AIDS Reviews 2006;8:179-90

Novel Drug Resistance Mutations in HIV: Recognition and Clinical Relevance

Carlo Federico Perno, Valentina Svicher and Francesca Ceccherini-Silberstein
Department of Experimental Medicine and Biochemical Sciences, University of Rome "Tor Vergata", Italy

Abstract

During its spread among humans, HIV-1 has developed an extraordinary degree of genetic diversity. The* pol *region encoding for viral enzymes such as the reverse transcriptase and the protease, and the* env *region encoding for the viral glycoprotein gp41 are subjected not only to natural variation, but also to the selection pressure imposed by the pharmacologic treatment. Under these conditions in HIV-1 infected people, the virus is able to escape from antiviral drugs by accumulating mutations, either alone or in clusters. The patterns of mutations accumulated by HIV-1 under drug pressure are quite variable, depending on the backbone of virus strains, the level and type of pharmacologic pressure, and the length of therapy. To date, a high number of mutations in protease, reverse transcriptase, and gp41 have been associated with reduced susceptibility to the antiretroviral drugs currently available. However, a number of studies continuously highlight the existence of additional mutations beyond those currently known to be involved in the development of drug resistance* in vivo*.

Most of these so-called "novel" mutations are involved in agonistic correlations with the classical drug resistance mutations on divergent evolutionary pathways, and are associated with an increased resistance to specific drugs. At the same time, the presence of some novel mutations at therapeutic failure has also been significantly associated with an increase of viremia, thus suggesting that they may also play a compensatory role leading to improved viral replication. Interestingly, some natural polymorphisms in drug-naive patients have been significantly associated with the development of drug resistance mutations at failure, thus suggesting their ability to decrease the genetic barrier to the development of drug resistance.

In contrast, other novel mutations are negatively associated with specific antiviral treatment, showing negative interactions with relevant drug resistance mutations, and are associated with increased susceptibility to specific drugs.

This article reviews the importance of recognition and the clinical relevance of novel mutations involved in resistance to the currently used antiretroviral drugs, discussing in particular the role of novel drug resistance mutations in the reverse transcriptase enzyme. Such novel mutations should be considered for improved prediction of clinical response to antiretroviral drugs and for assessing the efficacy of next-generation drugs. **(AIDS Reviews 2006;8:179-90)**

Corresponding author: Francesca Ceccherini-Silberstein, ceccherini@med.uniroma2.it

Key words

Antiretroviral therapy. HIV-1 Drug Resistance. Mutations. Genotype. Phenotype. Virologic response.

Correspondence to:
Francesca Ceccherini-Silberstein
Department of Experimental Medicine
University of Rome "Tor Vergata"
Via Montpellier 1
00133 Rome, Italy
E-mail: ceccherini@med.uniroma2.it

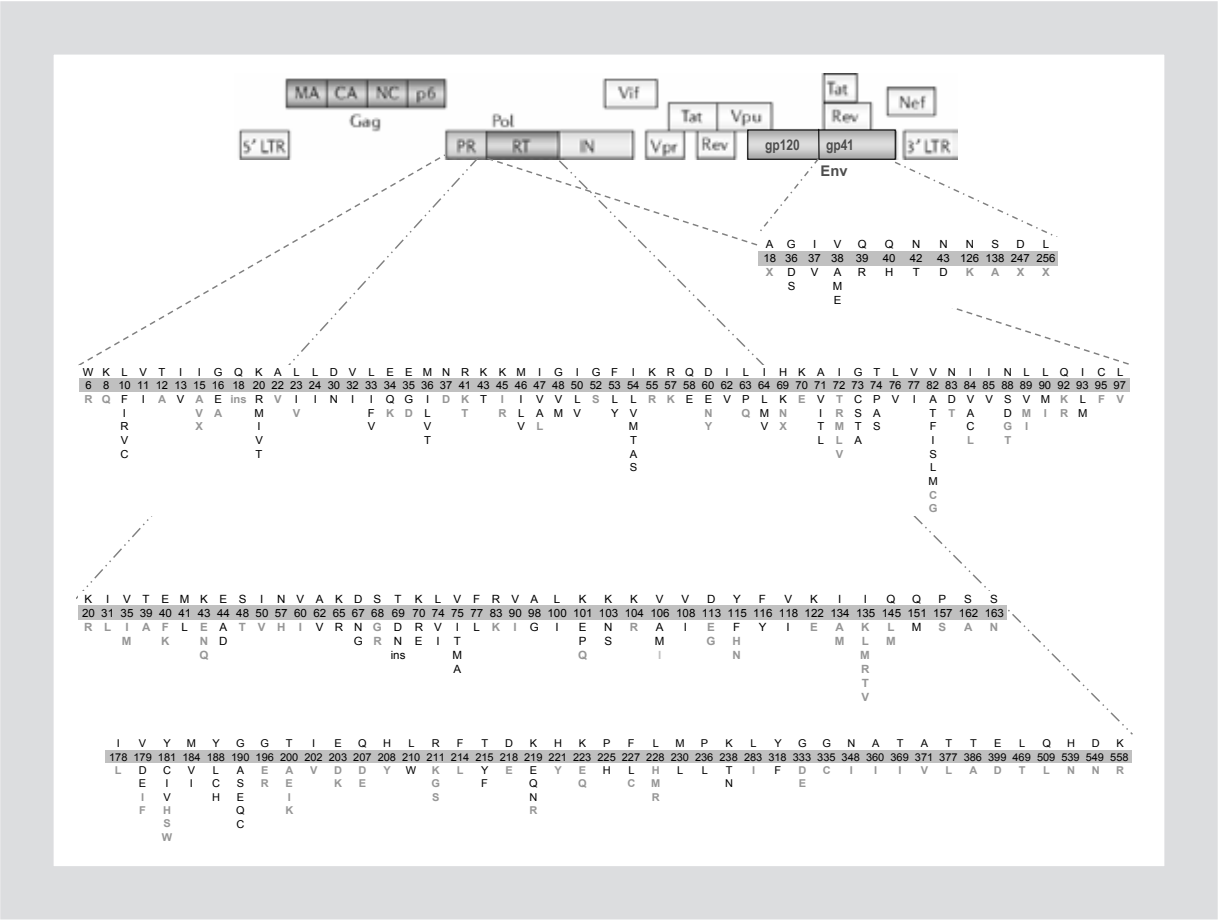

Figure 1. *Location of "novel" and "known" drug resistance mutations in the HIV-1 genome. The nine open-reading frames in the HIV-1 genome (Gag, Pol, Env, Tat, Rev, Vpu, Vif, Vpr and Nef) are depicted. Known and novel mutations in protease (PR), reverse transcriptase (RT), and gp41 associated with drug resistance to the antiretroviral drugs currently available are shown in black and grey, respectively.*

Introduction

Important progress has been made in the development and clinical use of drugs for the treatment of HIV-1 infection. To date, twenty-one drugs have been approved for the treatment of AIDS. Most of them target two viral enzymes: reverse transcriptase (RT) and protease, while one drug, enfuvirtide, targets the glycoprotein gp41 involved in viral entry. The combined use of these drugs has substantially improved the clinical management of HIV-1 infection in terms of delaying disease progression, prolonging survival, and improving quality of life. Nevertheless, when antiretroviral therapy fails to be fully suppressive, new viral variants emerge, thus allowing HIV-1 to escape from the drug by accumulating mutations, either alone or in multiple clusters.

An increasing number of mutations in protease, RT, and gp41 have been associated with reduced susceptibility to the antiretroviral drugs currently available[1-7] (Fig.1). The patterns of mutations accumulated by HIV-1 under drug pressure *in vivo* are quite complex and variable, depending not only on the intrinsic biochemical properties and mechanism of action of each drug, but also on the level of pharmacologic pressure, length of therapy, and the backbone of virus strains. Moreover, *in vivo* such variability is further increased by host selective pressure such as the immune system[8]. A better definition of the mutational pathways that regulate the complexity of drug resistance is therefore a key element for the design and management of effective anti-HIV chemotherapy.

In the light of such complexity, some additional mutations, defined as "novel", have been proposed by several *in vitro* and/or *in vivo* studies to contribute through different mechanisms to drug resistance (Figs. 1,2). While their role is still not completely defined, there is a growing interest, and convincing evidences on their relevance in clinical practice are accumulating. For instance, several novel mutations in HIV-1 protease have been associated either with a poorer or better virologic response to specific protease inhibitors (PI)[9-12]. For these reasons, they have been recently included in the set of mutations associated with re-

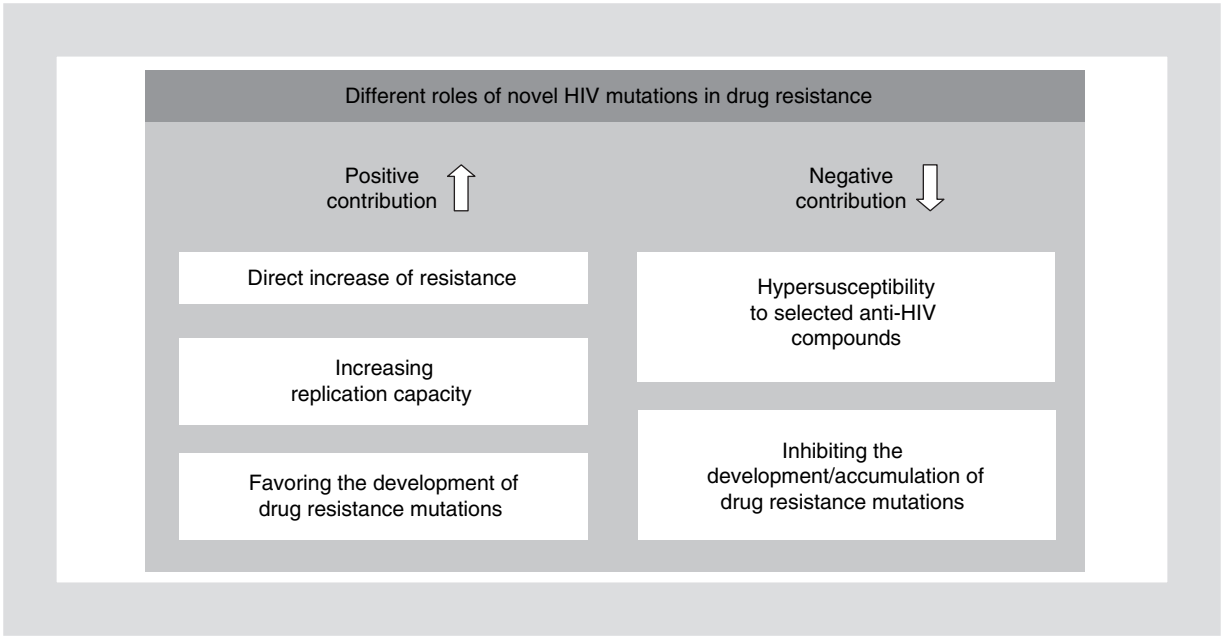

Figure 2. *Role of novel mutations in drug resistance. Positive and negative contributions of novel HIV-1 mutations in the development of resistance to anti-HIV drugs are shown.*

duced susceptibility to PI in different rules-based algorithms (http://hivdb.stanford.edu; http://assign.dci.uwa.edu.au/crest/crest5_table.asp). Some have also been recently included in the latest updated list of mutations reported by the International AIDS Society as associated with resistance to the latest generation of PI (atazanavir, tipranavir, and darunavir)[2].

In contrast, less information is available regarding the role and clinical relevance of novel RT mutations. Indeed, not many novel RT mutations are yet included either in the generation of new algorithms for improving prediction of clinical response to reverse transcriptase inhibitors (RTI), or in the updated list of RT mutations reported by the International AIDS Society. Therefore, in this review we specifically focused on the clinical significance of novel mutations in HIV-1 RT associated with resistance to the RTI currently available. We define these mutations as *novel* since they are not reported to be associated with drug resistance by the Stanford HIV Drug Resistance Database and by the International AIDS Society[1-2].

The contribution of novel mutations to resistance of all drug classes currently available has been already demonstrated by several groups, including ours. The most complex pattern of interactions, however, seems to be that of novel mutations involved in the resistance to RTI, in particular to nucleoside reverse transcriptase inhibitors (NRTI). This review will thus focus on this topic, crucial for a more sophisticated definition of the residual activity of, and the reciprocal HIV resistance to, NRTI in drug-failing patients.

Novel mutations involved in resistance to nucleoside reverse transcriptase inhibitors

Positive contribution to NRTI resistance

To date, 61 novel mutations at 44 positions have been positively associated *in vivo* and/or *in vitro* with NRTI treatment and resistance (Table 1)[13-45]. Among all novel NRTI mutations, only three mutations fall at two residues already associated with RTI resistance[1-2], whereas the others fall at new positions.

Novel mutations in RT can be categorized as follows:

– Some novel mutations are very rare (< 1% of frequency) or completely absent in isolates from drug-naive patients. In contrast, their prevalence significantly increases in patients failing an antiretroviral regimen containing NRTI[25,36,41-45]. Thus it is conceivable that these mutations play an important role in maintaining a good replication capacity, and that they may require the NRTI selective pressure for their emergence during virologic failure. Different studies show that these novel mutations occur principally in combination with several NRTI-resistance mutations, thus suggesting that they emerge after a prolonged NRTI exposure, i.e. when the virus has already accumulated a large number of NRTI-resistance mutations[27,38,44]. For instance, the novel mutation H208Y has been demonstrated to require as prerequisite the presence of at least M41L and T215Y in HIV-1 RT[27].

Table 1. Novel mutations associated with NRTI resistance

Mutations*	*in vivo; in vitro*	Drug†	References
Positive contribution to resistance			
K20R	*in vivo*	3TC, NRTI	Saracino, et al. 2006; Svicher, et al. 2006
V35M	*in vivo*	ABC, TDF	Svicher, et al. 2006
T39A	*in vivo*	ZDV, d4T, ddC, NRTI	Saracino, et al. 2006; Svicher, et al. 2006 Rhee, et al. 2005
E40F	*in vitro*	ZDV	Huigen et al. 2006
K43E/N/Q	*in vivo*	ZDV, d4T, ddC	Svicher, et al. 2006; Huigen, et al. 2006
N57H	*in vitro*	d4T, ddI	Bossi, et al. 1998
V60I	*in vivo*	ZDV, ddI	Precious, et al. 2000; Huigen, et al. 2006
S68G/**R**	*in vivo; in vitro*	ZDV, d4T, ddI, ABC, NRTI	Kavlick, et al. 1998; Roge, et al. 2003; Garcia-Lerma, et al. 2000
K104R	*in vitro*	NRTI	Huigen, et al. 2006
D113E/G	*in vitro*	ZDV	Larder, et al. 1989; Crespan, et al. 2005
Y115H/N	*in vitro*	ZDV	Larder, et al. 1989
K122E	*in vivo; in vitro*	ZDV, d4T, TDF	Svicher, et al. 2006
Q145L/M	*in vitro*	NRTI	Paolucci, et al. 2004
P157S	*in vitro*	3TC	Smith, et al. 2004
S162A	*in vitro*	NRTI	Huigen, et al. 2006
S163N	*in vitro*	ZDV	Jeeninga, et al. 2001
I178L	*in vivo*	3TC	Doualla, et al. 2004
G196E	*in vivo*	ZDV, 3TC, NRTI	Stoeckli, et al. 2002; Svicher, et al. 2006
T200**A**/E/I/K	*in vivo*	NRTI	De Luca, et al. 2006
I202V	*in vivo*	ZDV, ddI	Precious, et al. 2000
E203D/K	*in vivo*	ZDV, d4T, NRTI	Saracino, et al. 2006; Svicher, et al. 2006; Rhee, et al. 2005
Q207**E**/D	*in vivo*	ZDV	Stoeckli, et al. 2002; Lu, et al. 2005
H208Y	*in vivo; in vitro*	ZDV, d4T, ddI, ABC, NRTI	Sturmer et al. 2003; Marcelin, et al. 2005; Saracino, et al. 2006; Svicher, et al. 2006; Rhee, et al. 2005
R211**K**/G/S	*in vivo; in vitro*	ZDV, d4T, ddI	Sarmati, et al. 2001; Sturmer, et al. 2003; Marcelin, et al. 2005; De Luca, et al. 2006
F214L	*in vivo*	ZDV; d4T	Precious, et al. 2000; Sturmer, et al. 2003; Svicher, et al. 2006; De Luca, et al. 2006; Cozzi-Lepri, et al. 2006
D218E	*in vivo*	ZDV, ABC, ddC, ddI, NRTI	Saracino, et al. 2006; Svicher, et al. 2006; Rhee, et al. 2005
K219R	*in vivo*	NRTI	Yahi, et al. 1999; Hanna, et al. 2000
K223Q	*in vivo*	NRTI	Rhee, et al. 2005
L228H/M/R	*in vivo*	ddI, NRTI	Rhee, et al. 2005; Marcelin, et al. 2005

Table 1. Novel mutations associated with NRTI resistance *(Continuation)*

Mutations*	*in vivo; in vitro*	Drug†	References
G333**D**/E	*in vivo*	ZDV, d4T, 3TC, ddI	Kemp, et al. 1998; Coakley, et al. 2000; Gallego, et al. 2002; Sturmer, et al. 2003
G335C	*in vivo; in vitro*	ZDV	Nikolenko, et al. 2006
N348I	*in vivo; in vitro*	ZDV	Nikolenko, et al. 2006; Gupta, et al. 2006
A360I	*in vivo; in vitro*	ZDV	Nikolenko, et al. 2006
T369I	*in vivo; in vitro*	ZDV	Nikolenko, et al. 2006; Gupta, et al. 2006
A371V	*in vitro*	ZDV, 3TC, ABC, TDF	Brehm, et al. 2006
T377L	*in vivo*	d4t; ddC	Torti, et al. 2004
E399D	*in vivo; in vitro*	ZDV	Nikolenko, et al. 2006; Gupta, et al. 2006
L469T	*in vivo*	ZDV, d4T	Marcelin, et al. 2005
Q509L	*in vitro*	ZDV, 3TC, ABC, TDF	Brehm, et al. 2006
H539N	*in vivo*	ZDV	Nikolenko, et al. 2005
D549N	*in vivo*	ZDV	Nikolenko, et al. 2005
K558R	*in vivo*	ZDV, d4T	Marcelin, et al. 2005
Negative contribution to resistance			
V35I	*in vivo*	NRTI	Svicher, et al. 2006
I50V	*in vivo; in vitro*	3TC	Svicher, et al. 2006
R83K	*in vivo; in vitro*	ZDV, d4T, ddI	Svicher, et al. 2006

*Amino acid mutations in HIV-1 RT associated with NRTI resistance are reported using the clade B consensus sequence as a reference. According to the prevalence of mutations in the Stanford HIV Drug Resistance Database [www.hivdb.stanford.edu <http://www.hivdb.stanford.edu>], mutations occurring with a frequency < 1% in drug naive patients are shown in grey boldface, and polymorphisms present with a frequency > 10% in drug naive patients are shown in black boldface. Residues already associated with RTI resistance (Johnson, et al., 2006; Stanford HIV Drug Resistance Database [www.hivdb.stanford.edu]) are underlined.
†The drugs to which the novel mutation is associated are reported: ZDV: zidovudine; d4T: stavudine; 3TC: lamivudine; ddI: didanosine; ddC: zalcitabine; ABC: abacavir; TDF: tenofovir.

– Other novel mutations are common polymorphisms in drug-naive patients, whose prevalence significantly increases in isolates from NRTI-treated patients (Table 1)[25,36,37,44]. While their role in maintaining a good replicative capacity may be less relevant than those in the first group, they may contribute to a further increase in the level of resistance to NRTI, mainly to thymidine analogs (Table 1), thus amplifying the complexity of resistance and the degree of genetic barrier to resistance of these drugs.

It has been proposed that some novel mutations exert their contribution to zidovudine resistance by participating in one of the two divergent evolutionary pathways that induce resistance to thymidine analogs. It is known, in fact, that resistance to thymidine analogs is mediated by two specific sets of mutations collectively termed nucleoside analog mutations (NAM), defined by different mutation patterns (NAM-1: M41L, L210W, T215Y; and NAM-2: D67N, K70R, T215F, K219Q/E)[17,19,46]. These mutations confer resistance by promoting a phosphorolytic reaction that selectively removes the nucleoside analog from the terminated DNA chain[47-49]. The division of NAM into two distinct clusters has important clinical significance: zidovudine-resistant viruses carrying NAM-1 usually are cross-resistant to didanosine and tenofovir, whereas viruses carrying NAM-2 usually remain at least partially susceptible to those drugs[50,51].

Novel mutations E40F, K43E, V60I, K104R, K122E, S162A, and H208Y establish strong agonistic interactions with the NAM-1[27,38,41-46]. In particular, the co-presence of E40F, K43E, K122E, and H208Y, individually or combined, with NAM-1 increases the level of zidovudine resistance (up to 40-fold), and, although less extensive, that of stavudine[27,38,41,44]. Another study demonstrated that K43E, K122E, and H208Y (in addition to T39A and E203K) contribute to zidovudine resistance more than some classical zidovudine mutations such as T215F, K219Q, and K70R[38].

For H208Y, it was supposed that the appearance of such mutation, being proximal to the adenosine triphosphate (ATP)-binding site, may influence the geometry of the ATP-binding site, thus increasing the level of zidovudine resistance and maintaining the efficiency of the excision reaction even in the presence of M184V (that is known to decrease the ability of HIV-1 RT to carry out ATP-mediated removal of zidovudine or stavudine monophosphate from the terminated cDNA chain)[52].

Differently from the above-mentioned mutations, the novel mutations K20R and D218E established agonistic interaction with the NAM-2. Indeed, the co-presence of D218E with the NAM-2 cluster determined a 2.5-fold increase in zidovudine fold resistance[38,44].

Other potential advantages for the virus in the acquisition of novel NRTI mutations may come from an increase of viral replication *in vitro* and *in vivo*. For instance, the presence of mutations K43E, K122E, and H208Y at therapeutic failure has been also associated with higher values of viremia and lower values of CD4+ cell count[44]. In particular, the co-presence of K43E and H208Y with NAM-1 cluster was associated with an increase in viremia 3.7-fold higher than that observed with NAM-1 alone[44], confirming the *in vitro* ability of K43E to compensate the loss in viral fitness consequent to the emergence of E40F[41]. A recent study also showed the ability of other novel mutations (V60I, K104R, and S162A) to rescue HIV-1 replicative capacity impaired by the presence of some NAM such as M41L[42].

Another mutation that deserves attention is S68G. This has been observed in isolates from patients treated with stavudine, abacavir, and didanosine in combination with Q151M or K65R, drug resistance mutations known to reduce HIV-1 replication capacity[2,15,26,53-56]. The presence of S68G may improve the replication capacity of viruses having the Q151L mutation, the intermediate passage from glutamine to methionine, usually characterized by a dramatic loss of replicative capacity, thus facilitating the acquisition of Q151M[57]. A functional role of S68G has been hypothesized also in viruses harboring K65R in terms of increase viral fitness and/or of favoring the emergence of K65R; however further *in vitro* studies are necessary to confirm such hypotheses[26].

Taken all together, the findings support that novel mutations such as K43E, V60I, S68G, K122E, S162A, and H208Y may act as compensatory mutations leading to improved viral replication, especially if compromised by the presence of other NRTI-resistance mutations.

Regarding the specific impact and contribution of novel mutations on the virologic response to NRTI, little is known so far. Some novel mutations may affect the virologic response to specific NRTI and influence the evolution of relevant drug resistance mutations. In particular, a recent study showed that the baseline presence of the novel mutations H208Y, R211A/D/G/K/S, and L228H/R/M is associated with a poorer virologic response after four weeks of didanosine therapy[33]. In another study, some common polymorphisms in drug-naive patients predicted the subsequent evolution toward specific NAM patterns. In particular, T200A/E/I/K and R211G/S have been found to be associated with the on-treatment development of NAM-1 and NAM-2, respectively[40].

It is conceivable that such natural polymorphisms may represent crucial determinants for the course of resistance evolution, thus influencing the occurrence and accumulation of the classical drug resistance mutations. Further studies are urgently needed to provide insights regarding these important, but unfortunately still open, points.

In this context, the novel mutation F214L deserves additional attention. The F214L is a natural polymorphism observed in around 18% of drug-naive and NRTI-treated patients[37,44]. This polymorphism is involved in agonistic interaction with NAM-2, while it is rarely found concomitant with NAM-1[27,38,44,58]. There has recently been observed a trend for a greater frequency of NAM-2 at virologic failure if the 214L mutation (instead of 214F) was already present at baseline (unpublished work[40]), thus suggesting that this mutation may represent a determinant for the choice of a specific NAM pathway. It is conceivable that the structural vicinity of residue 214 with NAM-1 residues 210 and 215 may explain this clustering behavior. Recently we also provided evidence that the presence of polymorphism F214L may confer virologic benefit to patients starting zidovudine or stavudine as part of potent combination therapy. A short-term benefit (week-24 viral load reduction) was indeed observed both in antiretroviral therapy (ART)-naive and ART-experienced patients, and persisted during more extended follow-up in ART-naive patients, as evidenced by a lower rate of virologic failure (unpublished work[39]). This result extends the observation of a previous paper, showing the association of mutated position 214 with a stronger response to zidovudine-containing regimens at week 48[20].

Lack of compensatory novel mutations for M184V

To our knowledge, besides some indirect observations based on *in vivo* association[43], no novel or compensatory mutations have so far been described for M184V, a very common mutation associated with resistance to lamivudine and emtricitabine. The loss in fitness that characterizes such mutation has been well demonstrated by many *in vitro* and *in vivo* studies[59-61].

The lack of compensatory mutations to M184V may be related to its key positioning beside amino acids 110+185+186 that represent the catalytic triad of RT enzyme. Novel compensatory mutations to M184V do not exist or could not yet be described, but this point remains to be elucidated, due to its remarkable clinical relevance. Indeed, the maintenance of

lamivudine/emtricitabine as a fourth additional drug in new regimens may be of strategic relevance, with the purpose of providing the additional advantage of maintaining M184V and the associated loss of fitness.

Negative contribution to NRTI resistance

In clinical practice, mutations are associated with different levels of resistance. However, a new research field aims at defining whether some mutations can be associated with a lower appearance of primary mutations and/or with a lower rate of therapeutic failure.

Indeed, some novel mutations (V35I, I50V, R83K) in HIV-1 RT are negatively associated with NRTI failure (Table 1)[37-38,44]. These three mutations share peculiar characteristics. In fact, they are common polymorphisms in drug-naive patients with a frequency significantly decreased in isolates from patients who failed an antiretroviral regimen containing at least one NRTI. Moreover, these mutations have been rarely found in the presence of NRTI-resistance mutations and are not positively associated with any NRTI-resistance mutations. If anything, I50V and R83K show to be involved in antagonistic interactions with NRTI-resistance mutations (M184V and NAM, respectively), and are associated, when rarely present with M184V or NAM, respectively, with increased susceptibility to lamivudine, and thymidine analogs, respectively[44]. Based on these findings, it has been suggested that these mutations may be deleterious in terms of viral replication in the presence of NRTI-resistance mutations, thus contributing to increase the level of the genetic barrier to NRTI resistance.

Further insights regarding these natural polymorphisms may be quite relevant. In fact, the identification of specific mutations, such as I50V, R83K, and F214L, in patients before starting antiviral therapy opens new perspectives concerning the existence of "protective" polymorphisms that may interfere with the accumulation of drug resistance mutations. Therapy choice might benefit from taking such particular polymorphisms into account as potential contributors to the future course of resistance evolution and response to treatment, thus prolonging the benefits of the antiretroviral regimen and consequently slowing down the progression to AIDS.

Role of novel mutations in the C-terminus of the RT enzyme in NRTI resistance

Recently it has been proposed that mutations in the C-terminal portion of the RT enzyme (including the connection and the RNaseH domain) could also significantly contribute to an increase of RT resistance to thymidine analogs[34,62-65]. In particular, three mutations in the RNaseH domain (Q509L, H539N, D549N) have been shown to increase *in vitro* the level of zidovudine resistance when combined with the NAM, suggesting that the equilibrium between nucleoside excision and RNaseH activity may play an important role, not only in NRTI-mediated inhibition of HIV-1 replication, but also in NRTI drug resistance[62-63,65]. *In vivo,* other novel mutations at positions 469, 470, 554, and 558 of the RNaseH domain have been significantly associated with NRTI treatment and with the presence of NAM, even if their impact on zidovudine resistance has not yet been defined[34].

In a similar fashion, other mutations (G335C, N348I, A360I, A371V, E399D) in the connection domain of RT have been shown to increase up to 35-fold the level of zidovudine resistance[62,64-65].

These results may have important clinical implications for HIV-1 drug resistance, since most genotypic and phenotypic analyses exclude the connection and RNaseH domains of the RT enzyme, and this may result in an underestimation or misevaluation of drug resistance. Therefore, more studies are needed to understand better such molecular mechanism of resistance and its clinical significance.

Contribution to NRTI resistance in non-B subtype

Among novel NRTI mutations, those previously discussed have all been shown to be associated with drug resistance in HIV-1 B subtype. Few studies are available regarding the prevalence and role of novel RT mutations in non-B subtypes. In particular, a study has identified the novel mutation I178L potentially associated with M184V in isolates from HIV-1 C subtype-infected patients receiving lamivudine treatment, even if the exact role of this mutation in lamivudine resistance remains to be clearly elucidated[28]. The definition of resistance profile in non-B subtypes represents a critical issue for clinical management and drug resistance surveillance, since non-B subtype viruses are increasing in the Western countries and antiviral therapy is expanding also in areas where non-B subtypes predominate.

Novel mutations involved in resistance to nonnucleoside reverse transcriptase inhibitors

Positive contribution to NNRTI resistance

The resistance to nonnucleoside reverse transcriptase inhibitors (NNRTI) is generally mediated by the appearance of mutations at the hydrophobic NNRTI binding pocket that reduce the affinity of the inhibitor to the enzyme. To date 33 novel mutations at 22 positions in HIV-1 RT have been positively associated with NNRTI treatment and resistance (Table 2)[15,64,66-74]. Most novel mutations are located in the hydrophobic NNRTI

Table 2. Novel mutations associated with NNRTI resistance

Mutations*	*in vivo; in vitro*	Drug†	References
I31L	*in vitro*	TMC125	Vingerhoets, et al. 2005
E40K	*in vitro*	TMC125	Vingerhoets, et al. 2005
S48T	*in vivo*	EFV	Jeffrey, et al. 1998
A62V	*in vitro*	TMC125	Vingerhoets, et al. 2005
L74V	*in vitro*	TMC125	Vingerhoets, et al. 2005
V90I	*in vitro*	TMC125	Vingerhoets, et al. 2005
K101Q	*in vitro*	EFV, NVP	Svicher, et al. 2006
V106I	*in vitro*	NVP	Taylor, et al. 2000
I134A/M	*in vitro*	EFV	Sluis-Cremer, et al. 2005
I135**K**/L/M/R/**T**/V	*in vitro; in vivo*	EFV, NVP, DLV	Brown, et al. 2000; Vavro, et al. 2004; Svicher, et al. 2006
V179**F**/I	*in vivo*	EFV, NVP, TMC125	Turner, et al. 2004; Vingerhoets, et al. 2005; Svicher, et al. 2006
Y181H/S/W	*in vitro; in vivo*	NVP	Richman, et al. 1994; Sardana, et al. 1992
G196R	*in vitro*	NVP	Taylor, et al. 1996
H221Y	*in vitro; in vivo*	NVP	Saracino et al. 2006; Svicher, et al. 2006
K223E/Q	*in vitro; in vivo*	EFV, NVP	Saracino, et al. 2006; Svicher, et al. 2006
F227C	*in vitro*	TMC125	Vingerhoets, et al. 2005
L228H/R	*in vitro; in vivo*	EFV, NVP	Shafer, et al. 1995; Saracino, et al. 2006; Svicher, et al. 2006
L283I	*in vivo*	NVP, DLV	Brown, et al. 2000
N348I	*in vivo; in vitro*	EFV	Gupta, et al. 2006
T369I	*in vivo; in vitro*	EFV	Gupta, et al. 2006
T386A	*in vitro*	TMC125	Vingerhoets, et al. 2005
E399D	*in vivo; in vitro*	EFV	Gupta, et al. 2006

*Amino acid mutations in HIV-1 RT associated with NNRTI resistance are reported using the clade B consensus sequence as a reference. According to the prevalence of mutations in the Stanford HIV Drug Resistance Database [www.hivdb.stanford.edu <http://www.hivdb.stanford.edu>], mutations occurring with a frequency < 1% in drug naive patients are shown in grey boldface, and polymorphisms present with a frequency > 10% in drug naive patients are shown in black boldface. Residues already associated with RTI resistance (Johnson, et al. 2006; Stanford HIV Drug Resistance Database [www.hivdb.stanford.edu]) are underlined.
†The drugs to which the novel mutation is associated are reported: NVP: nevirapine; EFV: efavirenz; DLV: delavirdine.

binding pocket, and eight mutations are within five residues already associated with NNRTI resistance[1-2], whereas the others highlight new positions. Some novel mutations have been specifically associated with an increase in the level of nevirapine or efavirenz resistance. For instance, V106I, Y181H/S/W, G196R, and H221Y have been associated with an increase in nevirapine resistance[66,69,71,74], while S48T, K101Q, and mutations in positions 134 and 135 have been associated with efavirenz resistance[68,70,73].

Among novel NNRTI mutations, the I135M/T mutations surely deserve particular attention. We recently observed that I135M/T mutations occurred positively associated with the K103N mutation in NNRTI-failing patients, and their presence with K103N determined an increase in efavirenz resistance[74]. In a previous *in vitro* study it has been shown that I135T alone (and together with another novel mutation L283I) may reduce susceptibility to nevirapine and delavirdine[70], and the authors suggested that these two mutations may play a role in the

reduced susceptibility to NNRTI observed in individuals with primary HIV infection without the presence of other NNRTI mutations. Furthermore, I135T is a natural polymorphism in drug-naive patients that has been associated with the on-treatment development of NNRTI-resistance mutations[74-75], thus indicating that I135T polymorphism may represent a possible determinant of the future course of NNRTI-resistance evolution, reinforcing the hypothesis that some natural polymorphisms may represent crucial determinants for the course of resistance evolution, influencing the occurrence and accumulation of the classical drug resistance mutations. Finally, it is noteworthy that the position 135 has been shown to be the anchor position of the HLA-restricted B*5101 epitope in reverse transcriptase[76]. *In vitro* experiments demonstrated the ability of I135T to abrogate the epitope-HLA binding, thus contributing to the loss of cytotoxic T-lymphocyte responses *in vivo*[77]. Thus, the I135T mutation may allow HIV to escape from the HLA-restricted immune response and also from the inhibitory activity of the drugs, thus representing an interesting example of the synergistic interaction between immune pressure and drug pressure.

Interestingly, it has been recently proposed that some novel mutations in the connection domain of the RT enzyme may contribute to increase the level of NNRTI resistance. Among them, three mutations (N348I, T369I, E399D) have been associated with increased resistance to zidovudine and efavirenz[64]. It has been hypothesized that these mutations may modulate NNRTI resistance by affecting the dimerization between the p66 and p51 RT subunit.

The involvement of novel mutations in NNRTI resistance may represent a revolutionary concept, since it displaces the classical concept of one-step full resistance given by a single mutation. This may also have particular relevance in view of the upcoming approval of new, second-generation NNRTI, reported to be active against strains carrying few mutations conferring resistance to first-generation NNRTI, but whose efficacy is decreased by the presence of ≥ 3 NNRTI mutations. Studies are now ongoing to solve this crucial point.

Novel mutations involved in NNRTI hypersusceptibility

Hypersusceptibility to NNRTI is a recently described phenomenon that characterizes approximately 30% of HIV-1 isolates that are resistant to NRTI. These viral isolates exhibit greater phenotypic susceptibility to NNRTI than wild-type virus. The increased susceptibility to NNRTI has been associated with better virologic outcomes in several clinical trials and observational cohorts in which NNRTI-based regimens were used[78-80].

The genetic correlates leading to NNRTI hypersusceptibility may involve some classical NRTI-resistance mutations including M41L, V118I, L210W, and T215Y as well as the novel mutation H208Y[78,80-82]. In particular, *in vitro* experiments have shown that H208Y alone may confer an increased susceptibility to efavirenz, while H208Y + T215Y may confer an increased susceptibility to all three NNRTI[82]. This latter effect seems to require the concomitant presence of both mutations, since the presence of T215Y alone is not associated with any changes in NNRTI susceptibility[82]. This finding underlines the synergistic interaction of H208Y with the NAM-1 (in particular with T215Y) described in the previous paragraph. Thus, the novel mutation H208Y cooperates with the NAM-1 to increase the level of resistance to zidovudine and also to increase the level of susceptibility to NNRTI.

Overall findings support the complexity of resistance to RTI and highlight the significant interplay between NRTI and NNRTI resistance.

Novel mutations involved in resistance to new NNRTI

The NNRTI are important components of HAART as a consequence of their high potency and low toxicities. However, their use is hindered by the rapid emergence of viral strains that are resistant to all NNRTI as a class. This has promoted the research of novel NNRTI with improved binding to HIV-1 RT and higher thresholds against cross-resistance. Among them, TMC125 has recently entered clinical phase III trials. To date, several distinct pathways leading to TMC125 resistance have been identified[83]. The two key patterns selected from the wild-type virus involved the NNRTI-resistance-associated combinations Y181C + M230L and Y181C + G190E, together with mutations not previously associated with NNRTI resistance, such as I31L, E40K, A62V, L74V, V90I, V179IF, F227C, and T386A[83]. Interestingly, most of them such as L31I, E40K, A62V, L74V, V90I, and T386A are outside the NNRTI binding pocket, and some of them such as E40K, A62V, and L74V have been previously associated with resistance to NRTI. Among novel mutations involved in resistance to TMC125, V179I has been previously associated with treatment with the first generation NNRTI[74], and it has been shown to determine >10-fold decrease in TMC125 susceptibility when present with some classical NNRTI-resistance mutations[83].

Further *in vitro* and clinical studies are therefore necessary to confirm the efficacy and power of these new second-generation NNRTI, and to understand better the mechanisms underlying the development of their drug resistance.

Conclusions

In this review, we have described the role of novel mutations in the regulation of resistance to RT inhibitors. Many

Table 3. Novel RT mutations of potential clinical relevance

Mutations associated with impaired RTI efficacy*	Mutations associated with improved RTI efficacy*
K43E, S68G, K122E, H208Y, R211G/S/K, D218E, (NRTI)	I50V (3TC)
K101Q, I135T/M (NNRTI)	R83K, F214L (d4T, ZDV)
L228H/R (NRTI, NNRTI)	H208Y (NNRTI)

*In parenthesis the drugs or drug classes to whom the specific mutations are mostly associated.

novel mutations contribute to increase the level of resistance to specific RT inhibitors, mainly when combined with classical drug resistance mutations, and at the same time may also act as compensatory mutations leading to improved viral replication capacity. In contrast, other novel mutations such as R83K, I50V, and H208Y may have a negative impact on drug resistance, leading to increased susceptibility to specific RT inhibitors. However, the importance of novel mutations lies not only in their ability to modulate drug susceptibility, but also in their ability to affect the virologic response to specific RT inhibitors and/or influence the evolution of relevant drug resistance mutations as in the case of natural polymorphism F214L and I135T.

A summary of the most relevant novel mutations that will require more attention in clinical practice is shown in table 3.

Taken together, overall studies give evidence that novel mutations actively participate in the regulation of drug resistance *in vivo*. Such novel mutations should be considered for improving algorithms that predict clinical response to antiretroviral drugs and for assessing the efficacy of next-generation drugs. In addition, the identification of natural polymorphism in drug-naive patients able to modulate the genetic barrier to resistance strongly supports both the extension of wide genotyping to all patients that start an antiretroviral regimen, and the implementation of *in vitro* and *in vivo* studies aimed at shedding light upon the complex interactions between the virus, drugs, and the host.

Acknowledgements

The authors are grateful to Maria Mercedes Santoro, Salvatore Dimonte and Maria Trignetti for helpful comments and contributions to the manuscript.

References

1. Stanford HIV Drug Resistance Database [http://hivdb.stanford.edu].
2. Johnson V, Brun-Vezinet F, Clotet B, et al. Update of the drug resistance mutations in HIV-1: Fall 2006. Top HIV Med 2006;14:125-30.
3. Rhee S, Taylor J, Wadhera G, Ben-Hur A, Brutlag DL, Shafer RW. Genotypic predictors of human immunodeficiency virus type 1 drug resistance. Proc Natl Acad Sci USA 2006;103:17355-60.
4. Clotet B, Menendez-Arias L, Schapiro J, et al. Guide to management of HIV drug resistance, antiretrovirals pharmacokinetics and viral hepatitis in HIV infected subjects. "Fundació Lluita contra la SIDA" (FLS) publication, with the support of Roche, 2005. Fifth edition.
5. Su C, Melby T, DeMasi R, Ravindran P, Heilek-Snyder G. Genotypic changes in human immunodeficiency virus type 1 envelope glycoproteins on treatment with the fusion inhibitor enfuvirtide and their influence on changes in drug susceptibility *in vitro*. J Clin Virol 2006;36:249-57.
6. Xu L, Pozniak A, Wildfire A, et al. Emergence and evolution of enfuvirtide resistance following long-term therapy involves heptad repeat 2 mutations within gp41. Antimicrob Agents Chemother 2005;49:1113-9.
7. Svicher V, Ceccherini-Silberstein F, Erba F, et al. Novel human immunodeficiency virus type 1 protease mutations potentially involved in resistance to protease inhibitors. Antimicrob Agents Chemother 2005;49:2015-25.
8. John M, Moore C, James I, Mallal S. Interactive selective pressures of HLA-restricted immune responses and antiretroviral drugs on HIV-1. Antivir Ther 2005;10:551-5.
9. Marcelin A, Dalban C, Peytavin G, et al. Clinically relevant interpretation of genotype and relationship to plasma drug concentrations for resistance to saquinavir-ritonavir in human immunodeficiency virus type 1 protease inhibitor-experienced patients. Antimicrob Agents Chemother 2004;48:4687-92.
10. Vora S, Marcelin A, Gunthard H, et al. Clinical validation of atazanavir/ritonavir genotypic resistance score in protease inhibitor-experienced patients. AIDS 2006;20:35-40.
11. Bertoli A, Santoro M, Lorenzini P, et al. Different patterns of mutations involved in the genotypic resistance score for atazanavir boosted *versus* atazanavir unboosted in multiply failing patients. Presented at XV International HIV Drug Resistance Workshop 2006; Sitges, Spain. Antivir Ther 2006;11:S99.
12. Baxter J, Schapiro J, Boucher C, et al. Genotypic changes in human immunodeficiency virus type 1 protease associated with reduced susceptibility and virologic response to the protease inhibitor tipranavir. J Virol 2006;80:10794-801.
13. Larder B, Kemp S. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). Science 1989; 246:1155-8.
14. Bossi P, Yvon A, Mouroux M, Huraux J, Agut H, Calvez V. Mutations in the human immunodeficiency virus type 1 reverse transcriptase gene observed in stavudine and didanosine strains obtained by *in vitro* passages. Res Virol 1998;149:355-61.
15. Kavlick M, Wyvill K, Yarchoan R, Mitsuya H. Emergence of multidideoxynucleoside-resistant human immunodeficiency virus type 1 variants, viral sequence variation, and disease progression in patients receiving antiretroviral chemotherapy. J Infect Dis 1998; 177:1506-13.
16. Kemp S, Shi C, Bloor S, Harrigan P, Mellors J, Larder B. A novel polymorphism at codon 333 of human immunodeficiency virus type 1 reverse transcriptase can facilitate dual resistance to zidovudine and L-2',3'-dideoxy-3'-thiacytidine. J Virol 1998;72:5093-8.
17. Yahi N, Tamalet C, Tourres C, et al. Mutation patterns of the reverse transcriptase and protease genes in human immunodeficiency virus

type 1-infected patients undergoing combination therapy: survey of 787 sequences. J Clin Microbiol 1999;37:4099-106.
18. Coakley E, Gillis J, Hammer S. Phenotypic and genotypic resistance patterns of HIV-1 isolates derived from individuals treated with didanosine and stavudine. AIDS 2000,14:F9-15.
19. Hanna G, Johnson V, Kuritzkes D, et al. Patterns of resistance mutations selected by treatment of human immunodeficiency virus type 1 infection with zidovudine, didanosine, and nevirapine. J Infect Dis 2000;181:904-11.
20. Precious H, Gunthard H, Wong J, et al. Multiple sites in HIV-1 reverse transcriptase associated with virological response to combination therapy. AIDS 2000;14:31-6.
21. Jeeninga R, Keulen W, Boucher C, Sanders R, Berkhout B. Evolution of AZT resistance in HIV-1: the 41-70 intermediate that is not observed *in vivo* has a replication defect. Virology 2001;283:294-305.
22. Sarmati L, Nicastri E, Parisi S, et al. Failure of stavudine-lamivudine combination therapy in antiretroviral-naive patients with AZT-like HIV-1 resistance mutations. J Med Virol 2001;65:631-6.
23. Gallego O, Corral A, de Mendoza C, Rodes B, Soriano V. Prevalence of G333D/E in naive and pretreated HIV-infected patients. AIDS Res Hum Retroviruses 2002;18:857-60.
24. Stoeckli T, MaWhinney S, Uy J, et al. Phenotypic and genotypic analysis of biologically cloned human immunodeficiency virus type 1 isolates from patients treated with zidovudine and lamivudine. Antimicrob Agents Chemother 2002;46:4000-3.
25. Gonzales M, Wu T, Taylor J, et al. Extended spectrum of HIV-1 reverse transcriptase mutations in patients receiving multiple nucleoside analog inhibitors. AIDS 2003;17:791-9.
26. Roge B, Katzenstein T, Obel N, et al. K65R with and without S68: a new resistance profile *in vivo* detected in most patients failing abacavir, didanosine and stavudine. Antivir Ther 2003;8:173-82.
27. Sturmer M, Staszewski S, Doerr H, Larder B, Bloor S, Hertogs K. Correlation of phenotypic zidovudine resistance with mutational patterns in the reverse transcriptase of human immunodeficiency virus type 1: interpretation of established mutations and characterization of new polymorphisms at codons 208, 211, and 214. Antimicrob Agents Chemother 2003;47:54-61.
28. Doualla-Bell F, Gaseitsiwe S, Ndungu T, et al. Mutations and polymorphisms associated with antiretroviral drugs in HIV-1C-infected African patients. Antivir Chem Chemother 2004;15:189-200.
29. Paolucci S, Baldanti F, Maga G, et al. Gln145Met/Leu changes in human immunodeficiency virus type 1 reverse transcriptase confer resistance to nucleoside and nonnucleoside analogs and impair virus replication. Antimicrob Agents Chemother 2004;48:4611-7.
30. Smith R, Anderson D, Preston B. Purifying selection masks the mutational flexibility of HIV-1 reverse trascriptase. J Biol Chem 2004;279:26726-34.
31. Torti C, Quiros-Roldan E, Monno L, et al. HIV-1 resistance to dideoxynucleoside reverse transcriptase inhibitors: genotypic-phenotypic correlations. J Acquir Immune Defic Syndr 2004; 36:1104-7.
32. Crespan E, Locatelli G, Cancio R, Hubscher U, Spadari S, Maga G. Drug resistance mutations in the nucleotide binding pocket of human immunodeficiency virus type 1 reverse transcriptase differentially affect the phosphorolysis-dependent primer unblocking activity in the presence of stavudine and zidovudine and its inhibition by efavirenz. Antimicrob Agents Chemother 2005;49:342-9.
33. Marcelin A, Flandre P, Pavie J, et al. Influence of new reverse transcriptase mutations on virological response to didanosine in the didanosine add on Jaguar study. Presented at XIV International Drug Resistance Workshop, 2005; Quebéc, Canada; Antiviral Ther 2005;10:S25 .
34. Marcelin A, Roquebert B, Malet I, et al. Relationship between mutations in HIV-1 RNase-H domain and nucleoside reverse transcriptase inhibitors resistance mutations in experienced patients. Presented at XIV International Drug Resistance Workshop, 2005; Quebéc, Canada; Antiviral Ther 2005;10:S:98.
35. Lu J, Whitcomb J, Kuritzkes D. Effect of the Q207D mutation in HIV type 1 reverse transcriptase on zidovudine susceptibility and replicative fitness. J Acquir Immune Defic Syndr 2005;40:20-3.
36. Rhee S, Fessel W, Zolopa A, et al. HIV-1 Protease and reverse-transcriptase mutations: correlations with antiretroviral therapy in subtype B isolates and implications for drug resistance surveillance. J Infect Dis 2005;192:456-65.
37. Ceccherini-Silberstein F, Gago F, Santoro M, et al. High sequence conservation of human immunodeficiency virus type 1 reverse transcriptase under drug pressure despite the continuous appearance of mutations. J Virol 2005;79:10718-29.
38. Sing T, Svicher V, Beerenwinkel N, et al. Characterization of novel HIV drug resistance mutations using clustering, multidimensional scaling and SVM-based feature ranking, p. 285-296. In: Jorge A, Torgo L, Brazdil P, Camacho R, Gama J (ed). Knowledge Discovery in Databases: PKDD 2005. New York, Springer.
39. Cozzi-Lepri A, Ruiz L, Ceccherini-Silberstein F, et al. Mutation at reverse transcriptase codon 214 is antagonist to thymidine analogue mutations type 2 profiles and predicts virological response to thymidine analogue-containing cART regimens only if TAM type 1 profiles concomitantly detected. Presented at 13th CROI 2006; Denver, USA.
40. De Luca A, Perno C, Ceccherini- Silberstein F, et al. Polymorphisms in the viral reverse transcriptase predict the evolution towards distinct thymidine analogue mutational patterns: a longitudinal analysis. Presented at XV International Drug Resistance Workshop, 2006; Sitges, Spain; Antiviral Ther 2006;11:S157.
41. Huigen M, van Ham P, de Graaf L, Boucher C, Nijhuis M. Explaining the selection of a novel resistance (E40F) and a compensatory (K43R) substitution in HIV-1 reverse transcriptase. Presented at 4th European HIV Drug Resistance Workshop, 2006; Monte Carlo, Monaco.
42. Huigen M, Albert J, Lindström A, et al. Compensatory fixation explains long term persistence of the M41L in HIV-1 reverse transcriptase in a large transmission cluster. Presented at XV International Drug Resistance Workshop, 2006; Sitges, Spain; Antiviral Ther 2006;11:S113.
43. Saracino A, Monno L, Scudeller L, et al. Impact of unreported HIV-1 reverse transcriptase mutations on phenotypic resistance to nucleoside and non-nucleoside inhibitors. J Med Virol 2006;78:9-17.
44. Svicher V, Sing T, Santoro M, et al. Involvement of novel human immunodeficiency virus type 1 reverse transcriptase mutations in the regulation of resistance to nucleoside inhibitors. J Virol 2006;80:7186-98.
45. Geretti A, Sabin C, Dunn D, on behalf of the UK Collaborative Group on HIV Drug Resistance, and the UK Collaborative HIV Cohort (CHIC) Study Group. The H208Y mutations in reverse transcriptase is significantly associated with NRTI-exposure, M184V and TAM mutation pathways. Presented at XV International Drug Resistance Workshop, 2006; Sitges, Spain; Antivir Ther 2006;11:S158.
46. Cozzi-Lepri A, Ruiz L, Loveday C, et al. Thymidine analogue mutation profiles: factors associated with acquiring specific profiles and their impact on the virological response to therapy. Antivir Ther 2005;10:791-802.
47. Arion D, Kaushik N, McCormick S, Borkow G, Parniak M. Phenotypic mechanism of HIV-1 resistance to 3'-azido-3'-deoxythymidine (AZT): increased polymerization processivity and enhanced sensitivity to pyrophosphate of the mutant viral reverse transcriptase. Biochemistry 1998;37:15908-17.
48. Meyer P, Matsuura S, Schinazi R, So A, Scott W. Differential removal of thymidine nucleotide analogues from blocked DNA chains by human immunodeficiency virus reverse transcriptase in the presence of physiological concentrations of 2'-deoxynucleoside triphosphates. Antimicrob Agents Chemother 2000;44:3465-72.
49. Meyer P, Matsuura S, So A, Scott W. Unblocking of chain-terminated primer by HIV-1 reverse transcriptase through a nucleotide-dependent mechanism. Proc Natl Acad Sci USA 1998; 95:13471-6.
50. Marcelin A, Delaugerre C, Wirden M, et al. Thymidine analogue reverse transcriptase inhibitors resistance mutations profiles and association to other nucleoside reverse transcriptase inhibitors resistance mutations observed in the context of virological failure. J Med Virol 2004;72:162-5.

51. Miller M, Margot N, Lu B, et al. Genotypic and phenotypic predictors of the magnitude of response to tenofovir disoproxil fumarate treatment in antiretroviral-experienced patients. J Infect Dis 2004; 189:837-46.
52. Sarafianos S, Das K, Hughes S, Arnold E. Taking aim at a moving target: designing drugs to inhibit drug-resistant HIV-1 reverse transcriptases. Curr Opin Struct Biol 2004;14:716-30.
53. Schmit J, Van Laethem K, Ruiz L, et al. Multiple dideoxynucleoside analogue-resistant (MddNR) HIV-1 strains isolated from patients from different European countries. AIDS 1998;12:2007-15.
54. Segondy M, Montes B. Prevalence and conditions of selection of the K65R mutation in the reverse transcriptase gene of HIV-1. J Acquir Immune Defic Syndr 2005;38:110-1.
55. Doualla-Bell F, Avalos A, Brenner B, et al. High prevalence of the K65R mutation in human immunodeficiency virus type 1 subtype C-infected batswana patients treated with ddI-based regimens. Antimicrob Agents Chemother 2006;in press.
56. Trotta M, Bonfigli S, Ceccherini-Silberstein F, et al. Clinical and genotypic correlates of mutation K65R in HIV-infected patients failing regimens not including tenofovir. J Med Virol 2006;78:535-41.
57. Garcia-Lerma J, Gerrish P, Wright A, Qari S, Heneine W. Evidence of a role for the Q151L mutation and the viral background in development of multiple dideoxynucleoside-resistant human immunodeficiency virus type 1. J Virol 2000;74:9339-46.
58. Sing T, Svicher V, Beerenwinkel N, et al. Evolutionary and phenotypical characterization of novel NRTI-associated mutations. Basic principles and clinical implications. Presented at XIV International Drug Resistance Workshop, 2005; Quebec, Canada; Antiviral Ther 2005;10:S55.
59. Wei X, Liang C, Gotte M, Wainberg M. The M184V mutation in HIV-1 reverse transcriptase reduces the restoration of wild-type replication by attenuated viruses. AIDS 2002;16:2391-8.
60. Zaccarelli M, Perno C, Forbici F, et al. Using a database of HIV patients undergoing genotypic resistance test after HAART failure to understand the dynamics of M184V mutation. Antivir Ther 2003;8:51-6.
61. Castagna A, Danise A, Menzo S, et al. Lamivudine monotherapy in HIV-1-infected patients harbouring a lamivudine-resistant virus: a randomized pilot study (E-184V study). AIDS 2006;20:795-803.
62. Brehm J, Koontz D, Pathak V, Sluis-Cremer N, Mellors J. AZT selects mutations in the connection (A371V) and RNase H (Q509L) domains of RT that increase AZT resistance in combination with TAMs and reduce susceptibility to other NRTIs. Presented at XV International Drug Resistance Workshop, 2006; Sitges, Spain; Antiviral Ther 2006;11:S141.
63. Nikolenko G, Palmer S, Maldarelli F, Mellors J, Coffin J, Pathak V. Mechanism for nucleoside analog-mediated abrogation of HIV-1 replication: balance between RNase H activity and nucleotide excision. Proc Natl Acad Sci USA 2005;102:2093-8.
64. Gupta S, Fransen S, Paxinos E, et al. Infrequent occurrence of mutations in the C-terminal region of reverse transcriptase modulates susceptibility to RT inhibitors. Presented at XV International Drug Resistance Workshop, 2006; Sitges, Spain; Antiviral Ther 2006; 11:S143.
65. Nikolenko G, Frankenberry K, Palmer S, et al. The HIV-1 reverse transcriptase connection domain from treatment-experienced patients contributes to AZT resistance. Presented at XV International Drug Resistance Workshop, 2006; Sitges, Spain; Antiviral Ther 2006; 11:S142.
66. Richman D, Havlir D, Corbeil J, et al. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. J Virol 1994;68:1660-6.
67. Shafer R, Iversen A, Winters M, Aguiniga E, Katzenstein D, Merigan T. Drug resistance and heterogeneous long-term virologic responses of human immunodeficiency virus type 1-infected subjects to zidovudine and didanosine combination therapy. The AIDS Clinical Trials Group 143 Virology Team. J Infect Dis 1995;172:70-8.
68. Jeffrey S, Baker D, Tritch R, et al. A resistance and cross resistance profile for SUSTIVA (efavirenz, DMP 266). Presented at 5th CROI, 1998; Chicago, USA.
69. Taylor D, Ahmed P, Tyms A, et al. Drug resistance and drug combination features of the HIV inhibitor, BCH-10652 [(+/-)-2'-deoxy-3'-oxa-4'-thiocytidine, dOTC]. Antivir Chem Chemother 2000;11:291-301.
70. Brown A, Precious H, Whitcomb J, et al. Reduced susceptibility of human immunodeficiency virus type 1 (HIV-1) from patients with primary HIV infection to nonnucleoside reverse transcriptase inhibitors is associated with variation at novel amino acid sites. J Virol 2000;74:10269-73.
71. Sardana V, Emini E, Gotlib L, et al. Functional analysis of HIV-1 reverse transcriptase amino acids involved in resistance to multiple nonnucleoside inhibitors. J Biol Chem 1992;267:17526-30.
72. Turner D, Brenner B, Routy J, et al. Diminished representation of HIV-1 variants containing select drug resistance-conferring mutations in primary HIV-1 infection. J Acquir Immune Defic Syndr 2004; 37:1627-31.
73. Sluis Cremer N, Radzio J, Camarasa M, et al. Role of the β7-β8-loop in the 51kDa subunit of the HIV-1 reverse transcriptase in protein stability and in drug resistance. Presented at XIV International Drug Resistance Workshop, 2005; Quebéc, Canada; Antiviral Ther 2005; 10:S99.
74. Svicher V, Sing T, Santoro M, et al. Involvement of novel HIV-1 reverse transcriptase mutations in the regulation of NNRTI resistance. Presented at XV International Drug Resistance Workshop, 2006; Sitges, Spain; Antiviral Ther 2006;11:S165.
75. Vavro C, Florance A, Irlbeck D, Wine B, St Clair M, GlaxoSmithKline, Research Triangle Park, NC, USA. Mutations at codon 135 at baseline are associated with the accumulation of NNRTI-resistance mutations while on EFV-containing regimens. Presented at 11th CROI, 2004; San Francisco, USA.
76. Moore C, John M, James I, Christiansen F, Witt C, Mallal S. Evidence of HIV-1 Adaptation to HLA-Restricted Immune Responses at a Population Level. Science 2002;296:1439-43.
77. De Groot A, Jesdale B, Szu E, Schafer J, Chicz R, Deocampo G. An interactive Web site providing major histocompatibility ligand predictions: application to HIV research. AIDS Res Hum Retroviruses 1997;13:529-31.
78. Shulman N, Bosch R, Mellors J, Albrecht M, Katzenstein D. Genetic correlates of efavirenz hypersusceptibility. AIDS 2004;18:1781-5.
79. Haubrich R, Kemper C, Hellmann N, et al. The clinical relevance of non-nucleoside reverse transcriptase inhibitor hypersusceptibility: a prospective cohort analysis. AIDS 2002;16:F33-40.
80. Tozzi V, Zaccarelli M, Narciso P, et al. Mutations in HIV-1 reverse transcriptase potentially associated with hypersusceptibility to non-nucleoside reverse-transcriptase inhibitors: effect on response to efavirenz-based therapy in an urban observational cohort. J Infect Dis 2004;189:1688-95.
81. Delgado J, Shulman N. NNRTI hypersusceptibility. AIDS Read 2005; 15:28-30, 32-34.
82. Clark S, Shulman N, Bosch R, Mellors J. Reverse transcriptase mutations 118I, 208Y, and 215Y cause HIV-1 hypersusceptibility to non-nucleoside reverse transcriptase inhibitors. AIDS 2006; 20:981-4.
83. Vingerhoets J, Azijn H, Fransen E, et al. TMC125 displays a high genetic barrier to the development of resistance: evidence from *in vitro* selection experiments. J Virol 2005;79:12773-782.