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

Nucleoside and Nucleotide Analogue Reverse Transcriptase Inhibitors (nRTIs)[a]

69 Insertion Complex[b] (affects all nRTIs currently approved by the US FDA)

	M	A	▼	K	L	T	K
Multi-nRTI Resistance	41	62	69	70	210	215	219
	L	V	Insert	R	W	Y	Q
						F	E

151 Complex[c] (affects all nRTIs currently approved by the US FDA except tenofovir)

	A	V	F	F	Q
Multi-nRTI Resistance	62	75	77	116	151
	V	I	L	Y	M

Thymidine Analogue-Associated Mutations[d,e] (TAMs; affect all nRTIs currently approved by the US FDA other than emtricitabine and lamivudine)

	M	D	K	L	T	K
Multi-nRTI Resistance	41	67	70	210	215	219
	L	N	R	W	Y	Q
					F	E

	K	L	Y	M
Abacavir[f,g]	65	74	115	184
	R	V	F	V
	E			
	N			

	K	L
Didanosine[g,h]	65	74
	R	V
	E	
	N	

	K	M
Emtricitabine	65	184
	R	V
	E	I
	N	

	K	M
Lamivudine	65	184
	R	V
	E	I
	N	

	M	K	D	K	L	T	K
Stavudine[d,e,g,i,j,k]	41	65	67	70	210	215	219
	L	R	N	R	W	Y	Q
		E				F	E
		N					

	K	K
Tenofovir[l]	65	70
	R	E
	E	
	N	

	M	D	K	L	T	K
Zidovudine[d,e,j,k]	41	67	70	210	215	219
	L	N	R	W	Y	Q
					F	E

Nonnucleoside Analogue Reverse Transcriptase Inhibitors (NNRTIs)[a,m]

	L	K	K	V	V	Y	Y	G	P	M
Efavirenz	100	101	103	106	108	181	188	190	225	230
	I	P	N	M	I	C	L	S	H	L
			S			I		A		

	V	A	L	K	V	E	V	Y	G	M
Etravirine[n]	90	98	100	101	106	138	179	181	190	230
	I	G	I	E	I	A	D	C	S	L
				H		G	F	I	A	
				P		K	T	V		
						Q				

	L	K	K	V	V	Y	Y	G	M
Nevirapine	100	101	103	106	108	181	188	190	230
	I	P	N	A	I	C	C	A	L
			S	M		I	L		
							H		

	L	K	E	V	Y	Y	H	F	M
Rilpivirine[o]	100	101	138	179	181	188	221	227	230
	I	E	A	L	C	L	Y	C	I
		P	G		I				L
			K		V				
			Q						
			R						

MUTATIONS

Insertion

Amino acid wild-type — K
Amino acid position — 65
Amino acid substitution conferring resistance — R

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.

MUTATIONS IN THE PROTEASE GENE ASSOCIATED WITH RESISTANCE TO PROTEASE INHIBITORS[p,q,r]

	L	G	K	L	V	L	E	M	M	G	I	F	I	D	I	I	A	G	V	I	I	N	L	I
Atazanavir +/- ritonavir[s]	10	16	20	24	32	33	34	36	46	48	**50**	53	54	60	62	64	71	73	82	**84**	85	**88**	90	93
	I	E	R	I	I	I	Q	I	I	V	L	L	L	E	V	L	V	C	A	V	V	S	M	L
	F		M			F		L	L			Y	V			M	I	S	T					M
	V		I			V		V					M			V	T	T	F						
	C		T										T				L	A	I						
			V										A												
Darunavir/ ritonavir[t]			V		V	L			I		I		I					T		I		L		
			11		32	33			**47**		**50**		**54**					74	**76**	**84**		89		
			I		I	F			V		V		M					P	V	V		V		
													L											
Fosamprenavir/ ritonavir	L				V				M	I	I		I					G	L	V		L		
	10				32				46	47	**50**		54					73	76	**84**		90		
	F				I				I	V	V		L					S	V	V		M		
	I								L				V						A					
	R												M						F					
	V																		S					
																			T					
Indinavir/ ritonavir[u]	L		K	L	V			M	M				I				A	G	L	V	V	I		L
	10		20	24	32			36	**46**				54				71	73	76	77	**82**	**84**		90
	I		M	I	I			I	I				V				V	S	V	I	A	V		M
	R		R						L								T	A	I		F			
	V																				T			
Lopinavir/ ritonavir[v]	L		K	L	V	L			M	I	I	F	I		L		A	G	L		V	I		L
	10		20	24	**32**	33			46	**47**	50	53	54		63		71	73	**76**		**82**	84		90
	F		M	I	I	F			I	V	V	L	V		P		V	S	V		A	V		M
	I		R						L	A			L				T				F			
	R												A								T			
	V												M								S			
													T											
													S											
Nelfinavir[u,w]	L				D			M	M								A			V	V	I	N	L
	10				**30**			36	46								71			77	82	84	88	**90**
	F				N			I	I								V			I	A	V	D	M
	I								L								T				F		S	
																					T			
																					S			
Saquinavir/ ritonavir[u]	L			L						G		I			I		A	G		V	V	I		L
	10			24						**48**		54			62		71	73		77	82	84		90
	I			I						V		V			V		V	S		I	A	V		M
	R											L					T				F			
	V																				T			
																					S			
Tipranavir/ ritonavir	L					L	M		K	M	I		I	Q			H		T		V	N	I		L	
	10					33	36		43	46	**47**		54	**58**			69		**74**		**82**	**83**	**84**		89	
	V					F	I		T	L	V		A	E			K		P		L	D	V		I	
							L						M				R				T				M	
							V						V												V	

MUTATIONS IN THE ENVELOPE GENE ASSOCIATED WITH RESISTANCE TO ENTRY INHIBITORS

	G	I	V	Q	Q	N	N
Enfuvirtide[x]	**36**	**37**	**38**	**39**	**40**	**42**	**43**
	D	**V**	**A**	**R**	**H**	**T**	**D**
	S		**M**				
			E				

Maraviroc[y] See User Note

MUTATIONS IN THE INTEGRASE GENE ASSOCIATED WITH RESISTANCE TO INTEGRASE STRAND TRANSFER INHIBITORS[z]

	T	L	E	T	F	E	G	Y	S	Q	N	R
Dolutegravir[aa]					121	138	140			**148**	155	263
					Y	A	A			**H**	H	K
						K	S			**K**		
										R		
Elvitegravir[bb]	**66**		**92**	97	**121**				**147**	148	**155**	263
	I		**Q**	A	**Y**				**G**	**H**	**H**	K
	A		G							**K**		
	K									**R**		
Raltegravir[cc]		74	92	97	**121**	138	140	**143**	148	148	**155**	263
		M	Q	A	**Y**	A	A	**R**	**H**	**H**	**H**	K
						K	S	**H**	**K**	**K**		
								C	**R**	**R**		

User Notes

a. Some nucleoside (or nucleotide) analogue reverse transcriptase inhibitor (nRTI) mutations, like T215Y and H208Y,[1] may lead to viral hypersusceptibility to nonnucleoside analogue reverse transcriptase inhibitors (NNRTIs), including etravirine,[2] in nRTI-treated individuals. The presence of these mutations may improve subsequent virologic response to NNRTI-containing regimens (nevirapine or efavirenz) in NNRTI-naive individuals,[3-7] although no clinical data exist for improved response to etravirine in NNRTI-experienced individuals. Mutations at the C-terminal reverse transcriptase domains (amino acids 293-560) outside of regions depicted on the figure bars may prove to be important for nRTI and NNRTI HIV-1 drug resistance. The clinical relevance of these connection domain mutations arises mostly in conjunction with thymidine analogue–associated mutations (TAMs) and M184V and they have not been associated with increased rates of virologic failure of etravirine or rilpivirine in clinical trials.[8-10] K65E/N variants are increasingly reported in patients experiencing treatment failure with tenofovir, stavudine, or didanosine. K65E usually occurs in mixtures with wild type. K65N gives an approximately 4-fold decrease in susceptibility. Patient-derived viruses with K65E and site-directed mutations replicate very poorly in vitro; as such, no susceptibility testing can be performed.[11,12]

b. The 69 insertion complex consists of a substitution at codon 69 (typically T69S) and an insertion of 2 or more amino acids (S-S, S-A, S-G, or others). The 69 insertion complex is associated with resistance to all nRTIs currently approved by the US Food and Drug Administration (FDA) when present with 1 or more TAMs at codons 41, 210, or 215.[13] Some other amino acid changes from the wild-type T at codon 69 without the insertion may be associated with broad nRTI resistance.

c. Tenofovir retains activity against the Q151M complex of mutations.[13] Q151M is the most important mutation in the complex (ie, the other mutations in the complex [A62V, V75I, F77L, and F116Y] in isolation may not reflect multidrug resistance).

d. Mutations known to be selected by TAMs (ie, M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E) also confer reduced susceptibility to all currently approved nRTIs[14] except emtricitabine and lamivudine, which in fact reverse the magnitude of resistance and are recommended with tenofovir or zidovudine in the presence of TAMS. The degree to which cross-resistance is observed depends on the specific mutations and number of mutations involved.[15-18]

e. Although reverse transcriptase changes associated with the E44D and V118I mutations may have an accessory role in increased resistance to nRTIs in the presence of TAMs, their clinical relevance is very limited.[19-21]

f. The M184V mutation alone does not appear to be associated with a reduced virologic response to abacavir in vivo. When associated with TAMs, M184V increases abacavir resistance.[22,23]

g. As with tenofovir, the K65R mutation may be selected by didanosine, abacavir, or stavudine (particularly in patients with nonsubtype-B clades) and is associated with decreased viral susceptibility to these drugs.[22,24,25] Data are lacking on the potential negative impact of K65R on clinical response to didanosine.

h. The presence of 3 of the following mutations—M41L, D67N, L210W, T215Y/F, K219Q/E—is associated with resistance to didanosine.[26] The presence of K70R or M184V alone does not decrease virologic response to didanosine.[27]

i. K65R is selected frequently (4%–11%) in patients with some nonsubtype-B clades for whom stavudine-containing regimens are failing in the absence of tenofovir.[28,29]

j. The presence of M184V appears to delay or prevent emergence of TAMs.[30] This effect may be overcome by an accumulation of TAMs or other mutations.

k. The T215A/C/D/E/G/H/I/L/N/S/V substitutions are revertant mutations at codon 215 that confer increased risk of virologic failure of zidovudine or stavudine in antiretroviral-naive patients.[31,32] The T215Y mutant may emerge quickly from one of these mutations in the presence of zidovudine or stavudine.[33]

l. The presence of K65R is associated with a reduced virologic response to tenofovir.[13] A reduced response also occurs in the presence of 3 or more TAMs inclusive of either M41L or L210W.[13] The presence of TAMs or combined treatment with zidovudine prevents the emergence of K65R in the presence of tenofovir.[34-36] There are no data to indicate differences in resistance patterns between tenofovir disoproxil fumarate and tenofovir alafenamide because the active drug component in both formulations is tenofovir.

m. There is no evidence for the utility of efavirenz, nevirapine, or rilpivirine in patients with NNRTI resistance.[37]

n. Resistance to etravirine has been extensively studied only in the context of coadministration with ritonavir-boosted darunavir. In this context, mutations associated with virologic outcome have been assessed and their relative weights (or magnitudes of impact) assigned. In addition, phenotypic cutoff values have been calculated, and assessment of genotype-phenotype correlations from a large clinical database have determined relative importance of the various mutations. These 2 approaches are in agreement for many, but not all, mutations and weights.[38-40] The single mutations L100I, K101P, and Y181C/I/V have a high relative weight with regard to reduced susceptibility and reduced clinical response compared with other mutations.[41,42] The presence of K103N alone does not affect etravirine response.[42] Accumulation of several mutations results in greater reductions in susceptibility and virologic response than do single mutations.[43-45]

o. Fifteen mutations have been associated with decreased rilpivirine susceptibility (K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, H221Y, F227C, and M230I/L).[46-48] A 16th mutation, Y188L, reduces rilpivirine susceptibility 6 fold.[48] K101P and Y181I/V reduce rilpivirine susceptibility approximately 50 fold and 15 fold, respectively, but are not commonly observed in patients receiving rilpivirine.[49-51] Mutations at position 138 (most notably 138A) may occur as natural polymorphisms, especially in non-B subtypes.[52] K101E, E138K, and Y181C, each of which reduces rilpivirine susceptibility 2.5 fold to 3 fold, occur commonly in patients receiving rilpivirine. E138K and to a lesser extent K101E usually occur in combination with the nRTI resistance–associated mutation M184I, which alone does not reduce rilpivirine susceptibility. When M184I is combined with E138K or K101E, rilpivirine susceptibility is reduced about 7 fold and 4.5 fold, respectively.[51,53-55] The combinations of reverse transcriptase–associated mutations L100I plus K103N/S and L100I plus K103R plus V179D were strongly associated with reduced susceptibility to rilpivirine. However, for isolates harboring the K103N/R/S or V179D as single mutations, no reduction in susceptibility was detected.[48,56]

p. Often, numerous mutations are necessary to substantially impact virologic response to a ritonavir-boosted protease inhibitor (PI).[57] In some specific circumstances, atazanavir might be used unboosted. In such cases, the mutations that are selected are the same as with ritonavir-boosted atazanavir, but the relative frequency of mutations may differ.

q. Resistance mutations in the protease gene are classified as "major" or "minor." Major mutations in the protease gene (positions in bold type) are defined as those selected first in the presence of the drug or those substantially reducing drug susceptibility. These mutations tend to be the primary contact residues for drug binding and may also be associated with reductions in virologic responses to therapy. Minor mutations generally emerge later than major mutations and by themselves do not have a substantial effect on phenotype. They may improve replication of viruses containing major mutations. So minor mutations are present as common polymorphic changes in HIV-1 nonsubtype-B clades. Mutations in *gag* cleavage sites may

confer resistance to all PIs and may emerge before mutations in protease. A large proportion of virus samples from patients with confirmed virologic failure on a PI-containing regimen is not found to have PI resistance–associated mutations. Preliminary data from recent studies suggest that several mutations in the Gag protein[58] may be responsible for reduced PI susceptibility in a subset of these patients.

r. Ritonavir is not listed separately, as it is currently used only at low doses as a pharmacologic booster of other PIs.

s. Many mutations are associated with atazanavir resistance. Their impacts differ, with I50L, I84V, and N88S having the greatest effect. Higher atazanavir levels obtained with ritonavir boosting increase the number of mutations required for loss of activity. The presence of M46I plus L76V might increase susceptibility to atazanavir when no other related mutations are present.[59]

t. HIV-1 RNA response to ritonavir-boosted darunavir correlates with baseline susceptibility and the presence of several specific PI resistance–associated mutations. Reductions in response are associated with increasing numbers of the mutations indicated in the figure bar. The negative impact of the protease mutations I47V, I54M, T74P, and I84V and the positive impact of the protease mutation V82A on virologic response to ritonavir-boosted darunavir were shown in 2 data sets independently.[60,61] Some of these mutations appear to have a greater effect on susceptibility than others (eg, I50V vs V11I). The presence at baseline of 2 or more of the substitutions V11I, V32I, L33F, I47V, I50V, I54L or M, T74P, L76V, I84V or L89V was associated with a decreased virologic response to ritonavir-boosted darunavir.[62]

u. The mutations depicted on the figure bar cannot be considered comprehensive because little relevant research has been reported in recent years to update the resistance and cross-resistance patterns for this drug.

v. In PI-experienced patients, the accumulation of 6 or more of the mutations indicated on the figure bar is associated with a reduced virologic response to ritonavir-boosted lopinavir.[63,64] The product information states that accumulation of 7 or 8 mutations confers resistance to the drug.[65] However, there is emerging evidence that specific mutations, most notably I47A (and possibly I47V) and V32I, are associated with high-level resistance.[66-68] The addition of L76V to 3 PI resistance–associated mutations substantially increases resistance to ritonavir-boosted lopinavir.[59]

w. In some nonsubtype-B HIV-1, D30N is selected less frequently than are other PI resistance–associated mutations.[69]

x. Resistance to enfuvirtide is associated primarily with mutations in the first heptad repeat (HR1) region of the gp41 envelope gene. However, mutations or polymorphisms in other regions of the envelope (eg, the HR2 region or those yet to be identified) as well as coreceptor usage and density may affect susceptibility to enfuvirtide.[70-72]

y. The activity of CC chemokine receptor 5 (CCR5) antagonists is limited to patients with virus that uses only CCR5 for entry (R5 virus). Viruses that use both CCR5 and CXC chemokine receptor 4 (CXCR4; termed dual/mixed [D/M] virus) or only CXCR4 (X4 virus) do not respond to treatment with CCR5 antagonists. Virologic failure of these drugs is frequently associated with outgrowth of D/M or X4 virus from a preexisting minority population present at levels below the limit of assay detection. Mutations in HIV-1 gp120 that allow the virus to bind to the drug-bound form of CCR5 have been described in viruses from some patients whose virus remained R5 after virologic failure of a CCR5 antagonist. Most of these mutations are found in the V3 loop, the major determinant of viral tropism.[73] There is as yet no consensus on specific signature mutations for CCR5 antagonist resistance, so they are not depicted in the figure. Some CCR5 antagonist–resistant viruses selected in vitro have shown mutations in gp41 without mutations in V3[74]; the clinical significance of such mutations is not yet known.

z. In site-directed mutants and clinical isolates, the mutation F121Y has a profound effect on susceptibility to elvitegravir and raltegravir and to a lesser extent to dolutegravir. Mutation R263K can be selected in vivo during treatment with dolutegravir and raltegravir and results in a 2- to 5-fold reduction in susceptibility to dolutegravir, elvitegravir, and raltegravir.[75-80]

aa. Several mutations are required in HIV integrase to confer high-level resistance to dolutegravir.[81] Cross-resistance studies with raltegravir- and elvitegravir-resistant viruses indicate that Q148H/R and G140S in combination with mutations L74I/M, E92Q, T97A, E138A/K, G140A, or N155H are associated with 5-fold to 20-fold reduced dolutegravir susceptibility[82] and reduced virologic suppression in patients.[83-86]

bb. Seven elvitegravir codon mutations have been observed in integrase strand transfer inhibitor treatment–naive and –experienced patients in whom therapy is failing.[87-93] T97A, which may occur as a polymorphism,[94] results in only a 2-fold change in elvitegravir susceptibility and may require additional mutations for resistance.[92,93] The sequential use of elvitegravir and raltegravir (in either order) is not recommended because of cross-resistance between these drugs.[92]

cc. Raltegravir failure is associated with integrase mutations in at least 3 distinct, but not exclusive, genetic pathways defined by 2 or more mutations including (1) a signature (major) mutation at Q148H/K/R, N155H, or Y143R/H/C; and (2) 1 or more additional minor mutations. Minor mutations described in the Q148H/K/R pathway include L74M plus E138A, E138K, or G140S. The most common mutational pattern in this pathway is Q148H plus G140S, which also confers the greatest loss of drug susceptibility. Mutations described in the N155H pathway include this major mutation plus either L74M, E92Q, T97A, E92Q plus T97A, Y143H, G163K/R, V151I, or D232N.[95] The Y143R/H/C mutation is uncommon.[96-100] E92Q alone reduces susceptibility to elvitegravir more than 20 fold and causes limited (< 5 fold) cross-resistance to raltegravir.[101-103] N155H mutants tend to predominate early in the course of raltegravir failure, but are gradually replaced by viruses with higher resistance, often bearing mutations G140S plus Q148H/R/K, with continuing raltegravir treatment.[96]

References to the User Notes

1. Clark SA, Shulman NS, Bosch RJ, Mellors JW. Reverse transcriptase mutations 118I, 208Y, and 215Y cause HIV-1 hypersusceptibility to non-nucleoside reverse transcriptase inhibitors. *AIDS*. 2006;20(7):981-984.

2. Picchio G, Vingerhoets J, Parkin N, Azijn H, de Bethune MP. Nucleoside-associated mutations cause hypersusceptibility to etravirine. *Antivir Ther.* 2008;13(Suppl 3):A25.

3. Shulman NS, Bosch RJ, Mellors JW, Albrecht MA, Katzenstein DA. Genetic correlates of efavirenz hypersusceptibility. *AIDS*. 2004;18(13): 1781-1785.

4. Demeter LM, DeGruttola V, Lustgarten S, et al. Association of efavirenz hypersusceptibility with virologic response in ACTG 368, a randomized trial of abacavir (ABC) in combination with efavirenz (EFV) and indinavir (IDV) in HIV-infected subjects with prior nucleoside analog experience. *HIV Clin Trials*. 2008;9(1):11-25.

5. Haubrich RH, Kemper CA, Hellmann NS, et al. The clinical relevance of non-nucleoside reverse transcriptase inhibitor hypersusceptibility: a prospective cohort analysis. *AIDS*. 2002;16(15):F33-F40.

6. Tozzi V, Zaccarelli M, Narciso P, et al. Mutations in HIV-1 reverse transcriptase potentially associated with hypersusceptibility to nonnucleoside reverse-transcriptase inhibitors: effect on response to efavirenz-based therapy in an urban observational cohort. *J Infect Dis.* 2004;189(9): 1688-1695.

7. Katzenstein DA, Bosch RJ, Hellmann N, et al. Phenotypic susceptibility and virological outcome in nucleoside-experienced patients receiving three or four antiretroviral drugs. *AIDS*. 2003;17(6):821-830.

8. von Wyl V, Ehteshami M, Demeter LM, et al. HIV-1 reverse transcriptase connection domain mutations: dynamics of emergence and implications for success of combination antiretroviral therapy. *Clin Infect Dis*. 2010;51(5):620-628.

9. Gupta S, Vingerhoets J, Fransen S, et al. Connection domain mutations in HIV-1 reverse transcriptase do not impact etravirine susceptibility and virologic responses to etravirine-containing regimens. *Antimicrob Agents Chemother.* 2011;55(6):2872-2879.

10. Rimsky L, Van Eygen V, Vingerhoets J, Leijskens E, Picchio G. Reverse transcriptase connection domain mutations were not associated with virological failure or phenotypic resistance in rilpivirine-treated patients from the ECHO and THRIVE Phase III trials (week 96 analysis). *Antivir Ther.* 2012;17(Suppl 1):A36.

11. Fourati S, Visseaux B, Armenia D, et al. Identification of a rare mutation at reverse transcriptase Lys65 (K65E) in HIV-1-infected patients failing on nucleos(t)ide reverse transcriptase inhibitors. *J Antimicrob Chemother.* 2013;68(10):2199-2204.

12. Chunduri H, Crumpacker C, Sharma PL. Reverse transcriptase mutation K65N confers a decreased replication capacity to HIV-1 in comparison to K65R due to a decreased RT processivity. *Virology.* 2011; 414(1):34-41.

13. Miller MD, 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(5):837-846.

14. Whitcomb JM, Parkin NT, Chappey C, Hellman NS, Petropoulos CJ. Broad nucleoside reverse-transcriptase inhibitor cross-resistance in human immunodeficiency virus type 1 clinical isolates. *J Infect Dis.* 2003;188(7):992-1000.

15. Larder BA, Kemp SD. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). *Science.* 1989; 246(4934):1155-1158.

16. Kellam P, Boucher CA, Larder BA. Fifth mutation in human immunodeficiency virus type 1 reverse transcriptase contributes to the development of high-level resistance to zidovudine. *Proc Natl Acad Sci USA*. 1992;89(5):1934-1938.

17. Calvez V, Costagliola D, Descamps D, et al. Impact of stavudine phenotype and thymidine analogues mutations on viral response to stavudine plus lamivudine in ALTIS 2 ANRS trial. *Antivir Ther.* 2002; 7(3):211-218.

18. Kuritzkes DR, Bassett RL, Hazelwood JD, et al. Rate of thymidine analogue resistance mutation accumulation with zidovudine- or stavudine-based regimens. *JAIDS*. 2004;36(1):600-603.

19. Romano L, Venturi G, Bloor S, et al. Broad nucleoside-analogue resistance implications for human immunodeficiency virus type 1 reverse-transcriptase mutations at codons 44 and 118. *J Infect Dis.* 2002;185(7):898-904.

20. Walter H, Schmidt B, Werwein M, Schwingel E, Korn K. Prediction of abacavir resistance from genotypic data: impact of zidovudine and lamivudine resistance in vitro and in vivo. *Antimicrob Agents Chemother.* 2002;46(1):89-94.

21. Mihailidis C, Dunn D, Pillay D, Pozniak A. Effect of isolated V118I mutation in reverse transcriptase on response to first-line antiretroviral therapy. *AIDS.* 2008;22(3):427-430.

22. Harrigan PR**,** Stone C, Griffin P, et al. Resistance profile of the human immunodeficiency virus type 1 reverse transcriptase inhibitor abacavir (1592U89) after monotherapy and combination therapy. CNA2001 Investigative Group. *J Infect Dis*. 2000;181(3):912-920.

23. Lanier ER, Ait-Khaled M, Scott J, et al. Antiviral efficacy of abacavir in antiretroviral therapy-experienced adults harbouring HIV-1 with specific patterns of resistance to nucleoside reverse transcriptase inhibitors. *Antivir Ther.* 2004;9(1):37-45.

24. Winters MA, Shafer RW, Jellinger RA, Mamtora G, Gingeras T, Merigan TC. Human immunodeficiency virus 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(4):757-762.

25. Svarovskaia ES, Margot NA, Bae AS, et al. Low-level K65R mutation in HIV-1 reverse transcriptase of treatment-experienced patients exposed to abacavir or didanosine. *JAIDS.* 2007;46(2):174-180.

26. Marcelin AG, Flandre P, Pavie J, et al. Clinically relevant genotype interpretation of resistance to didanosine. *Antimicrob Agents Chemother*. 2005;49(5):1739-1744.

27. Molina JM, Marcelin AG, Pavie J, et al. Didanosine in HIV-1-infected patients experiencing failure of antiretroviral therapy: a randomized placebo-controlled trial. *J Infect Dis.* 2005;191(6):840-847.

28. Hawkins CA, Chaplin B, Idoko J, et al. Clinical and genotypic findings in HIV-infected patients with the K65R mutation failing first-line antiretroviral therapy in Nigeria. *JAIDS*. 2009;52(2):228-234.

29. Wallis CL, Mellors JW, Venter WD, Sanne I, Stevens W. Varied patterns of HIV-1 drug resistance on failing first-line antiretroviral therapy in South Africa. *JAIDS.* 2010;53(4):480-484.

30. Kuritzkes DR, Quinn JB, Benoit SL, et al. Drug resistance and virologic response in NUCA 3001, a randomized trial of lamivudine (3TC) versus zidovudine (ZDV) versus ZDV plus 3TC in previously untreated patients. *AIDS*. 1996;10(9):975-981.

31. Violin M, Cozzi-Lepri A, Velleca R, et al. Risk of failure in patients with 215 HIV-1 revertants starting their first thymidine analog-containing highly active antiretroviral therapy. *AIDS*. 2004;18(2):227-235.

32. Chappey C, Wrin T, Deeks S, Petropoulos CJ. Evolution of amino acid 215 in HIV-1 reverse transcriptase in response to intermittent drug selection. *Antivir Ther*. 2003;8:S37.

33. Garcia-Lerma JG, MacInnes H, Bennett D, Weinstock H, Heneine W. Transmitted human immunodeficiency virus type 1 carrying the D67N or K219Q/E mutation evolves rapidly to zidovudine resistance in vitro and shows a high replicative fitness in the presence of zidovudine. *J Virol.* 2004;78(14):7545-7552.

34. Parikh UM, Zelina S, Sluis-Cremer N, Mellors JW. Molecular mechanisms of bidirectional antagonism between K65R and thymidine analog mutations in HIV-1 reverse transcriptase. *AIDS*. 2007;21(11): 1405-1414.

35. Parikh UM, Barnas DC, Faruki H, Mellors JW. Antagonism between the HIV-1 reverse-transcriptase mutation K65R and thymidine-analogue mutations at the genomic level. *J Infect Dis.* 2006;194(5): 651-660.

36. von Wyl V, Yerly S, Böni J, et al. Factors associated with the emergence of K65R in patients with HIV-1 infection treated with combination antiretroviral therapy containing tenofovir. *Clin Infect Dis.* 2008;46(8):1299-1309.

37. 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(12):835-838.

38. Benhamida J, Chappey C, Coakley E, Parkin NT. HIV-1 genotype algorithms for prediction of etravirine susceptibility: novel mutations and weighting factors identified through correlations to phenotype. *Antivir Ther.* 2008;13(Suppl 3):A142.

39. Coakley E, Chappey C, Benhamida J, et al. Biological and clinical cut-off analyses for etravirine in the PhenoSense HIV assay. *Antivir Ther*. 2008;13(Suppl 3):A134.

40. Vingerhoets J, Tambuyzer L, Azijn H, et al. Resistance profile of etravirine: combined analysis of baseline genotypic and phenotypic data from the randomized, controlled Phase III clinical studies. *AIDS.* 2010;24(4):503-514.

41. Haddad M, Stawiski E, Benhamida J, Coakley E. Improved genotypic algorithm for predicting etravirine susceptibility: comprehensive list of mutations identified through correlation with matched phenotype. 17th Conference on Retroviruses and Opportunistic Infections (CROI). February 16-19, 2010; San Francisco, California.

42. Etravirine [prescribing information]. 2013. Titusville, NJ, Janssen Therapeutics.

43. Scherrer AU**,** Hasse B, Von Wyl V, et al. Prevalence of etravirine mutations and impact on response to treatment in routine clinical care: the Swiss HIV Cohort Study (SHCS). *HIV Med.* 2009;10(10):647-656.

44. Tambuyzer L, Nijs S, Daems B, Picchio G, Vingerhoets J. Effect of mutations at position E138 in HIV-1 reverse transcriptase on phenotypic susceptibility and virologic response to etravirine. *JAIDS.* 2011; 58(1):18-22.

45. Tudor-Williams G, Cahn P, Chokephaibulkit K, et al. Etravirine in treatment-experienced, HIV-1-infected children and adolescents: 48-week safety, efficacy and resistance analysis of the phase II PIANO study. *HIV Med*. 2014;15(9):513-524.

46. Rilpivirine [prescribing information]. 2015. Titusville, NJ, Janssen Therapeutics.

47. Azijn H, Tirry I, Vingerhoets J, et al. TMC278, a next-generation nonnucleoside reverse transcriptase inhibitor (NNRTI), active against wild-type and NNRTI-resistant HIV-1. *Antimicrob Agents Chemother.* 2010;54(2):718-727.

48. Picchio GR**,** Rimsky LT, Van Eygen V, Haddad M, Napolitano LA, Vingerhoets J. Prevalence in the USA of rilpivirine resistance-associated mutations in clinical samples and effects on phenotypic susceptibility to rilpivirine and etravirine. *Antivir Ther.* 2014;19(8):819-823.

49. Cohen CJ**,** Andrade-Villanueva J, Clotet B, et al. Rilpivirine versus efavirenz with two background nucleoside or nucleotide reverse transcriptase inhibitors in treatment-naive adults infected with HIV-1 (THRIVE): a phase 3, randomised, non-inferiority trial. *Lancet*. 2011; 378(9787):229-237.

50. Molina JM, Cahn P, Grinsztejn B, et al. Rilpivirine versus efavirenz with tenofovir and emtricitabine in treatment-naive adults infected with HIV-1 (ECHO): a phase 3 randomised double-blind active-controlled trial. *Lancet*. 2011;378(9787):238-246.

51. Rimsky L, Vingerhoets J, Van Eygen V, et al. Genotypic and phenotypic characterization of HIV-1 isolates obtained from patients on rilpivirine therapy experiencing virologic failure in the phase 3 ECHO and THRIVE studies: 48-week analysis. *JAIDS*. 2012;59(1):39-46.

52. Hofstra M, Sauvageot N, Albert J, et al. Transmission of HIV drug resistance and the predicted effect on current first-line regimens in Europe. *Clin Infect Dis*. 2016;62(5):655-663.

53. Kulkarni R, Babaoglu K, Lansdon EB, et al. The HIV-1 reverse transcriptase M184I mutation enhances the E138K-associated resistance to rilpivirine and decreases viral fitness. *JAIDS.* 2012;59(1):47-54.

54. Hu Z, Kuritzkes DR. Interaction of reverse transcriptase (RT) mutations conferring resistance to lamivudine and etravirine: effects on fitness and RT activity of human immuno deficiency virus type 1. *J Virol*. 2011;85(21):11309-11314.

55. Xu HT, Asahchop EL, Oliveira M, et al. Compensation by the E138K mutation in HIV-1 reverse transcriptase for deficits in viral replication capacity and enzyme processivity associated with the M184I/V mutations. *J Virol.* 2011;85(21):11300-11308.

56. Haddad M, Napolitano LA, Frantzell A, et al. Combinations of HIV-1 reverse transcriptase mutations L100I + K103N/S and L100I + K103R + V179D reduce susceptibility to rilpivirine [Abstract H-677]. 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). September 10-13, 2013; Denver, Colorado

57. Hirsch MS, Günthard HF, Schapiro JM, et al. Antiretroviral drug resistance testing in adult HIV-1 infection: 2008 recommendations of an International AIDS Society–USA panel. *Clin Infect Dis*. 2008;47(2): 266-285.

58. Fun A, Wensing AM, Verheyen J, Nijhuis M. Human immunodeficiency virus Gag and protease: partners in resistance. *Retrovirology.* 2012;9:63.

59. Young TP, Parkin NT, Stawiski E, et al. Prevalence, mutation patterns, and effects on protease inhibitor susceptibility of the L76V mutation in HIV-1 protease. *Antimicrob Agents Chemother.* 2010;54(11): 4903-4906.

60. De Meyer S, Descamps D, Van Baelen B, et al. Confirmation of the negative impact of protease mutations I47V, I54M, T74P and I84V and the positive impact of protease mutation V82A on virological response to darunavir/ritonavir. *Antivir Ther.* 2009;14(Suppl 1):A147.

61. Descamps D, Lambert-Niclot S, Marcelin AG, et al. Mutations associated with virological response to darunavir/ritonavir in HIV-1-infected protease inhibitor-experienced patients. *J Antimicrob Chemother*. 2009; 63(3):585-592.

62. Darunavir [prescribing information]. 2015. Titusville, NJ, Janssen Therapeutics..

63. Masquelier B, Breilh D, Neau D, et al. Human immunodeficiency virus type 1 genotypic and pharmacokinetic determinants of the virological response to lopinavir-ritonavir-containing therapy in protease inhibitor-experienced patients. *Antimicrob Agents Chemother*. 2002; 46(9):2926-2932.

64. Kempf DJ, Isaacson JD, King MS, et al. Identification of genotypic changes in human immunodeficiency virus protease that correlate with reduced susceptibility to the protease inhibitor lopinavir among viral isolates from protease inhibitor-experienced patients. *J Virol.* 2001;75(16):7462-7469.

65. Lopinavir/ritonavir [prescribing information]. 2015. Abbott Park, IL, AbbVie Inc.

66. Mo H, King MS, King K, Molla A, Brun S, Kempf DJ. Selection of resistance in protease inhibitor-experienced, human immunodeficiency virus type 1-infected subjects failing lopinavir- and ritonavir-based therapy: mutation patterns and baseline correlates. *J Virol.* 2005; 79(6):3329-3338.

67. Friend J, Parkin N, Liegler T, Martin JN, Deeks SG. Isolated lopinavir resistance after virological rebound of a ritonavir/lopinavir-based regimen. *AIDS.* 2004;18(14):1965-1966.

68. Kagan RM, Shenderovich M, Heseltine PN, Ramnarayan K. Structural analysis of an HIV-1 protease I47A mutant resistant to the protease inhibitor lopinavir. *Protein Sci*. 2005;14(7):1870-1878.

69. Gonzalez LM, Brindeiro RM, Aguiar RS, et al. Impact of nelfinavir resistance mutations on in vitro phenotype, fitness, and replication capacity of human immunodeficiency virus type 1 with subtype B and C proteases. *Antimicrob Agents Chemother.* 2004;48(9):3552-3555.

70. Reeves JD, Gallo SA, Ahmad N, et al. Sensitivity of HIV-1 to entry inhibitors correlates with envelope/coreceptor affinity, receptor density, and fusion kinetics. *Proc Natl Acad Sci USA*. 2002;99(25):16249-16254.

71. Reeves JD, Miamidian JL, Biscone MJ, et al. Impact of mutations in the coreceptor binding site on human immunodeficiency virus type 1 fusion, infection, and entry inhibitor sensitivity. *J Virol.* 2004;78(10):5476-5485.

72. 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(3):1113-1119.

73. Maraviroc [prescribing information]. 2015. Research Triangle Park, NC, ViiV Healthcare**.**

74. Anastassopoulou CG, Ketas TJ, Sanders RW, Klasse PJ, Moore JP. Effects of sequence changes in the HIV-1 gp41 fusion peptide on CCR5 inhibitor resistance. *Virology.* 2012;428(2):86-97.

75. Malet I, Gimferrer AL, Artese A, et al. New raltegravir resistance pathways induce broad cross-resistance to all currently used integrase inhibitors. *J Antimicrob Chemother.* 2014;69(8):2118-2122.

76. Cahn P, Pozniak AL, Mingrone H, et al. Dolutegravir versus raltegravir in antiretroviral-experienced, integrase-inhibitor-naive adults with HIV: week 48 results from the randomised, double-blind, non-inferiority SAILING study. *Lancet*. 2013;382(9893):700-708.

77. Quashie PK, Mesplede T, Han YS, et al. Characterization of the R263K mutation in HIV-1 integrase that confers low-level resistance to the second-generation integrase strand transfer inhibitor dolutegravir. *J Virol.* 2012;86(5):2696-2705.

78. Souza Cavalcanti J, Minhoto LA, de Paula Ferreira JL, da Eira M, de Souza Dantas DS, de Macedo Brigido LF. In-vivo selection of the mutation F121Y in a patient failing raltegravir containing salvage regimen. *Antiviral Res.* 2012;95(1):9-11.

79. Margot NA, Hluhanich RM, Jones GS, et al. In vitro resistance selections using elvitegravir, raltegravir, and two metabolites of elvitegravir M1 and M4. *Antiviral Res.* 2012;93(2):288-296.

80. Brenner BG, Lowe M, Moisi D, et al. Subtype diversity associated with the development of HIV-1 resistance to integrase inhibitors. *J Med Virol*. 2011;83(5):751-759.

81. Frantzell A, Petropoulos C, Huang W. Dolutegravir resistance requires multiple primary mutations in HIV-1 integrase [CROI Abstract 121]. In Special Issue: Abstracts From the 2015 Conference on Retroviruses and Opportunistic Infections. *Top Antivir Med.* 2015;23(e-1):51.

82. Kobayashi M, Yoshinaga T, Seki T, et al. In vitro antiretroviral properties of S/GSK1349572, a next-generation HIV integrase inhibitor. *Antimicrob Agents Chemother.* 2011;55(2):813-821.

83. Raffi F, Rachlis A, Stellbrink HJ, et al. Once-daily dolutegravir versus raltegravir in antiretroviral-naive adults with HIV-1 infection: 48 week results from the randomised, double-blind, non-inferiority SPRING-2 study. *Lancet*. 2013;381(9868):735-743.

84. Eron JJ, Clotet B, Durant J, et al. Safety and efficacy of dolutegravir in treatment-experienced subjects with raltegravir-resistant HIV type 1 infection: 24-week results of the VIKING Study. *J Infect Dis*. 2013;207(5):740-748.

85. Seki T, Suyama-Kagitani A, Kawauchi-Miki S, et al. Effects of raltegravir or elvitegravir resistance signature mutations on the barrier to dolutegravir resistance in vitro. *Antimicrob Agents Chemother.* 2015;59(5):2596-2606.

86. DeAnda F, Hightower KE, Nolte RT, et al. Dolutegravir interactions with HIV-1 integrase-DNA: structural rationale for drug resistance and dissociation kinetics. *PLoS One.* 2013;8(10):e77448.

87. Goodman D, Hluhanich R, Waters J, et al. Integrase inhibitor resistance involves complex interactions among primary and second resistance mutations: a novel mutation L68V/I associates with E92Q and increases resistance. *Antivir Ther.* 2008;13(Suppl 3):A15.

88. Waters J, Margot N, Hluhanich R, et al. Evolution of resistance to the HIV integrase inhibitor (INI) elvitegravir can involve genotypic switching among primary INI resistance patterns. Fort Myers, FL. *Antivir Ther.* 2009;14(Supp 1):A137.

89. Doyle T, Dunn DT, Ceccherini-Silberstein F, et al. Integrase inhibitor (INI) genotypic resistance in treatment-naive and raltegravir-experienced patients infected with diverse HIV-1 clades. *J Antimicrob Chemother*. 2015;70(11):3080-3086.

90. Sax PE, DeJesus E, Mills A, et al. Co-formulated elvitegravir, cobicistat, emtricitabine, and tenofovir versus co-formulated efavirenz, emtricitabine, and tenofovir for initial treatment of HIV-1 infection: a randomised, double-blind, phase 3 trial, analysis of results after 48 weeks. *Lancet*. 2012;379(9835):2439-2448.

91. DeJesus E, Rockstroh J, Henry K, et al. Co-formulated elvitegravir, cobicistat, emtricitabine, and tenofovir disoproxil fumarate versus ritonavir-boosted atazanavir plus co-formulated emtricitabine and tenofovir disoproxil fumarate for initial treatment of HIV-1 infection: a randomised, double-blind, phase 3, non-inferiority trial. *Lancet*. 2012;379(9835):2429-2438.

92. Abram ME, Hluhanich RM, Goodman DD, et al. Impact of primary elvitegravir resistance-associated mutations in HIV-1 integrase on drug susceptibility and viral replication fitness. *Antimicrob Agents Chemother*. 2013;57(6):2654-2663.

93. White K, Kulkarni R, Miller MD. Analysis of early resistance development at the first failure timepoint in elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate-treated patients. *J Antimicrob Chemother*. 2015;70(9):2632-2638.

94. Scherrer AU, Yang WL, Kouyos RD, et al. Successful prevention of transmission of integrase resistance in the Swiss HIV Cohort Study. *J Infect Dis.* 2016;214(3):399-402.

95. Hazuda DF, Miller MD, Nguyen BY, Zhao J, 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:S10.

96. Gatell JM, Katlama C, Grinsztejn B, et al. Long-term efficacy and safety of the HIV integrase inhibitor raltegravir in patients with limited treatment options in a Phase II study. *JAIDS.* 2010;53(4):456-463.

97. Fransen S, Gupta S, Danovich R, et al. Loss of raltegravir susceptibility by human immunodeficiency virus type 1 is conferred via multiple nonoverlapping genetic pathways. *J Virol.* 2009;83(22):11440-11446.

98. Hatano H, Lampiris H, Fransen S, et al. Evolution of integrase resistance during failure of integrase inhibitor-based antiretroviral therapy. *J Acquir Immune Defic Syndr.* 2010;54(4):389-393.

99. Wittkop L, Breilh D, Da Silva D, et al. Virological and immunological response in HIV-1-infected patients with multiple treatment failures receiving raltegravir and optimized background therapy, ANRS CO3 Aquitaine Cohort. *J Antimicrob Chemother*. 2009;63(6):1251-1255.

100. Armenia D, Vandenbroucke I, Fabeni L, et al. Study of genotypic and phenotypic HIV-1 dynamics of integrase mutations during raltegravir treatment: a refined analysis by ultra-deep 454 pyrosequencing. *J Infect Dis.* 2012;205(4):557-567.

101. Cooper DA, Steigbigel RT, Gatell JM, et al. Subgroup and resistance analyses of raltegravir for resistant HIV-1 infection. *N Engl J Med*. 2008;359(4):355-365.

102. Malet I, Delelis O, Valantin MA, et al. Mutations associated with failure of raltegravir treatment affect integrase sensitivity to the inhibitor in vitro. *Antimicrob Agents Chemother*. 2008;52(4):1351-1358.

103. Blanco JL, Varghese V, Rhee SY, Gatell JM, Shafer RW. HIV-1 integrase inhibitor resistance and its clinical implications. *J Infect Dis.* 2011;203(9):1204-1214.