

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 to include recently published data on the effects of the V106M mutation on nonnucleoside reverse transcriptase inhibitors (NNRTIs).¹

The figures presented here (March 15, 2003) replace the November/December 2002 versions published in this journal and at www.iasusa.org.

New Addition to the Mutations Figures

This updated version of the figures includes the addition of the V106M mutation to all NNRTI bars—nevirapine, delavirdine, and efavirenz. The V106M mutation has also been added to the first multi-NNRTI resistance bar on the figures.

In the recently published study¹, investigators performed genotypic analysis to ascertain prevalence of V106 (GTG) and 106M (ATG) codons in clinical isolates. Most subtype B isolates harbored GTA (valine) at codon 106 (97%), and the GTG (valine) polymorphism was generally present in clade C viruses (94%).

In addition, cell-based phenotypic assays demonstrated that under conditions of efavirenz (but not nevirapine or delavirdine) pressure in tissue culture, clade C isolates developed the V106M mutation (GTG←ATG), conferring high-level (100- to 1000-fold IC₅₀ change) cross-resistance to all NNRTIs.¹

As stated in the new User Note 12, this mutation has been observed only in HIV

clade C clinical isolates. However, site-directed mutagenesis indicates that the V106M mutation confers cross-resistance to all NNRTIs in HIV clade B virus.

The IAS–USA Mutations Figures are available on a pocket-sized folding card.

Copies of the card can be ordered by:

- **Calling the IAS–USA at (415) 544-9400**
- **Visiting www.iasusa.org/resistance_mutations/index.html, and printing out the request form and faxing to (415) 544-9401 or mailing to IAS–USA, 425 California Street, Suite 1450, San Francisco, CA, 94104-2120**
- **E-mailing resistance@iasusa.org for ordering information**

Also in this new version of the figures, enfuvirtide (T-20) is no longer listed as available only through expanded access. The drug was approved by the US Food and Drug Administration in March 2003, marking the first approval in the fusion inhibitor class of anti-HIV agents.

Comments?

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 or by fax at (415) 544-9401. Please include your name and institution.

Reference

1. Brenner B, Turner D, Oliveira M, et al. A V106M mutation in HIV-1 clade C viruses exposed to efavirenz confers cross-resistance to nonnucleoside reverse transcriptase inhibitors. *AIDS*. 2003;17:F1-F5.

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

Nucleoside and Nucleotide Reverse Transcriptase Inhibitors

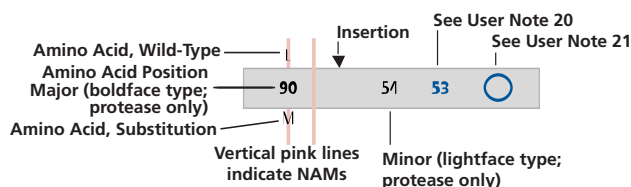
Multi-nRTI Resistance: 151 Complex	A		V		F	F		Q		
	62		75		77	116		151		
	V		I		L	Y		M		
Multi-nRTI Resistance: 69 Insertion Complex ¹	M	A	D	▼	K	L		T	K	
	41	62	67	69	70	210		215	219	
	L	V	N	insert	R	W		Y	Q	
						F		E		
Multi-nRTI Resistance: NAMs ²	M	D	K	V		L		T	K	
	41	44	67	70	118		210		215	219
	L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

Nonnucleoside Reverse Transcriptase Inhibitors

Multi-NNRTI Resistance ^{11,12}	K V				Y			
	103 106				188			
Multi-NNRTI Resistance: Accumulation of Mutations ¹³	N M				L			
	L	V			Y	G	M	
	100	106			181	190	230	
	I	A			C	S A	I	
Nevirapine	L	K	V	V	Y	Y	G	
	100	103	106	108	181	188	190	
	I	N	A	I	C	C	A	
			M		I	L	I	
Delavirdine ¹⁴	K V				Y	Y	P	
	103 106				181	188	236	
	N	M			C	I	L	
Efavirenz ¹⁴⁻¹⁶	L	K	V	V	Y	Y	G	P
	100	103	106	108	181	188	190	225
	I	N	M	I	C	L	S	I
					I	A		

MUTATIONS IN THE PROTEASE GENE ASSOCIATED WITH RESISTANCE TO PROTEASE INHIBITORS**Protease Inhibitors¹⁷**

Multi-PI Resistance: Accumulation of Mutations ¹⁸	-					M		I		V	I	L					
	10					46		54		82	84	90					
	F					I		V		A	V	M					
	I					L		M		E							
	R							L		T							
	V									S							
Indinavir ¹⁹	-	K	L		V	M	M	I	A	G	V	V	I	L			
	10	20	24		32	36	46	54	71	73	77	82	84	90			
	I	M	I		I	I	L	V	V	S	I	A	V	M			
	R	R							T	A		E					
	V											T					
Ritonavir	-	K			V	L	M	M	I	A		V	V	I	L		
	10	20			32	33	36	46	54	71		77	82	84	90		
	F	M			I	F	I	L	V	V		I	A	V	M		
	I	R							L	T			E				
	R												T				
	V											S					
Saquinavir	-							G	I	A	G	V	V	I	L		
	10							48	54	71	73	77	82	84	90		
	I							V	V	V	S	I	A	V	M		
	R								L	T							
	V																
Nelfinavir	-			D		M	M		A		V	V	I	N	L		
	10			30		36	46		71		77	82	84	88	90		
	F			N		I	L		V		I	A	V	D	M		
	I								T			E		S			
												T					
											S						
Amprenavir	-			V		M	I	I	I		G		I		L		
	10			32		46	47	50	54		73		84		90		
	F			I		L	V	V	L		S		V		M		
	I								M								
	R																
	V																
Lopinavir/ Ritonavir ^{20,21}	L	K	L		V	L	M	I	I	F	I	L	A	G	V	I	L
	10	20	24		32	33	46	47	50	53	54	63	71	73	82	84	90
	F	M	I		I	F	L	V	V	L	V	P	V	S	A	V	M
	I	R											T		E		
	R														T		
	V													S			
Atazanavir ²² (expanded access)				V		M	I	I	A		V	I	N	L			
				32		46	50	54	71		82	84	88	90			
				I		I	L	L	V	A	V	S	M				

MUTATIONS IN THE GP41 ENVELOPE GENE ASSOCIATED WITH RESISTANCE TO ENTRY INHIBITORS**Enfuvirtide²³****MUTATIONS**

For each amino acid residue, the letter above the bar indicates the amino acid associated with wild-type virus and the letter(s) below indicate the substitution(s) that confer viral resistance. The number shows the position of the mutation in the protein. Mutations selected by protease inhibitors in Gag cleavage sites are not listed because their contribution to resistance is not yet fully defined. HR1 indicates first heptad repeat; NAMs indicates nRTI-associated mutations; nRTI indicates nucleoside reverse transcriptase inhibitor; NNRTI indicates nonnucleoside reverse transcriptase inhibitor; PI indicates protease inhibitor. The figures were last published in *Topics in HIV Medicine* in November/December 2002.

Amino acid abbreviations: A, alanine; C, cysteine; D, aspartate; E, glutamate; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

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. The V106M mutation confers high-level resistance in vitro to nevirapine, delavirdine, and efavirenz (Brenner et al, *AIDS*, 2003). This mutation has been observed only in HIV clade C clinical isolates, although site-directed mutagenesis indicates that V106M confers cross-resistance to all NNRTIs in HIV clade B virus.

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

14. 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.

15. 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.

16. 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 labora-

tory strains of HIV-1, their presence in a clinical isolate may indicate prior selection for efavirenz-resistant variants.

17. 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.

18. 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).

19. 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.

20. 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.

21. 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 IC₅₀ 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.

22. 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).

23. 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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