UPDATE

New patterns of HIV-1 resistance during HAART

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HIV-1 resistance and subsequent virologic failure occur in a substantial proportion of HIV-infected patients receiving HAART regimens. In the present article, we summarize new data on resistance to current and forthcoming antiretroviral drugs which will help in the interpretation of the results of resistance tests and the individualization of therapy. Nucleoside analog mutations (NAMs) (M41L, D67N, K70R, L210W, T215Y/F and K219Q/E) are associated with reduced susceptibility to most nucleoside analogs and the nucleotide tenofovir. This recently approved drug has shown a reduced virologic response in the presence of three or more NAMs, including M41L or L210W, as well as in the presence of T69 insertions. Hypersusceptibility ($IC_{50} < 0.5$) to non-nucleoside reverse transcriptase inhibitors (NNRTIs) has recently been described in association with increased resistance to nucleoside analogs, and it seems to enhance the immunologic and virologic reponses in patients receiving efavirenz-containing regimens. New protease inhibitors (PIs) have a lower cross-resistance profile, although more clinical data are needed to establish appropriate PI sequencing to promote sustained virologic success. Cross-resistance between amprenavir (APV) and lopinavir (LPV/r) in the presence of only four APV-related mutations has been described, suggesting that phenotypic tests should be applied before prescribing LPV/r to APV-experienced patients. Resistance to the new entry inhibitor class compound T-20 (enfuvirtide) has also been detected.

Keywords HIV resistance, mutational patterns, antiretrovirals

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The introduction of highly active antiretroviral therapy (HAART) in the management of HIV disease has led to a sustained suppression of viral replication, a partial restoration of the immune system, and a sharp decrease in the incidence of opportunistic complications and mortality. However, persistence of viral replication with HIV-1 resistance and subsequent virologic failure still occur in a substantial proportion of patients, due to subtherapeutic drug levels related to poor adherence or other factors, such as genetic variability, P-glycoprotein mechanisms, and impaired absorption [1,2]. HIV resistance is a complex issue, and even in patients with plasma viral loads below 50 copies/mL, the presence of HIV mutations has been observed, although their clinical significance is not known [3].

Despite several important limitations, genotypic and phenotypic resistance tests may be helpful tools in guiding antiretroviral therapy, especially in certain populations such as recently infected or previously failing patients [4–7].

Our knowledge of HIV-1 resistance is continuously evolving. The aim of the present paper is to summarize new data regarding resistance to currently existing and forthcoming antiretroviral drugs, which may aid in interpreting the results of resistance tests and in correctly individualizing therapy.

NRTIs (NUCLEOSIDE ANALOG REVERSE TRANSCRIPTASE INHIBITORS)

(Table 1.) Nucleoside-analog mutations (NAMs) comprise a set of mutations including M41L, D67N, K70R, L210W, T215Y/F and K219Q/E,

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Table 1 Main mutations associated with NRTIs and NNRTIs[a]

Mutations	Comments
M184V	3TC
	ABC, partial resistance
	Modifies susceptibility to ZDV and TDF impaired by other mutations
K65R	ABC, ddI, selected in vivo
	TDF, selected principally in vitro
NAMs: M41L, D67N, K70R,	Mutations associated with ZDV resistance and
L210W, T215Y/F, K219Q	most other nucleotides (except for 3TC) and TDF
>3 NAMs + M41L and/or L210W	Reduced susceptibility to TDF
E44D/A, V118I	Increase levels of ZDV resistance and enhance viral fitness
Q151M complex	High level of resistance to all NRTIs. No TDF resistance
T69 insertion	Multi-NRTI and TDF resistance
K103N, Y188L	High level of NNRTI resistance
Two or more: L100I, V106A, Y181C, G190A/S, P225H, M230L, P236L	Resistance to NNRTIs

ABC, abacavir; 3TC, lamivudine; d4T, stavudine; ddI, didanosine; TDF, tenofovir; ZDV, zidovudine.
[a]For more complete information, consult Visible Genetics or other Guidelines.

which were originally named zidovudine (ZDV) resistance mutations, and which are now widely recognized as having a role in resistance to other nucleosides, except for lamivudine (3TC) [8–11]. NAMs seem to act through a novel mechanism, by improving the enzymatic efficiency of the reverse transcriptase (RT) in several ways [12]. NAMs are principally selected by ZDV, and accumulate in a stepwise manner, from an eight-fold reduction with the first mutation to over 100-fold later in the course of treatment [13,14]. Although V75T is the major in vitro stavudine (d4T) mutation, a significant reduction in the response of d4T-treated patients has been observed when three or more NAMs are present, especially 41L, 210W and 215Y/F [9]. As the drugs more commonly associated with this cluster of mutations are ZDV and d4T, the term thymidine analog mutations (TAMs) is also frequently used to refer to these six mutations.

The appearance of NAMs depends in part on the analog–d4T combination: these mutations have been sequenced at an earlier stage from clinical isolates of patients treated with d4T–didanosine (ddI) than from d4T−3TC samples, suggesting that the presence of the 184V mutation impaired viral fitness and led to reduced replication [15]. However, the clinical impact of these data is uncertain. Although NAMs emerged at comparable rates in HIV-1 during ZDV- or d4T-based therapy, in one study there was a trend towards a greater risk of developing >2 NAMs in ZDV/3TC-treated patients, probably due to the more frequent emergence of the M41L mutation [16].

Two recently discovered mutations, which are frequently associated with NAMs, especially with T215Y, are the E44D and V118I mutations, which appear to compensate and improve replicative competence in isolates from patients treated with ZDV [17,18]. These mutations have also been observed in ddI and zalcitabine (ddC)-treated patients.

NAMs plus the 3TC-associated mutation M184V reduce susceptibility to abacavir (ABC) 10-fold, but M184V alone does not appear to be associated with a reduced virologic response [19]. In addition, archived mutations resulting from prior exposure to ZDV have been implicated in early virologic failure in patients treated with ABC-containing regimens [20,21]. M184V may help to partially reverse ZDV resistance associated with M41L and T215Y [22]. However, the clinical significance of this effect is not known, and, in addition, it may be overridden by an accumulation of NAMs.

Multinucleoside resistance mutations

While the Q151M mutation causes only partial resistance to current NRTIs, the Q151M complex (Q151M, A62V, V75I, F77L and F116Y) is associated with high-level resistance to the whole class of drugs, except tenofovir [23]. It acts by a mechanism of drug discrimination, and almost always appears in patients with current or prior ddI-containing regimens [24].

T69 insertions (T69S, T69SSG, T69SSX)

These consist of a set of mutations involving a primary codon change, T69S, with a six-base in-frame insertion to yield two extra amino acids between codons 69 and 70. T69SSX is the most common insertion [16]. This mutation, unlike the Q151M complex, confers a >20-fold loss of susceptibility to tenofovir that is partially reversed to six-fold in the presence of M184V [25]. T69 insertion has a low prevalence, and appears with prolonged exposure to NRTIs. It is associated with multi-NRTI resistance with a background of one or more NAMs, especially T215Y. The mechanisms of resistance seem to be related to enhanced pyrophosphorolysis or to structural alteration in the dNTP-binding pocket [26].

NUCLEOTIDE ANALOGS

Tenofovir, the first approved nucleotide analog, is a potent compound with an excellent resistance profile. In vitro, an HIV variant derived from different passages in increasing concentrations of tenofovir developed the K65R mutation on RT, which confers high resistance to the drug. However, less than 1% of patients develop it during tenofovir treatment [25,27]. Recent data from pooled analyses of the effect of NRTI-associated resistance in patients enrolled in the 902 and 907 trials showed that specific NAMs diminished the virologic response in patients treated with tenofovir. Although a statistically significant mean reduction in plasma HIV-1 RNA (−0.6 log copies/mL) from baseline to week 48 was achieved in this study, in patients with three or more NAMs that included either M41L or L210W mutations ($n = 86$; mean −0.21 log copies/mL), and in those with the L210W mutation in any context, a modest virologic response was observed. In contrast, in those patients with other mutations such as D67N, K70R or K219Y, the response to tenofovir was not affected. Importantly, the presence of the 69XX insertion confers high-level resistance to this drug.

In the 902 and 907 studies, a greater than 3.8-fold decrease in susceptibility to tenofovir was observed in the phenotypic test in patients with resistance mutations together with low virologic response at 48 weeks, when compared with the wild-type virus. In genotypic assays, the presence of the K65R mutation, the 69 double insertion or multiple NAMs (including M41L and L210W in the majority of cases) was detected [28] (Figure 1). Some data showed that the presence of M184V could partially reverse the effect of the thymidine mutations [25].

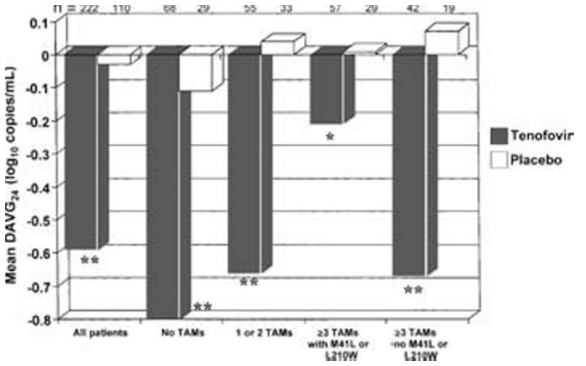

Figure 1 Studies 902 and 907. Effect of type and number of TAMs on response (intent to treat). $^{*}P = 0.013$, $^{**}P < 0.0001$.

NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NNRTIs)

These drugs achieve high plasma concentrations, but few mutations are required before high-level resistance across the entire class is observed ('low genetic barrier'). The presence of either K103N or Y188L alone produces high-level resistance to all NNRTIs [29]. Moreover, two or more of mutations L100I, V106A, Y181C, G190S or M230L substantially reduce the clinical utility of all approved NNRTIs [30].

Some authors have suggested that nevirapine-treated patients showing early virologic failure with the sole presence of mutation Y181C could be switched to efavirenz, again suppressing viral replication [31]. Although this strategy may prove beneficial in selected cases, overall sequencing of NNRTIs appears not to be an optimal clinical strategy [32].

Recently, a systematic search of a large phenotypic–genotypic database (Virco) linked Y318F substitution with an overall >10-fold decrease in NNRTI susceptibility in 85% of clinically derived isolates [33].

Enhanced susceptibility or hypersusceptibility ($IC_{50} < 0.5$), to NNRTIs has recently been described in association with increased resistance to nucleoside analogs [34,35]. NNRTI hypersusceptibility occurs in approximately 20% of NRTI-experienced patients [34]. The primary NRTI

mutational pattern associated with this phenomenon was M41L, M184V and T215Y, usually with L210W [36]. Although this is a controversial issue, recent studies have suggested that this NNRTI-associated exclusive phenomenon may be associated with better virologic and CD4 responses [37].

Another NNRTI mutation, G190A/S, can cause delavirdine hypersusceptibility, as well as resistance to nevirapine and efavirenz [29].

SECOND-GENERATION NNRTIs

TMC-125 is a new diaminopyrimidine NNRTI with potent activity against both wild-type virus and virus that is resistant to currently available NNRTIs. Its antiviral power is equivalent to that of a five-drug HAART regimen [38]. Phase II trials and in vitro assays with recombinant virus harboring K103N, Y181C, L100I, Y188L and G190A/S mutations have demonstrated an antiviral activity similar to that of wild-type virus at a TMC-125 stable concentration of 1 nM [39].

PROTEASE INHIBITORS (PIs)

Resistance to PIs is driven by the selection of primary mutations probably due to selective non-optimal drug pressure. Later, secondary mutations are selected in order to compensate for the initial impairment of viral fitness. These secondary mutations seem to be common to all the PIs, and facilitate the resistance to the whole class. The International AIDS Society (IAS)–USA expert panel has recently redefined primary mutations as major mutations and secondary mutations as minor mutations [29].

Resistance to PIs is a relative phenomenon, in which raising drug trough levels (by boosting PIs) may overcome the IC_{50} of mutant isolates [1].

Owing to the occurrence of partial cross-resistance between PIs, strategic sequencing of PI-containing regimens may allow long-term suppression of viral replication. It has therefore been suggested that nelfinavir's resistance pattern (with the exclusive D30N as the most frequent primary mutation associated with an impairment in viral fitness) favors its use as the first PI [40,41].

Recently available or forthcoming PIs, such as amprenavir, atazanavir and tipranavir, seem to have a lower PI cross-resistance profile. More clinical data are needed to clarify a hierarchy in PI sequencing, in order to obtain sustained virologic success.

Amprenavir

In vitro experiments with increasing APV concentrations identified an isoleucine-to-valine substitution at position 50 as a key marker of resistance, leading to a three-fold decrease in APV susceptibility [42]. Further development of M46I/L and/or I47L could lead to a greater than ten-fold reduction in APV susceptibility. Sequenced mutations at Gag positions L449 and P453 were associated with I50V enhanced resistance to APV, and also improved in vitro viral fitness [43].

Data from antiretroviral-experienced patients rescued with APV showed four group mutations: I50V, I54L/I54M, I84V, and V32I + I47V. The association of I50V and I84V produced the greatest reduction of susceptibility to APV, although these mutations conferred little PI cross-resistance [44]. In vitro data showed that certain APV-selected mutations conferred greater than 10-fold cross-resistance to LPV/r: L10F/M46I/I47V/I50V-GagL449F. These data suggest that phenotypic tests should be used before prescribing LPV/r to APV-experienced patients [45].

Lopinavir/ritonavir (LPV/r)

LPV/r is a PI co-formulation that has shown significant antiviral potency in naive and pretreated patients and currently constitutes a major option in the treatment of heavily treated failing patients. This antiviral potency depends on the drug's extremely high genetic barrier and also on its pharmacokinetic profile.

In naive patients treated with LPV/r, no major PI mutations were sequenced in isolates during virologic rebound. It is likely that the combined pharmacokinetic and pharmacodynamic characteristics of LPV/r (high in vivo IQ, small resistance step size, and rapid decline of LPV/r concentrations during missed doses) contribute to the clinical observation of lack of resistance development in naive patients treated with this co-formulation [46].

A panel of viral isolates from PI-failing patients was used to show that 11 amino acid mutations—10, 20, 24, 46, 54, 63, 71, 82, 84, 90—(LPV/r mutation score) in the protease were associated with reduced sensitivity to LPV/r [47]. Virologic

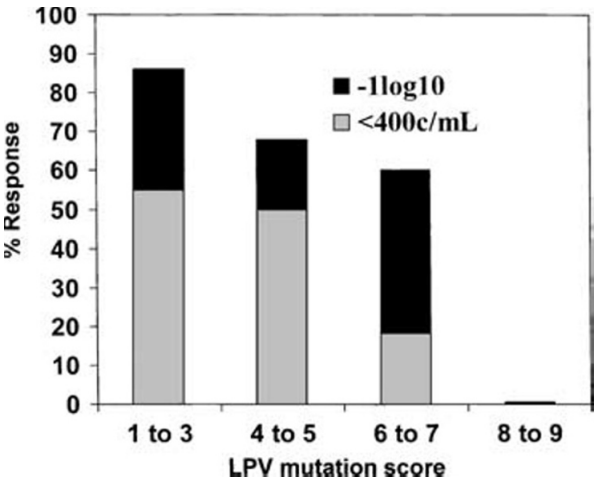

Figure 2 Virologic response to LPV/r-containing regimen accordIng to the LPV mutatIon score (n = 65).

response (HIV-1 RNA < 400 copies/mL) has been observed in 33% of patients with more than eight mutations but in 76% of those with up to seven mutations [48].

Data from a prospective study in PI-experienced patients rescued with LPV/r showed that a mutation LPV/r score >5, the presence of the I54V mutation at baseline, a high number of previous PIs, prior therapy with indinavir or ritonavir and a lower LPV/r trough concentration were independently associated with virologic failure in this cohort [49]. In Figure 2, differences in virologic response according to the number of PI mutations are shown.

Cross-resistance between APV and LPV/r in the presence of only four APV-related mutations has previously been mentioned.

Atazanavir (ATV)

Atazanavir is a once-daily PI which is currently in phase III clinical trials and which has shown activity against viruses resistant to other PIs. The specific mutation appearing in early in vitro studies is the I50L substitution, followed by mutations at positions N88S, V32I, A71V, L33F and I84V with increasing ATV concentration [50].

The presence of I50L increases HIV susceptibility to other PIs, including APV. Interestingly, both APV and ATV produced a different mutation at the same codon; thus, I50V confers resistance to APV but leaves the virus susceptible to ATV and other PIs.

On the other hand, isolates from PI-experienced patients who received combination therapy with ATV plus Saquinavir (SQV) showed emergence of the I84V mutation, which is associated with broad cross-resistance to other PIs [51]. Data are needed to clarify whether patients with ATV-resistant isolates harboring only the I50L mutation may be successfully treated with other PI-containing regimens.

Tipranavir (TPV)

Tipranavir is the first of a novel class of non-peptidic protease inhibitors. It has demonstrated broad in vitro antiviral activity, with a uniquely robust resistance profile against HIV isolates resistant to multiple PIs. It binds to the protease active site with fewer hydrogen bonds than other PIs, allowing great flexibility [52,53].

In a phase II trial, 41 multiple PI-experienced patients received ritonavir-boost TPV. Over 50% of patients had more than 10 PI mutations, and all expressed a high level of resistance to other PIs; however, a 2.34 log RNA HIV mean viral load reduction was observed. Overall, 16–20 PI mutations at baseline were associated with subsequent reductions in TPV susceptibility, but V82T and L33I/V frequently emerged during TPV 48-week treatment [54]. It is likely that four mutations (V32I, L33F, V82T, I84V) within or in close proximity to the enzyme active site determined the reduced susceptibility to TPV [55].

FUSION INHIBITORS

T-20 (enfuvirtide) is a peptide mimetic of the heptide residue (HR-1) domain of HIV-1 gp41 which has been defined as a fusion inhibitor. Mutations in gp41 in the region of amino acids 36–45 have been identified in in vitro assays: G36S, V38M, V38A, Q39H, Q40H, and N43D [56].

In a subgroup of 18 HIV-1 heavily treated patients rescued with T-20-containing regimens, those with continuous viral replication presented mutations detected in a highly conserved region across M group subtypes—Q40H, N43S, L45M—and in sequences previously described as polymorphisms—A30T, S35A [57].

No classical resistance-associated mutations in response to T-20 were identified in HIV-1 group M subtypes (A–G), although two new mutations in B isolates in a region critical for T-20 activity, I37V

and Q39R, were sequenced in naive patients; however, the impact on T-20 sensitivity is not known [58,59].

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