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HIV-1 Genotypic Drug Resistance Interpretation Rules – 2009 Spanish Guidelines

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Abstract

Interpreting the results of drug resistance tests for HIV-1 is one of the most difficult tasks for both clinicians and virologists. There are many amino acid changes in viral proteins influencing the susceptibility to specific drugs, causing loss of activity or conversely hypersusceptibility. Moreover, the results of interactions derived from complex mutational patterns are difficult to predict. Different interpretation algorithms have been developed to facilitate the translation of information obtained in the genotypes to clinicians. Controversy exists, however, regarding the impact of genotypic changes over the activity of many antiretroviral drugs. Based on virologic outcomes, scientific literature, and expert opinion, the Drug Resistance Platform of the Spanish AIDS Research Network (RIS, Red de Investigación en SIDA) has developed over the last years its own interpretation system. Herein, we present the 2009 guidelines, in which special efforts have been made to standardize the criteria for interpreting resistance mutations for compounds within the same drug family and to facilitate the clinical interpretation of HIV-1 resistance genotypes. **(AIDS Rev. 2009;11:39-51)**

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Key words

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Introduction

Drug resistance testing has proved to be useful to make treatment decisions in HIV-infected patients[1,2], especially helping the choice of subsequent treatments in antiretroviral-experienced individuals[3,4]. The Havana trial demonstrated that expert advice improved the performance of HIV-1 genotyping interpreted by a software package in HIV patients failing antiretroviral therapy and switched to a new salvage regimen[5]. Different interpretation systems and algorithms have been developed to facilitate the clinical meaning of drug resistance mutations in HIV-1. These are updated periodically, incorporating new mutations and information regarding resistance to new drugs. However, interpretation of some mutational patterns is often difficult due to poor phenotypic correlates, disparity with clinical virologic outcomes, unexpected interactions between changes, and differential impact on viral fitness. Resistance interpretation systems can be derived from lists of mutations which have been associated with reduced antiviral activity *in vivo* and/or which have demonstrated *in vitro* to impact phenotypically for a given drug(s)[6]. Alternatively, algorithms can be more sophisticated and be based on expert rules, with or without clinical validation[7-11]. The presentation and format could differ, being available at web sites or linked to commercial drug resistance tests. The antiretroviral drug resistance interpretation systems more frequently used are Stanford HIVRT&PR (HIVdb program)[11,12], geno2pheno[13], Retrogram[14], Rega[15], ANRS[16], Trugene (Siemens)[17] and ViroSeq (Abbott)[18].

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Nucleoside reverse transcriptase inhibitors (pol gene, reverse transcriptase sequence positions)

M	A	K	D	T	K	L	V	F	Y	F	Q	M	L	T	K
41	62	65	67	69	70	74	75	77	115	116	151	184	210	215	219

Nonnucleoside reverse transcriptase inhibitors* (pol gene, reverse transcriptase sequence positions)

V	A	L	K	K	V	V	E	V	Y	Y	G	P	F	M	K
90	98	100	101	103	106	108	138	179	181	188	190	225	227	230	238

Protease inhibitors (pol gene, protease sequence positions)

L	V	L	V	L	M	K	M	I	G	I	F	I	Q	A	G	L	T	V	N	I	N	L	L
10	11	24	32	33	36	43	46	47	48	50	53	54	58	71	73	74	76	82	83	84	88	89	90

Entry inhibitors (env gene, gp41 sequence positions)

G	I	V	Q	Q	N	N	L	L	N	N	S
36	37	38	39	40	42	43	44	45	126	137	138

Integrase inhibitors (pol gene, integrase sequence positions)

T	L	E	T	E	G	Y	S	Q	V	N	E	G	I	S
66	74	92	97	138	140	143	147	148	151	155	157	163	203	230

Figure 1. *Amino acid positions that should appear in HIV drug resistance reports.*
**Mutations Y318F, N348I, A376S and E399D seem to reduce the susceptibility to some nonnucleoside reverse transcriptase inhibitors. Commercial drug resistance assays do not amplify these positions[6,56].*

The Platform for Drug Resistance of the Spanish AIDS Research Network (Red de Investigación en SIDA, RIS) was built in 2006, and periodically has released national guidelines for interpreting HIV-1 drug resistance genotypes. The need for local rules was justified by the perception that different use of antiretroviral agents and criteria for combinations and switches of drugs, as well as the rate of non-B subtypes, might determine a different proportion of mutational patterns or, more rarely, different mutational resistance pathways in comparison with other regions. Information derived from virologic outcomes, expert opinion, and updated scientific literature have been taken into account to build the 2009 interpretation rules, and a particular effort has been made to facilitate their interpretation for clinicians. The clinical relevance of distinct mutational patterns has been discussed, considering their rate in the Spanish HIV drug resistance database, which currently includes more than 5,000 HIV-1 genotypes derived from both antiretroviral-naive and treatment-experienced patients[19-21].

The Spanish HIV drug resistance interpretation rules require a minimum list of mutations that should be considered in any drug resistance report (Fig. 1), to ensure that the information received from the labs is complete to make any further interpretation reliable. The 2009 interpretation rules consider all currently available antiretroviral drug families and apply to all HIV-1 variants, excluding HIV-2 and HIV-1 group O and N. To avoid sophisticated interpretation approaches, which often are subject to modification, uniform criteria are proposed to assess the impact of distinct resistance mutations over drugs within the same family, even when this approach may occasionally be too simple and potentially less accurate. Conversely, it facilitates understanding by clinicians. For nucleos(t)ide reverse transcriptase inhibitors (NRTI), nonnucleoside

reverse transcriptase inhibitors (NNRTI), and protease inhibitors (PI), three categories of resistance have been defined: resistance (R), intermediate resistance (I), and susceptible (S). Single drug resistance mutations are scored based on their impact on distinct drugs. In this way, there are mutations scoring 3 points, mutations with 2 points, and finally mutations with 1 point. Mutations considered to result in hypersusceptibility are scored with a value of –1 point. At the end, "R" for a given drug is considered when mutations score ≥ 5 points, "I" if they add 3-4 points, and "S" if ≤ 2 points. For entry inhibitors and integrase inhibitors, the current interpretation is mainly derived from the information obtained in clinical trials[22], with slight modifications.

Drug resistance to nucleoside reverse transcriptase inhibitors

Table 1 records all mutations that have been associated with resistance to NRTI, including those leading to multi-NRTI resistance. Distinct mutations may impact to a different degree on a given NRTI. Moreover, the same mutations may reduce the susceptibility to distinct NRTI in a different extent. In this way, the panel recommends that in the presence of T215Y/F, zidovudine should be avoided, even in the absence of other thymidine-associated mutations (TAM). The same rule applies to L74V for didanosine, K65R for tenofovir, and M184V/I for lamivudine and emtricitabine. Although the impact of all these mutations but M184V/I is considered to lead to intermediate phenotypic resistance, the virologic response *in vivo* is poor and generally transient[23-25].

K65R is the primary resistance mutation selected under tenofovir-containing regimens in viruses lacking TAMs. K65R has been associated with reduced virologic response to tenofovir *in vivo*[25,26]. Besides tenofovir, K65R may be selected when failing didanosine and abacavir *in vitro*[25] and more rarely *in vivo*[27,28].

Mutations M41L, D67N, K70R, L210W, T215Y/F, and K219E/N/Q/R are known as TAMs. They are selected upon failure to thymidine analogs, such as zidovudine and stavudine[29], and lead to decreased susceptibility to all NRTI to different degrees[30]. The activity of zidovudine and stavudine is most largely affected by TAMs; conversely, TAMs impair lamivudine and emtricitabine activity only slightly. Thus, while T215Y/F should be interpreted as "R" for zidovudine and stavudine, almost all TAMs should be present before considering "R" to lamivudine/emtricitabine. It should be noted that other amino acid changes may appear at codons displaying TAMs. Although they generally do not affect drug susceptibility, some changes at codon 215 (C/D/E/I/S/V) may represent revertants. They are signature mutations for transmission of a drug-resistant virus in the primary infection event[31]. Although by themselves they do not produce resistance, they are prone to more rapid selection of resistance (e.g. T215Y/F), given that only one nucleotide change is required to switch to the resistant variant[32].

Mutation M184V is one of the most prevalent resistance mutations in patients failing antiretroviral therapy[21]. It causes high-level resistance (> 100-fold) to lamivudine and emtricitabine, and emerges rapidly in patients exposed to lamivudine monotherapy[33] or failing virologically under any lamivudine/emtricitabine-containing regimen. The impact of M184V over the rest of the NRTI depends very much on the presence of other resistance changes at the HIV-1 reverse transcriptase. Characteristically, M184V leads to hypersusceptibility to zidovudine, stavudine, and tenofovir, and reduces the impact of M41L and/or T215Y over zidovudine and stavudine, and of K65R over tenofovir efficacies[24,30]. Mutation M184V alone renders lamivudine/emtricitabine ineffective but does not significantly compromise the response to abacavir or didanosine[34-36]. However, M184V in combination with TAMs or changes at positions 65, 74, or 115 may significantly increase abacavir and didanosine resistance[30,37-39]. For this reason, the Spanish rules score M184V with 1 point when considering abacavir or didanosine.

Multi-NRTI resistance patterns refer to four different genotypic changes: 67 deletions, 67/69 insertions, Q151M complex, and ≥ 5 TAMs. The 69 insertion complex as well as deletions between codons 67 and 69 are associated with resistance to all NRTI when present together with one or more TAM[40,41]. In contrast, viruses harboring Q151M may retain some activity against tenofovir, lamivudine, and emtricitabine[42,43]. The presence of ≥ 5 TAMs compromises the response to all NRTI available[30].

Drug resistance to nonnucleoside reverse transcriptase inhibitors

Three NNRTI are currently available for the treatment of HIV infection in Europe: nevirapine, efavirenz, and etravirine. Delavirdine is also approved in other regions, although rarely used. The NNRTI display a low genetic

Table 1. Drug resistance interpretation for nucleos(t)ide reverse transcriptase inhibitors

Drug	Zidovudine	Stavudine	Didanosine	Abacavir	Tenofovir	Lamivudine	Emtricitabine
Group 3 *(3 points)*	T215F/Y M41L	T215F/Y	L74I/V K65R	K65R L74I/V	K65R	M184V/I	M184V/I
Group 2 *(2 points)*	K70R L210W	M41L V75M/T/S	T69D/G K70E T215F/Y	K70E Y115F M184I/V T215F/Y	M41L K70E L210W	K65R	K65R
Group 1 *(1 point)*	D67E/G/N K70G T215C/D/E/I/V/S K219E/N/Q/R/T/W	K65R D67E/G/N T69D/G/N K70R V75A/I L210W T215C/D/E/I/V/S K219E/N/Q/R/T/W	M41L K65N D67E/G/N T69N V75A/IM/T/S M184V L210W	M41L K65N K70R L210W	K65N D67E/G/N K70R T215F/Y	K65N K70E T215F/Y	K65N K70E T215F/Y
Hypersusceptibility *(–1 point)*	**K65R** **M184V**	**M184V**			**M184V**		
Multidrug resistance:							
Del 67 / 69	R	R	R	R	I	R	R
69 insert	R	R	R	R	R	I	I
Q151M/L (complex)	R	R	R	R	I	I	I
≥ 5 TAMs	R	R	R	R	R	I	I
Interpretation	≥ 5 = Resistant (R) 4 – 3 = Intermediate resistance (I) ≤ 2 = Susceptible (S)						

Gray: Mutations that when present itself lead in a significant reduction on the susceptibility. Black: Mutations that produce hypersusceptibility and subtract 1 point to the final value. Q151M complex: A62V, V75I, F77L, F116Y, Q151M. Thymidine-associated mutation (TAMs): M41L, D67N, K70R, L210W, T215Y/F, K219Q/E.

Table 2. Drug resistance interpretation for nonnucleoside reverse transcriptase inhibitors

Drug	Nevirapine	Efavirenz	Etravirine*
Group 3 *(3 points)*	L100I K103N/S/T V106A/M Y181C/I/V/S Y188C/L G190A/C/E/G/S/V/T M230L K238T/N Y318F	L100I K103N/S/T V106A/M Y181C/I/V Y188L G190A/C/E/Q/S/V/T M230L	
Group 2 *(2 points)*	K101E/P V179F Y188H F227C	K103I/P Y181S Y188C/H P225H	Y181C/I/V L100I K101P M230L
Group 1 *(1 point)*	A98G L100V K101H/N K103Q/E V106L V108I E138K V179D/E/M F227L/Y	A98G L100V K101E/H/N K103Q/E V106L V108I E138K V179D/E/F/M K238T/N F227C Y318F	V90I A98G L100V K101E/H V106A/M/I E138A/K V179D/E/F/M/T Y181S Y188C/H/L G190A/C/E/Q/S/V/T P225H F227C/L
Interpretation		≥ 3 points = Resistant (R) ≤ 2 points = Susceptible (S)	

**Poveda E, et al.[37]; Hirsch M, et al.[21]*
Commercial assays do not amplify position 399 located at the C-terminal of the reverse transcriptase region. Recent studies suggest that the presence of mutation E399D in addition to one additional mutation associated with resistance to etravirine lead to resistance to the drug[23,37]. Frequent polymorphism in HIV-1 non-B subtypes: A98S; V179I.

barrier for resistance, especially nevirapine and efavirenz. Accordingly, resistance to NNRTI is subject to a dichotomous interpretation (susceptible or resistant), excluding the intermediate category (Table 2). Although *in vitro* data have provided intermediate levels of resistance for some particular changes with respect to either nevirapine or efavirenz, there is uniform rationale that these changes must be interpreted as "R" in the clinical setting, as any benefit in virologic response tends to be transient, rapidly arousing other mutations that annul any residual activity of NNRTI.

The extent of cross-resistance within the NNRTI family is high, especially for nevirapine and efavirenz[44]. Accordingly, the long-term virologic response to sequential NNRTI is rather poor, even for rescue interventions with efavirenz upon recent failure on nevirapine and exclusive selection of Y181C[45,46], despite *in vitro* data showing only limited impact of this mutation on efavirenz susceptibility[47]. So, this panel does not recommend the sequential use of first generation NNRTI in any order. It should be noted that HIV-1 non-B subtypes may select for alternative resistance changes upon failure under NNRTI, such as V106M under efavirenz in HIV-1 subtypes C and G[48].

Etravirine is a second generation NNRTI. It displays a higher genetic barrier for resistance than nevirapine and efavirenz[49]. Based on information derived from the registrational DUET trials[50-53], a total of 17 mutations located at 10 different positions along the reverse transcriptase (V90I, A98G, L100I, K101E/H/P, V106I, E138A, V179D/F/T, Y181C/I/V, G190A/S and M230L) have been shown to impact on etravirine

susceptibility. Overall, the presence of three or more of these mutations results in a reduced virologic response to etravirine. Interestingly, K103N is the most prevalent NNRTI mutation in patients who have failed NNRTI, especially efavirenz[21], and does not seem to compromise etravirine susceptibility. By contrast, Y181C is often selected upon nevirapine failure and is one of the changes that compromise etravirine activity more extensively. In fact, its presence along with another etravirine-associated resistance mutation must be interpreted as "R"[6]. Vingerhoets, et al.[54] recently weighted the 17 etravirine-associated resistance mutations. In this analysis changes at position 100, 101 and 181 were associated with a blunted virological response. Although the presence of ≥ 3 had the greatest effect on etravirine activity, other mutations located at the reverse transcriptase C-terminus domain (N348I, T369I and E399D) may also significantly lead to reduced etravirine activity, as has been shown for nevirapine and efavirenz[55,56]. Several mutations in the connection subdomain of the reverse transcriptase might modulate NNRTI resistance by affecting dimerization of p66/p51 heterodimers. Given that commercial tests for HIV drug resistance genotyping do not cover this region, it seems worthwhile to know the real prevalence of these changes in patients who have failed nevirapine or efavirenz, since failure to consider these changes may result in under-interpretation of etravirine resistance[56].

In the Spanish algorithm, "R" to NNRTI is considered for viruses scoring ≥ 3 points. For nevirapine and efavirenz the most frequent NNRTI resistance mutations are always scored with three points, based on phenotypic data and virologic outcomes. In contrast, the presence of Y181C/I, M230L, L100I or K101P in addition to another etravirine resistance mutation or the presence of at least three of these changes is considered to render the virus as resistant to etravirine.

Drug resistance to protease inhibitors

The widespread use of ritonavir-boosted PIs has dramatically changed the interpretation of genotypic resistance to PIs. Although it is important to check the number of protease resistance mutations in a "quantitative" manner[57], the "quality" of mutations and their weight must be considered separately for each drug within this family. Many algorithms designed to predict the virologic response to ritonavir-boosted PIs incorporate a simple list of protease mutations specific for each PI. When a minimum number of these changes are present, "R" is assumed for a given PI. However, for the last generation of PIs, tipranavir and darunavir, weighted mutation scores have been proposed. It is assumed that specific changes influence susceptibility to each PI and not all mutations impact to the same extent. Many of these PI resistance scores have been built based on virologic outcomes in patients with prior failure to other PIs. In this way, primary resistance changes for a new PI are generally absent in patients with prior failure to other PIs and algorithms tend to ignore these capital changes[58-65], which must be added separately. This is the case of G48A/M/S/T/V for saquinavir, I50L for atazanavir, I50V for fosamprenavir, or I47A for lopinavir[66,67].

Table 3 depicts the mutation scoring system for ritonavir-boosted PIs, in which the signature mutations selected *in vitro* or in PI-naive patients are highlighted[6,66-68], along with other protease mutations categorized by their different impact on drug susceptibility. When signature mutations are present and alternative treatment options exist, it is advisable to avoid that specific PI since a maximal response must not be expected. On the other hand, in the Spanish interpretation algorithm, mutations causing hypersusceptibility to some PIs are taken into account, counterbalancing the impact of other resistance changes[69,70]. This is the case of I50L, which significantly impairs the susceptibility to atazanavir but enhances the activity of all other PIs[70].

Resistance to tipranavir and darunavir merits particular discussion. The most accurate tipranavir resistance mutation score has been built based on data derived from the RESIST trials[71]. A re-analysis of data has provided a weighted list of mutations[72], which recently has been validated in a separate database and has permitted to improve the accuracy of the tipranavir resistance score[73]. Briefly, a total of 13 mutations are associated with reduced susceptibility to tipranavir; conversely, four mutations lead to hypersusceptibility. The highest impact on tipranavir resistance has been recognized for T74P and I47V, which score +6 points, followed by 58E and V82L/T, which score +5. On the other hand, increased responses are observed in the presence of I54L, I50V/L, L24I and I76V, which accordingly are scored with –7, –4, –2 and –2, respectively. This weighted mutation score has been simplified in the Spanish rules, following the principles applied to other PIs. In this regard, all hypersusceptible mutations are scored with –1 point.

Darunavir is the other new PI with the highest genetic barrier for resistance. As for tipranavir, the most

Table 3. Drug resistance interpretation for protease inhibitors

Drug	Indinavir	Saquinavir	Atazanavir*	Fosamprenavir	Lopinavir	Tipranavir	Darunavir
				Boosted with *ritonavir*			
Group 3 *(3 points)*	V82A/F/S/T	G48A/M/S/T/V L90M I84A/V	I50L N88S	I50V I84A/V	I47A	V82L/T	I50V
Group 2 *(2 points)*	M46I/L L76V V82M I84A/C/V L90M	I54V I84C	G48V/M V82A/F/S/T I84A/C/V L90M	V32I M46I/L I47A/V I54L/M L76V I84C L90M	M46I/L I50V I54L/M/V V82A/F/S/T I84V	I47V Q58E T74P N83D	I54L/M L76V I84V
Group 1 *(1 point)*	V32I M46V G48M/V I54A/L/M/S/T/V V82C/L N88S	I54A/L/M/S/T G73C/S/T V82A/F/L/M/T N88D	V32I M46I/L/V I54A/M/S/T/V V82L/M N88D/G/T	M46V I54A/T/V V82A/F/L/M/S/T	V32I M46V I47V G48M/V I54A/S/T L76V V82C/L/M I84C L90M	M36I K43T M46I/L/V I54A/V V82A/C/M/F/S I84V	V32I L33F M46I/L/V 47A/V V82A/F/S/T/M/L/C I84A/C
Hypersusceptibility *(–1 point)*	**I50L**	**I50L** **L76V**	**L76V**	**N88S**	**I50L**	**L24I** **I50L/V** **I54L** **L76V**	**I50L** **N88S**
Interpretation	≥ 5 points = Resistant (R) 4 – 3 points = Intermediate resistance (I) ≤ 2 points = Susceptible (S)						

*Drug resistance to atazanavir without boosting with ritonavir will be considered from 4 points.

Gray: Mutations that itself lead to a significant reduction of susceptibility to the drug. Black: Mutations that produce hypersusceptibility and subtract 1 point to the final value. Compensatory mutations: L10I/F/R/V/Y; V11I; L24I; L33F; F53L/Y; A71I/T/V; G73A/C/S/T; L89I/M/T/V. Only in the case of intermediate resistance with 4 points could increase the level of resistance to 5 points. Frequent polymorphisms in HIV-1 non-B subtypes: L10V/I; K20I/R; M36I; V82I; L89M/I.

accurate mutation score has been designed with information derived from registrational trials, POWER and DUET including 11 substitutions at 10 positions (V11I, V32I, L33F, I47V, I50V, I54L/M, T74T, L76V, I84V and L89V)[74-76]. In addition, the impact of baseline protease resistance mutations on darunavir response has been examined in clinical cohorts, although the main limitation of these studies is the relatively small size of the study population, the absence of control arms, and the lack of subsequent validation on separate databases[80]. With these limitations in mind, mutations contributing to darunavir resistance have been scored in three different categories, the highest impact being for I50V. Given this fact, a certain degree of cross-resistance between darunavir and fosamprenavir exists[68]. In contrast, most mutations conferring tipranavir resistance do not overlap with those reducing darunavir activity; instead, some of them produce hypersusceptibility.

There is a group of accessory or compensatory resistance mutations at the protease (e.g. at codons 10, 11, 24, 33, 53, 71, 73, and 89). By themselves they do not produce PI resistance; however, they compensate for the decreased viral fitness resulting from the selection of other PI resistance mutations[78] and/or further increase the level of PI resistance[79,80]. These changes are scored in the Spanish rules with +1 point only when present along with other PI resistance mutations that sum +4 points.

Finally, HIV-1 non-B subtypes often display natural polymorphisms at the protease, some of them at codons involved in PI resistance. However, these are changes generally at sites of accessory PI resistance mutations in clade B viruses[81,82]. Controversy exists regarding the potential role of these polymorphisms influencing the susceptibility to PIs and how they should be interpreted[83]. However, the large body of evidence favor that HIV-1 non-B clades behave similarly to subtype B in terms of PI resistance with minor special considerations, and perhaps an overall slightly lower genetic barrier for PI resistance than subtype B, as recently shown for tipranavir in some clade F viruses[84]. However, there is no need to build a different resistance interpretation algorithm for HIV-1 non-B variants[85].

There is overwhelming evidence that *gag* mutations also impact PI activity. They usually restore the replicative capacity impaired by protease mutations, but can also increase the level of resistance in particular cases. However, *gag* is not routinely sequenced and is not considered in the present guidelines.

Drug resistance to HIV-1 entry inhibitors

Fusion inhibitors

Resistance to enfuvirtide is mainly associated with the selection of changes at amino acids 36 to 45 (GIVQQQNNLL) within the HR1 region of gp41[86-89] (Fig. 2a). Enfuvirtide exhibits a low genetic barrier for resistance, and selection of a single mutation can confer high-level resistance to the drug (> 10-fold). Changes in HR2 have also been observed in patients with prolonged failure under enfuvirtide therapy, although in the majority of cases they do not follow a recognizable pattern. However, substitutions at positions 126 (N126K) and 138 (S138A) in HR2 have been observed in patients who had failed enfuvirtide at rates of 17 and 14%, respectively[90]. *In vitro* studies have shown that these HR2 changes generally only result in slight reductions in enfuvirtide susceptibility. Only when selected along with HR1 mutations N42T and N43K can their impact be recognized, increasing significantly the level of enfuvirtide resistance. In patients failing enfuvirtide, mutation S138A is almost always selected together with or after the selection of other mutations within the HR1 region[91]. Selection of S138A generally results in a further increase in enfuvirtide resistance[88,92], suggesting that it behaves as a secondary/compensatory resistance mutation.

CCR5 antagonist

Treatment with CCR5 antagonists (e.g. maraviroc and vicriviroc) requires previous assessment of viral tropism, since these drugs are not active against HIV-1 isolates with X4R5 dual tropic or X4 viruses[93-95]. In antiretroviral-experienced patients with advanced HIV disease, the prevalence of X4R5 dual tropic or X4 viruses can be around 50%[96]; conversely it is below 20% in antiretroviral-naive recent HIV seroconverters[97,98]. Two main resistance pathways have been shown to allow HIV-1 to evade CCR5 antagonists. The first is an outgrowth of X4 viruses that preexist as a minority population below the level of assay detection. The second mechanism results from the selection of mutations in the HIV-1 gp120 molecule, which allows the virus to bind the CCR5 coreceptor despite the presence of CCR5 antagonists (Fig. 2b)[99-101]. Some of these mutations have been identified, but the pattern of amino acid changes differs considerably between patients. Interestingly, shifts in the 50% inhibitory concentration (IC_{50}), a common parameter used to measure the

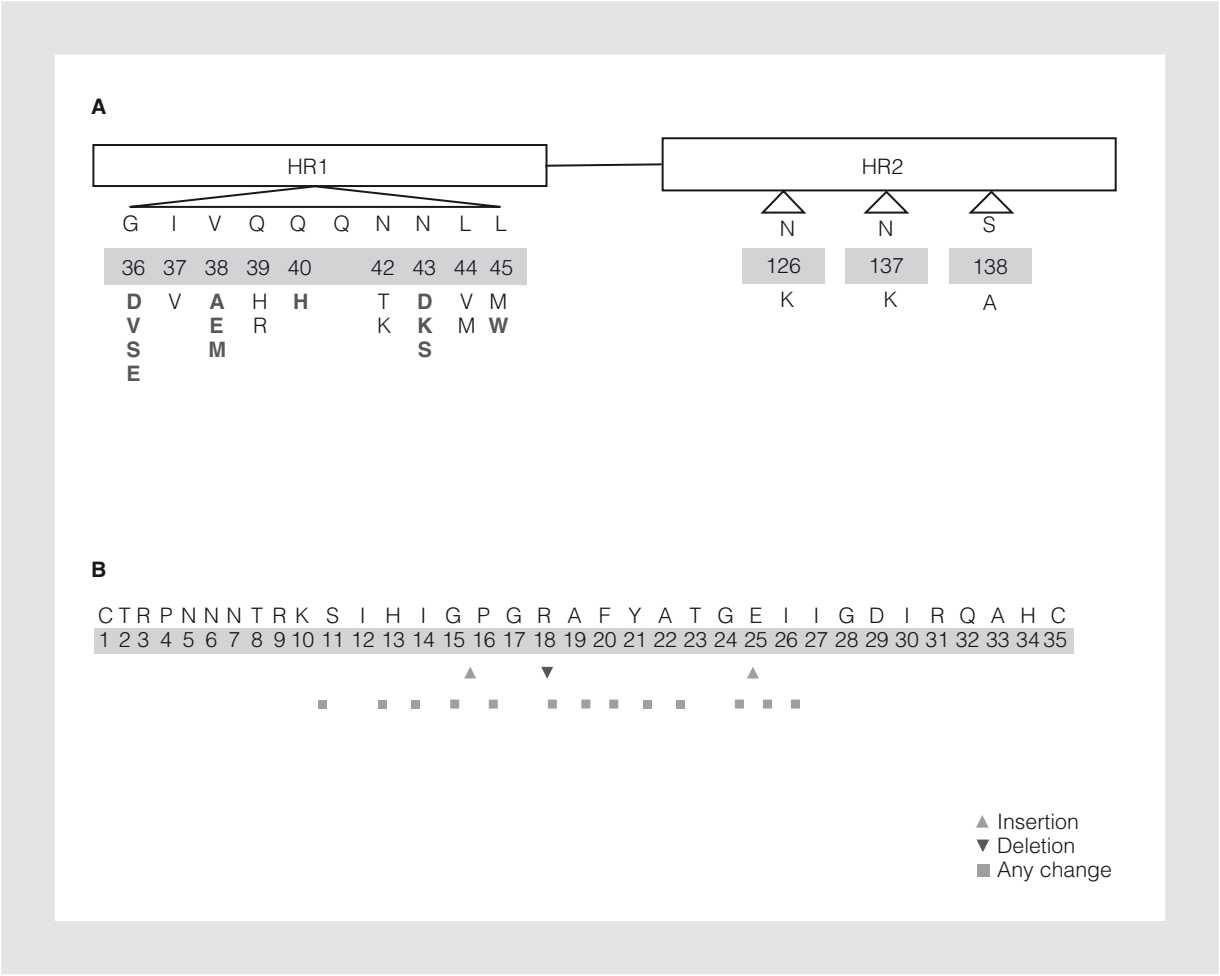

Figure 2. *Resistance mutations to entry Inhibitors.* ***A:*** *gp41 changes associated with resistance to enfuvirtide;* ***B:*** *changes at V3 region associated with resistance to maraviroc. Overall, maraviroc resistance is complex and the information derives from a relatively low number of patients recruited in the MOTIVATE 1 and 2 clinical trials[94,95].*

level of resistance to other antiretroviral agents, are generally not recognized in patients failing CCR5 antagonists with R5 viruses. Instead, plateaus in the maximal percentage of inhibition are observed. Therefore, the phenotypic behavior of resistance to CCR5 antagonists seems to rely more on a reduced capacity of inhibition rather than an increased IC_{50}. Overall, the resistance profile for CCR5 antagonists is expected to be complex, given the large variability in the *env* gene across HIV variants.

Drug resistance to HIV-1 integrase inhibitors

Raltegravir is so far the only the integrase inhibitor approved for the treatment of HIV infection. Information about raltegravir resistance mutations mainly derives from registrational clinical trials[102,103]. Figure 3 summarizes the changes associated with resistance to raltegravir and their impact on elvitegravir, an experimental drug within this family in the last steps of clinical development. In the BENCHMRK studies[102,103], 41 patients in the raltegravir arm experienced virologic failure. Three signature mutations were identified in 32 of these subjects: N155H, Q148K/R/H and, less frequently, Y143R/C. No mutations were detected in the remaining nine patients. In a phase IIb study with raltegravir, Grinsztejn, et al.[104] examined 35 patients who experienced virologic failure on raltegravir. Two signature mutations at the integrase gene were identified: N155H or Q148K/R/H. These changes were mutually exclusive

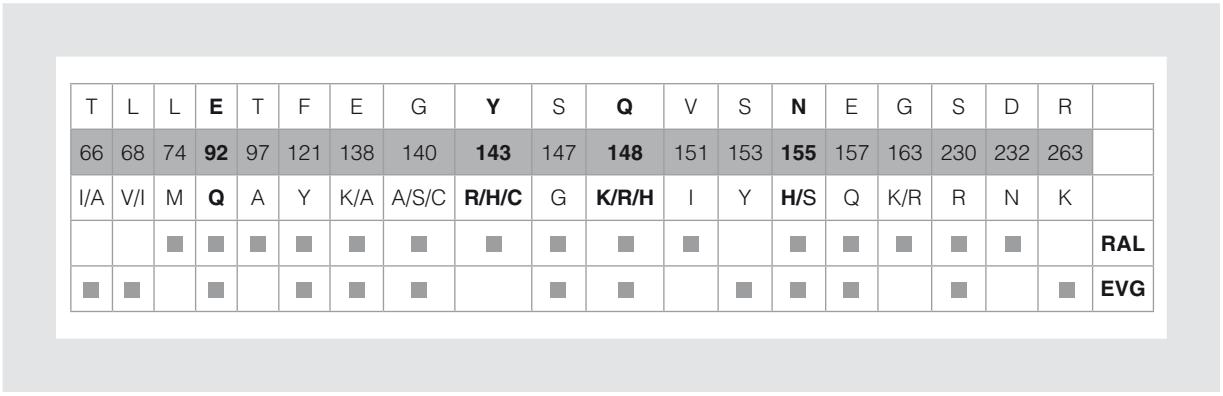

Figure 3. *Integrase changes associated with drug resistance to integrase inhibitors. RAL: raltegravir; EVG: elvitegravir.*

and conferred phenotypic resistance to raltegravir *in vitro*, with Q148 resulting in measurably larger reductions in susceptibility than N155H (25-fold and 10-fold, respectively). It is interesting that these mutations are directly associated to the catalytic site of the HIV integrase[105]. With persistent viral replication under RAL there is a shift from N155H to Q148K/R/H restoring the replicative capacity. At this time, factors influencing the selection of these mutations as well as their full clinical implications are uncertain[106].

Much of the current information on elvitegravir resistance derives from analysis carried out on patients experiencing virologic failure in the Gilead study 105, a phase II, randomized, dose-finding trial conducted in highly antiretroviral-experienced patients[107]. Integrase genotyping was performed in 28 out of 30 patients who experienced virologic failure under ritonavir-boosted elvitegravir by week 24. The most common integrase mutations seen in these patients were E92Q, E138K, Q148R/K/H, and N155H. Other changes were observed less frequently, including S147G and T66I/A/K. Phenotypic analysis confirmed the impact of these mutations on elvitegravir susceptibility. Moreover, it showed that extensive cross-resistance exists between elvitegravir and raltegravir, despite the different structure of these compounds.

Given that the HIV protease, reverse transcriptase, and integrase share the same polyprotein precursor, the Gag-Pol, it has been hypothesized that interactions between changes at these genes may occur, complicating the interpretation of single resistance changes[108]. However, this has not been confirmed by a recent study, which has not found any recognizable relationship between genotypic changes at distinct *pol* genes when testing heavily antiretroviral-experienced patients with multiple resistance mutations at the protease and reverse transcriptase genes[109]. Moreover, the recognition of naturally occurring polymorphisms at the integrase in HIV-1 non-B subtypes[109-111] does not seem to impair the activity of integrase inhibitors across HIV variants, including HIV-2[112,113]. It should be noted that the catalytic domain of the integrase of HIV-1 group M subtypes displays a high conservation, being polymorphisms mainly recognizable at other residues[110].

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References

1. Clavel F, Hance A. HIV drug resistance. N Engl J Med. 2004;350: 1023-35.
2. Anderson J, Jiang H, Ding X, et al. Genotypic susceptibility scores and HIV type 1 RNA responses in treatment-experienced subjects with HIV type 1 infection. AIDS Res Hum Retroviruses. 2008;24:685-94.
3. Vray M, Maynard J, Dalban C, et al. Predictors of the virological response to a change in the antiretroviral treatment regimen in HIV-1 infected patients enrolled in a randomized trial comparing genotyping, phenotyping and standard of care (Narval trial, ANRS088). Antivir Ther. 2003;8:427-34.
4. von Hentig N, Babacan E, Staszewski S, et al. Predictive factors for response to a boosted dual HIV-protease inhibitor therapy with saquinavir and lopinavir in extensively pre-treated patients. Antivir Ther. 2007;12:1237-46.
5. Tural C, Ruiz L, Holtzer C, et al. Clinical utility of HIV-1 genotyping and expert advice: The HAVANA trial. AIDS. 2002;16:209-18.
6. Johnson V, Brun-Vezinet F, Clotet B, et al. Update of the Drug Resistance Mutations in HIV-1: December 2008. Top HIV Med. 2008;16:138-45.
7. Beerenwinkel N, Schmidt B, Walter H, et al. Diversity and complexity of HIV-1 drug resistance: a bioinformatics approach to predict phenotype from genotype. Proc Natl Acad Sci USA. 2002;99:8271-6.
8. Wang K, Samudrala R, Mittler J. Antivirogram or Phenosense: a comparison of their reproducibility and analysis of their correlation. Antivir Ther. 2004;9:703-12.
9. Sevin A, de Gruttola V, Nijhuis M, et al. Methods for investigation of the relationship between drug-susceptibility phenotype and HIV type 1 genotype with applications to AIDS clinical trials group 333. J Infect Dis. 2000;182:59-67.
10. Wang D, Larder B. Enhanced prediction of lopinavir resistance from genotype by use of artificial neural networks. J Infect Dis. 2003;188:653-60.
11. Shafer R, Schapiro J. HIV-1 drug resistance mutations: an updated framework for the second decade of HAART. AIDS Rev. 2008;10:67-84.
12. Rhee S, Taylor J, Wadhera G, et al. Genotypic predictors of HIV type 1 drug resistance. Proc Natl Acad Sci USA. 2006;103:17355-60.
13. Beerenwinkel N, Däumer M, Oette M, et al. Geno2pheno: Estimating phenotypic drug resistance from HIV-1 genotypes. Nucleic Acids Res. 2003;31:3850-5.
14. De Luca A, Cingolani A, di Giambenedetto S, et al. Variable prediction of antiretroviral treatment outcome by different systems for interpreting genotypic HIV-1 drug resistance. J Infect Dis. 2003;187:1934-43.
15. Vercauteren J, Vandamme A. Algorithms for the interpretation of HIV-1 genotypic drug resistance information. Antiviral Res. 2006;71:335-42.
16. De Luca A, Cozzi-Lepri A, Perno C, et al. Variability in the interpretation of transmitted genotypic HIV-1 drug resistance and prediction of virologic outcomes of the initial HAART by distinct systems. Antivir Ther. 2004;9:743-52.
17. Nogales C, Serrano C, Bernal S, et al. Study of resistance using the TRUGENE HIV-1 genotyping system and analysis of agreement between rule-based algorithms and virtual phenotyping. Enferm Infecc Microbiol Clin. 2005;23:149-55.
18. Eshleman S, Crutcher G, Petrauskene O, et al. Sensitivity and specificity of the ViroSeq HIV-1 genotyping system for detection of HIV-1 drug resistance mutations by use of an ABI PRISM 3100 genetic analyzer. J Clin Microbiol. 2005;43:813-7.
19. De Mendoza C, Rodriguez C, Colomina J, et al. Resistance to nonnucleoside reverse transcriptase inhibitors and prevalence of HIV type 1 non-B subtypes are increasing among persons with recent infection in Spain. Clin Infect Dis. 2005;41:1350-4.
20. Martínez-Picado J, Gutierrez C, de Mendoza C, et al. Surveillance of drug resistance and HIV subtypes in newly diagnosed patients in Spain during year 2004. Antivir Ther. 2005;10:S131.
21. De Mendoza C, Garrido C, Corral A, et al. Changing rates and patterns of drug resistance mutations in antiretroviral-experienced HIV-infected patients. AIDS Res Hum Retroviruses. 2007;23:879-85.
22. Dalmau D, Klimkait T, Telenti A. Resistance to new anti-HIV agents: problems in the pathway of drug registration. Antivir Ther. 2005;10: 867-72.
23. Cozzi-Lepri A, Ruiz L, Loveday C, et al. Thymidine analog mutation profiles: factors associated with acquiring specific profiles and their impact on the virologic response to therapy. Antivir Ther. 2005;10: 791-802.
24. Winters M, Shafer R, Jellinger R, et al. HIV type 1 reverse transcriptase genotype and drug susceptibility changes in infected individuals receiving dideoxyinosine monotherapy for 1 to 2 years. Antimicrob Agents Chemother. 1997;41:757-62.
25. Miller M. K65R, TAMs and tenofovir. AIDS Rev. 2004;6:22-33.
26. Barrios A, de Mendoza C, Martin-Carbonero L, et al. Role of baseline HIV genotype as a predictor of viral response to tenofovir in heavily pretreated patients. J Clin Microbiol. 2003;41:4421-3.
27. Vallejo A, Olivera M, Rubio A, et al. Genotypic resistance profile in treatment-experienced HIV-infected individuals after abacavir and efavirenz salvage regimen. Antiviral Res. 2004;61:129-32.
28. Svarovskaia E, Margot N, Bae A, et al. Low-level K65R mutation in HIV-1 reverse transcriptase of treatment-experienced patients exposed to abacavir or didanosine. J Acquir Immune Defic Syndr. 2007;46:174-80.
29. De Mendoza C, Soriano V, Briones C, et al. Emergence of zidovudine resistance in HIV-infected patients receiving stavudine. J Acquir Immune Defic Syndr. 2000;23:279-81.
30. Whitcomb J, Parkin N, Chappey C, Hellmann N, Petropoulos C. Broad nucleoside reverse transcriptase inhibitor cross-resistance in HIV type 1 clinical isolates. J Infect Dis. 2003;188:992-1000.
31. Shafer R, Rhee S, Pillay D, et al. HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance. AIDS. 2007;21: 215-23.
32. Garcia-Lerma J, Nidtha S, Blumoff K, et al. Increased ability for selection of zidovudine resistance in a distinct class of wild-type HIV-1 from drug naïve persons. Proc Natl Acad Sci USA. 2001;98:13485-7.
33. Ingrand D, Weber J, Boucher C, et al. Phase I/II study of 3TC (lamivudine) in HIV-positive, asymptomatic or mild AIDS related complex patients: sustained reductions on viral markers. AIDS. 1995;9:1323-9.
34. Miller V, Sturmer M, Staszewski S, et al. The M184V mutation in HIV-1 reverse transcriptase (RT) conferring lamivudine resistance does not result in broad cross-resistance to nucleoside analogue RT inhibitors. AIDS. 1998;12:705-12.

35. Marcelin A, Flandre P, Pavie J, et al. Clinically relevant genotype interpretation of resistance to didanosine. Antimicrob Agents Chemother. 2005;49;1739-44.
36. Blanco J, Biglia A, de Lazzari E, et al. Antiretroviral activity of didanosine in patients with different clusters of reverse transcriptase mutations. AIDS. 2006;20:1891-2.
37. Svedhem V, Bergroth T, Lidman K, Sönnerborg A. Presence of M184I/V in minor HIV-1 populations of patients with lamivudine and/or didanosine treatment failure. HIV Med. 2007;8:504-10.
38. Llibre J, Bonjoch A, Iribarren J, et al. Targeting only reverse transcriptase with zidovudine/lamivudine/abacavir plus tenofovir in HIV-1 infected patients with multidrug-resistant virus: a multicentre pilot study. HIV Med. 2008;62:909-13.
39. McColl D, Chappey C, Parkin N, Miller M. Prevalence, genotypic associations and phenotypic characterization of K65R, L74V and other HIV-1 RT resistance mutations in a commercial database. Antivir Ther. 2008; 13:189-97.
40. Cases-González C, Franco S, Martínez M, Menéndez-Arias L. Mutational patterns associated with the 69 insertion complex in multi-drug-resistant HIV-1 reverse transcriptase that confer increased excision activity and high-level resistance to zidovudine. J Mol Biol. 2007;365:298-309.
41. Villena C, Prado J, Puertas M, et al. Relative fitness and replication capacity of a multinucleoside analogue-resistant clinical HIV type 1 isolate with a deletion of codon 69 in the reverse transcriptase coding region. J Virol. 2007;81:4713-21.
42. 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.
43. Gallego O, de Mendoza C, Labarga P, et al. Long-term outcome of HIV-infected patients with multinucleoside resistant genotypes. HIV Clin Trials. 2003;4:372-81.
44. Zhang Z, Hamatake R, Hong Z. Clinical utility of current NNRTIs and perspectives of new agents in this class under development. Antivir Chem Chemother. 2004;15:121-34.
45. Briones C, Soriano V, Dona C, et al. Can early failure with nevirapine be rescued with efavirenz? J Acquir Immune Defic Syndr. 2000;24:76-8.
46. Antinori A, Zaccarelli M, Cingolani A, et al. Cross-resistance among nonnucleoside reverse transcriptase inhibitors limits recycling efavirenz after nevirapine failure. AIDS Res Hum Retroviruses. 2002;18:835-8.
47. Bacheler L, Jeffrey S, Hanna G, et al. Genotypic correlates of phenotypic resistance to efavirenz in virus isolates from patients failing nonnucleoside reverse transcriptase inhibitor therapy. J Virol. 2001;75:4999-5008.
48. Brenner B, Turner D, Oliveira M, et al. A V106M mutation in HIV-1 clade C viruses exposed to efavirenz confers cross-resistance to non-nucleoside reverse transcriptase inhibitors. AIDS. 2003;17:F1-5.
49. 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;79:12773-82.
50. Madruga J, Cahn P, Grinsztejn B, et al. Efficacy and safety of TMC125 (etravirine) in treatment experienced HIV-1 infected patients in DUET-1: week results from a randomised, double-blind, placebo controlled trial. 24 week results from a randomized, double-blind, placebo-controlled trial. Lancet. 2007;370:29-38.
51. Lazzarin A, Campbell T, Clotet B, et al. Efficacy and safety of TMC-125 (etravirine) in treatment experienced HIV-1 infected patients in DUET-2: 24 week results from a randomised, double-blind, placebo controlled trial. Lancet. 2007;370:39-48.
52. Nadler J, Berger D, Blick G, et al. Efficacy and safety of etravirine (TMC125) in patients with highly resistant HIV-1: primary 24 weeks analysis. AIDS. 2007;21:F1-10.
53. Vingerhoets J, Buelens A, Peeters M, et al. Impact of baseline NNRTI mutations on the virological response to TMC125 in the phase III clinical trials DUET-1 and DUET-2. Antivir Ther. 2007;12(Suppl):34.
54. Vingerhoets J, Peeters M, Azijn H, et al. An update of the list of NNRTI mutations associated with decreased virologic response to etravirine: multivariate analyses on the pooled Duet-1 and Duet-2 clinical trial data. Antivir Ther. 2008;13:A26.
55. Poveda E, Garrido C, de Mendoza C, et al. Prevalence of etravirine (TMC-125) resistance mutations in HIV-infected patients with prior experience of non-nucleoside reverse transcriptase inhibitors. J Antimicrob Chemother. 2007;60:1409-10.
56. Poveda E, de Mendoza C, Pattery T, et al. Phenotypic impact of resistance mutations on etravirine susceptibility in HIV patients with prior failure to nonnucleoside analogues. AIDS. 2008;22:2395-8.
57. De Mendoza C, Valer L, Ribera E, et al. Performance of six different ritonavir-boosted protease inhibitor-based regimens in heavily antiretroviral-experienced HIV-infected patients. HIV Clin Trials. 2006;7:163-71.
58. Marcelin A, Flandre P, de Mendoza C, et al. Clinical validation of saquinavir/ritonavir genotypic resistance score in protease-inhibitor-experienced patients. Antivir Ther. 2007;12:247-52.
59. Marcelin A, Chazallon C, Gérard L, et al. External validation of atazanavir/ritonavir genotypic score in HIV-1 protease inhibitor-experienced patients. J Acquir Immune Defic Syndr. 2006;42:127-8.
60. Vora S, Marcelin A, Günthard H, et al. Clinical validation of atazanavir/ritonavir genotypic resistance score in protease inhibitor-experienced patients. AIDS. 2006;20:35-40.
61. Dronda F, Antela A, Pérez-Elias M, et al. Rescue therapy with once-daily atazanavir based regimens for antiretroviral-experienced HIV-infected patients. J Acquir Immune Defic Syndr. 2006;42:258-9.
62. King M, Rode R, Cohen-Codar I, et al. Predictive genotypic algorithm for virologic response to lopinavir-ritonavir in protease inhibitor-experienced patients. Antimicrob Agents Chemother. 2007;51:3067-74.
63. Maillard A, Chapplain J, Tribut O, et al. The use of drug resistance algorithms and genotypic inhibitory quotient in prediction of lopinavir-ritonavir treatment response in HIV type 1 protease inhibitor-experienced patients. J Clin Virol. 2007;38:131-8.
64. Marcelin A, Lamotte C, Delaugerre C, et al. Genotypic inhibitory quotient as predictor of virological response to ritonavir-amprenavir in HIV type 1 protease inhibitor-experienced patients. Antimicrob Agents Chemother. 2003;47:594-600.
65. Masquelier B, Assoumou K, Descamps D, et al. Clinically validated mutation scores for HIV-1 resistance to fosamprenavir/ritonavir. J Antimicrob Chemother. 2008;61:1362-8.
66. De Mendoza C, Valer L, Bacheler L, et al. Prevalence of the HIV-1 protease mutation I47A in clinical practice and association with lopinavir resistance. AIDS. 2006;20:1071-4.
67. Colonno R, Rose R, McLaren C, et al. Identification of I50L as the signature atazanavir (ATV)-resistance mutation in treatment-naive HIV-1-infected patients receiving ATV-containing regimens. J Infect Dis. 2004; 189:1802-10.
68. Delaugerre C, Mathez D, Peytavin G, et al. Key amprenavir resistance mutations counteract dramatic efficacy of darunavir in highly experienced patients. AIDS. 2007;21:1210-3.
69. Martinez-Picado J, Wrin T, Frost S, et al. Phenotypic hypersusceptibility to multiple protease inhibitors and low replicative capacity in patients who are chronically infected with HIV type 1. J Virol. 2005;79:5907-13.
70. Sista P, Wasikowski B, Lecocq P, et al. The HIV-1 protease resistance mutation I50L is associated with resistance to atazanavir and susceptibility to other protease inhibitors in multiple mutational contexts. J Clin Virol. 2008;42:405-8.
71. Baxter J, Schapiro J, Boucher C, et al. Genotypic changes in HIV protease associated with reduced susceptibility and virological response to the protease inhibitor tipranavir. J Virol. 2006;80:10794-801.
72. Scherer J, Boucher C, Baxter J, et al. Improving the prediction of virologic response to tipranavir: the development of a tipranavir weighted mutation score. 6th European drug resistance workshop. March, 2008. Budapest [abstract 94].
73. Scherer J, Schapiro J, Maggiolo F, et al. Improved tipranavir weighted score predicts virologic response in diverse treatment-experienced patient populations. 7th European HIV Drug Resistance Workshop. Stockholm, Sweden. March 2009 (in press)
74. De Meyer S, Azijn H, Surleraux D, et al. TMC114, a novel HIV type 1 protease inhibitor active against protease inhibitor-resistant viruses, including a broad range of clinical isolates. Antimicrob Agents Chemother. 2005;49:2314-21.
75. De Meyer S, Vangeneugden T, van Baelen B, et al. Resistance profile of darunavir: combined 24-week results from the POWER trials. AIDS Res Hum Retroviruses. 2008;24:379-88.
76. De Meyer S, Dierynck I, Lathouwers E, et al. Phenotypic and genotypic determinants of resistance to darunavir: analysis of data from treatment experience patients on power 1, 2, 3 and DUET-1 and 2. Antivir Ther. 2008;13:A33.
77. Pellegrin I, Wittkop L, Joubert L, et al. Virological response to darunavir/ritonavir-based regimens in antiretroviral-experienced patients (PREDIZISTA study). Antivir Ther. 2008;13:271-9.
78. Martinez-Picado J, Savara A, Sutton L, D'Aquila R. Replicative fitness of protease inhibitor-resistant mutants of HIV-1. J Virol. 1999; 73:3744-52.
79. Velazquez-Campoy A, Muzammil S, Ohtaka H, et al. Structural and thermodynamic basis of resistance to HIV-1 protease inhibition: implications for inhibitor design. Curr Drug Targets Infect Disord. 2003;3: 311-28.
80. Blanco J, Biglia M, Arnedo M, et al. Evolution of resistance mutations pattern in HIV-1-infected patients during intensification therapy with a boosted protease inhibitor. AIDS. 2005;19:829-31.
81. Kantor R, Katzenstein D. Polymorphisms in HIV-1 non-subtype B protease and reverse transcriptase and its potential impact on drug susceptibility and drug resistance evolution. AIDS Rev. 2003;5:25-35.
82. Kantor R, Katzenstein D, Efron B, et al. Impact of HIV-1 subtype and antiretroviral therapy on protease and reverse transcriptase genotype: results of a global collaboration. PLoS Med. 2005;2:e112.
83. Snoeck J, Kantor R, Shafer R, et al. Discordances between interpretation algorithms for genotypic resistance to protease and reverse transcriptase inhibitors of HIV are subtypes dependent. Antimicrob Agents Chemother. 2006;50:694-701.
84. Poveda E, de Mendoza C, Parkin N, et al. Evidence for different susceptibility to tipranavir and darunavir in patients infected with distinct HIV-1 subtypes. AIDS. 2008;22:611-6.
85. Vergne L, Stuyver L, Van Houtte M, et al. Natural polymorphism in protease and reverse transcriptase genes and in vitro antiretroviral drug susceptibilities of non-B HIV-1 strains from treatment-naive patients. J Clin Virol. 2006;36:43-9.

86. Cabrera C, Marfil S, García E, et al. Genetic evolution of gp41 reveals a highly exclusive relationship between codons 36, 38 and 43 in gp41 under long-term enfuvirtide-containing salvage regimen. AIDS. 2006; 20:2075-80.
87. Poveda E, Rodés B, Labernardière J, et al. Evolution of genotypic and phenotypic resistance to enfuvirtide in HIV-infected patients experiencing prolonged virologic failure. J Med Virol. 2004;74:21-8.
88. Poveda E, Rodés B, Lebel-Binay S, et al. Dynamics of enfuvirtide resistance in HIV-infected patients during and after long-term enfuvirtide salvage therapy. J Clin Virol. 2005;34:295-301.
89. Holguin A, Rodriguez de Arellano E, Soriano V. Amino acid conservation in the gp41 transmembrane protein and natural polymorphisms associated with enfuvirtide resistance across HIV-1 variants. AIDS Res Hum Retroviruses. 2007;23:1067-74.
90. Loutfy M, Raboud J, Montaner J, et al. Assay of HIV gp41 amino acid sequence to identify baseline variation and mutation development in patients with virologic failure on enfuvirtide. Antiviral Res. 2007;75:58-63.
91. Poveda E, Briz V, Paraskevis D, et al. Dynamics of drug-resistant HIV-1 in plasma and peripheral blood cells in patients during and after enfuvirtide therapy. AIDS Res Hum Retroviruses. 2007;23:1078-82.
92. 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.
93. Soriano V, Geretti A, Perno C, et al. Optimal use of maraviroc in clinical practice. AIDS. 2008;22:2231-40.
94. Gulick R, Lalezari J, Goodrich J, et al. Maraviroc for previously treated patients with R5 HIV-1 infection. N Engl J Med. 2008;359:1429-41.
95. Fätkenheuer G, Nelson M, Lazzarin A, et al. Subgroup analyses of maraviroc in previously treated R5 HIV-1 infection. N Engl J Med. 2008; 359:1442-55.
96. Wilkin T, Su Z, Kuritzkes D, et al. HIV type 1 chemokine coreceptor use among antiretroviral-experienced patients screened for a clinical trial of a CCR5 inhibitor: AIDS Clinical Trial Group A5211. Clin Infect Dis. 2007; 44:591-5.
97. Brumme Z, Goodrich J, Mayer H, et al. Molecular and clinical epidemiology of CXCR4-using HIV-1 in a large population of antiretroviral-naive individuals. J Infect Dis. 2005;192:466-74.
98. De Mendoza C, Rodriguez C, Garcia F, et al. Prevalence of X4 tropic viruses in patients recently infected with HIV-1 and lack of association with transmission of drug resistance. J Antimicrob Chemother. 2007; 59:698-704.
99. Westby M, Smith-Burchnell C, Mori J, et al. Reduced maximal inhibition in phenotypic susceptibility assays indicates that viral strains resistant to the CCR5 antagonist maraviroc utilize inhibitor-bound receptor for entry. J Virol. 2007;81:2359-71.
100. Westby M, Lewis M, Whitcomb J, et al. Emergence of CXCR4-using HIV-1 variants in a minority of HIV-1-infected patients following treatment with the CCR5 antagonist maraviroc is from a pretreatment CXCR4-using virus reservoir. J Virol. 2006;80:4909-20.
101. Moncunill G, Armand-Ugón M, Pauls E, Clotet B, Esté J. HIV-1 escape to CCR5 coreceptor antagonism through selection of CXCR4-using variants in vitro. AIDS. 2008;22:23-31.
102. Steigbigel R, Cooper D, Kumar P, et al. Raltegravir with optimized background therapy for resistant HIV-1 infection. N Engl J Med. 2008;359: 339-54.
103. Cooper D, Steigbigel R, Gatell J, et al. Subgroup and resistance analyses of raltegravir for resistant HIV-1 infection. N Engl J Med. 2008;359: 355-65.
104. Grinsztejn B, Nguyen B, Katlama C, et al. Safety and efficacy of the HIV-1 integrase inhibitor raltegravir (MK-0518) in treatment-experienced patients with multidrug-resistant virus: a phase II randomised controlled trial. Lancet. 2007;369:1261-9.
105. Pommier Y, Johnson A, Marchand C. Integrase inhibitors to treat HIV/AIDS. Nat Rev Drug Discov. 2005;4:236-48.
106. Hazuda D, Miller M, Nguyen B, for the P005 Study Team. Resistance to the HIV-integrase inhibitor raltegravir: analysis of protocol 005, a Phase II study in patients with triple-class resistant HIV-1 infection. Antivir Ther. 2007;12(Suppl):10.
107. Goethals O, Clayton R, Van Ginderen M, et al. Resistance mutations in HIV type 1 integrase selected with elvitegravir confer reduced susceptibility to a wide range of integrase inhibitors. J Virol. 2008;82: 10366-74.
108. Ceccherini-Silberstein F, Malet I, D'Arrigo R, Antinori A, Marcelin A, Perno C. Characterization and structural analysis of HIV-1 integrase conservation. AIDS. Rev [in press].
109. Garrido C, Geretti A, de Mendoza C, Booth C, Strang A, Soriano V. Polymorphisms at the integrase gene may influence the susceptibility to integrase inhibitors in distinct HIV populations. 6th European drug resistance workshop. 26-28 March, 2008. Budapest [abstract 12].
110. Hackett J, Harris B, Holzmayer V, et al. Naturally occurring polymorphisms in HIV-1 group M, N, and O integrase: implications for integrase inhibitors. 15th CROI. February 3-6, 2008; Boston, MA [abstract 872].
111. Loizidou E, Kousiappa I, Zeinalipour-Yazdi C, et al. Implications of HIV-1 M group polymorphisms on integrase inhibitor efficacy and resistance: genetic and structural in silico analyses. Biochemistry. 2009;48:4-6.
112. Xu L, Anderson J, Ferns B, et al. Genetic diversity of integrase sequences in antiretroviral treatment-naive and treatment-experienced HIV type 2 patients. AIDS Res Hum Retroviruses. 2008;24:1003-7.
113. Roquebert B, Damond F, Collin G, et al. HIV-2 integrase gene polymorphisms and phenotypic susceptibility of HIV-2 clinical isolates to the integrase inhibitors raltegravir and elvitegravir in vitro. J Antimicrob Chemother. 2008;62:914-20.