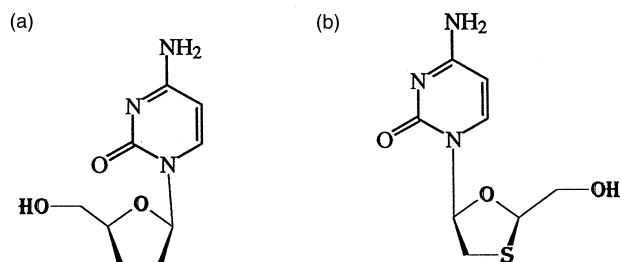


Fig. 1. The structures of (a) zalcitabine and (b) lamivudine.

Genetic diversity and selection

In common with many RNA viruses, HIV displays a high degree of genetic diversity, even within individual patients at any given time [17]. The very high rate of turnover of the virus [18,19] and the high rate of incorporation of errors by the HIV RT (an estimated *in vivo* forward mutation rate of 3.4×10^{-5}) [20] are key to the continual generation of diversity. Indeed, it has been suggested that there may be 'mutation-prone regions' present in the HIV RT gene with higher rates of error incorporation [20,21] that are not (as with human DNA polymerases) 'edited out' because HIV RT does not possess detectable proof-reading exonuclease activity [22].

Such is the level of diversity that each infected individual is thought to harbour a very large, dynamic reservoir of virus quasispecies [23]. A large proportion of lymphoid CD4+ cells harbour HIV, mostly in latency [24]. However, it is estimated that of the order of 10^7 CD4+ lymphocytes are productively infected even in individuals with asymptomatic infection [25]. It is also noted that this approximates to the daily turnover of CD4+ lymphocytes in infected patients [18,19]. In addition, the half-life of plasma HIV-1 is thought to be of the order of 2 days or less and the daily turnover of virus is calculated to be at least 10^8 – 10^9 virions [18,19,26]. Together with the rate of error incorporation, this indicates a very high level of potential daily variability. One implication of this is the likelihood of mutations related to drug resistance being present in viral populations prior to therapy [27]. This makes the virus highly susceptible to positive selection.

Under normal circumstances such selection is driven by the immune response. However, the dynamic response of the virus to external pressures such as vaccination or antiviral intervention provides a major barrier against the successful control of HIV-1 infection [28,29]. This is exemplified by the well-documented selection of HIV-1 variants resistant to antiviral agents in patients during therapy [30]. Selection pressure from the non-nucleoside RT inhibitor nevirapine can result in a complete replacement of wild-type with single-substitution highly resistant mutant within 14 days of therapy [19]. Other therapies require more prolonged treatment before resistant virus becomes predominant. For example, resistance to SQV is an infrequent event even after 1 year of therapy, particularly when combined with other agents, despite the requirement of only one or two mutations to establish resistance [31]. Resistance to other compounds such as ZDV, ritonavir and indinavir may require accumulation of several mutations before significant changes in median inhibitory concentration (IC_{50}) are observed. However, the number of mutations required may not be predictive of 'time to resistance' in the clinic. Factors that may delay the appearance of drug-resistant virus include the following: (i) the extent of pre-treatment polymorphism at the critical mutation points; (ii) the influence of resistance mutations selected for by prior and concomitant agents; (iii) the effect of resistance mutations on viral replication competence; (iv) the selective pressure applied by a regimen; and (v) the extent of residual viral turnover during therapy. Rational therapy should, therefore, involve a very potent combination of anti-retroviral drugs with non-overlapping resistance profiles that are known to significantly reduce viral load and delay resistance to fellow components of the regimen. In addition, the different profiles of resistance selected by antiviral agents in monotherapy and combination use should be carefully considered to limit narrowing of future therapeutic options.

Structural basis for resistance to inhibitors of HIV RT

HIV RT is a heterodimer composed of p66 and p51 subunits. The p51 subunit is a proteolytic cleavage product of p66. It comprises the amino-terminal 440 amino acids of p66. This region corresponds with the polymerase domain. The subdomains within this are designated fingers, palm, thumb and connection (Fig. 2) [32]. The additional carboxy-terminal 120 amino acids of p66 makes up an RNaseH domain.

The RNA template-primer complex binds to a cleft between the subdomains of the polymerase region of p66 such that the 3'-OH terminus of the primer lies close to the polymerase active site. In p51, the binding cleft is absent, the essential aspartic acid residues of the active site are buried and the polymerase appears to be inactive. However, it should be noted that the muta-

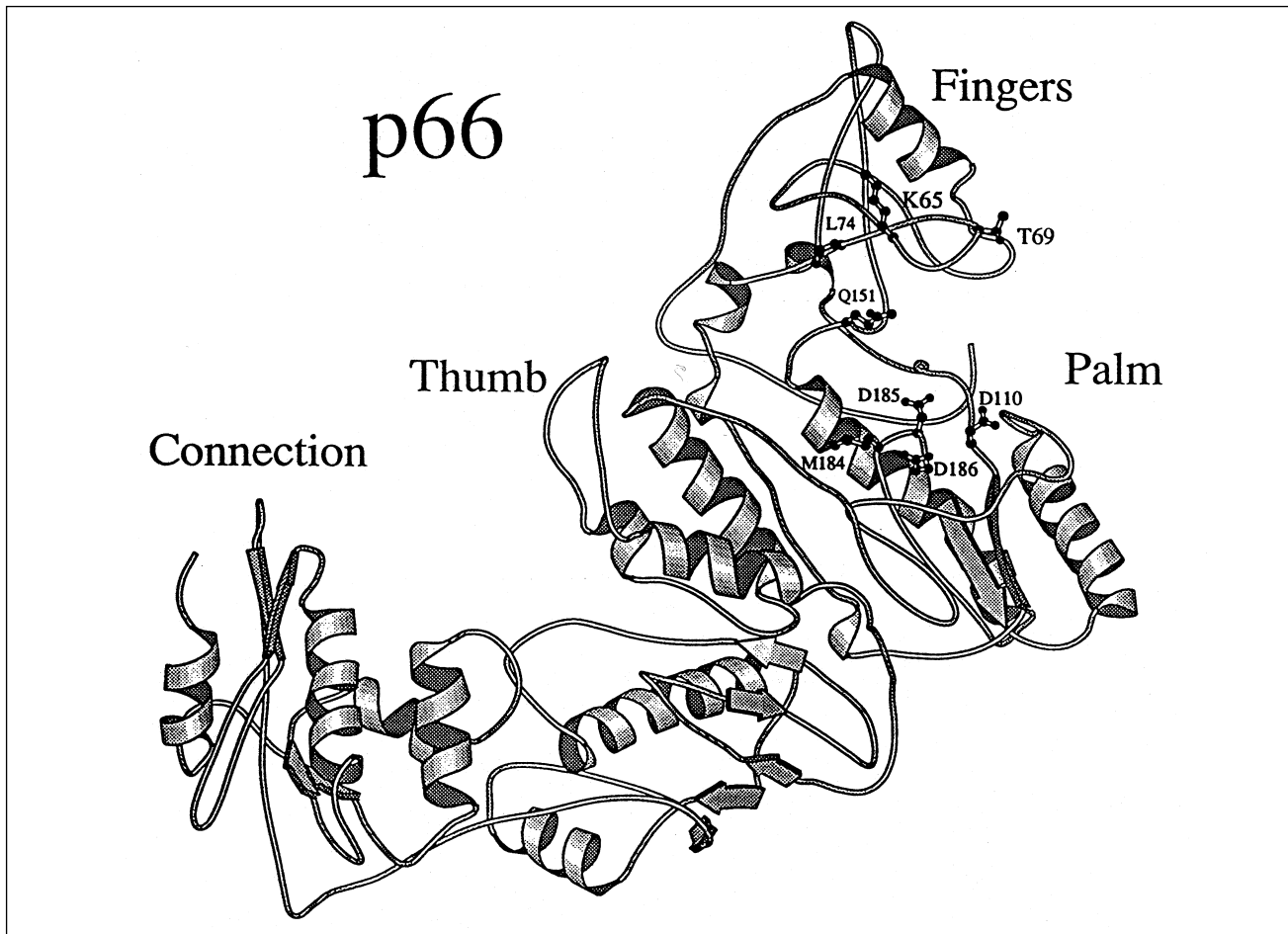


Fig. 2. The structure of the p66 subunit of HIV reverse transcriptase. The fingers, palm, thumb and connection domains are shown. In addition, the catalytic aspartic acid (D) residues and the main sites implicated in the generation of resistance to zalcitabine are indicated. Reprinted with the kind permission of Drs A. Kroehn and B. Sherborne (Roche Discovery Welwyn, Welwyn Garden City, Hertfordshire, UK).

tions that give rise to reduced sensitivity to inhibitors of the polymerase function are likely to be present in both p66 and p51.

The main features of the structure of the p66 subunit of HIV RT are shown in Fig. 2. Nucleoside analogues and dNTP bind at a site in the palm domain of p66, beside the 3'-terminus of the primer. This site includes the catalytic aspartic acids (Asp₁₁₀, Asp₁₈₅, Asp₁₈₆) [33,34]. Gln₁₅₁ is located close to the template strand and may be involved in base-pair recognition [35]. More recent data confirm that Gln₁₅₁ is involved in dNTP binding and further implicate it in the nucleotidyl transferase catalytic step [36]. An essential role for this residue is suggested by the highly conserved nature of the sequence Leu-Pro-Gln-Gly (residues 149–152) among 26 different retroviruses including HIV, simian immunodeficiency virus and other animal retroviruses [37,38]. A conserved motif Ile₆₃-Lys₆₄-Lys₆₅-Lys₆₆, which is involved with both

binding dNTP and the enzyme template–primer interaction [32,39], is also present. A monoclonal antibody targeted to amino acids 65–69 has been found to neutralize RT activity [39], suggesting an essential role for these residues.

Resistance to ddC

High-level (>100-fold changes in IC₅₀) resistance to ddC has not been reported to date either from *in vitro* studies or from clinical trials. Furthermore, the development of resistance to both ddI and ddC seems to occur less frequently than to ZDV [40], possibly in part related to the greater similarity of these compounds to natural substrate compared with that of ZDV (or 3TC).

Interpretation of changes in IC₅₀ *in vitro* should be made with consideration of the inherent variability of

the assays (fourfold) [41]. In addition, because both ddC and ddI are most active in resting cells and assays generally use phytohaemagglutinin-stimulated lymphocytes, standard systems may not accurately reflect *in vivo* conditions [42].

Five individual mutations have been identified as being primarily involved in the generation of reduced sensitivity to ddC: Lys₆₅Arg, Thr₆₉Asp, Leu₇₄Val, Gln₁₅₁Met, Met₁₈₄Val/Ile. It is notable that all these are situated within or close to conserved motifs near to the catalytic site.

Lys₆₅Arg

Low-level resistance to ddC has been demonstrated following *in vitro* selection carried out with both MT4 cells and cord blood peripheral blood mononuclear cells (PBMC). A mutation (Lys₆₅Arg) was found to confer 12-fold reduced sensitivity to ddC [43]. Reduced sensitivity (5–10-fold) was confirmed in a molecular clone and the mutation was identified in clinical specimens from four out of 11 patients treated with ddC for more than 2 months [43].

Reduced chain termination of HIV RNA transcription by RT was reported in Lys₆₅Arg mutant RT in the presence of ddC, which was consistent with the increased IC₅₀ measured in tissue culture with mutant virus generated by site-directed mutagenesis of a molecular clone [44]. Cross-resistance with dNTPs, 3TC-triphosphate (TP) and ZDV-TP was demonstrated in these studies by chain-termination measurements [44,45]. However, IC₅₀ determinations in tissue culture indicated that although cross-resistance to ddC, ddI, 3TC and 9-(2-phosphonylmethoxyethyl)adenine (PMEA) could be demonstrated, reduction in sensitivity to ZDV was not observed [45–47]. This lack of correlation is not surprising since many factors may influence the sensitivity of HIV to compound in tissue culture. It has been reported that the choice of cell line may have a profound influence on results from this type of study [48]. In one study with recombinant mutant virus, cross-resistances of 10-fold against ddC, 20-fold against 3TC and threefold against ddI were reported [43]. Interestingly, a recombinant virus with two mutations (Lys₆₅Arg and Met₁₈₄Val) did not show increased levels of resistance to ddC over those found with the Lys₆₅Arg mutant alone [43].

Thr₆₉Asp

The change Thr₆₉Asp requires a double mutation (ACT to GAT). It was first reported in a patient after long-term treatment (>80 weeks) with ddC [49]. It appears to be specific to ddC and does not confer cross-resistance to ZDV or ddI. This is surprising since mutation of the adjacent amino acid (Lys₇₀Arg) reduces sensitivity to ZDV eightfold, also without effect on sensitivity of virus to ddC [50,51]. The Thr₆₉Asp muta-

tion produced a fivefold reduction in the ddC sensitivity of a molecular clone compared with the 'wild-type' clone [49].

Isolates from a different patient receiving alternating therapy with ZDV and ddC for approximately 2.5 years were found to have mutations at codons 41, 67, 210 and 215 (ZDV-associated) and 69 (ddC-associated) [52]. When the mutations were assessed in a molecular clone, it was confirmed that the Thr₆₉Asp mutation produced a fivefold reduction of sensitivity to ddC, but it was also reported that the mutation at codon 41 antagonized ddC resistance, increasing sensitivity to ddC by a factor of three- to fourfold (IC₅₀, 1.48 μM reduced to 0.39 μM) [52]. Despite this, resistance to ddC was present in the clinical isolates with mutations at both codons 41 and 69. Therefore, other differences between these isolates and the wild-type molecular clone have compensated for this antagonism. Six months of follow-up treatment with ddI monotherapy did not result in reversion of the genotype, nor did resistance to ddI develop [52].

The mutation at codon 69 has also been reported infrequently during ZDV–ddC combination therapy [53].

Leu₇₄Val

This mutation was first described in virus isolates from an individual who had received therapy with ddI [54]. It does not appear to represent a favoured route *in vivo* for ddC resistance. When introduced into a molecular clone, 10- and 15-fold reductions of sensitivity to ddI and ddC, respectively, were found. In combination therapy with ZDV, it should be noted that the incidence of this mutant is very low even after 48 weeks therapy (ZDV–ddC, one in 29; ZDV–ddI, one in 39) [55]. In contrast, the frequency is much higher (17 out of 26) after 1 year of monotherapy with ddI [56].

In a study of the effects of stereochemistry, it was reported that a virus prepared from a molecular clone with the mutation Leu₇₄Val was eightfold less sensitive to β-D-ddC but did not show significant cross-resistance with the unnatural enantiomers, β-L-ddC, (–)-2'3'-dideoxy-5-fluoro-3'-thiacytidine (FTC) or (–)-β-L-2'3'-dideoxy-5-fluorocytidine (5-F-β-L-ddC) [57]. This suggests a role for the sugar moiety in the mechanism of resistance.

Gln₁₅₁Met

In a recent study, virus was analysed from a patient who had received ZDV–ddC alternating weekly for 41 months. Dual ZDV and ddC resistance was observed [40]. Genotypic analysis revealed that this patient had developed a series of substitutions not previously reported with either ZDV or ddC. In all, seven substitutions were found, of which five are thought to contribute to resistance: Ala₆₂Val, Val₇₅Ile, Phe₇₇Leu, Phe₁₁₆Tyr, Gln₁₅₁Met.

The Gln₁₅₁Met mutation was unexpected in view of its apparent essential role in the RT enzyme (see above). The multiple mutant virus showed a 200-fold increase in ZDV IC₅₀, 40-fold increase in ddC IC₅₀, and 4.5-fold increase in ddI IC₅₀. Further analysis of sequential isolates from this patient have demonstrated that the Gln₁₅₁Met mutation occurred first, at which point there was a concomitant increase in viraemia. This was then followed by Phe₁₁₆Tyr and Phe₇₇Leu, and finally Ala₆₂Val and Val₇₅Ile, which again heralded increased viraemia [58]. Virus with these five mutations has been more extensively reported in persons receiving ZDV-ddI therapy [56,59,60].

Site-directed mutagenesis studies with the HXB2 virus indicate that the Gln₁₅₁Met mutation confers a >22-fold reduction in sensitivity to ddC, a 10-fold reduction to ZDV and a fivefold reduction to ddI [58]. The five-substitution mutant also reduced sensitivity to d4T and partially to 3TC [59]. However, although the addition of the four further mutations in a molecular clone increased the level of resistance to ZDV and ddI to >320- and >43-fold, respectively, resistance to ddC was further increased by only a factor of two [58]. In other studies, mutations were introduced to the molecular clone NL4-3. With this genetic background, all five mutations produced only a 10-fold reduction in sensitivity to ddC [61]. Interestingly, the mutation at codon 77 was found to confer reduced replicative capacity, although this was compensated for by the mutation at codon 75. On examination of sequential isolates from two patients, the mutation at codon 77 was found to follow that at codon 75 [61]. The finding by others of the occurrence of a mutation at codon 77 before that at codon 75 suggests that such a variant remains viable in the clinic [58].

Met₁₈₄Val/Ile

In a preliminary *in vitro* study with serial passage of virus in the presence of ddI, a variant with cross-resistance to ddC was isolated and found to have the mutation Met₁₈₄Val [62]. When introduced into recombinant virus, it was found to produce a fivefold reduction of sensitivity to both ddI and ddC, but with no effect on ZDV [62]. The mutation was reported in five out of seven patients who had received long-term (4–14 months) ddI therapy combined with ZDV [62]. Further studies have suggested a lower prevalence: one out of 39 patients treated with ZDV-ddI for 48 weeks, and two out of 29 in a group receiving ZDV-ddC for the same period [55].

The mutation was first observed after serial passage of HIV-1 in PBMC and MT-2 cells in the presence of (–)-FTC and (–)-BCH-189 (3TC) [63]. The production of HIV-1 variants highly resistant to 3TC and FTC was found after only two to five cell passages [63]. High-level resistance of both variants for the (+) and

(–) enantiomers of both compounds was reported. Susceptibility to ZDV was unaltered, but a sixfold reduction in sensitivity to ddC was observed [63]. Two mutations, Met₁₈₄Val and Met₁₈₄Ile, were strongly implicated. The finding was confirmed in studies with (–)-FTC and recombinant virus, although in this laboratory strain, the observed resistance to ddC was of the order of only threefold [64]. This mutation is adjacent to two of the three catalytic aspartic acid residues and may influence enzyme fidelity [65] but does not appear to compromise RT activity [66].

In vitro, the mutation Met₁₈₄Ile seems to arise more readily than the Met₁₈₄Val mutation [63,67]. This may be attributed to the higher frequency of the DNA base change G→A (required for Met→Ile) compared with the base change A→G (required for Met→Val) [68]. However, whereas Met₁₈₄Val confers little growth disadvantage over wild-type virus, Met₁₈₄Ile is quickly outgrown in competition experiments with Met₁₈₄Ile/Val mixtures [68]. This may explain why the Met₁₈₄Val mutation is most commonly observed during both mono- and combination therapy with 3TC, although it may be preceded by the transient appearance of the Ile-substituted variant [1,3,69].

These mutations have been shown to occur rapidly and at high frequency in patients treated with 3TC monotherapy or in ZDV–3TC combination therapy. Resistance has been documented within 1 week of monotherapy, and increased in prevalence to 100% of patients within 3 weeks [3]. A reduction of sensitivity of the order of 1800- to 5500-fold to 3TC with associated reductions of sensitivity to ddC (4.5- to 9.3-fold) and ddI (1.2- to 4.9-fold) are typical in clinical isolates [1]. This level of resistance to 3TC is associated with at least partial loss of antiviral effect, but not inevitable return of viral load to baseline values. The partial maintenance of antiviral effect despite high-level resistance to 3TC has been suggested to be related to the influence of mutations at codon 184 on viral fitness *in vivo*, as would be predicted from mathematical modelling [70].

It has been noted *in vitro* that the Met₁₈₄Val mutation can, depending on the genetic background of the resistant isolate, at least partially confer ZDV sensitivity on ZDV-resistant virus (IC₅₀ 1.26 μM reduced to 0.17 μM) [64]. An increase in sensitivity of ZDV-resistant virus (e.g., 2.5 to 0.29 μM; 0.59 to 0.042 μM) has also been found in some persons after 3TC treatment in the clinic [1]. In addition, although combination treatment results in the rapid acquisition of Met₁₈₄Val, there is some evidence that it will produce a delay in the appearance of ZDV resistance [71,72].

However, there is now evidence both *in vitro* and from clinical trials that RT may adapt to the ZDV–3TC

regimen and produce virus with double resistance [73,74]. Although it is unclear whether additional mutations are required for dual resistance, preliminary data implicates codons 333–416 (part of the RT connection domain), or codon 558 [75]. In a clinical study of 41 patients in which 3TC was added into combination with ZDV after at least 6 months prior therapy with this agent, after 12 weeks 34 patient isolates showed resistance to 3TC. Of 10 patients with ZDV resistance at baseline who developed 3TC resistance, four virus isolates showed suppression of the ZDV-resistance phenotype, whereas six displayed dual phenotypic resistance [76].

The inevitability of selection for these mutations at codon 184 during 3TC therapy has raised concerns that prior therapy with this agent may limit future therapeutic benefit from ddC or ddI [77]. A small clinical trial is now planned to test this possibility.

Frequency of resistance to ddC and possible clinical implications

Although it would appear from the previous sections that there are various options open to the virus to generate low-level resistance to ddC *in vitro*, it is apparent that these are infrequently taken up by the virus during combination therapy.

No series assessing ddC resistance from monotherapy studies have been reported. In treatment-naïve patients, three studies have examined phenotypic ddC resistance during ZDV–ddC therapy. In study BW34,225-02 [55], no change in phenotypic susceptibility to ddC after 48 weeks therapy was observed in 10 isolates, although two out of 17 isolates from patients treated with ZDV–ddI showed resistance to ddI. Neither ddC or ddI were noted to influence the development of phenotypic resistance to ZDV [55]. Similarly, in the AIDS Clinical Trials Group study ACTG 106, ddC resistance was not found in 11 isolates from patients treated for up to 48 weeks or in seven patients treated for up to 112 weeks [78]. Furthermore, in the virological subset from the Delta study, a subset of ZDV-naïve patients studied after 112 weeks therapy with ZDV alone, ZDV–ddC or ZDV–ddI were found not to have any mutations associated with resistance to ddI or ddC [79]. In a study of ZDV-experienced patients, isolates from 41 patients who received 48 weeks of ZDV–ddC therapy, Lys₆₅Arg and Leu₇₄Val mutations (which can give cross-resistance to ddI) were not found, although five isolates were mutated at codon 69 [53,80].

ZDV–ddC, particularly as initial therapy, provides significant delay in clinical disease progression compared with ZDV monotherapy [12–14] and similar responses in viral load and CD4 counts over 24 weeks to those

observed with ZDV–ddI and ZDV–3TC [81]. The benefits of this combination may arise from both synergy with ZDV against both ZDV-sensitive and -resistant strains [10], and sustained viral sensitivity to ddC. Use of this combination with SQV delays the development of SQV resistance *in vivo* relative to SQV monotherapy [31]. SQV has been noted to delay ZDV resistance *in vivo* [31]; therefore, the combination of ZDV–ddC–SQV currently used in clinical trials includes agents that delay resistance to each of the component agents, a factor that may be important in the more substantial and sustained effects on activity markers observed with this combination compared with two-drug combinations [82].

The influence of ddC resistance on clinical outcome or activity has not been investigated. The presence of ZDV resistance is independently associated with increased risk of disease progression. However, knowledge of viral sensitivity is only one aspect in individualizing patient therapy, principally useful in guiding drug choice. Measurement of other variables such as CD4 cell count, the presence of a syncytium-inducing viral phenotype and viral load remain vital, and represent markers of both risk of future progression and therapeutic response. Furthermore, because no trials investigating treatment sequencing have been reported, the extent of influence of small changes in *in vitro* sensitivity to a compound on clinical activity of subsequent agents is unclear. Measurement of viral sensitivity to an antiviral drug provides no information on other aspects of viral behaviour that may be influenced by mutations in RT such as replication capacity, enzyme fidelity and cytopathogenicity.

Discussion

A number of mutations that generate resistance to ddC have been described. However, the relatively low frequencies of their occurrences (in particular in combination with ZDV), the low levels of sensitivity changes observed and the limited cross-resistance patterns attained represent potentially useful properties of ddC therapy. Indeed, it would appear that benefit from ddC therapy is more at risk of being compromised by the selection of cross-resistant virus through the use of ddI or 3TC than *vice versa* [83]. Another finding encouraging the early use of ddC is the apparent impotence of multiple mutations to induce high-level resistance [58].

It has been suggested that the optimal sequence for nucleoside analogue therapy would place ddC ahead of ddI [84] on the basis of both activity in treatment-naïve and experienced patients and cross-resistance profiles. The rationale being that cross-resistance selected by ddI at codon 74 is likely to reduce the benefit of ddC ther-

apy. Similarly, it might be argued that use of 3TC should be reserved for use after ddI as its induction of cross-resistance to both ddC and ddI would rapidly compromise the potential benefit of these two options in a high proportion of patients [77,85].

Although most investigators have not noted cross-resistance between ZDV and ddC, in 48 isolates from ddC and ddI-naïve patients treated with ZDV, a significant correlation was found between the development of resistance to ZDV and reduced sensitivity to ddC and ddI [86,87]. The molecular basis of these findings remains unknown, but it is apparent that the antiviral activity of ZDV-ddC therapy is greater in patients not showing resistance to ZDV [53].

A combination of two nucleoside analogues potentially with a third agent from a different class represents the current standard of antiretroviral therapy in most developed countries. The combination of ZDV-ddC appears to provide similar clinical efficacy in treatment-naïve patients to ZDV-ddI [12–14] and similar effects on activity markers to ZDV-3TC [81]; it is therefore difficult to choose between these agents on the basis of relative efficacy. The incidence of adverse events in comparative studies with ZDV-ddI at a variety of disease stages is similar [12–14], and the only comparative study with ZDV-3TC, a three-arm 24-week study (extended to 52 weeks) of 254 ZDV-experienced persons with baseline CD4 cell counts $>100 \times 10^6/l$, found no differences in the incidence of adverse events [88]. Overall, these findings suggest that the ZDV-ddC combination in patients with relatively preserved CD4 cell counts represents an acceptably well-tolerated option for first-line therapy. Other nucleoside analogues, which may perform better in ZDV-experienced patients [12–14,88] and possess greater potential for selecting cross-resistant virus, could be considered as second-line options.

Loss of antiviral effect of the ZDV-ddC (or ZDV-ddI) combination appears to be associated with resistance to ZDV but not ddC. Therefore, it would seem rational to substitute the ZDV component of the regimen for an agent of non-overlapping resistance profile whilst continuing ddC. However, as more agents become available most physicians may prefer to change both members of the regimen.

In treatment-experienced patients, ddC appears best used in combination with SQV (or possibly other protease inhibitors) as this combination provides a substantial survival advantage over ddC monotherapy [15] and ddC may delay resistance to SQV [31].

Such is the dynamic nature of HIV that the prevalent strains of virus might be expected ultimately to change in their sensitivities to the main anti-HIV antiviral agents.

Indeed, in view of the incidence of ZDV resistance in seroconverting patients, which appears to be increasing, there is a case for commencing therapy with non-ZDV-based combinations. ddC in combination with SQV represents an option with proven clinical benefit [15] and a low potential for generating cross-resistance to both other RT and protease inhibitors [83].

In conclusion, patterns of resistance and cross-resistance should be considered when designing long-term antiretroviral treatment strategies, with the aim of achieving maximal therapeutic benefit whilst maintaining the widest possible base of future therapeutic options. These criteria appear to be satisfied by the combination of ZDV and ddC. Additional information on the frequency of resistance during therapy with drug combinations, pathogenicity and replicative capacity of mutant strains, and the influence of drug resistance on disease progression and benefit of future agents is required to help inform therapy individualization.

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