Current knowledge of HIV-1 reverse transcriptase mutations selected during nucleoside analogue therapy: the potential to use resistance data to guide clinical decisions

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Massive viral turnover and the high error rate of reverse transcriptase create the potential for drug-resistant viral variants to appear rapidly under the selective pressure of antiretroviral therapy. Loss of antiviral effect in treatment compliant persons is most commonly coincident with the appearance of viral mutants with reduced drug sensitivity. Thus, detection of viral resistance may represent an early marker of therapy failure. Similarly, substantial reduction in viral replication in the plasma compartment, to below quantification of a viral load assay, is associated with a sustained therapeutic response and delayed development of viral resistance. Information on patterns of resistance to and cross-resistance between antiretroviral agents are increasingly well characterized and represents an important consideration when deciding how to combine and/or sequence antiretrovirals to achieve optimal antiviral effects. When switching therapy, the change of several agents in the treatment regimen is currently recommended. The use of novel means of evaluating resistance, such as a genotypic probe, may guide clinicians in choosing an agent to which the patient’s dominant viral quasispecies remains sensitive, potentially increasing the chances of achieving a therapeutic response. However, no studies using resistance to guide clinical decision making have yet been reported and available sequencing studies have focused largely on switching or adding therapies to patients who have received zidovudine monotherapy. Thus, no resistance driven treatment algorithm is currently available.

Introduction

Human immunodeficiency virus (HIV) infection is characterized by high rates of viral turnover throughout the disease process eventually leading to CD4+ T-cell depletion and disease progression.1,2 The goal of antiretroviral therapy is, therefore, to achieve both substantial and sustained control of viral replication. Prolonged antiviral activity of drugs currently available or in advanced development is limited principally by the development of viral and, with nucleoside analogues, cellular resistance, leading to therapeutic failure. Therefore, achievement of sustained viral control is likely to involve the sequential use of therapies, generally, combinations of two or more antiretrovirals. Choice of initial and subsequent therapy should, therefore, be made on a rational basis and knowledge of resistance and cross-resistance patterns is vital in guiding these decisions and thus avoiding the squandering of future therapy options through selection of cross-resistant virus. A assessment of resistance-associated mutations at the time of therapy modification, by a genotypic probe for example, potentially provides valuable information for guiding decision making. Additionally, the increasing frequency of transmission of zidovudine resistant virus, up to 25% in some urban populations, suggests assessment of resistance before commencement of treatment may also be a prudent approach.

Massive viral turnover, coupled with failure of HIV reverse transcriptase to correct transcription errors by exonucleolytic proofreading, with an estimated in-vivo forward mutation rate of $3.4 \times 10^{-5}$ per base pair per cycle,3 results in the rapid establishment of extensive genotypic variation. Although some template positions or base pair substitutions may be more error prone,3,4 mathematical modelling suggests every possible single point mutation may occur more than 10,000 times/day in infected individuals.5 Some mutations leave HIV unviable and so are eliminated, whilst others cause insignificant changes in the resistance profile.
gene product and thus have no effect on viral behaviour. Nucleotide sequencing has exhibited differences of >25% between different strains of HIV-1, with multiple variants or ‘quasispecies’ existing in infected patients. Mutations at critical sites, such as genes that encode for structural and regulatory proteins or enzymes, may affect a number of aspects of viral behaviour including virulence, replication capacity and competence, cytotoxicity and response to antiretroviral therapy.

For resistance to an antiretroviral agent to occur, the target enzyme must be changed yet preserve its function in the presence of the inhibitor. Point mutations leading to an amino acid substitution may result in change in shape, size or charge of the enzyme’s active site, substrate binding site or surrounding regions which will affect the ability of the enzyme to select or reject an incoming potential substrate. Mutants resistant to antiretroviral agents have been detected at low levels before the initiation of therapy.

However, as these mutant strains represent only a small proportion of the total viral load, they presumably have a replication or competitive disadvantage compared with wild-type virus. The selective pressure of antiretroviral therapy provides these drug-resistant mutants with a competitive advantage and thus they eventually come to represent the dominant quasispecies. With some agents this may occur extremely rapidly, for example, the complete replacement of wild-type virus by drug-resistant variants which has been reported within 14 days of initiation of nevirapine monotherapy.

Mathematical modelling suggests that, as drug-resistant mutants do not replicate as well as wild-type virus, it is to be expected that steady state levels of mutant virus will be lower than those of wild-type. A small decrease in the selective advantage or replicative fitness of a mutant will substantially increase the time required for this mutant to become dominant. The gradual and stepwise emergence of resistance to zidovudine, ritonavir, or indinavir may represent this phenomenon with continued viral replication allowing mutation of the first selected quasispecies (usually a single-point mutant with low level sensitivity changes) towards an increasingly fit viral population of multi-point mutants with higher level resistance. Reductions in plasma HIV RNA load to below the quantification level of a sensitive viral load assay (2–500 copies/mL) appears to be associated with a substantial delay in the outgrowth of drug resistant mutants, and thus represents an attractive therapeutic goal. However, resistant virus may arise through continued viral replication, including: that in sites, such as the central nervous system (CNS) and genital tract, which are poorly penetrated by drugs; following drug interactions; and, most commonly, secondary to episodic failure of patient compliance.

The emergence of HIV-resistance is a dynamic process with multiple strains of virus of varying sensitivities to antiretroviral agents frequently coexisting, both in a single site and at different sites within patients. Drug-resistant mutants may persist either as the dominant quasispecies or at low levels after the selective pressure of antiretroviral therapy has been removed, suggesting that some mutants have replication competence approaching that of wild-type virus.

Patterns of mutations in the pol gene of HIV-1 are well described for the principal nucleoside analogues, non-nucleoside reverse transcriptase inhibitors (NNRTIs) and, increasingly, with protease inhibitors. In general, genotypic changes correlate well with phenotypic measurements of sensitivity, although in some cases at least partial reversal of resistance has been reported with the inclusion of an additional mutation in a previously resistant background. However, no studies using resistance to guide clinical decision making have been reported yet and sequencing studies have focused largely on switching or adding therapies to patients experienced with zidovudine monotherapy. Thus, no resistance driven treatment algorithm is currently available. The British HIV Association, a clinical group in the UK, has, however, recently proposed principles of antiretroviral treatment which include consideration of resistance and cross-resistance in clinical decision making.

Resistance to antiretrovirals may be assessed phenotypically and genotypically. Phenotypic assessment currently takes weeks, is expensive, and requires sufficient quantities of virus to establish in cell culture. Genotypic probes, such as the Line Probe Assay (LiPA) for HIV-1 RT mutations which has recently become available in Europe, represent a rapid (same day) means of assessing multiple genotypic changes in reverse transcriptase (RT). The principle of this assay is based on polymerase chain reaction (PCR) technology, methodology now familiar to and feasible in most virology laboratories. This assay has a detection sensitivity of <1000 copies/mL of virus with 5% of mutant gene and high specificity at the single nucleotide level used in conjunction with other disease markers, such as viral load, CD4+ T-cell count and clinical status, this type of probe could potentially aid physicians in making rational treatment decisions based on knowledge of mutations in RT associated with nucleoside analogue resistance and cross-resistance patterns.

I will here review the current knowledge of resistance patterns selected by nucleoside analogues and discuss the potential of genotypic probe assays in the clinical setting; I will also provide information regarding interpretation of the first generation LiPA HIV-1 RT assay (Table I).

Reverse transcriptase inhibitors

Five nucleoside analogue HIV reverse transcriptase inhibitors (zidovudine (ZDV: Retrovir, Glaxo Wellcome, Uxbridge, U.K.), zalcitabine (ddC: HIVID, Hoffman-
HIV-1 reverse transcriptase mutations

Table I. Interpretation of the LiPA HIV-1 RT assay

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Compound</th>
<th>Cross-resistance</th>
<th>Reversal of resistance</th>
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<tr>
<td></td>
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<td></td>
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<tr>
<td>Single mutations</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>M 41L</td>
<td>ZDV (&lt;four-fold)</td>
<td>ddC (69D)</td>
<td></td>
</tr>
<tr>
<td>T 69D</td>
<td>ddC (&lt;ten-fold)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K 70R</td>
<td>ZDV (&lt;four-fold)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L 74V</td>
<td>ddl (&lt;20-fold)</td>
<td>ddC, 1592U 89</td>
<td>ZDV (215F/41L)²</td>
</tr>
<tr>
<td>M 184V</td>
<td>3TC (&gt;500-fold)</td>
<td>ddC, ddl, 1592U 89</td>
<td>ZDV (215F/41L)³</td>
</tr>
<tr>
<td>A bsent M 184</td>
<td>3TC (184)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T 215F or Y</td>
<td>ZDV (&lt;16-fold)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A bsent T 215</td>
<td>ddC (215C may arise on prior 215Y)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Combinations of mutations (which have been reported in vitro or in vivo)

| 41L + 215F/41 | ZDV (<60-fold) | ddC, ddl |            |
| 41L + 215F/41 + 184 | 3TC | ddC, ddl | ZDV²       |
| 41L + 215F/41 + 70R | ZDV (>100-fold) | ddC, ddl |            |
| 41L + 215F/41 + 74V | ddl | ddC | ZDV       |
| 41L + 215F/41 + 69D | ZDV + ddC |          |            |
| 41L + 69D | ZDV | ddC |            |

The line probe assay for HIV-1 RT mutations is the first commercially available mutation assay in Europe. It both sensitively and specifically detects mutations classically associated with four of the available nucleoside analogue RTIs (ZDV, ddC, ddl, and 3TC). This table provides data to guide clinicians in the interpretation of LiPA results.

PMEA, 9-(2-phosphonyl-methoxypropyl) adenine.

²74 + 215/41 rarely observed in vivo.

³Dual resistance also reported with mutant 184 + 215/41.

⁴Other non-resistance conferring mutations may also explain an absent band.

⁵Poor viral responses reported in absence of more than four-fold phenotypic change.

⁶Dual resistance also reported with mutant 184 + 215/41.

⁷Rarely observed in vivo.

Considerations in interpreting of genotypic probes

The presence of viral resistance, whether assessed geno- or phenotypically, requires interpretation in the context of other treatment markers such as viral load and CD4 cell count as well as the clinical well-being of the patient. Issues to consider in interpretation include:

Compartmentalization

Sensitive, partially resistant and highly resistant quasi-species of HIV may coexist simultaneously in plasma and within different body compartments, for example blood and CSF. In particular, the brain may have a distinct virus population, an issue that may necessitate continued use of a CNS-penetrating compound in a regimen despite the presence of virus resistant to it in the plasma. Although ZDV has the highest CSF/plasma ratio of the available drugs, ZDV-resistant virus has been isolated from both CSF and brain.

LaRoche, Basle, Switzerland), didanosine (ddl: Videx, Bristol-Meyers Squibb, Syracuse, NY, USA), stavudine (d4T: Zerit, Bristol-Meyers Squibb, Syracuse, NY, USA), and lamivudine (3TC: Epivir) are currently licensed in Europe and the USA. Additionally, two NNRTIs, nevirapine (Viramune, Boehringer Ingelheim, Ingelheim am Rhein, Germany) and delavirdine (Rescriptor, Pharmacia & Upjohn, Kalamazoo, MI, USA), are licensed in the USA. Further members of these drug classes, including the nucleoside analogue 1592U 89 (abacavir) and the NNRTI DMP 266, are in clinical development. All these agents have demonstrated at least short-term antiviral activity and, therefore, it is not surprising that, as they exert a selective pressure on HIV, drug-resistant mutants arise during therapy. Whilst these drugs are normally used in combination regimens, many of the available resistance data arise from phase I/II monotherapy studies. Mutations observed during monotherapy may not accurately reflect mutations responsible for resistance that develops in the presence of pressure from several agents acting at the same site and, hence, on the same gene.
However, as nucleoside analogues are active after intracellular triphosphorylation, extracellular concentrations have not been shown to correlate well with activity. Therefore, the value of CSF/plasma ratios as a means of assessing activity in the CNS is not currently established. The genital tract may also represent a body compartment with a distinct viral population.

Failure despite sensitive virus

In persons compliant with their therapy, failure of therapy may occur secondary to drug-drug interactions, intercurrent illness (e.g., gastrointestinal disease) influencing drug absorption, and changes in drug metabolism. For nucleoside analogues changes in intracellular phosphorylation, sometimes called cellular resistance, have also been reported.

Intracellular phosphorylation of ZDV has been proposed as a potential marker for individualizing therapy decisions, based on data which demonstrate a relationship to therapy response, disease stage and CD4 cell count. ZDV phosphorylation not only shows variability between patients, but also diminishes over time resulting in declining intracellular levels of the active triphosphate during long-term therapy. This cellular ‘resistance’ appears to result from a decline in thymidine kinase activity, but does not seem to lead to ‘resistance’ to ddC or ddl. However, as d4T is phosphorylated by the same mechanism as ZDV, it is possible that the activity of this drug may be similarly influenced.

While most investigators have not reported in-vitro cross-resistance between ZDV and ddC or ddl, patients whose HIV has the codon 215Y or F mutations have diminished virological responses to the addition of ddl or ddC relative to persons with wild-type virus codon 215. These data support the role of genotypic assessment of resistance at time of therapy modification, and suggest caution in translating in-vitro data directly into the in-vivo context without consideration of its limitations.

No consistent mutation pattern has been reported to be associated with d4T resistance or loss of virological response to this compound. The mechanism of failure of d4T therapy is, therefore, not currently established.

Persistent activity despite resistance

Virus resistant to an antiviral drug is likely to be compromised relative to wild type, only becoming the dominant quasispecies in the presence of drug selective pressure. However, HIV-2 is naturally resistant to NNRTIs suggesting that RT can be highly replication competent in the presence of some genetic variation that leads to drug resistance. The ZDV-resistant phenotype appears to be reasonably stable in vivo, with resistant virus sometimes being detected up to 1 year after cessation of therapy, and despite treatment with ddl, suggesting good replication competence. However, the presence of HIV with the Met184Val or Ile mutations associated with >500-fold reductions in sensitivity to 3TC does not result in return in viral load to pre-treatment levels even during 3TC monotherapy. It has been suggested that this persistence of effect of 3TC relates to the extent of viral compromise. This hypothesis is consistent with mathematical models of resistance selection and is therefore likely to be true for a range of mutations or combinations of mutations.

Increasing drug concentrations to overcome resistance

How the in-vitro IC₅₀ or IC₉₅ relate to plasma or intracellular drug levels is not well understood and in-vitro studies are generally only performed in 10% plasma using PHA-stimulated cell lines. Considerations in interpreting these data include the extent and tightness of plasma protein binding (with protease inhibitors), interpatient variability in drug metabolism, activity of agents in different cell lines (e.g. active or resting cells, PBMCs or macrophages) and the potential to achieve intracellular drug concentrations above the in-vivo viral inhibitory concentration. Increasing drug concentrations either by dose escalation (as reported with nevirapine) or secondary to metabolic interactions (such as combining saquinavir with a second protease inhibitor) may enable suppression of a virus that was resistant to lower drug concentrations.

Switching therapy may be beneficial independent of resistance

A correlation between development of ZDV resistance, both phenotypic (IC₅₀ > 1.0 µM) and genotypic (presence of codon 215 and 41 mutations), and disease progression was reported in ACTG study 116B/117. The presence of the two mutations produced a relative hazard of 2.5 for disease progression (95% confidence interval (CI) 1.02-3.26) and 5.42 for death (CI 1.92-15.30) relative to wild-type virus, when controlling for other factors. Importantly, the increased risk of progression and death with ZDV resistance was independent of the benefits associated with switching to ddl in this trial. Similarly, switching to ddl, in this study, provided clinical benefit regardless of the presence or absence of ZDV resistance.

Novel mutations

Whilst patterns of genotypic mutations associated with changes in phenotypic resistance to the leading reverse transcriptase inhibitors (RTIs) are established from both in-vitro and in-vivo work, other, rarely reported, resistance mutations may arise occasionally during clinical studies. Isolates with a unique pattern of amino acid substitutions at codons 62, 75, 77, 116, and 151 have been identified in
patients receiving prolonged combination therapy with ZDV plus ddl or ddC; these isolates are resistant to both drugs and there is cross-resistance to stavudine and partial cross-resistance to 3TC. This mutation pattern was not observed in large cohorts including those in BW 34,225–02 or ACTG 106 trials. Additionally, data on resistance patterns arising in persons receiving three RTIs or new combinations of RTIs such as d4T/3TC are currently lacking. No consistent genotypic change has been associated with phenotypic d4T resistance or, indeed, loss of virological effect of this compound.

Undetectable mutations

Both phenotypic and genotypic assays have detection limitations. A range of viral quasispecies, in dynamic flux, exists within all HIV-infected persons. Phenotypic assays generally assess the in vitro IC\(_{50}\) or IC\(_{95}\) of the dominant quasispecies. Genotypic probes may detect non-dominant quasispecies potentially providing evidence of evolution of resistance. The lower limit of detection of the LIPA probe is 5% of variant virus. Thus, whilst wild-type virus may be the only genotype detected in an individual, viral variants may exist at proportions below 5%, which, on initiation of therapy rapidly emerge to become the dominant quasispecies. This has been strikingly demonstrated with nevirapine monotherapy, with a mathematical model suggesting the prevalence of the 181Cys mutant before nevirapine therapy being between only 7 and 133 per 10,000 copies.\(^{42}\)

Mutations reported to nucleoside analogue RT inhibitors

Zidovudine

HIV variants with decreased susceptibility to ZDV were first reported in 1989; in some isolates, greater than 100-fold increases in the concentration of ZDV were required to inhibit viral replication by 50%.\(^{43}\) The ZDV-resistant phenotype appears to be reasonably stable in vivo, with resistant virus sometimes being detected up to 1 year after cessation of therapy,\(^{16}\) and despite treatment with didanosine.\(^{37}\)

Nucleotide sequencing of HIV RT has revealed a number of mutations which can influence viral sensitivity to ZDV and which may be used as genotypic markers for the presence of ZDV resistance.\(^{14,44,45}\) A range of mutants with increasing levels of resistance appear in an ordered manner, with the sequential appearance of these mutations being associated with incremental reductions in viral sensitivity to ZDV.\(^{16,46}\) A substitution at codon 70 (A\(\Rightarrow\)T) may be transiently dominant and appears critical to virological failure during ZDV monotherapy.\(^{47}\)

Continued ZDV therapy selects for a further mutation at codon 215, which appears to be a more stable variant, though both Thr215\(\Rightarrow\)Tyr and Thr215\(\Rightarrow\)Phe substitutions have been described and may coexist.\(^{48}\) Virus with additional mutations may then appear, most commonly a substitution at codon 41 (M\(\Rightarrow\)F), followed by further additional mutations at codons 67 (A\(\Rightarrow\)S) and 219 (L\(\Rightarrow\)G) or the reappearance of the codon 70 mutation.\(^{16}\)

Site-directed mutagenesis techniques have been used to assess the interactions resulting from the different mutations.\(^{46}\) These demonstrated that high-level resistance to ZDV (IC\(_{50}\) > 1 \(\mu M\)) is typically associated with the presence of multiple mutations. Although frequently synergistic, mutations may also be antagonistic. For example, a mutation at codon 74 (Leu\(\Rightarrow\)Val) observed during therapy with ddl or ddC has been noted to be antagonistic to the ZDV 215 mutation in vitro, reducing the degree of resistance to ZDV.\(^{49}\) An antagonism of the 215 mutation in vitro has also been reported by the codon 181 mutation selected for by most NNRTIs and the mutation at codon 184 seen with lamivudine and, less frequently, ddC and ddI.\(^{50–54}\) However, novel mutation patterns or additional ‘compensatory’ mutations may be observed in vivo during combination therapy facilitating dual or multi-drug resistance (see below).

Viral strains resistant to ZDV exhibit cross-resistance to other nucleoside analogues containing the 3'-azido group such as 3'-azido-2',3'-dideoxyuridine (AZU).\(^{56}\) Cross-resistance to stavudine, a thymidine-based analogue which lacks a 3'-azido moiety, has also been reported by one group in both a laboratory strain of HIV and one of 11 clinical isolates.\(^{56}\) Most investigators have found no evidence that mutations selected for during ZDV monotherapy influence sensitivity to ddl, ddC or 3TC.\(^{19,37,55–57}\) However, resistance to ddl has been rarely reported after prolonged ZDV therapy.\(^{54,58}\) and one report has suggested that, for each ten-fold decrease in ZDV sensitivity in clinical isolates, there is a corresponding 2.2-fold reduction in susceptibility to ddl and two-fold decrease in sensitivity to ddC.\(^{59}\) Furthermore, patients with ZDV resistance at baseline are significantly less likely to achieve a RNA response after the addition of ddC or ddl than those with wild-type virus at baseline.\(^{34,35}\)

Zalcitabine and didanosine

High-level resistance (>100-fold increase in IC\(_{50}\)) to ddl or ddC has not yet been reported and, indeed, diminished susceptibility to ddC in clinical isolates has not been extensively documented.\(^{56–59}\) This may, in part, be related to the greater similarity of these compounds, as compared with ZDV, to natural nucleosides. A ditionally, these compounds are more active in resting cells\(^{64}\) and assays generally use PHA-stimulated lymphocytes, standard systems may not accurately reflect in-vivo conditions.

Resistance to ddl is mediated through a Leu74\(\Rightarrow\)Val mutation which produces a six-fold to 26-fold reduction in sensitivity, but may partially restore susceptibility to ZDV.
in vitro by antagonism of the codon 215 mutation. This mutation also reduces sensitivity to ddC by around tenfold. This is the most likely explanation for the data documenting the development of phenotypic ddC resistance in four of seven patients treated with ddl, but never after ddC therapy. The frequency of the codon 74 mutation has been reported to have increased from zero at the start of therapy to 56% at week 24 in a group of 64 persons with a mean baseline CD4 cell count of 129/mm$^3$, which switched to ddl having previously received ZDV. Similarly, in a mixed population of both treatment-naive and ZDV-experienced patients with CD4 cell counts of 200–500/mm$^3$, who received ddl monotherapy in the ACTG 143 study, 17 of 26 isolates had mutations at codon 74 at 1 year. Mутант codon 74 arose in only two of the 55 patients in this study who received ZDV/ddl combination therapy.

Virus with a mutation at codon 65 (Lys65→Arg) has been isolated from several patients receiving long-term treatment with ddI or ddC. This is associated with a three- to five-fold increase in the IC$_{50}$ of ddl with a five- to ten-fold reduction in ddC sensitivity and a 20-fold reduction in susceptibility to 3TC. A mutation at codon 69 (Thr69→Asp), which leads to a five-fold reduction in sensitivity to ddC but does not appear to result in cross-resistance to other nucleoside analogues, is the most frequent mutation selected for by ddC in vivo. The development of resistance to ddC has recently been reviewed elsewhere.

Combinations of zidovudine with zalcitabine or didanosine

Combination therapy with ZDV/ddC or ZDV/ddI may influence the rate of emergence of resistance and may suppress some of the mutations observed during monotherapy but may result in the appearance of novel (and hence possibly more compromised) mutational patterns.

In ACTG 106, ddC resistance was not observed in 11 therapy-naive patients receiving combination therapy with ZDV and ddC for up to 48 weeks or in seven patients on this combination for up to 112 weeks. The BW 34,225–02 study involved patients with advanced HIV infection (CD4 cell counts <300/mm$^3$) who had received less than 4 weeks’ previous ZDV. During ZDV/ddC or ZDV/ddI combination therapy for up to 48 weeks no phenotypic resistance to ddC was observed in ten isolates, with 2/17 ZDV/ddI recipients showing ddl resistance. Mutations associated with reduced susceptibility to ddl (2/27 with Met184→Val) or ddl (one with 184Val and one with 74Leu→Val in 39 samples) were infrequently observed. No delay in ZDV resistance was reported.

In ACTG 143, patients commencing therapy with ZDV/ddl or adding ddl to established ZDV therapy were followed for 2 years. A variety of viral responses were observed, with patients with a poor virological response (less than ten-fold decrease in plasma RNA) being noted to develop ZDV resistance more frequently, including dual ZDV/ddI resistance in two of the 39 patients. These were the only patients in which ddl resistance was observed and, notably, the codon 74 mutation was not observed. This suggests both that the mutations selected for when ddC or ddl are used as monotherapies are not compatible with functional reverse transcriptase if added to the typical ZDV resistance pattern, and that the prolonged therapeutic efficacy seen with these regimens may be in part related to continued viral sensitivity to at least one of the combination agents. Alternating regimens may select more readily for resistance than simultaneous combination therapy. For example, monthly alternating therapy with ZDV plus ddC for 2.5 years has been found to select for a mutant that is resistant to both agents (with several mutations including codons at 215, 41, and 69).

Novel mutation patterns may emerge during combination therapy. Isolates with a unique pattern of amino acid substitutions at codons 62, 75, 77, 116, and 151 have occasionally been identified in patients receiving prolonged combination therapy with ZDV plus ddI or alternating ZDC/ddC: these are resistant to both drugs and confer cross-resistance to stavudine and partial cross-resistance to 3TC. The frequency in persons treated for >1 year with ZDV/ddI ranges from 0 to >10%. Mutations selected by 3TC (184Val) and nevirapine (181Cys) may readily be added to this background in vitro and 184Val and 103Asp (for loviride resistance) being reported in vivo. While these virus mutations appear to be replication competent in the presence of drug, the likely reason these novel mutations are not seen during monotherapy probably relates to their failure to compete with those mutants that become dominant.

Lamivudine

Resistance to 3TC occurs rapidly in vivo with a substitution at codon 184 (most commonly Met184→Val) being observed during both monotherapy and combination therapy and its appearance being temporally associated with at least partial virological failure. This mutation leads to high-level resistance to 3TC (500-fold to 1000-fold increase in IC$_{50}$), as well as some cross-resistance to both ddl and ddC (four- to eight-fold reductions in susceptibility) raising the concern that 3TC use may limit the subsequent value of these agents. In vitro this mutation may antagonize ZDV resistance mediated through the 215 and 41 codon mutations thus restoring phenotypic sensitivity to ZDV, although dual ZDV/3TC resistance has been reported both in vivo and in clinical isolates. Other, possibly compensatory, mutations such as at codon 135 or 333 may be required for dual ZDV/3TC resistance, an issue that is currently under investigation. When 3TC was added to patients pre-treated with ZDV in study NUC A 3002, phenotypic 3TC resistance developed in 82% of 33 patients by week 12. Of the ten patients with ZDV resistance at baseline (as defined by an IC$_{50}$ >0.2 mM) who...
HIV-1 reverse transcriptase mutations

Developed 3TC resistance, four had isolates that were more sensitive to ZDV whilst six patients had dual ZDV/3TC resistance, suggesting that viral resensitization to ZDV is not universal in vivo. A available clinical data argue for the use of 3TC later than ddC or ddl in the treatment sequence. In particular, 3TC appears active and well tolerated in a range of clinical contexts where ddC and ddl may be less active. Data from the recently reported CAESAR study showed significant improvements in survival in individuals mostly already on antiretroviral therapy, including ZDV monotherapy or ZDV/ddI or ZDV/ddC combinations, and with advanced immunodeficiency. The 184Val mutation has been reported to arise both in vitro and in vivo with ddC and ddI (and in vitro with abacavir; see below), suggesting it also provides a competitive advantage for HIV in the presence of these compounds.

Stavudine

In-vitro selection of HIV resistant to d4T, confirmed by site-directed mutagenesis, has identified a mutation at codon 75 (Val75→Thr) which confers a seven-fold increase in IC50, as well as reduced susceptibility to both ddl and ddC. A mutation at codon 50 leading to a 30-fold reduction in d4T sensitivity, but which does not appear to confer cross-resistance to other nucleoside analogues has also been observed in vitro. In vivo, however, a range of amino acid changes, including the codon 75 mutation but not the codon 50 substitution, have been reported. The maximum decrease in sensitivity to d4T seen in 13 ZDV-naive patients followed for 18 to 22 months was 12-fold. However, five patients developed nine-fold to 176-fold reductions in ZDV sensitivity and three subjects developed seven-fold to 29-fold decreases in susceptibility to ddl, suggesting use of d4T may limit subsequent therapeutic options in some patients. No consistent mutation pattern for resistance to d4T has, therefore, been established.

Abacavir

In-vitro passage data have been reported with this new carbocyclic nucleoside analogue. A abacavir is active in vitro in a range of cell lines and against ZDV-resistant HIV. The first mutation to arise in vitro is 184Val, as observed with 3TC, although sensitivity changes to abacavir are less than five-fold. Further passage results in accumulation of mutations at codons 65 (previously reported to confer resistance to ddC, ddl, and 3TC), 74 (typical of ddl resistance), and 115, and high fold sensitivity changes. Passage with a ZDV-resistant mutant also selected for 184Val. The emergence of 184Val with abacavir raises concerns that this agent will be less active in 3TC-experienced persons. A s 65, 74, 115, and 184 codon mutations are rarely observed after ZDV in combination with ddC or ddl, abacavir may be best used after these drugs.

Clinical significance of resistance

Choice of initial and subsequent therapy for HIV infection should be uncompromising in terms of activity but also planned and based rationally on knowledge of resistance and cross-resistance patterns to maintain a wide base of future therapy options. Table II provides a list of treatment options after initial therapy with widely used nucleoside analogue combinations to assist in the choice of initial nucleoside analogue treatment. Table III provides suggestions for potential treatment options based on resistance data which would be reported using the LiPA HIV-1 RT assay.

However, analysis of the influence of drug resistance on clinical outcome in HIV infection is complicated by a number of factors including severe immunosuppression, high viral load, viral phenotype (syncytium-inducing (SI) versus non-syncytium-inducing (NSI)). Detection of phenotypic resistance or specific genotypic mutations using polymerase chain reaction (PCR) probes represent potential useful markers, both in trials and clinical practice, for assessing and guiding an individual’s need to switch or add further agents. However, individualization of therapy will also require the use of other markers of disease progression such as viral load and CD4 cell count as well as clinical data and must be viewed in terms of cost versus benefit issues.

The presence of phenotypic ZDV resistance or the codon 70, 41, or 215 mutations in patients receiving ZDV is

<table>
<thead>
<tr>
<th>Initial combination</th>
<th>Probable treatment options</th>
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<tbody>
<tr>
<td>ZDV/ddC</td>
<td>d4T/ddI, d4T/3TC, ZDV/3TC, abacavir</td>
</tr>
<tr>
<td>ZDV/ddl</td>
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<tr>
<td>d4T/3TC</td>
<td>?ZDV/ddC or ddl</td>
</tr>
</tbody>
</table>
associated with a more rapid decline in CD4 cell count and increased viral load. Similarly, the presence of ddI resistance (mediated through the codon 74 mutation) has also been reported to be associated with a more rapid decline in CD4 cell count and increase in viral load in patients with advanced HIV infection switched to ddI after prolonged ZDV therapy. Clinical studies have also shown a temporal relationship between the development of resistance to NNRTIs or protease inhibitors and a decline in efficacy, as measured by CD4 cell count or viral load responses. However, less is known concerning the relationship between resistance to antiretrovirals and clinical outcome.

The presence of ZDV-resistance does appear to be an independent predictor of subsequent disease progression in both adults and children with HIV infection, independent of therapy change to ddI. The multicentre Canadian AZT study found a correlation between development of ZDV resistance (more than 30-fold increase in IC₅₀) and progression to symptomatic disease, with a relative risk of 1.98 (95% CI 1.36–2.89) for those with resistant virus. A similar correlation was reported between the development of phenotypic (IC₅₀ >1.0 µM) or genotypic (presence of 215 and 41 mutations) ZDV resistance and disease progression in ACTG 116B/117. The presence of the two mutations produced a relative hazard of 1.82 for disease progression (CI, 1.02–3.26) and 5.42 for death (CI, 1.92–15.30) when controlling for other factors. Lower levels of phenotypic resistance were not associated with an increased risk of progression. Importantly, the increased risk of progression and death with ZDV resistance was independent of the benefits associated with switching to ddI in this trial. Patients with ZDV-resistant virus were at increased risk of disease progression whether they continued on ZDV or switched to ddI implying that highly ZDV resistant virus may be less well suppressed by ddI than ZDV sensitive virus. In-vitro observations of increased cytopathogenicity and increased replicative capacity of ZDV-resistant virus may help explain these findings. Quantitative assessment of plasma HIV RNA with or without the 215 mutation has shown that addition of ddI to ZDV therapy results in a decrease in wild-type RNA, despite the mutant virus being sensitive to ddI in vitro. Similarly, patients with ZDV-resistant virus are significantly less likely to achieve a virological response after the addition of ddC than those with wild-type virus at baseline, data in keeping with reports of ZDV-resistance leading to cross-resistance to ddI and ddC.

These data suggest that ZDV resistance has negative consequences for patients, both in terms of disease progression and limitation of treatment options with nucleoside analogues. This has important implications for the choice of co-therapies with ZDV, timing of modification of antiretroviral therapy, particularly when to stop ZDV, and for how the available drugs should be sequenced.

Table III. Choice of nucleoside analogues in the presence of resistance as detected by LiPA HIV-1 RT assay (based on current practice of using ZDV or d4T as combination base with ddC, ddI, or 3TC as co-therapy)

<table>
<thead>
<tr>
<th>Mutations (drug(s) to which HIV may be resistant)</th>
<th>Possible treatment options</th>
</tr>
</thead>
<tbody>
<tr>
<td>70R, or 215F/41L (ZDV alone)</td>
<td>d4T based combinations (best option d4T/3TC)</td>
</tr>
<tr>
<td>184V (3TC, ? abacavir)</td>
<td>ZDV or d4T, other agents may not be active</td>
</tr>
<tr>
<td>74V (ddl, /ddc)</td>
<td>ZDV or d4T with 3TC</td>
</tr>
<tr>
<td>69D (ddC)</td>
<td>Any non-ddC containing combination</td>
</tr>
<tr>
<td>215F/41L/184V (?ZDV/3TC)</td>
<td>d4T, other agents may not be active</td>
</tr>
<tr>
<td>215F/41L/74V (ZDV/ddI)</td>
<td>d4T plus ddC or 3TC</td>
</tr>
<tr>
<td>215F/41L/69D (ZDV/ddC)</td>
<td>d4T plus ddl or 3TC</td>
</tr>
<tr>
<td>41L/69D (ZDV alone)</td>
<td>d4T plus ddC, ddl or 3TC</td>
</tr>
</tbody>
</table>

There is currently no consensus regarding appropriate therapy once ZDV resistance is established. Protease inhibitors, which act on a different enzyme, may be of better clinical value than nucleoside analogues. A data suggest that transmission of ZDV-resistant virus may be common, with ZDV resistance-associated mutations being detected in up to 10–25% of seroconverting patients in urban populations, one of these agents may also be best included in initial therapy regimens. Delayed appearance of resistance to ddC and ddI has been reported during combination therapy with ZDV, coincident with an observed prolongation of immunological and virological response compared with monotherapy, suggesting the potential of including or maintaining a drug in a combination regimen to help prolong another agent’s activity. Replication-competent virus which is resistant to multiple agents has been reported, although such viruses probably have a selective disadvantage in the absence of therapy. It is not known whether these mutants have other altered
behavioural characteristics or an unfavourable impact on disease outcome as has been reported with ZDV-resistant virus.

**Conclusions**

The genetic basis of resistance to the nucleoside analogues ZDV, ddC, ddI, and 3TC is well characterized. Genotypic assays, such as the LiPA HIV-1 RT probe, represent a rapid and relatively simple means of detecting the key or classical mutations to these agents. Results from genotypic probes require interpretation in the context of other disease markers and appreciation of their limitations. However, they hold potential for guiding drug choice both at therapy initiation and at the time of therapy modification.

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HIV-1 reverse transcriptase mutations


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