Drugs 1999 Mar; 57 (3): 337-361
0012-6667/99/0003-0337/$25.00/0

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Managing Resistance to Anti-HIV Drugs

An Important Consideration for Effective Disease Management

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Abstract

Current recommendations for the treatment of HIV-infected patients advise highly active antiretroviral therapy (HAART) consisting of combinations of 3 or more drugs to provide long-term clinical benefit. This is because only a complete suppression of virus replication will be able to prevent virus drug resistance, the main cause of drug failure. Virus drug resistance may remain a cause of concern in patients who have already received suboptimal mono- or bitherapy, or for patients who do not experience complete shut-down of virus replication under HAART. For these patients, replacement of one combination therapy regimen by another at drug failure, taking into account the existing resistance profile, will be needed. The development of new drugs will remain necessary for those patients who have failed to respond to all currently available drugs, as will be the institution of more effective and less toxic HAART regimens.

During the past 10 years significant progress has been made in the treatment of HIV-infected patients, in part due to the development and clinical use of an increasing number of anti-HIV drugs. Three classes of drugs have been approved by the Food and Drug Administration (FDA) (table I). They are targeted at only 2 events in the HIV replication cycle: (i) reverse transcription of the viral RNA into double-stranded proviral DNA by the viral reverse transcriptase (RT), and (ii) processing of the viral precursor gag-pol protein by the viral protease (PRO). Both the RT and PRO are virus-specific enzymes and essential for replication. They are therefore excellent targets for antiviral therapy.

Two classes of RT inhibitors are being success-

Table I. Efficacy of FDA-approved drugs and drugs under expanded or early access. Data in this table are derived from published data.[1-17] Schinazi et al.[5] list all resistance mutations currently observed *in vitro* and *in vivo*

Drug[a]	Log_{10} virological efficacy in monotherapy (peak drop in RNA viral load[b]	Time to resistance with monotherapy	Most common resistance mutations in the target protein after monotherapy[c]	Increase in resistance of observed phenotype (-fold)
Nucleoside and nucleotide analogue reverse transcriptase inhibitors (NRTI)				
Zidovudine (AZT)	0.5	Months to years	M41L, D67N, K70R, L210W, T215Y/F, K219Q	>120[d]
Didanosine (ddI)	0.4-0.7	Years	K65R, L74V, M184V, (V75T)	4-10[e]
Zalcitabine (ddC)	0.3-0.5	Years	K65R, T69D, V75T, M184V, (L74V)	4-20[e]
Stavudine (d4T)	0.5-1.0	Years? (not enough *in vivo* data)	V75T	7
Lamivudine (3TC)	1.0-1.3	Weeks	M184I/V, (K65R)	>100[f]
Abacavir (1592U89)	1.0-1.5	Months? (not enough *in vivo* data)	L74V, Y115F, M184V, (K65R)	8[d]
Adefovir dipivoxil [bis(POM)-PMEA]	0.5-0.9	Years? (not enough *in vivo* data)	K70E, (K65R)	7-10[e]
Non-nucleoside analogue reverse transcriptase inhibitors (NNRTI)				
Nevirapine (BI-RG-587)	0.5-2.4	Weeks	A98G, L100I, K103N, V106A, V108I, Y181C/I, Y188C, G190A~	>100[g]
Delavirdine (BHAP, U-90152)	0.9-1.4	Weeks to months	K103N/T, Y181C, P236L	>100[g]
Efavirenz (DMP 266)	2.0	Weeks? (not enough *in vivo* data)	L100I, K101E/Q, K103N, V106A, V108I, Y181C, Y188C/H/L, G190E/A/S, P225H	>100[d]
Protease inhibitors (PI)				
Saquinavir (Ro-31-8959)	0.9-1.3	Months	L10I, **G48V**, I54V, L63P, A71V, G73S, V82A, **L90M**	>50[d]
Ritonavir (ABT-538)	0.8-1.9	Months	K20R, L33F, M36I, M46I, I54L/V, L63P, A71V, **V82A/F/S/T**, I84V, L90M	>40[d]
Indinavir (MK-639)	1.3-1.9	Months	L10I/R/V, K20R/M, L24I, L63P **M46I/L**, I54V, A71T/V, G73S, **V82A/F/T**, I84V, L90M	>30[d]
Nelfinavir (AG-1343)	1.4-1.7	Months	**D30N**, M36I, M46I, L63P, A71V, V77I, N88D, L90M	>30[d]
Amprenavir (141W94)	1.5-2.0	Months? (not enough *in vivo* data)	L101, L33F, M46I, I47V, **I50V**, 154V, I84V	>10[d]

a Abacavir and adefovir dipivoxil are presently available under the expanded access programme. Amprenavir is available under an "early access" programme.

b Peak drop in viral load is dependent on baseline characteristics.

c Protease inhibitor resistance mutations shown in bold are primary mutations, others are secondary mutations. Mutations in parentheses are cross-resistance mutations selected during therapy with another drug.

d Combined mutations.

e Single mutations.

f Single 184 mutation.

g Single *or* combined mutations.

fully used at present: nucleoside analogue RT inhibitors (NRTIs), acting as competitive inhibitors and chain terminators, and non-nucleoside RT inhibitors (NNRTIs), exerting an allosteric effect by binding to a hydrophobic pocket close to the active site.[18] The PRO inhibitors (PI) in current use are targeted at the active site of the enzyme.[19]

In monotherapy all these drugs rapidly select for virus drug resistance (table I), which emerges within a few weeks for the most potent NRTIs and

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NNRTIs[12,20-24] and in several months to a few years for most NRTIs and PIs.[13,25-32] The emergence of drug resistance in HIV-1 correlates with the presence of point mutations in the targeted protein (table I). Although the more potent drug combination therapies may be able to delay the emergence of virus drug resistance, it still remains to be assessed whether combination therapies can prevent resistance indefinitely. We are thus left with the difficult task of managing HIV drug resistance, and this will probably remain the most critical issue in HIV therapy for the next few years.

1. Mechanism of Resistance

During the asymptomatic phase of untreated HIV infection, there is continuing active replication in the lymph nodes and a rapid turnover of both free virus in the plasma and of CD4+ target cells.[33-36] The relatively stable viral load and CD4+ cell count in the peripheral blood are the result of a dynamic equilibrium. This is attained by a daily production of 10^8 to 10^{10} virions involved in the daily destruction and trapping of 10^9 CD4+ cells on the one hand, and by daily production of new CD4+ cells and clearance of the virus with a half-life of less than 1 day on the other. Since the mutation rate of the viral RT is in the range of 10^{-3} to 10^{-5} misincorporations per nucleotide per site per replication cycle, due to the absence of proofreading, every base of the 10^4-nucleotide–long genome of HIV may be prone to mutation every day. Therefore, HIV does not have a fixed genomic sequence, but rather exists as a mutant swarm called quasispecies, in which some point mutations are more likely to be present than others.[37] Because of the dynamics of HIV replication, pre-existing replication-competent virus with a single point mutation displaying a reduced sensitivity to an anti-HIV drug will become the predominant genotype after viral replication comes under the selective pressure of that drug. The time needed for this shift in quasispecies is dependent on such factors as mutant frequency at the time of treatment initiation, the fitness of the mutant and the magnitude of the selective pressure (i.e. the potency of the drug), factors which can differ among patients.[38,39] For example, the M184V mutant selected for under lamivudine (3TC) monotherapy can appear in some patients within 2 weeks.[12]

It is statistically very unlikely that 3 or more resistance mutations will be present in a single virus of the initial swarm of genotypes. Therefore, they may only be selected when antiviral drug pressure allows residual virus replication. The extent of residual virus replication during treatment is then an additional factor in the time required to develop resistance. Any residual virus replication will result in a gradual build-up of one mutation after the other, until the virus acquires high level resistance to the drug pressure, be it monotherapy or combination therapy. Thus, development of resistance will be slower when an increasing number of mutations is required for high-level resistance to one drug or a combination of drugs, and when residual virus replication is lower due to more potent therapy.

The HIV RT is a heterodimer with a p66 and a p51 subunit. The p66 subunit resembles a right hand with structural features called ‘palm’, ‘thumb’ and ‘fingers’. The template-primer passes through the cleft between ‘fingers’ and ‘thumb’, where the catalytic site is located (fig. 1a).[43,44] NRTIs inhibit replication through competitive inhibition and subsequent chain termination following their intracellular phosphorylation to the 5′-triphosphate form. To do this they must bind to the enzyme-template-primer complex. The natural substrates, deoxynucleoside triphosphates (dNTPs), have a higher binding affinity for this complex than do the 5′-triphosphates of the non-natural (dideoxy) nucleoside analogues (ddNTPs). All NRTI resistance mutations seem to influence either the dNTP binding site or the template-primer binding site on the enzyme (fig. 1a; residues shown in dark blue, light blue, green and magenta). Most of these mutations seem to increase the enzymatic specificity for natural dNTP binding over that of the ddNTPs, thereby reducing the sensitivity of the RT to the ddNTPs.[44-47] This mechanism could in part ex-

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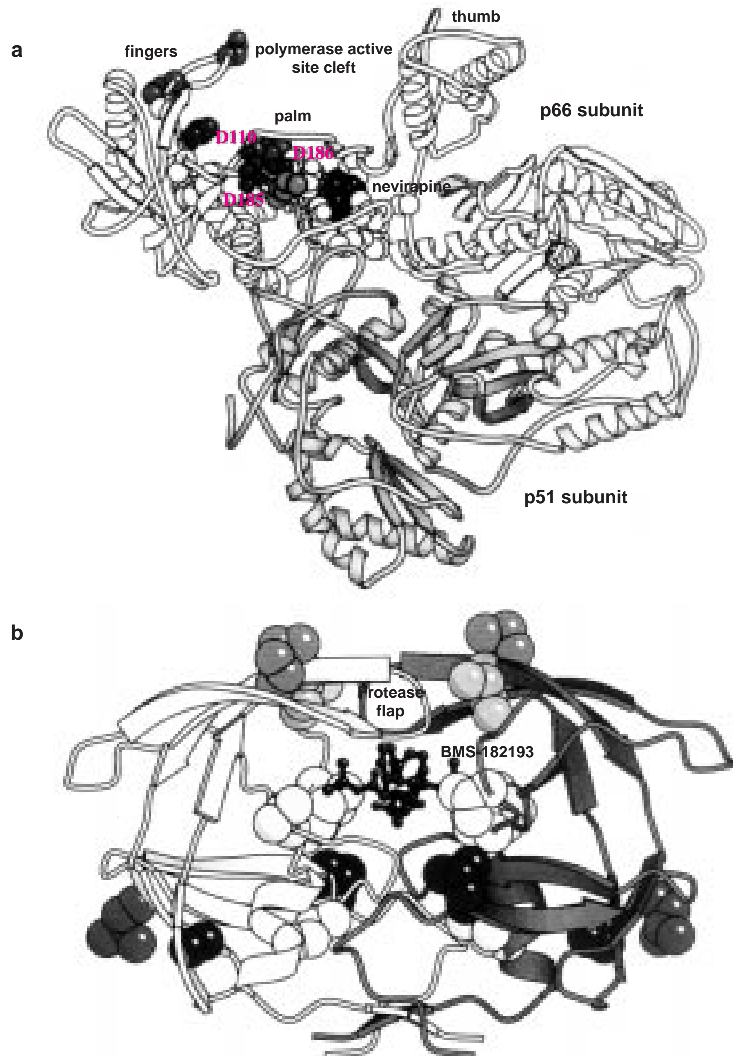

Fig. 1. Secondary structures, inhibitor binding and resistance mutations of HIV-1 reverse transcriptase (RT) and protease (PRO). (**a**) RT with a bound non-nucleoside RT inhibitor (NNRTI) [nevirapine] and resistance mutations. The heterodimer of RT is shown with the p66 subunit in pale grey (top half) and the p51 subunit in dark grey beneath. Nevirapine is shown as the dark grey Corey-Pauling-Koltun (CPK) model sitting in the NNRTI-binding site at the base of the polymerase active site cleft[37] [Protein Data Base (PDB) code 1VRT]. The p66 subunit resembles a right hand with structural features called the palm, thumb and fingers, as indicated. The catalytic aspartate residues are shown in red. Dark blue side-chains show the positions of the zidovudine resistance mutations D67N, K70R, T215Y/F and K219Q. The pale blue side-chains show the positions of the multinucleoside resistance mutations A62V, V75I, F77L, F116Y and Q151M. The green side-chain shows the site of the lamivudine resistance mutation M184V, and the magenta side-chains show the site of the didanosine resistance mutation L74V. Yellow side-chains correspond to the residues that are associated with NNRTI resistance.[5] (**b**) PRO showing a bound inhibitor (BMS-182193) and resistance mutations. The subunits of the protease dimer are shown in different shades of grey. BMS-182193 is shown as a dark grey ball-and-stick model sitting in the active site of the enzyme[38] (PDB code 1ODW). The positions of different residues involved in resistance against protease inhibitors[5] are shown by coloured side-chains (red, L10I; pink, D30N; dark green, M46I/L; orange, I54V; dark blue, L63P; magenta, A71V; light blue, V82A/F/S/T; yellow, I84V; light green, L90M). These figures were drawn using BobScript.[39]

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plain the cross-resistance to NRTIs observed in some of these mutants.[48,49]

The enzymatic basis of zidovudine resistance is not entirely clear. *In vitro* enzymatic assays using RT carrying zidovudine resistance mutations did not reveal reduced inhibition by zidovudine 5′-triphosphate, the active metabolite of zidovudine. Zidovudine-resistant enzyme carrying mutations at residues 215 and/or 219 has an increased binding affinity for the template-primer and increased processivity, while the mutations D67N and K70R have an enhanced pyrophosphorolysis activity.[50,51] Together, these mutations could result in selective pyrophosphorolytic cleavage of chain-terminated DNA and enhanced processivity to compensate for this interrupted synthesis.

The NNRTIs bind to a hydrophobic pocket in the palm (as shown for nevirapine in fig. 1a). Binding in the pocket appears to inhibit RT function by an allosteric mechanism whereby the catalytic Asp residues are moved relative to the polymerase binding site as a whole.[52] All NNRTI resistance mutations, including E138K in the p51 subunit, which is responsible for resistance against the experimental NNRTI TSAO,[53,54] are located in this binding pocket. These mutations contribute to resistance by disturbing the interaction between the NNRTI and the pocket and thereby reducing the affinity of the inhibitor for the enzyme (fig. 1a, yellow residues). Although specific amino acid residues interact with specific NNRTIs,[44,55] the fact that all NNRTIs bind to the same pocket explains why considerable cross-resistance is seen with this class of inhibitors.

The HIV PRO is a C_2-symmetric dimer with the active site in a cleft at the interface between the 2 monomers (fig. 1b). Currently used PIs bind to the active site using 2 strategies: either (i) they mimic the transition state during peptide cleavage, or (ii) they are designed to fit the active site either as a steric complement or as a symmetric inhibitor of the C_2-symmetric dimer,[19] as indicated for the experimental drug BMS-182193 in figure 1. PI resistance mutations are located: (a) in the cleft of the active site at the catalytic site (residues 21 to 32) or in the substrate-binding site (residues 78 to 88) [fig. 1b; light blue, yellow and pink residues], where they directly interfere with inhibitor binding;[56,57] or (b) in the flap region (residues 46 to 56) [fig. 1b; dark green and orange residues], in the β-sheets (residues 56 to 78) [fig. 1b; dark blue and magenta residues] or at amino acid residues close to the dimer interface (fig. 1b; red and light green residues) that are involved in the coordinated movements that occur upon complex formation.[57,58]

2. Correlation Between Phenotypic and Genotypic Resistance

Not all mutations observed after anti-HIV therapy contribute to a reduced sensitivity of the virus to the drugs. Clear answers on the significance of each mutation can only be provided by *in vitro* experiments including site-directed mutagenesis, where the phenotype of every single mutation or combination of mutations is investigated. A complete list of all resistance-related mutations and their effect is given in Schinazi et al.[5] The most common resistance-related mutations arising during monotherapy, and the phenotypes correlated therewith, are given in table I. The level of resistance to these drugs is roughly proportional to the number of mutations in the target gene. There are currently 22 NRTI, 35 NNRTI and 42 PI resistance mutations known from *in vitro* and *in vivo* data on clinically used and experimental drugs. In particular, the PRO, a 99-amino-acid protein, has an amazing flexibility; half of its amino acid residues have already been reported to be involved in resistance.

The resistance phenotype can be measured using replication-based or enzyme-based assays.[59-61] The phenotype can be monitored as the IC_{50}, the concentration of drug required to reduce enzymatic activity by 50%, or the EC_{50}, the concentration of drug required to reduce virus replication by 50%. Extent of resistance (-fold) is generally calculated as the ratio of the EC_{50} for the resistant isolate to the EC_{50} for the sensitive isolate. The value thus obtained does not necessarily correspond with the

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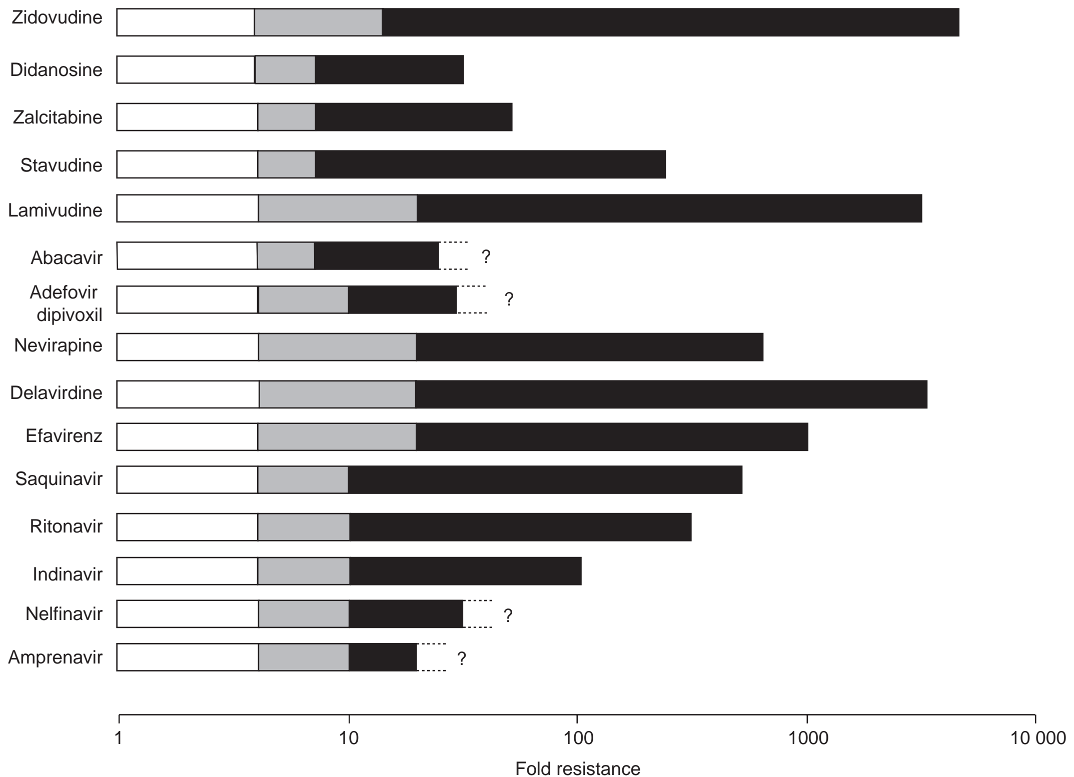

Fig. 2. Schematic presentation of the level of clinically observed phenotypic resistance against approved drugs and drugs under expanded or early access for the treatment of HIV infection. The white boxes indicate the variation in sensitivity observed in patients harbouring wild-type virus. Grey boxes represent low level of resistance for which interpretation is difficult. Black boxes represent high level resistance usually corresponding to drug failure. Not enough *in vivo* data are available for abacavir, adefovir dipivoxil, nelfinavir and amprenavir. The black boxes presented here for didanosine, zalcitabine, stavudine, abacavir, adefovir dipivoxil and nelfinavir are the result of cross-resistance data. The data used in this figure are derived from published studies.[11,13,30,31,48,62-81]

value obtained for the ratio of the respective EC_{90} values.

Owing to the high sequence variability of HIV, wild-type (WT) virus is different in every single patient, and this is associated with a variation in drug sensitivities in patient-derived WT virus (white boxes in fig. 2). Additionally, sensitivities may also vary with the subtype of the HIV-1 strain.[82] This variation is smaller for NRTIs and larger for NNRTIs.[23,83] Therefore, interpretation of a particular phenotype as being drug-resistant will depend on the drug evaluated. High-level resistance (black boxes in fig. 2) associated with drug failure generally correlates with a >20-fold reduced sensitivity to, for example, nevirapine,[84] but with a sensitivity reduced only 7-fold to, for example, zalcitabine.[28] Between WT and high-level resistance there is a grey zone that is difficult to interpret (hatched boxes in fig. 2). The interpretation of resistance should depend on: (a) the level of phenotypic resistance that has been observed for mutant viruses; (b) the selectivity index [SI; the ratio of the 50% cytotoxic concentration (CC_{50}) to the EC_{50}]; and (c) the drug concentrations that can be achieved in patients. For example, the grey zone of NNRTIs can extend over levels of phenotypic resistance that would be considered high-level resistance for NRTIs. The SI of NNRTIs is high, and

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drug concentrations considerably higher than the EC_{50} can be readily reached in patients.[4] The SI of zalcitabine and didanosine is low, and high dosages cannot be used in patients because of toxicity. The level of phenotypic resistance seen for mutant virus selected under monotherapy with zalcitabine or didanosine (table I) is generally between 4- and 20-fold, and this is partly located in the grey zone (hatched boxes in fig. 2). It is therefore very difficult to interpret the level of resistance to these drugs in patients.

In patient isolates, a given set of resistance-related mutations can be associated with a 5- to 10-fold range in phenotypic resistance.[85] It is not clear whether the phenotypic range associated with a certain set of resistance mutations is due only to the inherent sequence variability of patient strains, or also to the relatively low reproducibility of phenotypic assays (typical variabilities of 2- to 5-fold) or to the lack of sensitivity for mixed genotypes of the sequencing assays used. The generally used direct polymerase chain reaction (PCR) sequencing strategies can only reliably detect mutant strains when they constitute more than 25 to 50% of the isolate.[60,86,87] To what extent mixed genotypes influence the observed phenotype has only been partially evaluated.[88]

Another difficulty associated with genotypic assays is that some mutations work synergistically (e.g. in cross-resistance), and others antagonistically. The antagonistic effect has been best documented in the RT gene and is interesting since it can result in a, usually transient, beneficial effect of some resistance mutations. The best known of these antagonistic mutations is the lamivudine resistance mutation M184V, which resensitises zidovudine-resistant virus to zidovudine.[89] The level of resensitisation is dependent on the number of zidovudine-resistance mutations: dual resistance can be observed in the presence of M184V and 3 or more zidovudine resistance mutations.[90] Thus, the loss of response seen in patients upon adding lamivudine to a zidovudine-containing regimen may be related to the extent of pre-existing zidovudine resistance rather than the acquisition of the M184V mutation.[91] Upon prolonged exposure to both drugs, dual resistance is usually seen. Other antagonistic mutations in the RT gene that suppress zidovudine resistance, known from *in vitro* studies, are the L74V mutation occasionally selected during didanosine therapy,[64] and the L100I and Y181C mutations selected during NNRTI therapy.[92] The P236L mutation, which can be selected during delavirdine therapy, antagonises the Y181C mutation associated with the use of other NNRTIs.[93] Another interesting example of antagonism is that between the zidovudine and foscarnet resistance mutations.[94] Foscarnet is a drug that has been approved for the treatment of some herpesvirus infections. It is also active against HIV and used in HIV-infected patients. Thus, combining drugs that select for antagonising mutations may be considered as a useful strategy to afford at least transient clinical benefit.[95]

Combination therapy with NRTIs or NNRTIs can select for different, and sometimes new, mutations compared with monotherapy, and this may result in cross-resistant virus.[96] For example, it has been reported that combinations of NRTIs can select for a new set of resistance mutations (A62V, V75I, F77L, F116Y and Q151M) [light blue residues in fig. 1a] not seen under monotherapy or in *in vitro* studies.[70,97-99] They confer cross-resistance to all currently used NRTIs, and the levels of resistance to didanosine, zalcitabine, abacavir and stavudine are significantly higher than the levels observed upon monotherapy. In fact, in figure 2, the highest resistance (black zone) for these 4 drugs has only been seen with the multinucleoside resistance genotype. Preliminary data suggest that other new mutations contribute to cross-resistance to zidovudine and lamivudine (R211K, L214F, G333E/D)[100] and to cross-resistance to zidovudine, didanosine, zalcitabine, lamivudine and stavudine (T69SSS or T69SSA).[101,102] New combinations might select for yet newer cross-resistance mutations that cannot be interpreted independently from the phenotype.

Cross-resistance to PIs has also been observed in patients. The initial mutations generally seen with

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the different inhibitors in therapy-naïve patients are more or less specific and often in the active site of the enzyme:[103] for saquinavir, G48V and L90M; ritonavir, V82A/F/T; indinavir, M46I/L and V82A/F/T; nelfinavir, D30N; amprenavir; I5OV (shown in bold in table I). However, upon acquisition of secondary mutations, varying degrees of cross-resistance to all PIs are observed,[13,75,104] ritonavir and indinavir showing the greatest level of cross-resistance. Therefore, the longer the treatment with one PI, the higher the likelihood of developing increasing levels of cross-resistance to other PIs.[66,105] When PIs are used in pairs, these same mutations are seen and high-level cross-resistance is obtained.[106] It seems that for each class of inhibitors, NRTI, NNRTI and PI, long-term and combination treatment may result in cross-resistant virus. Patient-derived isolates that have resistance mutations to both NRTIs and NNRTIs, as well as PIs, have already been reported.[71] These isolates are resistant to all available drugs, and it is very hard to find a 'salvage' therapy for this group of patients.

Phenotypic resistance of the virus is always a result of genotypic changes. Yet, for all the reasons mentioned above, correctly predicting the resistance phenotype from genotypic information is not always straightforward in a clinical setting. The practical utility of genotypic data will therefore require further extensive clinical studies designed to predict phenotypic properties from genotypic ones. The establishment of generally accessible databases which link patient-derived genotypes with the associated phenotypes will be very useful in this respect.

3. Fitness of Resistant Virus

From the point of view of virus dynamics, it seems logical that the most abundant virus is the most fit virus. In the absence of selective pressure from drugs, the most fit virus would be expected to be the WT virus. In the presence of selective pressure from drugs, resistant mutant-type (MT) is the most fit virus. Therefore, resistant MT virus is expected to be less fit than WT virus in the absence of drug selective pressure.[7] For some initial resistance-related mutations, this is indeed the case. Several initial NRTI resistance mutations result in virus with reduced fitness. The didanosine-selected L74V mutation showed 11% loss of fitness compared with WT virus in the absence of drug pressure.[107] The lamivudine-selected M184I and M184V mutations result in an RT with slightly reduced processivity relative to that of WT enzyme.[108] This correlates with reduced replication of the resistant variant in lymphocytes.[109] The Q151M mutation, found as the initial mutation for multinucleoside resistance,[70] results in a virus with reduced fitness.[110] Additionally, some of these mutant viruses show increased fidelity of the RT enzyme. The mutations M184V, L74V, E89G (which confer resistance to the pyrophosphate analogue foscarnet *in vitro*) and Q151M all result in enzymes with increased fidelity.[111-114] This observation suggests a correlation between the mechanism of NRTI resistance and the mechanism of mutagenesis for some mutants: increased specificity for correct dNTP binding results both in increased drug resistance and in increased fidelity through decreased affinity for the non-natural substrates, i.e. nucleoside analogue inhibitors (ddNTPs) and wrong natural nucleotides (dNTPs that are not complementary to the template), respectively. For these mutants, and especially for the M184V variant, reduced processivity and increased fidelity translate into reduced replication efficiency and consequently reduced viral load of the resistant virus. Under the selective pressure of lamivudine, the viral load initially rapidly drops and then rises upon acquisition of the resistance mutation M184V, but remains below pretreatment levels.[12] This might not necessarily be beneficial for the patient. The increased fidelity results in production of fewer lethal mutations, thereby resulting in increased infectivity of the mutant virus.[115]

It was originally argued that the increased fidelity of resistant mutants would result in slower development of resistance mutations to other drugs,[111] but this proved not to be the case.[115-117] This suggests that the high replication rate of HIV

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has a greater effect on the speed of resistance development than the low fidelity of the RT. To delay resistance, it is thus more important to reduce the viral load than to increase the fidelity of RT. Since the HIV mutation rate is probably near the error threshold, an increased error rate of the RT might push the virus over this threshold, resulting in genetic breakdown and loss of viral viability. Resistance mutations resulting in decreased fidelity might therefore be more valuable in controlling HIV replication than mutations resulting in increased fidelity.[118]

Some NNRTI resistance mutations also confer a reduced replication capacity to the virus. The most typical mutation in this respect is G190E, obtained after *in vitro* selection with the experimental drugs S-2720 (a quinoxaline derivative) and U-95133 (a delavirdine analogue), which reduced the activity of the RT enzyme to <10%, and greatly affected the replicative capacity of the virus.[119,120] Mutations at residue 190 have also been found in patients on nevirapine therapy. More modest replication disadvantages have also been observed for the L100I mutation.[121]

For many PI-resistant variants, the loss of replication capacity has been well documented *in vitro*. PI-resistant virus carrying the V32I, M36I, M46I, G48V, V82T/A/F, I84V or L90M mutations in varying combinations has reduced processing activity and replication capacity.[121-128] However, upon continuing resistance selection, secondary mutations such as K20R, I54V, L63P and A71V accumulate, which compensate for the loss of PRO activity and increase replication efficiency, with or without effect on the resistance level.[31,121,123,124,128-133] Particularly interesting is the fact that cleavage site mutations (e.g. in the Gag region) can also compensate for the reduced fitness of PRO mutants.[127,134] Worrying in this respect is that although the initial resistance mutations are different for the different PI inhibitors, as mentioned previously, the compensatory mutations are similar for all PI inhibitors. The efficacy of changing from one PI to another might be greatly compromised when the virus has had the opportunity to develop compensatory mutations, even when no phenotypic cross-resistance is observed.[103]

The development of compensatory mutations has also been observed for multinucleoside resistance. Although the Q151M mutation displays reduced replication capacity,[110] the concomitant accumulation of the A62V, V751, F772 and F116Y mutations results in a virus that has a replication advantage compared with the WT virus (in the absence of drugs).[70] Furthermore, HIV that has had the opportunity to accumulate multiple zidovudine resistance mutations in patients under prolonged therapy shows no or only a very slow reversion to WT virus after cessation of zidovudine.[135,136] Virus with mutations at RT residues 67, 70, 215 and 219 was even shown to have a replication advantage in the absence of drugs.[137]

For some drugs, resistance mutations have been observed to appear in an ordered fashion. For zidovudine, it has been reported that the first mutation to appear is at codon 70, albeit transiently. Subsequently, the mutations at codon 215 and 41 develop, with reappearance sometimes of the mutation at codon 70. Later, mutations at codons 67, 210 and 219 are added.[25,138-141] For PIs, ordered appearance of resistance mutations has been observed for ritonavir.[31,124] The first mutation to appear is at codon 82, followed by mutations at codons 54, 71 and 36. For the other PIs, the order of appearance is not so strict but, generally, primary mutations appear first, followed by secondary mutations, as indicated in table I. This ordered appearance is probably a consequence of the combination of the level of resistance and of viral fitness associated with the respective mutations. This ordered appearance of mutations offers the possibility of specific testing for early genotypic changes associated with resistance.

Exploiting the phenomenon of reduced virus fitness upon acquisition of resistance mutations will require a change in therapy, before compensatory and potentially cross-resistant mutations arise. It might therefore be argued that changing therapy immediately after drug failure is more efficient than waiting until the viral load is back to pretreat-

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ment levels. Allowing replication of these initial resistant variants in the presence of drug selective pressure would give the virus the opportunity to increase its resistance and cross-resistance level, and to develop compensatory mutations restoring viral fitness. Any change of drug after recovery of the virus will exert selective pressure on the fit resistant variant and induce additional resistance mutations, possibly resulting in virus that is cross-resistant to the previous and the current therapy. Any change of drug before the virus fully recovers might leave the unfit virus variant at selective disadvantage compared with the WT variant, and could cause a reversion to WT virus upon subsequent active therapy.[71,87] Although resistant provirus might not disappear from the body, the new treatment would exert its effect on predominantly WT virus thus avoiding or delaying cross-resistance. Additionally, especially for PIs, early change of therapy, when the viral load is still low, may be more effective since it gives the virus less opportunity to develop cross-resistance mutations.[142] A rapid change might therefore leave more options for later salvage therapy.

4. Clinical Relevance of Virus Drug Resistance

The lack of clinical benefit for a particular anti-HIV therapy is experienced by patients and clinicians as a steady progression of the disease (as measured by opportunistic infections) despite therapy. The main cause of this progression is continuing virus replication, resulting in deterioration of the host immune system. The correlation between virus replication (and hence viral load) and clinical progression has been convincingly demonstrated.[143-145] Therefore, despite some criticism,[146] the efficiency of therapy is increasingly measured by its ability to reduce viral load, while drug failure can be monitored by a rebound in viral load. The reason for continuing viral replication can be drug intolerance, insufficient drug absorption or metabolism, unfavourable drug interactions, inaccessibility of sanctuary sites, poor compliance of the patient or lack of potency of the antiviral therapy. The potency of the therapy is dependent on the drug combination used: more potent drugs will result in a larger clinical benefit, but even potent drugs will lose their effect if the virus becomes resistant. It seems logical, therefore, to think that virus drug resistance has immediate impact on disease progression, since resistance is accompanied with a loss of drug potency. In some specific studies, a correlation between zidovudine resistance and clinical progression has been shown.[147-150] Several preliminary reports indicate that the baseline resistance profile influences subsequent response to therapy.[151-156] Yet the clinical significance of HIV drug resistance in general has not yet been unequivocally demonstrated. Resistance is usually correlated with an increase in viral load and decrease in CD4+ counts.[14,18,39,70,151] In lamivudine monotherapy, resistance mutations seem to precede the rise in viral load,[12] while in zidovudine monotherapy, the rise seems to start before the appearance of drug-resistant mutations.[152] Thus, the effects of resistance following drug monotherapy are not always clearcut. With powerful drug combination therapies, no resistance was seen after 1 year in patients with no evidence of virus replication. In patients with poor compliance, virus replication and resistance was observed.[153] Thus, although both monotherapy and combination therapy point to a correlation of virus replication with resistance development, it is at present unclear whether virus drug resistance is the cause or the consequence of a rise in viral load. It is difficult to address this question since, in many therapies, the development of virus drug resistance is a slow process with a gradual shift from WT to resistant virus populations due to the sequential accumulation of resistance mutations.[31,154-156] Additionally, the fitness of resistant strains is often lower than that of WT strains, and this may raise speculations as to the benefit of acquiring drug-resistant mutations (see section 3).

With our current view on virus dynamics, these seemingly conflicting interpretations can easily be understood. Virus replication is necessary to obtain the genetic variability from which resistant mu-

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tants can be selected under drug pressure. Thus, without virus replication, there will be no drug resistance. On the other hand, active therapy will reduce viral load and impose selective pressure on the replicating virus. Any drug-resistant mutants present at that time will experience less inhibition of their replication and will outgrow the WT virus, with a resulting rise in viral load. Thus, virus replication is both a cause and a consequence of virus drug resistance. Theoretically, any residual virus replication under drug selective pressure should be predictive of the development of resistance, and drug resistance should be predictive of a rise in viral load and thus drug failure. To verify this, there is a need for studies that specifically address the predictive value of genotypic or phenotypic resistance.

Preliminary studies provide an idea of the clinical significance of some resistance-related mutations.[157-162] Most evidence is obtained for the zidovudine resistance–related mutation at codon 215 of the RT gene, which seems to be a predictor of virological response or of disease progression in zidovudine-treated patients.[163-167] The fact that clinical drug resistance and drug failure are not always due to viral drug resistance will remain a confounding factor in these studies. A rise in viral load can, for example, also be due to poor compliance, drug intolerance or poor drug absorption. Additionally, nucleoside analogues that must be metabolised to the active triphosphate derivative,[168] and that are relatively toxic, have been reported to select for cellular resistance accompanied by loss of efficacy in the patient, without any resistance of the virus itself.[169-172]

5. Delaying Virus Resistance

Since virus drug resistance results in a loss of drug potency, strategies to delay resistance may be expected to prolong therapeutic effectiveness. The more potent a therapy is, the higher the selective pressure and the more clearcut the relation between the development of resistance and a rise in viral load. Considering the fact that virus replication is both cause and consequence of virus resistance, it is possible that more potent antiviral therapy can either precipitate or delay viral resistance, depending on the minimal viral replication required to maintain enough genomic variability from which resistance mutants can be readily selected. In this respect it is logical that an increased selective pressure, even with combination therapy, will give an opportunity for resistant mutant virus to outgrow the WT virus, provided that the mutations preexisted as initial quasispecies, e.g. as single mutants, or that the viral load is not reduced below the threshold that guarantees sufficient genetic variability for multiple mutations to arise. For drug regimens that require only single mutations for high-level resistance, the resistant strain is most probably present as an initial variant, readily selected under drug pressure. For drug regimens that are not powerful enough to decrease viral replication below the threshold, the development of resistance will be faster with increasing potency of the combination, as has been modelled by Stilianakis et al.[173]

Several observations confirm these theoretical assumptions. When using monotherapy with nevirapine, which requires only a single mutation for drug resistance, higher dosages, able to induce a larger virological benefit than lower dosages, did not delay the emergence of nevirapine resistance.[4] In the Delta trial, zidovudine resistance developed earlier in the more potent zidovudine plus didanosine or zidovudine plus zalcitabine combination arms than in the zidovudine monotherapy arm,[174] although the viral load was lower in the combination arms. Similarly, resistance against didanosine was seen earlier in patients receiving hydroxyurea in combination with didanosine than in the didanosine monotherapy arm.[175] In this combination arm, the increased potency of didanosine is due to a hydroxyurea-induced decrease in the levels of dATP, the cellular competitor of the active form of didanosine, ddATP.[176] In these cases, the residual viral replication with the more potent therapy as judged by the viral load during the period of maximal virus suppression was still high: 1000 to 10 000 HIV

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RNA copies per millilitre of plasma, ensuring sufficient residual virus replication to maintain a high level of genomic variability.

On the other hand, as mentioned in section 1, resistance will develop more slowly when high-level resistance requires increasing numbers of mutations. This has been confirmed by clinical observations: in monotherapies with similar potency, the development of resistance is slower with PIs, which require several mutations to develop high-level resistance, than with nevirapine and lamivudine, which require only single mutations for the same level of resistance (table I).

Several authors have demonstrated that it generally takes a triple-drug combination therapy to reduce viral load sufficiently to delay viral resistance. Zidovudine resistance developed earlier in the zidovudine plus didanosine arm than in the zidovudine plus didanosine plus nevirapine arm of the INCAS trial.[177] Viral load was reduced to less than 20 HIV RNA copies per millilitre of plasma in half of the patients in the triple combination arm. Similarly, delavirdine resistance is delayed during triple therapy with zidovudine plus didanosine plus delavirdine, compared with the bitherapy arms zidovudine plus delavirdine or didanosine plus delavirdine,[177] and lamivudine resistance is delayed in triple-drug combination therapies that contain that drug.[178]

Combining data from several trials, Jacobsen et al.[29] showed that a delay in the development of resistance was observed in the triple combination arm (zidovudine plus saquinavir plus zalcitabine) but not in the double combination arms (zidovudine plus zalcitabine) or monotherapy arms (zidovudine or saquinavir). This delay of resistance was linked to a significantly larger viral load reduction in the triple combination (fig. 3). These triple-drug combinations, exerting a powerful antiretroviral effect, are called highly active antiretroviral therapy (HAART). Additionally, combination drug therapies always require several mutations for high-

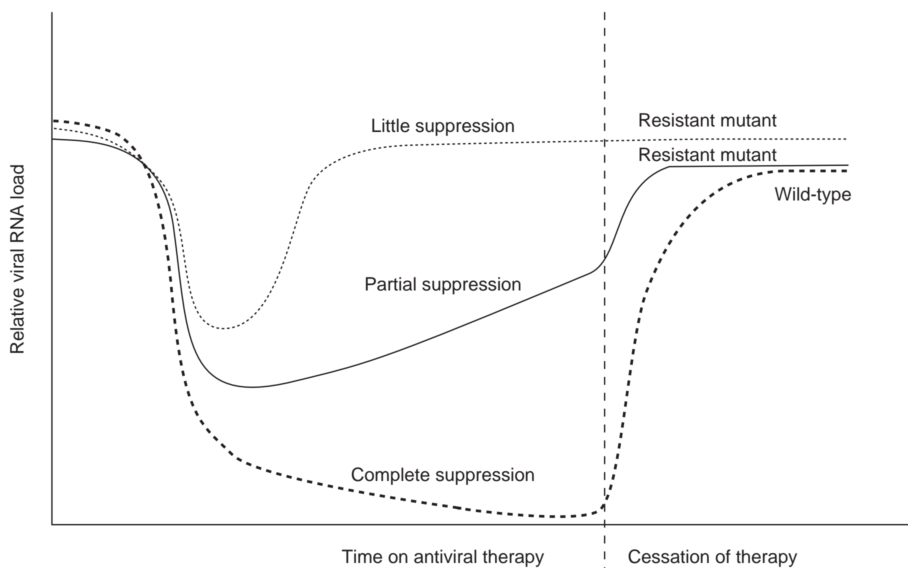

Fig. 3. Delay or prevention of resistance using antiviral therapy against HIV. Suboptimal therapies that provide only little suppression of virus replication, as measured by the HIV RNA viral load, will result in a rapid development of resistant mutant virus. Powerful therapy that provides a substantial but only partial suppression of virus replication will delay resistance but will eventually also result in resistant mutant virus. Only complete suppression of virus replication can prevent resistance, but to date has not been shown to eradicate the virus. Wild-type virus replication will recover as soon as therapy is interrupted (figure drawn according to an idea by Coffin[179]).

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level resistance to develop to the combination regimen. This can explain the delay of resistance, even against lamivudine which requires only one mutation for high-level resistance against lamivudine monotherapy.[180]

Results from Günthard et al.[153] and Wong et al.[181] suggest that even though resistance can be delayed by HAART, it cannot be prevented if there is residual virus replication. Their data seem to suggest that we will need to consider the use of ultrasensitive viral load assays (detection limit around 20 to 50 HIV RNA copies per millilitre of plasma) instead of the currently used assays with a detection limit of around 500 HIV RNA copies per millilitre of plasma, and that the goal of HAART should be to obtain undetectable viral load with these ultrasensitive assays. Only if virus replication is completely suppressed will the virus have no opportunity to develop resistance (fig. 3). Upon cessation of therapy, however, WT virus replication recovers,[179] arguing in favour of continuous treatment. Since the majority of the infected CD4+ cells are trapped in the lymphoid tissue, virus must be cleared not only from the plasma but also from the lymph nodes and other sanctuary sites. In some patients, HAART resulted in reduction of virus replication in the lymph nodes.[182,183] Since monitoring of lymph node viral load is not an established procedure, individual patient follow-up will for now be forced to rely on plasma viral load as a marker for residual virus replication.

To block virus replication in order to prevent resistance, it is also very important that patients are compliant with the powerful but difficult HAART regimens. The accumulated adverse effects and sometimes drug intolerance as a result of HAART and the accompanying therapies for opportunistic infections may seriously affect the quality of life of these patients.[184] Unfavourable drug interactions and antagonistic anti-HIV drug combinations such as zidovudine plus stavudine should be avoided.[185] Compliance should be stressed and monitored. Poor compliance, even for a few days, gives the virus the opportunity to replicate, and this was found to be responsible for the development of resistance.[177]

6. Clinical Use of Genotypic and Phenotypic Resistance Testing

For many patients, optimising anti-HIV treatment will require resistance testing. The resistance phenotype can be measured using replication-based or enzyme-based assays.[60,61,171] For phenotypic testing of patient isolates, the only assays that are sensitive enough are those based on HIV replication. The fastest and most reproducible among these is the recombinant virus assay, which still requires at least 4 weeks.[60,76,186,187] Phenotypic tests measure the average sensitivity towards the tested drugs. They are not able to identify minor populations of highly resistant virus.[88] Their big advantage is that they measure the effective sensitivity resulting from known or unknown resistance-related mutations and their interactions.

Genotypic tests feasible for patient isolates include direct PCR sequencing, selective PCR, point mutation assays and hybridisation assays such as the Line Probe Assay (LiPA).[60,155,188,189] Results from these assays can be made available within a few days. Sequencing has the advantage of detecting all possible resistance-related mutations, but it is not very sensitive in detecting mixed genotypes.[86,87,190] Specific assays such as selective PCR or LiPA are much more sensitive to detect minor populations of resistant virus (down to 1 to 5% for LiPA),[87] but they do not cover all possible resistance-related mutations and, because of background sequence variability, some codons cannot be typed in some patient isolates.

As explained above in section 2, because differences in genetic environment in patient-derived strains can influence the resistance phenotype, and because new combination treatments might select for new mutations with unknown phenotype or unknown interactions with other resistance-related mutations, the correlation between genotype and phenotype is not always clearcut. The most frequently used phenotypic test, the recombinant virus assay, and all the genotypic tests, depend on

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the ability to amplify the RT and/or PRO gene from a patient isolate. Therefore, genetically divergent strains are sometimes difficult to amplify.

For these tests, the source of genetic material should be plasma RNA rather than circulating lymphocyte DNA. Their detection limit is thus comparable with the detection limit of template amplification-based viral load assays. Plasma RNA is used because resistance development is delayed in proviral DNA from peripheral blood mononuclear cells,[153,191] mainly for 2 reasons: (i) the turnover of infected lymphocytes is much slower than the turnover of free virus – it takes longer to replace all WT proviral DNA by resistant genotypes; and (ii) the majority of virus turnover is in lymphoid tissue from which virus is spilled in circulating blood, and resistance is thus anticipated to occur first in lymphoid tissues. However, in cases of prolonged drug exposure it might be informative to investigate resistance mutations in the proviral DNA. Resistance reversal might result in WT viral RNA while resistance might still be lingering in the proviral DNA, ready to reappear if selective pressure for this drug is renewed. Unfortunately, in most cases it is not practically feasible to test both viral RNA in the plasma and proviral DNA in the lymphocytes, and so viral RNA should be the source of genetic material for resistance testing. For this reason, the recommendations are never to recycle a drug that was part of a failing regimen.

The use of resistance testing in individual patient follow-up is still vigorously debated, especially since the clinical validation of each test has not yet been performed.[192] In our opinion, the optimal usage of resistance testing to guide drug choice in individual patient follow-up is depicted in figure 4. This should be practised with the advice of experts on HIV drug resistance, since interpretation of resistance results is very complicated and direct clinical benefit has not yet been proven. Although still controversial, we think that drug resistance testing at the initiation of therapy, even in drug-naïve patients, should be considered. Transmission of drug-resistant virus has been increasingly documented and can reach up to 15% in some countries.[194-196] Additionally, some resistance mutations may be present as natural variants (quasispecies) in untreated patients.[197,198] Resistance testing might also be used to prevent the transmission of resistant virus, e.g. to guide the drug choice in postexposure prophylaxis or in prevention of vertical transmission. Therefore, combination therapy in pregnant women should be considered, especially in mothers harbouring zidovudine-resistant virus.[193,199]

Phenotypic assays can be used when predominant virus populations need to be evaluated and when the results are not extremely urgent, such as before the initiation of therapy. Genotypic tests could be employed when results are urgently requested, as is the case in postexposure prophylaxis. Direct PCR sequencing will map all known mutations in the predominant virus population. Specific assays that can detect minor variants (e.g. selective PCR or LiPA) are useful in cases where particular mutations are expected to appear (e.g. M184V in lamivudine-containing drug regimens). They will be able to detect these mutations earlier than is the case with sequencing strategies, and before their effect is measurable in phenotypic tests. In many studies, both phenotypic and genotypic tests are used. To guide salvage therapy, resistance testing might be the only option left to select a possibly active triple-drug therapy. Drugs to which the virus is not phenotypically resistant could be combined and potential antagonistic effects of drug resistance mutations could be maximally exploited.

Although resistance testing is very expensive, its use to guide therapy can be cost effective, since the administration of inactive drugs represents a huge waste of money. Performing HIV resistance mutation assays or sequencing with commercial kits (which are becoming available) costs $US150 to $US300 per sample, whereas phenotypic assays are at present 3 to 7 times more expensive. The costs of antiviral therapy are around $US500 per month per patient.[200]

On the basis of the observations and arguments in this review, the development of drug resistance mutations should be predictive for drug failure.

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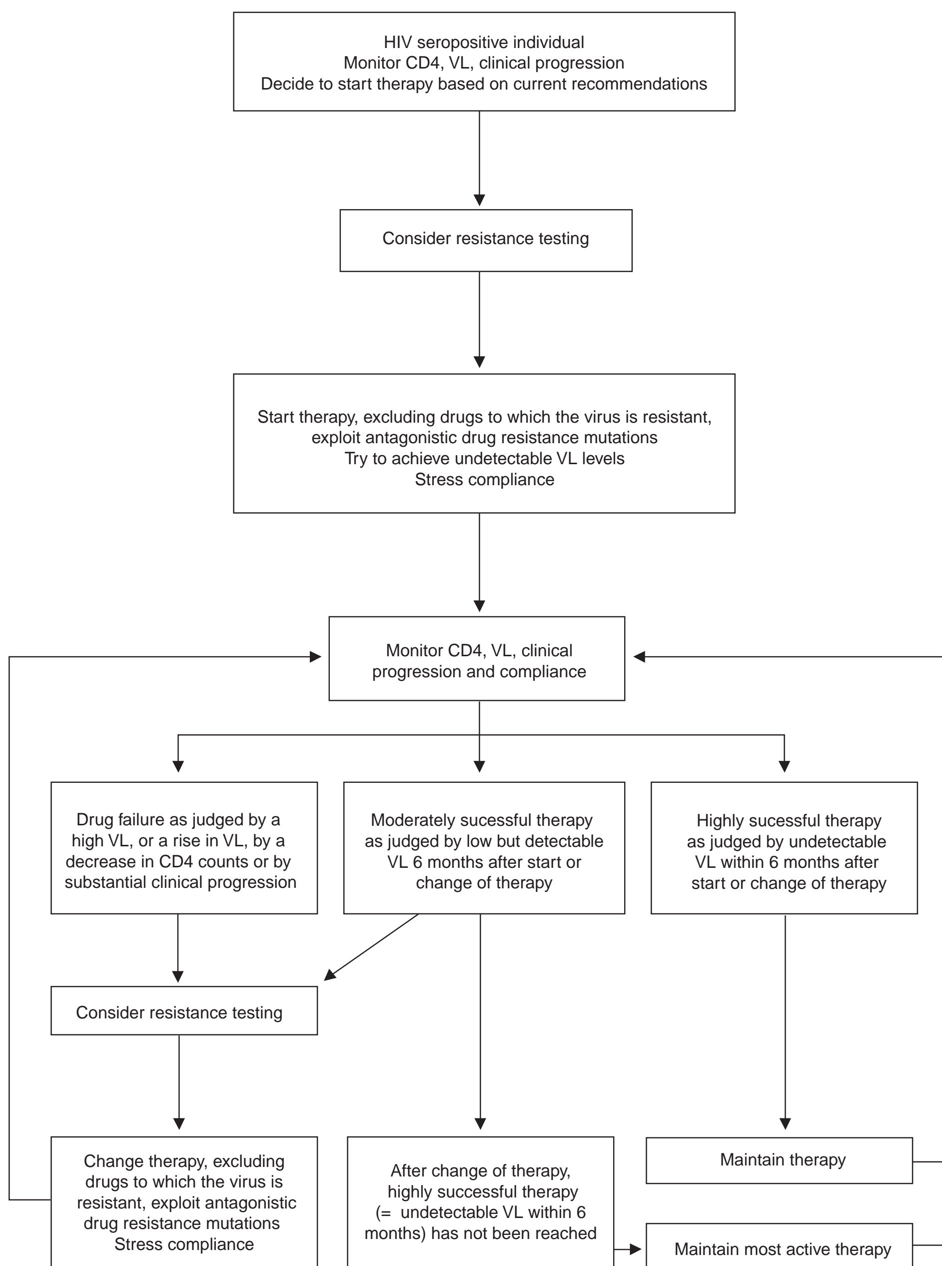

Fig. 4. Algorithm for a possible strategy for managing HIV-1 drug resistance. Current recommendations to start or change anti-HIV therapy based on viral load (VL), CD4+ cell count (CD4) or clinical progression are reviewed in Fauci et al.[193] Failure of anti-HIV therapy can be due to insufficient concentrations of active drug (e.g. poor compliance, poor drug absorption, cellular resistance), drug intolerance or virus drug resistance.

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Thus, theoretically, changing therapy on the basis of resistance testing in addition to virological, immunological and clinical markers could anticipate drug failure. The facts that resistance mutations have the tendency to appear in an ordered fashion with initial mutations often displaying reduced viral fitness, and that the level of phenotypic resistance and cross-resistance gradually increases with the number of mutations added, suggest that genotypic resistance testing may be able to detect imminent drug failure earlier than phenotypic resistance testing or viral load monitoring. Early switch of therapy, based on genotypic resistance development, could ensure a more prolonged benefit of anti-HIV therapy. It would include resistance testing every 3 months along with viral load measurement during patient follow-up, and would only be feasible and affordable with fast, relatively cheap genotypic tests of which the 'gold standard' is still sequencing. Since changing therapy on the basis of genotypic resistance data has never been evaluated, the decision to change therapy in figure 4 does not include resistance testing. Clinical studies to validate the use of genotypic resistance testing to decide when to change therapy are urgently needed before we can recommend a change of therapy based on genotypic testing.

7. Strategies for Managing Drug Resistance

The delay and possibly prevention of viral resistance is entirely dependent on the power of antiviral therapy to reduce or even block viral replication. If therapy is initiated, based on viral load, CD4+ cell counts and clinical progression (for guidelines see Fauci et al.[193]), the goal should be to obtain a viral load that is undetectable with the most sensitive assay available. Resistance testing may help in the initial choice of drugs (fig. 4).[185] For compliant drug-naïve patients without pre-existing resistance mutations, HAART should be able to keep the viral load at least temporarily below the detection limit.[193,201] For patients who started on suboptimal bi- or monotherapies, a change to HAART might be considered even before viral load starts to rise. This is because suboptimal therapies will in most cases result in drug-resistant virus. Viral load under HAART seems to follow a biphasic decline, with an initial rapid fall within 2 to 4 weeks and a slower second phase declining to maximum suppression within 6 months.[193,201] If undetectable viral load cannot be reached within this period, changing antiviral therapy, for example by adding a fourth drug, might be necessary to prevent the development of resistance (fig. 4). To minimise residual replication in sanctuary sites such as the central nervous system (CNS), it is recommended that at least 1 drug be included that can gain access to the CNS, such as zidovudine or stavudine.[202] Compliance should be stressed and viral load, CD4+ cell counts and clinical progression should be monitored every 3 months.[185]

If drug failure is imminent, as judged by a high or rising viral load, by declining CD4+ cell counts or by substantial clinical progression, patients should change to a new HAART combination. Since changes in CD4+ counts and clinical progression might be delayed compared with changes in viral load, and because of the scientific rationale for controlling virus replication, the decision to change therapy should be mainly based on viral load data.[193] Theoretically, changing therapy as soon as viral RNA becomes detectable should be beneficial, considering the reduced virus fitness of many initial drug-resistant mutants and the increasing levels of cross-resistance when additional mutations arise during virus recovery, as explained in section 3 on fitness of resistant virus. Even though a possible reversion to the more fit WT virus after a very early change in therapy might not eliminate resistant proviral copies in quiescent lymphocytes, the new therapy would exert its selective pressure on replicating WT virus, avoiding the accumulation of mutations which would result in virus cross-resistant to the previous and the changed therapy.

If resistance testing is proven to be predictive for therapy failure, its use in patient follow-up in the decision when to change therapy, implying the need to test every 3 months, might in the future become an acceptable strategy for predicting ther-

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apy failure and managing drug resistance. Present recommendations recognise the need for an early change of therapy based on a detectable viral load, but practical considerations may result in further observation in patients with a detectable but low viral load.[193] Resistance testing is, however, not currently recommended in routine use.[193] In the absence of resistance testing, the new drug combination should exclude all previously experienced drugs.

Alternatively, resistance testing might permit the re-use of drugs to which the virus is still sensitive, especially when previous suboptimal therapies limit the choice of agents (fig. 4). Resistance testing additionally gives the clinician the opportunity to exploit antagonistic drug resistance mutations.

After a change of therapy, the goal for the new combination is again to achieve undetectable viral load. In some drug-experienced patients, HAART may decrease viral load, but undetectable levels may never be reached, even after a change of therapy. In these cases, the most active HAART should be maintained (fig. 4) and patients should be carefully monitored, since the ongoing low-level HIV replication increases the likelihood of drug failure.[153]

Recent findings suggest that even after 30 months of HAART and undetectable viral load, patient-derived lymphocytes that are actively producing virus can be cultured *in vitro*.[203-205] The recovered virus did not contain resistance-related mutations, indicating that virus replication had indeed been greatly suppressed. Therefore, for lack of proof to the contrary, at present we must assume that to survive HIV infection, patients will require permanent HAART. Long-term treatment might ultimately result in multidrug-resistant virus, leaving few options for so-called ‘salvage therapy’. Therefore, the development of new drugs, active against these resistant viruses, will remain an important strategy for the management of drug resistance. Additionally, new and more powerful drugs that elicit fewer adverse effects and have greater access to sanctuary sites will allow more powerful and easier combinations with less opportunity for residual virus replication and better compliance.

To stay a step ahead of resistance, new and more active PRO and RT inhibitors should be developed. Some of the new-generation NNRTIs, which require multiple mutations in the viral RT to evoke high-level resistance, might give anti-HIV treatment a new lease of life.[206-210] Drugs inhibiting other targets of the HIV replication cycle would be most effective against current resistant strains, and would probably allow more potent combination strategies. A particularly attractive new class of compounds is the bicyclams, which are targeted at the chemokine receptor CXCR4 and inhibit virus-cell fusion.[211] Promising new targets such as proviral DNA integration and HIV gene regulation[212,213] should also be further explored and exploited. Possibly, gene therapy approaches can help to target infected cells, whether resistant or not.[214]

8. Conclusion

HIV drug resistance is currently the major factor responsible for antiviral drug failure in patients. The important issues in managing resistance are shown in table II. With current HAART the selective pressure on the virus is increasing, with no evidence of virus eradication. Any residual replication will eventually result in resistant virus that has accumulated multiple drug-resistant mutations. The resistance problem will therefore grow worse if we do not develop treatment strategies that can anticipate future resistance. The best strategy to prevent resistance is to achieve a maximal reduction of virus replication by HAART and to change therapy to a new highly active combination, if possible, as soon as viral replication can be detected.

Resistance testing can help us to make intelligent decisions instead of blind choices for HAART. Possibly, imminent drug failure due to developing resistance can be detected earlier with a genotypic assay able to detect minor resistance populations than with phenotypic assays or viral load determinations. However, to justify the utility of genotypic resistance testing for the decision on when to

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Table II. Important issues in managing HIV drug resistance

HIV does not have a fixed genome but exists as a quasispecies. Because of the high replication rate, single mutants are produced daily and resistant single mutants are readily selected under drug selective pressure
In many cases, resistant single mutants are less fit than WT virus in absence of drug. Continuing drug selective pressure may give the virus the opportunity to recover which can eventually result in virus that is as fit as or more fit than the WT virus
The longer the selective pressure with one drug, the higher the likelihood of cross-resistance to the other drugs of the same class (NRTI, NNRTI or PI)
Resistance is delayed when multiple mutations are needed for high-level resistance and when virus replication is suppressed below a threshold, reducing the genetic variability to such an extent that it takes longer for the virus to accumulate resistance mutations. Both conditions are achieved in powerful combination therapies
Resistance will only be prevented when virus replication in the circulation, lymphoid tissues and sanctuary sites is completely suppressed. Whether this is possible has still to be assessed
Delaying and possibly preventing drug resistance will require continuous HAART able to reduce viral load below the detection limit of the most sensitive viral load assays. Compliance with the regimen is crucial
Drug failure due to resistance should be recognised as soon as possible. The faster the change in therapy after the emergence of resistance, the higher the likelihood of reversion to the more fit WT virus and the lower the cross-resistance to other drugs
Drug choices should be intelligent, preferably based on resistance testing
Continuing efforts should be devoted to new drug development. New drugs are the only alternative for patients carrying multiple drug-resistant viruses

HAART = highly active antiretroviral therapy; **NRTI** = nucleoside analogue reverse transcriptase inhibitors; **NNRTI** = non-nucleoside analogue reverse transcriptase inhibitors; **PI** = protease inhibitors; **WT** = wild-type.

change therapy, there is an urgent need for clinical studies specifically designed to measure the predictive value of specific virus drug resistance mutations. When these goals have been achieved, cost-efficiency studies can further promote the use of resistance testing.

The drugs currently available do not leave enough treatment options for patients experiencing drug failure under HAART. Unabated efforts in developing new drugs to fight resistant virus, including those that are active at targets other than the HIV RT or PRO, will remain essential for the next decade.

Acknowledgements

The original investigations of the authors are supported, in part, by the Biomedical Research Programme of the European Commission (EC BIOMED2 grant BMH4-CT-95-1634), the Flemish Fonds voor Wetenschappelijk Onderzoek (FWO grant G.3304.96) and the Belgian Geconcerteerde Onderzoeksacties (GOA 95/5). We thank Dr Robert Esnouf for preparing figure 1.

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