

# Drug Resistance Mutations in HIV-1

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The International AIDS Society–USA (IAS–USA) Drug Resistance Mutations Group reviews new data on HIV drug resistance with the goal of maintaining a current list of mutations associated with clinical resistance to HIV. This list, presented as the IAS–USA Mutations Figures, has been revised based on data from the 11th International HIV Drug Resistance Workshop in Seville, Spain, in July 2002, and other recent conferences. The figures have been updated since they were last published in the May/June 2002 issue of this journal.

## New Drugs Added to the Mutations Figures

The most notable change in this update is the addition of the HIV entry inhibitor class. The first agent added to the figure is enfuvirtide, a fusion inhibitor that is not approved by the US Food and Drug Administration (FDA) but is available through an expanded access protocol. Resistance mutations in the gp41 envelope gene have been identified primarily at positions 36 to 45 of the first heptad repeat (HR1) region. The mutations listed on the figure—G36D/S, I37V, V38A/M, Q39R, N42T, and N43D—are from preliminary data<sup>1-3</sup>; further research is needed to evaluate the clinical relevance of these and other mutations.

Atazanavir, also only available through an expanded access protocol, has been added to the protease inhibitor (PI) category. The mutations included for atazanavir (V32I, M46I, I50L, I54L, A71V, V82A, I84V, N88S, and L90M) are based on recent data from studies using the drug as the initial PI, and as a subsequent PI in combination with saquinavir.<sup>4</sup>

## Other Revisions

In light of the expanding information offered in the notes accompanying the figures, the name “Footnotes” has been changed to “User Notes.”

The E44D and V118I mutations are now included as nRTI-associated mutations (NAMs). The NAMs (M41L, E44D, D67N, K70R, V118I, L210W, T215Y/F, and K219Q/E) are associated with cross-resistance to nucleoside reverse transcriptase inhibitors (nRTIs) and are represented in the figures by vertical pink lines (see user note 2). In this revision, the NAMs lines have been extended to lamivudine in recognition of data that indicate that the E44D and V118I mutations confer resistance to lamivudine only in the presence of multiple other NAMs.<sup>5</sup>

The IAS–USA Mutations Figures are available on a pocket-sized folding card. To order copies, please contact the IAS–USA at (415) 561-6720 (phone) or [resistance@iasusa.org](mailto:resistance@iasusa.org).

In the user notes, the discussion of revertant mutations in codon 215 has been updated. New data indicate that an expanded list of substitutions at 215 confer increased risk of virologic failure of zidovudine and stavudine in antiretroviral-naïve adults starting therapy with these drugs.<sup>6,8</sup> The T215Y mutant may emerge quickly from these mutations in the presence of zidovudine or stavudine.

The IAS–USA Drug Resistance Mutations Group welcomes comments on the mutations figures and user notes. Please send your evidence-based comments, including relevant reference citations, to the IAS–USA at [resistance@iasusa.org](mailto:resistance@iasusa.org) or by fax at (415) 561-6740. Please include your name and institution.

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**MUTATIONS IN THE REVERSE TRANSCRIPTASE GENE ASSOCIATED WITH RESISTANCE TO REVERSE TRANSCRIPTASE INHIBITORS**

**Nucleoside and Nucleotide Reverse Transcriptase Inhibitors**

Multi-nRTI Resistance: 151 Complex	A	V	F	F	Q					
	<b>62</b>	<b>75</b>	<b>77</b>	<b>116</b>	<b>151</b>					
	V	I	L	Y	M					
Multi-nRTI Resistance: 69 Insertion Complex <sup>1</sup>	M	A	D	▼	K			L	T	K
	<b>41</b>	<b>62</b>	<b>67</b>	<b>69</b>	<b>70</b>			<b>210</b>	<b>215</b>	<b>219</b>
	L	V	N	insert	R			W	Y	Q
								F	E	
Multi-nRTI Resistance: NAMs <sup>2</sup>	M	E	D	K	V			L	T	K
	<b>41</b>	<b>44</b>	<b>67</b>	<b>70</b>	<b>118</b>			<b>210</b>	<b>215</b>	<b>219</b>
	L	D	N	R	I			W	Y	Q
								F	E	
Zidovudine <sup>3,4</sup>	M	E	D	K	V			L	T	K
	<b>41</b>	<b>44</b>	<b>67</b>	<b>70</b>	<b>118</b>			<b>210</b>	<b>215</b>	<b>219</b>
	L	D	N	R	I			W	Y	Q
								F	E	
Stavudine <sup>3,5</sup>	M	E	D	K	V			L	T	K
	<b>41</b>	<b>44</b>	<b>67</b>	<b>70</b>	<b>118</b>			<b>210</b>	<b>215</b>	<b>219</b>
	L	D	N	R	I			W	Y	Q
								F	E	
Didanosine <sup>6,7</sup>			K	L						
			<b>65</b>	<b>74</b>						
			R	V						
Zalcitabine			K	T	L					M
			<b>65</b>	<b>69</b>	<b>74</b>					<b>184</b>
			R	D	V					V
Abacavir <sup>8</sup>			K	L	Y					M
			<b>65</b>	<b>74</b>	<b>115</b>					<b>184</b>
			R	V	F					V
Lamivudine <sup>9</sup>	E				V					M
	<b>44</b>				<b>118</b>					<b>184</b>
	D				I					V
										I
Tenofovir <sup>3,10</sup>			K							
			<b>65</b>							
			R							

**Nonnucleoside Reverse Transcriptase Inhibitors**

Multi-NNRTI Resistance <sup>11</sup>			K		Y				
			<b>103</b>		<b>188</b>				
			N		L				
Multi-NNRTI Resistance: Accumulation of Mutations <sup>12</sup>		L	V		Y	G		M	
		<b>100</b>	<b>106</b>		<b>181</b>	<b>190</b>		<b>230</b>	
		I	A		C	S		L	
					I	A			
Nevirapine	L	K	V	V	Y	Y	G		
	<b>100</b>	<b>103</b>	<b>106</b>	<b>108</b>	<b>181</b>	<b>188</b>	<b>190</b>		
	I	N	A	I	C	C	A		
					I	L	H		
Delavirdine <sup>13</sup>			K		Y	Y		P	
			<b>103</b>		<b>181</b>	<b>188</b>		<b>236</b>	
			N		C	L		L	
Efavirenz <sup>13-15</sup>	L	K	V		Y	Y	G	P	
	<b>100</b>	<b>103</b>	<b>108</b>		<b>181</b>	<b>188</b>	<b>190</b>	<b>225</b>	
	I	N	I		C	L	S	H	
					I	A			



## User Notes

The IAS–USA Drug Resistance Mutations Group reviews new data on HIV drug resistance in order to maintain a current list of mutations associated with clinical resistance to HIV. This list, presented as the IAS–USA mutations figures, includes mutations that may contribute to a reduced virologic response to a drug. These mutations have been identified by one or more of the following criteria: (1) in vitro passage experiments; (2) susceptibility testing of laboratory or clinical isolates; (3) genetic sequencing of viruses from patients in whom the drug is failing; (4) correlation studies between genotype at baseline and virologic response in patients exposed to the drug. Drugs that have been approved by the US Food and Drug Administration (FDA) or are available through expanded access protocols are included. Additional information on the mutations is provided, where necessary, in these user notes.

1. The 69 insertion complex, consisting of a mutation at codon 69 (typically T69S) and followed by an insertion of 2 or more amino acids (S-S, S-A, S-G, or others), is associated with resistance to all FDA-approved nRTIs. The 69 insertion complex is often accompanied by mutations at other sites. Some other amino acid changes from the wild-type T in codon 69 without the insertion may also be associated with broad nRTI resistance.

2. The nRTI-associated mutations (NAMs), including M41L, E44D, D67N, K70R, V118I, L210W, T215Y/F, and K219Q/E, are associated with cross-resistance to nRTIs and are represented by vertical pink lines. Zidovudine and stavudine select for these mutations, and as such, the positions and mutations are indicated on the bars along with the pink lines. For other nRTIs, the NAMs are not commonly selected by those drugs, but the presence of the NAMs confers cross-resistance to the drugs. This is represented by pink lines only at the positions.

The E44D and V118I mutations are listed as NAMs. In a recent study, the E44D and V118I mutations were more common in virus from patients treated with zidovudine and lamivudine, and were associated with higher-level resistance to zidovudine (Kuritzkes et al, *Antimicrob Agents Chemother*, in press). When present together with other NAMs, the E44D and V118I mutations confer resistance to lamivudine.

Analysis from the AIDS Clinical Trials Group (ACTG) study 136 has shown that the V118I mutation is commonly selected by a zidovudine/didanosine regimen (Shafer et al, *J Infect Dis*, 1995). Findings from ACTG study 241 have shown that the E44D mutation is commonly selected by zidovudine/didanosine (Hanna et al, *J Infect Dis*, 2002) and that the E44D mutation is associated with a significantly worse response to treatment with zidovudine and didanosine, with or without nevirapine (Precious et al, *AIDS*, 2000). The significance of E44D or V118I when each occurs in isolation is unknown (Romano et al, *J Infect Dis*, 2002; Walter et al, *Antimicrob Agents Chemother*, 2002; Girouard et al, *Antivir Ther*, 2002).

3. The M184V mutation may enhance susceptibility to zidovudine, stavudine, or tenofovir. This effect may be overcome by an accumulation of NAMs or other mutations. The clinical significance of this effect is not known.

4. Recent data on revertant mutations in codon 215 indicate that the T215D/C/S/E/N/A/V substitutions confer increased risk of virologic failure of zidovudine and stavudine in antiretroviral-naïve adults starting therapy with these drugs (Riva et al, *Antivir Ther*, 2002). In vitro studies and preliminary clinical studies suggest that the T215Y mutant may emerge quickly from these mutations in the presence of zidovudine or stavudine (Garcia-Lerma et al, *Proc Natl Acad Sci U S A*, 2001; Lanier et al, *Antivir Ther*, 2002; Riva et al, *Antivir Ther*, 2002).

5. Mutations at codon 75 (V75T/M/S/A) have been observed in vitro and may confer a low-level change in susceptibility to stavudine (Lacey et al, *Antimicrob Agents Chemother*, 1994).

6. The K65R mutation or the L74V mutation, alone or in combination with the NAMs and/or T69D/N can lead to didanosine resistance.

7. Based on preliminary, yet-unpublished data, the M184V mutation does not appear to have a negative impact on in vivo responses to didanosine, even though the mutation reduces susceptibility in vitro. (Winters et al, *Antivir Ther*, 2002; Eron et al, *Antivir Ther*, 2002; Pozniak et al, *Antivir Ther*, 2002).

8. When present with NAMs, the M184V

mutation contributes to reduced susceptibility to abacavir and is associated with impaired response in vivo. However, when present alone, the M184V mutation does not appear to be associated with a reduced virologic response to abacavir in vivo (Harrigan et al, *J Infect Dis*, 2000).

9. The E44D and V118I mutations were reported to confer low-level resistance to lamivudine when accompanied by several other nRTI-associated mutations (M41L, D67N, L210W, T215Y/F, K219Q/E) in the absence of a concurrent M184V mutation (Hertogs et al, *Antimicrob Agents Chemother*, 2000). Data presented but not yet published (D'Arminio-Monforte et al, 8th CROI, 2001), reported no association over the short term between E44D or V118I and virologic response to a lamivudine-containing combination regimen. (See also user note 2.)

10. The accumulation of NAMs (M41L, D67N, K70R, L210W, T215Y/F, K219Q/E [note: data here do not include E44D and V118I]) increases resistance to tenofovir. Mutations M41L and L210W contribute more than others. Therefore, the number and type of NAMs will determine the degree of reduced response. T69D/N/S may also contribute to a reduced response to tenofovir (Miller et al, *Antivir Ther*, 2002; Lu et al, *Antivir Ther*, 2002; Masquelier et al, *Antivir Ther*, 2002).

11. The K103N or Y188L mutation alone can substantially reduce the clinical utility of all currently approved NNRTIs.

12. Accumulation of 2 or more of these mutations substantially reduces the clinical utility of all of the currently approved NNRTIs.

13. The prevalence of the Y318F mutation in clinical isolates along with mutations K103N, Y181C, or P236L was approximately 5%, 2%, and 15%, respectively (Kemp et al, *Antivir Ther*, 2001). In vitro this mutation confers resistance to nevirapine, delavirdine, and efavirenz.

14. The Y181C/I mutation is not selected by efavirenz, but its presence contributes to low-level cross-resistance to the drug. Clinical impact of this mutation may be overcome with a fully active antiretroviral combination regimen, although no clinical trial data yet address this question.

15. V108I and P225H each contribute to efavirenz resistance when present in combination with other NNRTI-associated mutations. Although V108I or P225H alone does not confer measurable resistance in laboratory strains of HIV-1, their presence in a clinical isolate may indicate prior selection for efavirenz-resistant variants.

16. Resistance mutations in the protease gene are classified as either "major" or "minor" (if known).

**Major:** In general, major mutations are either (1) selected first in the presence of the drug; or (2) shown at the biochemical or virologic level to lead to an alteration in drug binding or an inhibition of viral activity or viral replication. By themselves, major mutations have an effect on phenotype. In general, these mutations tend to be the major contact residues for drug binding.

**Minor:** In general, minor mutations appear later than major mutations, and by themselves do not have a significant effect on phenotype. In some cases, their effect may be to improve replicative fitness of virus carrying major mutations.

17. Accumulation of 4 or more of these mutations is likely to cause multi-PI resistance (Palmer et al, *AIDS*, 1999; Shafer et al, *Ann Intern Med*, 1998).

18. For indinavir, the mutations listed as major may not be the first mutations selected, but they are present in most clinical isolates in combination with other mutations.

19. Major and minor mutations have not been designated for lopinavir/ritonavir-associated resistance since currently there are no clear data defining degrees of influence with this drug combination. The accumulation of 6 or more of these mutations is associated with a diminished response to lopinavir/ritonavir. The product information states that accumulation of 7 or 8 mutations confers resistance to the drug. However, recent data suggest as few as 4 mutations can be associated with such high-level resistance (Prado et al, *AIDS*, 2002). Further clinical experience and research are needed to better define the mutations that affect the clinical effectiveness of lopinavir/ritonavir. It is reasonable to consider phenotyping to assess this in individual cases.

20. Protease mutation L63P is common in viruses that have never been exposed to PIs (Kozal et al, *Nat Med*, 1996) and may be more prevalent in viruses from patients in whom a PI-containing regimen has failed. However, by itself, L63P does not cause any appreciable increase in the IC<sub>50</sub> for any PI. L63P is listed for lopinavir/ritonavir (and not any other PI) because studies have shown that this mutation, when present with multiple other mutations, is associated with clinical failure.

21. Atazanavir is currently available through an expanded access protocol and is not approved by the US FDA. When administered to patients as the initial PI, atazanavir selects for the mutations I50L and A71V (Colonna et al, *Antivir Ther*, 2002). When used as a subsequent PI in combination with saquinavir, atazanavir selects for I54L and I84V (Colonna et al, *Antivir Ther*, 2002). In vitro, atazanavir selects for V32I, M46I, I84V, and N88S (Gong et al, *Antimicrob Agents Chemother*, 2000). Although other major mutations, such as V82A and L90M, have not been selected for by atazanavir either in vitro or in vivo, these mutations have been shown to confer cross-resistance to atazanavir, particularly when present in combination with each other or with other known PI resistance mutations (Colonna et al, *Antivir Ther*, 2000).

22. Enfuvirtide is currently available through an expanded access protocol and is not approved by the US FDA. To date, resistance mutations in the gp41 envelope gene have been identified primarily at positions 36 to 45 of the first heptad repeat (HR1) region. These mutations have been identified in viruses from patients treated with the drug and have been shown to confer resistance or reduced susceptibility (Wei et al, *Antimicrob Agents Chemother*, 2002; Sista et al, *Antivir Ther*, 2002; Mink et al, *Antivir Ther*, 2002). It is important to note that wild-type viruses in this region show a 500-fold range in susceptibility, and mutations in other regions in the envelope may affect susceptibility to enfuvirtide. Further research is needed to evaluate the clinical relevance of these mutations.

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